

Proceedings of

# The 4<sup>th</sup> International Seminar on Livestock Production and Veterinary Technology

“TECHNOLOGY INNOVATIONS AND COLLABORATIONS IN  
LIVESTOCK PRODUCTION FOR SUSTAINABLE  
FOOD SYSTEMS”

Virtual seminar: September 6-7<sup>th</sup>, 2021



**IAARD  
PRESS**

**Proceedings of**  
**The 4<sup>th</sup> International Seminar**  
**on Livestock Production**  
**and Veterinary Technology**

**"Technology Innovations and Collaborations in Livestock  
Production for Sustainable Food Systems"**  
**Bogor, September 6-7<sup>th</sup>, 2021**



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**“Technology Innovations and Collaborations in Livestock  
Production for Sustainable Food Systems”  
Bogor, September 6-7<sup>th</sup>, 2021**



Indonesian Center for Animal Research and Development  
in collaboration with  
Faculty of Animal Husbandry, University of Mataram

THE 4<sup>th</sup> INTERNATIONAL SEMINAR ON LIVESTOCK PRODUCTION  
AND VETERINARY TECHNOLOGY

“Technology Innovations and Collaborations in Livestock Production for  
Sustainable Food Systems”, Bogor, September 6-7<sup>th</sup>, 2021

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“Technology Innovations and Collaborations in Livestock Production for Sustainable Food Systems”

Bogor, September 6-7<sup>th</sup>, 2021

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## Preface

Indonesian Centre for Animal Research and Development (ICARD), in collaboration with the Faculty of Animal Science at the University of Mataram, has held the 4<sup>th</sup> International Seminar on Livestock Production and Veterinary Technology virtually on September 6-7<sup>th</sup>, 2021. This seminar brings the topic on “Technology Innovations and Collaborations in Livestock Production for Sustainable Food Systems”, which faces current livestock conditions in Indonesia and is highly strategic to deliver the innovation to users.

The seminar is an important strategy to share information on the latest technology innovation on livestock and veterinary aspects and build scientific networks nationally and worldwide with researchers, academicians, and practitioners. Currently, ICARD focus on cross-ministerial collaboration to influence stakeholders and the public community to implement and adopt research findings. In a research system era, collaboration among broad spectrum channels is necessary to be conducted to get feedback from users. Therefore, research should be driven by the market and the invention and development of comprehensive agricultural technology innovations are expected to increase agricultural production and productivity competitively through good collaboration.

The seminar is officially opened by the Indonesian Minister of Agriculture, who deliver a keynote speech, followed by the Director General (DG) of the Indonesian Agency for Agricultural Research and Development to welcome a speech for all distinguished participants. There were seven invited speakers from the USA, Australia, New Zealand, France, China, and Indonesia. There are 56 submitted papers invited, evaluated, and selected by the committee from 83 papers from universities, research institutions, and local livestock services. The supporting paper was classified into four sub-themes, *i.e.* Livestock Production, Veterinary Science, Agricultural Social Economics and Policy, and Livestock Nutrition and Feed Technology. There are 486 participants from all provinces in Indonesia who attended this seminar.

We hope that this venue will bring us to a broader opportunity to strengthen our capacity and ensure that we will build a better productive food system for the nation.

Bogor, December 2021

Director of ICARD,

Dr. drh. Agus Susanto, M.Si.



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## **Report from Chairman of Committee The 4<sup>th</sup> International Seminar on Livestock Production and Veterinary Technology**

- His Excellency Indonesian Minister of Agriculture, also as the Keynote Speaker, **Dr. H. Syahrul Yasin Limpo, S.H., M.H.**
- Director General of Indonesian Agency for Agricultural Research and Development, **Dr. Ir. Fadry Djufry, M.Si.**
- Rector of University of Mataram, **Prof. Dr. Lalu Husni, S.H., M.Hum.**
- All invited speakers:
- Participants as experts, researchers, observers on animal husbandry and veterinary and also practitioners.

**Assalaamu'alaikum Warohmatullaahi Wabarokaatuh**  
**Good morning, ladies and gentlemen**

First of all, let us thank Allah Almighty for being merciful and blessing to all of us to gather in good health and be assembled here in the Opening Ceremony of the fourth International Seminar on Livestock and Veterinary Technology. On behalf of the organizing committee, I would like to welcome you all to this Seminar held virtually. A deep appreciation is extended to you, especially to the overseas participants who have been willing to participate in this seminar.

As the Chairman of the Organizing Committee, I am proud to organize this International Seminar on Livestock Production and Veterinary Technology with the main theme is *“Technology Innovations and Collaborations in Livestock Production for Sustainable Food Systems”*.

**His excellency, distinguished guest, ladies and gentlemen**

As the committee, I would like to report that more than 600 participants are attending this seminar. Fifty-six submitted papers were invited, evaluated and selected by the committee from 83 papers. The overseas participants are from universities and research institutions from America, Australia, New Zealand, France, and China. The Indonesian participants come from universities in Indonesia, research institutions under the Ministry of Agriculture; Ministry of Education, Culture and Research Technology; Indonesian Institute of Science; private institutions and associations.

There will be seven invited speakers; one comes from Indonesia and six others from overseas. The Indonesian Minister of Agriculture will give a keynote speech that relates to the theme of this Seminar.

His excellency, distinguished guest, ladies, and gentlemen

As is always the case, nothing in the world is perfect. On behalf of the organizing committee, I apologize for any shortcomings in organizing this seminar. I would like to thank the steering committee for the direction and all organising committee members, both from the Indonesian Centre for Animal Research and Development and University of Mataram, for their hard work, which has made it possible for us to be here today.

Finally, please allow me to request His Excellency, The Indonesian Minister of Agriculture, to give the keynote speech and officially open this Conference.

Wassalamu'alaikum Warahmatullaahi Wabarakaatuh

Bogor, September 6<sup>th</sup>, 2021

Chairman of Organizing Committee,

Dr. Ir. Endang Romjali, MSc.

## **Remarks from The Rector, University of Mataram**

The honorable Minister of Agriculture **Dr. Syahrul Yasin Limpo**,  
The Director General of the Indonesian Agency of Agricultural Research and  
Development **Dr. Ir. Fadjry Djufray, M.Si.**

The invited speakers from Australia, New Zealand, USA, France and China  
All presenters and seminar participants

Welcome to the 4th International Seminar on Livestock Production and  
Veterinary Technology.

It is my pleasure to welcome you all and to thank you for your contribution to  
this international seminar.

This seminar is an important activity for our university because dissemination  
of research results is one of our mandates.

This is a great opportunity of all scientist, practitioners and students to  
exchange ideas and learn from each other to further develop our livestock  
industry.

This is the first collaborative international seminar between University of  
Mataram and the Indonesian Centre of Agricultural Research and  
Development, Ministry of Agriculture republic of Indonesia. I hope we can  
continue our collaboration in the future.

Please enjoy this academic event and I hope you can benefit from the  
interactions between scientists from many different countries participating in  
this seminar.

Thank you

Mataram, September 6, 2021  
The Rector of University of Mataram

Prof. Dr. Lalu Husni, S.H., M.Hum.

## **Welcome Remarks from Director General of Indonesian Agency for Agricultural Research and Development**

- His Excellency Indonesian Minister of Agriculture, also as the Keynote Speaker, Dr. H. Syahrul Yasin Limpo, S.H.
- All invited speakers from Australia, USA, France, New Zealand, China as well as from Indonesia
- In particular, I warmly welcome the participants consisting of experts, researchers, observers on animal husbandry and veterinary and also practitioners

### **Assalaamu'alaikum warahmatullaahi wabarakaatuh**

First of all, let us thank Allah Almighty for merciful and blessing to all of us so that this morning we can attend the 4<sup>th</sup> International Seminar on Livestock Production and Veterinary Technology.

### **Good morning, ladies and gentlemen**

Indonesian Centre for Animal Research and Development is one of the Centres within the IAARD with their tasks of producing inventions and innovations related to livestock and animal health.

One of their mandates is to generate a new breed of livestock and or a new variety of forages with superior productivity and well adapted to the changing climate.

Not only that, research institutes within the coordination of ICARD are encouraged to obtain supporting technologies for better livestock productivity such as animal health, nutrition and reproduction of poultry and ruminants.

### **Colleagues, ladies and gentlemen,**

Once the invention has been produced, it is time to deliver to many stake holders such as local government, private sectors, industry as well as small holder farmers

Those invention in livestock and animal health are meant to support the food system especially from the livestock sub sector.

Those invention too, will be used to welfare the small holder farmers, because on their backbone, the production of meat, eggs and milk are relied on. Since 96% of our animal-based protein source are produced by small holder farmers.

**Distinguished ladies and gentlemen,**

This international seminar is a very challenging opportunity, an avenue to meet and greet colleagues worldwide.

Through this occasion, we are delighted to collaborate with other institutions to strengthen our output and impact.

Let us work together to strengthen our capacity and ensure that we will build a better productive food system for the nation.

**Distinguished participants,**

I would like to express my gratitude to the invited speakers from Australia, USA, France, New Zealand, China, and Indonesia. I would also like to thank all participants who have submitted their papers. There are 59 submitted papers were invited, evaluated and selected by the committee

I hope this conference will be a media to exchange information among participants from different countries and share the experience of the technology application towards modern agriculture and sustainability.

**Colleagues, ladies and gentlemen,**

At this very special occasion, I look forward to hearing your discussions and learning from your insights.

Finally, please allow me to request His Excellency, The Indonesian Minister of Agriculture, to give the keynote speech and officially open this Seminar.

Wassalamu'alaikum wa rohmatullahi wa barokatuh.

Jakarta, September 6, 2021

Director General of IAARD

Dr. Ir. Fadjry Djufry, M.Si.

## **Keynote Speech Indonesian Minister of Agriculture**

**Assalaamu'alaikum warahmatullaahi wa barakaatuh**

**Distinguished Invited Speakers,**

**Ladies and Gentlemen,**

First of all, let us thank Allah Almighty for being merciful and blessing to all of us to attend the 4th International Seminar on Livestock Production and Veterinary Technology this morning. This seminar is an annual agenda of the Indonesian Agency for Agricultural Research and Development (IAARD), which will be held in collaboration with the University of Mataram. During the Covid-19 pandemic, which is entering its second year, I am grateful that this scientific activity can still be held virtually.

**Distinguished Ladies and Gentlemen,**

This international seminar in 2021 brings the theme on "*Technology Innovation and Collaboration in Livestock Production for Sustainable Food System*", is very important to be held to convey research results and exchange the latest information and knowledge nationally and globally.

**Distinguished Ladies and Gentlemen,**

The year 2021 is a meaningful time, as the unfavourable global and national economic conditions, partly due to the Covid-19 pandemic, which is estimated to continue until 2022. Of the various sectors contributing to economic growth, the agricultural sector still grows around 14% until the second half of 2020. Meanwhile, in line with the COVID-19 outbreak that is starting to be under control, it is expected that the Indonesian economy can grow and be positive in the second quarter of 2021, even though in the first quarter of 2021, it is still minus 0.6-0.9 per cent year to year basis. However, the government's efforts have to continue to be carried out to prevent a second wave of Covid-19 attacks.

The existence of restrictions on large-scale population activities, followed by restrictions on micro-scale activities, results in changes in consumption behavior and impacts the availability of employment opportunities, which impact income, and finally on the ability to select food.

**Distinguished Ladies and Gentlemen,**

The medium-term development target of 2020-2024 is to achieve an independent, advanced, equitable and prosperous Indonesian society through accelerating development in various fields. This is done by emphasizing the

establishment of a solid economic structure based on comparative and competitive advantages in various economic sectors and regions. This national development is supported by the wealth of natural resources, qualified human resources, advanced technology and consolidated institutions that are able to produce competitive and sustainable products.

The development of agriculture and food is directed to actualize advanced, independent and modern agriculture in order to increase economic growth and farmers' welfare. Advanced agriculture can be interpreted by improving the quality of agricultural human resources that are able to increase work productivity and welfare. Meanwhile, independent agriculture can be interpreted that agricultural development is based on domestic capabilities in accordance with community conditions. Modern agriculture means that innovation-based agricultural development is in line with the 4.0 industrial revolution.

#### **Distinguished Ladies and Gentlemen,**

In line with the efforts to fulfil sustainable food, the Indonesian government has designed a food estate program based on food commodities, horticulture, plantations and livestock in various locations. In 2020 food estates have been developed in 3 provinces, namely Central Kalimantan, North Sumatra and West Nusa Tenggara and will be extended to 8 other provinces next year. Local governments are also expected to participate in building food estates at the provincial and district levels. The involvement of the private sector in food estates as actors and off-takers of the products produced is expected to build a sustainable food system in Indonesia. The breakthrough was made in response to the FAO's warning of a possible food crisis in the world.

Innovative livestock technology invented by academics must be able to answer the challenge of fulfilling food from livestock. The formation of superior livestock strains that are adaptive to climate change, the use of local materials as a source of feed processed and stored for a long term, and the use of plant-based feed additives and natural microbes are expected to increase livestock productivity. Utilization of this biomass can increase the region's capacity to support the increasing livestock population with superior genetic resources, and it is expected to achieve Food Sovereignty.

#### **Distinguished Ladies and Gentlemen,**

The success of sustainable agricultural development cannot be separated from the obvious role of farmers because they contribute about 95% of food production for Indonesia's 272 million population. The development of farmer

corporations in the area of food economic resources is expected to be able to provide a strong position for farmers.

Farmer's corporation is an institutional model of economic cooperation of a group of farmers with an agribusiness orientation through land consolidation into one overlay, while ensuring the ownership of each farmer's land. It is expected that through farmer corporations, resource management will be more optimal because it is carried out in a more integrated, consistent, and sustainable form that builds more efficient, effective, and have high-quality standards of business to encourage economic growth in rural areas.

In line with the establishment of a farmer corporation, the implementation of digitalization will improve the position of farmers, both in providing production inputs, marketing products, and networking with food processors.

**Distinguished Ladies and Gentlemen,**

From this seminar, I hope that the latest ideas and technologies that can contribute to accelerating sustainable agricultural development can be informed so that the vision of Indonesia's agricultural development can be achieved. Academic colleagues from abroad who join in this international seminar are expected to join hands to build an updated research climate.

**Distinguished Ladies and Gentlemen,**

I am highly welcome and put high hopes for the results of this seminar. Let us work together earnestly, not easily give up and sincerely, to build Indonesian agriculture from the livestock sub-sector, which is expected to achieve animal food sovereignty. God willing, Allah SWT will provide guidance, the best solution, His mercy, and blessing for all of us. Aamiin.

By saying Bismillahhirohmannirrohim, I declare that the 4<sup>th</sup> International Seminar on Livestock Production and Veterinary Technology in 2021 is officially commenced.

**Wassalamu'alaikum wa rohmatullahi wa barokatuh.**

Jakarta, September 6, 2021

Minister of Agriculture

Dr. H. Syahrul Yasin Limpo, S.H., M.H.



## **Summary of The 4<sup>th</sup> International Seminar of Livestock Production and Veterinary Technology**

### **INTRODUCTION**

The success of sustainable agricultural development cannot be separated from the obvious role of farmers because they contribute about 95% of food production for Indonesia's 272 million population. The development of agriculture and food is directed to actualize advanced, independent, and modern agriculture to increase economic growth and farmers' welfare. Advanced agriculture can be interpreted by improving the quality of agricultural human resources that can increase work productivity and welfare.

In the years to come, this would be in the form of Farmer's Corporation is an institutional model of economic cooperation of a group of farmers with an agribusiness orientation through land consolidation into one overlay. It is expected that resource management will be more optimal due to being carried out in a more integrated, consistent, and sustainable form that builds more efficient, effective, and have high-quality standards of business to encourage economic growth in rural areas.

To achieve food security, livestock enterprise and industry efficiency must increase as the global population increases. Climate change requires cattle in the tropics to tolerate some challenges in improving their productivity and reducing greenhouse gas emissions. Some technologies must be implemented by smallholder beef and dairy farmers through improved value chains that reward farmers for the quality of their products. The formation of new multi-organizational, multi-disciplinary collaborative partnerships will assist in such transformation.

ICARD and its implementation unit have the mandate to produce inventions and have to be delivered to stakeholders such as local government, private sectors, the industry as well as smallholder farmers. Those inventions in livestock and animal health are meant to support the food system especially from the livestock sub-sector and in turn, will enhance farmers' welfare. The production of meat, eggs, and milk has relied on small-holder farmers since 96% of our animal-based protein sources are produced by them.

Indonesian Centre for Animal Research and Development in collaboration with the Faculty of Animal Science, University of Mataram has held virtually the 4th International Seminar on Livestock Production and Veterinary Technology on September 6-7th, 2021. The seminar brings the theme on

“Technology Innovations and Collaborations in Livestock Production for Sustainable Food Systems” and officially opened by Indonesian Minister of Agriculture, Dr. H. Syahrul Yasin Limpo, S.H., M.H. A total of 57 papers were presented, including 7 papers of invited speakers from the USA, France, Australia, New Zealand, China, and Indonesia as well. Contributed papers were categorized into 4 areas, i.e. Livestock Production, Veterinary Science, Agricultural Social Economics and Policy, and Livestock Nutrition and Feed Technology.

## LIVESTOCK PRODUCTION

Technological developments in the field of molecular genetics related to reproduction include: The haplotype from block 1 (SNP 1 and SNP 2 combination) could be used for further association analysis with reproduction traits in Sragen and Jabres cattle; Identification of SNP BTA10 to Twinning Birth Trait of Simmental - Ongole Grade Crossing; The diversity of CAST|AluI genes can be used as a source of basic information in the selection of superior livestock related to the tenderness of meat in beef cattle; association of prolactin gene with Laying Traits in Merawang and KUB-2 Chicken were found that the same SNP at Merawang and KUB-2 chicken. Regarding the domesticated indigenous chickens, which were derived from the Red Junglefowl subspecies *Gallus gallus spadiceus* distributed in southwestern China, northern Thailand, and Myanmar, it is recommended to develop reliable genomic markers for monitoring the impact and efficiency of genetic improvement for indigenous chickens.

Increased livestock productivity to produce more meat has been carried out, among others for beef cattle with the introduction of Belgian Blue cattle crossed with FH cows had shown the highest birth weight and body size among Ongole grade, Simmental, Brahman, and Limousine. This indicates that the birth weight and body size of crossbred Belgian Blue calves were affected by genotypes. Likewise, Dorper sheep were crossed with local Garut sheep, showed offspring with better production performance, able to utilize low-quality of feed, adaptability to heat stress, and resistance to parasites.

Reproductive aspects are also a lot of attention. Observation of reproductive performance of dairy farming under 5 KUD's in West Java had shown that the reproductive performances, *i.e.* calving interval, average first calving, parity, service per conception, body condition score, and body weight of the cows considered in good condition, as for the differences in the reproductive performance of cows between locations could be due to differences in mating management. Nerve Growth Factor levels in the seminal plasm positively

correlated with sperm motility, related to sperm fertility. There was no effect of sexual dimorphism on body weight, scrotal circumference, and testosterone levels Bali bull age of 1,5 to 2 years. The performance of PE goats in the breeding source area needs to be improved in quality, namely by introducing reproduction and management technology. No significant difference of both in Nulliparous Sapera Goat treated with GnRH and without the addition of GnRH on litter size, colostrum, and milk production that synchronized by PGF2 $\alpha$ . The germ line chimeras were obtained, which was indicated by the presence of PGCs in the recipient embryo. The black plumage quail's external quality and reproductive efficiency were higher than the brown plumage quail.

In comparison to Indonesia, the dairy industry in New Zealand is mostly under a pastoral-based production system and cooperative industry structure. The New Zealand dairy industry is an integrated system, encompassing the production, manufacturing, and marketing of milk products with the industry is based on cooperative, farmer-owned structures wherein milk processors (e.g. Fonterra) focus on maximizing financial return to shareholder farmers. Genetic selection and herd recording are carried out by breeding companies, the largest being livestock improvement corporation (a farmer-owned co-op), along with research and extension from dairy, which is paid for by an industry levy on each kg milk.

Research results related to post-harvest, including processing chicken meat and eggs to improve product quality and added value

## **VETERINARY SCIENCE**

One of the most common zoonotic infections in the world is brucellosis. Control of human brucellosis depends on controlling its source which requires a 'One Health' approach with actors in both animal and human health working together. Another thing that still gets attention is Avian Influenza, showing that the most pathogenic AI-H5N1-clade 2.3.2 was Wates isolates, followed by the Lamongan and Karawang (clade 2.1.3).

Cases of parasites in livestock such as helminthiasis distributed highly in areas examined (Malang, Banten, Waingapu, and Denpasar)

Drug residues in livestock products such as enrofloxacin were found as a residue in liver samples of a broiler so that proper action should be taken to avoid the emergence of antibiotic resistance in consumers as well as in the environment. Trenbolone acetate hormone residues were detected on meat from the slaughter house and cold storage in Jakarta, West Java, and Banten.

High nitrite was detected in the edible bird nest. Several other research results, namely chemical agent (deltamethrin), traditional medicine, and the effect of the housing system in disease control.

## **AGRICULTURAL SOCIO-ECONOMICS AND POLICY**

The pandemic of Covid-19 in Indonesia generally significantly affects the beef price but does not affect broiler and egg prices. In the case of the traditional market in Manado, the income of the broiler business was influenced by the volume of broiler sold, selling price, and market cost. Alabimaster-1 Agrinak duck development is considered economically liable as the breeding practices of duck farmers. Hence, it is prospective to be developed massively and intensively.

In beef cattle farming, analysis of daily Income Over Feed Cost (IOFC) suggests that growth rates can be increased and a system for formulating rations to supply a certain amount of nutrients or target a specific growth rate on a least-cost basis would increase IOFC. A least-cost ration (LCR) formulation system provides a way of formulating rations to increase the ME content at the least cost. Potential for growth declines from EuroxOngole to Ongole to Bali cattle but the IOFC for bulls of different breeds may not differ very much. An LCR process that accommodates feed price fluctuations can be used to formulate rations that promote high growth rates to increase IOFC.

Regarding human resources, it shows that do not directly affect the development of smallholder beef cattle farming but through various aspects of resources that support these development efforts.

## **LIVESTOCK NUTRITION AND FEED TECHNOLOGY**

Research on livestock nutrition, the use of feed ingredients derived from waste and agricultural by-products is still being carried out, including: The treatment of palm fronds on rations had a significant effect on the daily weight gain for Bali cattle and as a result, it was also able to increase the BCS value and conception rate due to improved feed ration formula; Rice bran and other rejected rice in the ration can replace the use of commercial chicken ration and show economic efficiency of the ration; The whole-plant corn has good quality as ruminants feed; the provision of fermented maize stalks; combined silage of grass and Indigofera and concentrate gives Bali cows better performance than other treatments; The combination of cashew nut shell by product as feed demonstrate the ability to reduce methane emission; the utilization of noni and clove oil did not show significant effect to the increasing size of internal

organs of broiler duck compared to the control; The best method to encapsulate gambir was one stage method and 10% gambir in EG could be protected 70% at pH 2 solution; The inclusion of Palm Kernel Cake in laying hens diet reduced the egg production and impaired the FCR, and supplementation of DGDH enzyme slightly improved the egg production and FCR; The optimal liquid monosodium glutamate by-product (MBP) supplementation in cattle-concentrate feed based on agricultural by-products was 8%. Other research results are: the substitution of Indigofera flour to the egg production of Sensi and KUB local chicken showed that it increased the egg production, reduce feed cost and in turn increase the farmer's income; The supplementation of Bioport was not sufficient to prevent the impact of the stress response of the Anpera goat; Drying methods can be used in preserving BSF larvae as a source of dietary protein for farm animals

Other research that is still attracting attention is about the reduction of methane gas in ruminants, among others: One possible strategy to reduce green house gas emissions is dietary modifications that include feeding tannin-rich diets to cattle and other ruminants. GHG reduction strategies should be established to increase ruminant production efficiency while minimizing losses of  $CH_4$  and volatile organic compounds from animal agriculture. However, the low level on the use of tannin concentration has not been able to reduce methane emissions.



# Invited Papers



## **Opportunities and Challenges to Increasing Productivity in Cattle Farmed by Smallholders in Asia and Africa**

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### **ABSTRACT**

The global population is expected to grow rapidly by 2050, with most growth occurring in Africa and Asia. To achieve food security, livestock enterprise and industry efficiency must increase by 2.0-2.5% per annum, equivalent to doubling outputs from constant resources over 30 years. Due to pressures on agriculture in developed countries, most increased production must occur in regions of greatest need (Africa and Asia) and the face of greater competition for land, water, grain, and labour, leading to higher costs of production. Climate change adds to the challenge, requiring livestock that are productive under hotter and drier climates and, in the tropics, requiring cattle that tolerate increased parasite burdens and vector-borne diseases. Productivity of cattle herds must therefore be significantly improved and greenhouse gas emissions reduced, but using less grain and water, while animals tolerate more extreme climates and disease stressors. To double outputs from constant resources, new, cost-effective and transformational technologies must be implemented by smallholder beef and dairy farmers through improved value chains that reward farmers for the quality of their product. The formation of new multi-organizational, multi-disciplinary collaborative partnerships will assist in such transformation. This paper examines several emerging technologies for their potential to deliver the productivity improvements required from beef and dairy cattle in smallholder farmer herds in Africa and Asia. It also examines the potential for transformational approaches to support new markets, value chains, and collaborations across public and private sector partnerships.

**Keywords:** Improved productivity, Beef and dairy cattle, Smallholder farmers in Asia and Africa

### **INTRODUCTION**

Although major differences exist between the productivity of cattle producers in advanced and low-middle-income countries (LMICs), several very significant challenges need to be overcome by all cattle farmers,

regardless of their location, if they are to capture new opportunities that also exist.

The world's population is expected to increase from 7 billion people in 2011 to 9 or 10 billion by 2050, with most of that growth occurring in Africa and Asia (Gerland et al. 2014). The incomes of many people in LMICs are now increasing and, with rising incomes, the demand for meat and dairy products is also increasing (Delgado et al. 1999). To achieve food security by 2050, livestock enterprise and industry efficiency as measured by Total Factor Productivity need to increase by 2.0-2.5% per annum. This is the equivalent of doubling outputs from constant resources through to 2050 (Mullen 2012). Due to pressures on agriculture in developed countries, a significant proportion of that increased production must occur in the regions of greatest need *i.e.* in Africa and Asia.

This increased demand for food is leading to greater competition for inputs such as land, water, grain, and labour, driving up the cost of beef and dairy production. Climate change will add to this challenge (Hughes 2003), requiring livestock that are productive under hotter and drier climates and, in the tropics and sub-tropics, requiring cattle that can tolerate significant increases in ecto-parasitic and endo-parasitic burdens and vector-borne diseases. There is therefore an urgent need to greatly increase the productivity of cattle herds but using less grain and water, whilst the cattle simultaneously tolerate more extreme climates and disease stressors and reduce their methane emissions per kg of product. An added benefit of improving production efficiency is that emissions intensity decreased for most livestock species globally between 2000 and 2018 because of increased production efficiency [5]. Those authors also showed that improving production efficiency, particularly in countries in Asia, South America, and Africa, has much greater mitigation effects than removing livestock products from global human diets (Chang et al. 2021).

The opportunities to significantly improve the productivity of livestock systems are greatest for extensive or pastoral production systems in tropical and sub-tropical environments, including those in Africa and Asia. These systems utilize land resources with few alternative uses, including urbanization. In addition, they capitalize on the strengths of ruminants, which utilize low-quality pastures that are not suitable for humans or mono-gastric livestock species. The pastoral livestock industries are also far less likely than the intensive livestock industries to face inequitable demands about their production systems from urban populations, as has occurred over recent years in the intensive livestock industries.

Hence, to double outputs from constant resources as will be required for global food security by 2050, cattle farmers in the tropics and sub-tropics

will need to adopt new, cost-effective and transformational technologies for use in animals that are well adapted to their production environments.

Traditional technologies which deliver incremental changes will assist in improving productivity, in the same way as they have in the past. By way of example, one study demonstrated that through the use of long-established technologies such as animal breeding and animal nutrition, US dairy farmers now require 21% fewer cows, 23% less feed, 65% less water, and 90% less land to produce 1 billion kg of milk than they did in 1944, with simultaneous large reductions in waste (Capper et al 2009). However, these traditional technologies are no longer sufficient to deliver the major productivity increases now required.

This paper, therefore, examines several emerging technologies for their potential to deliver the productivity improvements needed from cattle raised in pastoral production environments in the tropics and sub-tropics. It considers only animal-based technologies, although the author readily acknowledges that alternative technologies such as technologies to significantly increase the shelf life of beef and dairy products, improved meat and milk processing methods, and possibly production of artificial meat will also contribute significantly to improved industry productivity and production through to 2050.

## **THE NEED FOR ADAPTED CATTLE BREEDS**

Maximizing beef and dairy production and profitability require the breed to be matched to the particular production environment. Indigenous livestock which evolved in stressful tropical environments has a range of unique adaptive traits enabling them to survive and be productive in those environments (Devendra 1987). A detailed summary of a wide range of environmental stressors experienced by beef cattle grazed at pasture in tropical environments, the impacts of those stressors, and the methods used to measure an animal's resistance to them is provided by Burrow (2014). Those environmental stressors are similar to those experienced by dairy cattle grazed at pasture in the tropics, although in high-input intensive dairy production systems found in developed countries, the stressors are controlled for example through the use of supplementary feeding, cooling systems to control cattle heat stress and treatments to control parasites. In this paper, only dairy cattle that are grazed at pasture in low-input production systems are considered.

In the tropics and sub-tropics, the differences that exist between cattle breeds in temperate environments for traits such as growth, milking ability, reproduction, and product quality are masked by the effects of the

environmental stressors (Burrow et al. 2001; Burrow 2006). Hence in tropical and sub-tropical environments, breeds are best categorized into breed types to compare their performance across environments. Those broad breed groupings (Payne and Hodges 1997) include:

*Bos taurus* (British and Continental), which have good growth and reproductive rates and high-quality products when reared in the absence of environmental stressors but which are poorly adapted to tropical environments;

- *Bos indicus* breeds (e.g. Brahman, Nellore, Ongole, Sahiwal, Sindhi) are the best adapted to tropical environments, but this is at the expense of productive traits, except when they are reared in the most severe tropical environments;
- Tropically adapted taurine breeds (e.g. southern African Sanga breeds, West African humpless breeds, and the Criollo breeds of Latin America and the Caribbean), which retain the productive attributes of *B. taurus* and are also relatively well adapted to tropical and sub-tropical environments, although their resistance to environmental stressors is generally below that of pure *B. indicus*;
- Additionally, and of direct relevance to Indonesia, *Bos javanicus* (Bali and Banteng cattle) evolved independently of these other breed types (Copland 1996). Bali cattle are different from all other species of cattle and have a different number of chromosomes than *B. taurus* and *B. indicus*. They can be crossed with *B. taurus* and *B. indicus*, though the male offspring are reputed to be infertile (Jellinek et al. 1980). There are no known direct breed comparisons between *B. javanicus* and other breed types but their performance can be inferred from (Copland 1996) to be similar to that of the tropically adapted taurine breeds; and
- Synthetic tropical breeds based on *B. indicus* and/or tropically adapted taurine breeds and which have been inter-bred for several generations. They have attributes that are similar to those of the tropically adapted taurine breeds.

The availability of diverse breed resources with large productive and adaptive differences allows the breed types to be matched to different environments, management practices, and markets, maximizing the opportunity for high productivity. Generally though, unless very valuable niche markets exist for the poorly adapted cattle breeds, cattle producers aiming to maximize productivity and profitability of cattle grazed at pastures in tropical and sub-tropical environments should use breeds that are well adapted to the production environment rather than attempting to modify the environment to better suit poorly adapted cattle. For most

tropical and sub-tropical environments, optimal levels of productivity, adaptation, and cattle welfare will be achieved using a combination of multiple breed types (*i.e. B. indicus*, tropically adapted taurine, British, and/or Continental). In Indonesia, this could also include Bali cattle and their crosses, particularly if crossbreeding amongst first and subsequent breed crosses proves feasible (*i.e.* that the crosses are fertile).

## **TRANSFORMATIONAL TECHNOLOGIES TO IMPROVE HERD PRODUCTIVITY**

Achieving the productivity improvements needed to ensure global food security by 2050 requires the application of transformational technologies in cattle herds that are already well adapted to their production environments. This section examines several technologies which, based on the author's perspectives, offer the potential to achieve the required impacts by 2050. It does not provide a comprehensive review of all possible technologies, instead of focusing only on those technologies that have the potential to be effectively used over the next three decades by smallholder beef and dairy cattle

farmers in tropical and sub-tropical environments to achieve significant productivity improvements.

### **Genomics**

Genetic improvement is already an important method for the beef and dairy industries in developed countries to achieve productivity gains and directly address the challenges that need to be overcome to ensure global food security by 2050. However, traditional genetic improvement programs based on measuring large numbers of pedigreed animals in well-defined cohort groups for the full range of economically important productive and adaptive traits are generally not possible for smallholder farmers in LMICs. Hence, where artificial insemination (AI) is possible, they have sometimes used AI to introduce new breed(s) or genetically superior bull(s) of the same breed as their cows to attempt to genetically improve their herds.

Now the opportunity to use genomic (DNA-based) data in conjunction with alternative animal measurements obtained by use of information and communication technologies (see section 3.2) provides very significant new opportunities to increase the rates of genetic gain by crossbreeding and within-breed selection to improve all economically important traits. A similar approach can also be used to develop new plant varieties that tolerate environmental stressors to improve crop and pasture quantity and quality in

tropical and sub-tropical environments. Other technologies based on genomic data (*e.g.* genome editing, where base pairs at specific locations in the genome are deleted, changed, or added) have the potential to transform animal and plant breeding even further as causal mutations are found (Hickey et al 2016).

In addition, the use of genomic information is providing new opportunities to optimize the management of individual animals or groups of cattle to best meet market specifications and to create value-based marketing systems that reward farmers, dairies, feedlots, abattoirs, wholesalers, and retailers for delivery of cattle that meet specific market requirements (see section 4). Genomic data can also be used to develop new treatments (*e.g.* vaccines against parasites and disease, live microbial or bio-active products to reduce methane emissions and improve feed efficiency – see section 3.1.3) aimed at increasing production and reducing costs.

The scientific opportunity to achieve large productivity gains in all of these areas arises due to recent gains in the underlying genomic and associated technologies, for example:

- Very rapidly reducing costs of full genome sequencing (Goddard & Hayes 2009) down from US\$60 million/animal in 2005 to US\$14,000/animal in 2010, to ~US\$1,000/animal in 2016 and now ~US\$300/animal;
- Momentous reductions in the time taken to sequence an entire genome, down from close to 13 years (October 1990 to April 2003) for the first human genome sequence (National Human Genome Research Institute 2021) to now being achieved in a single day (Votintseva et al. 2017). Research is ongoing to achieve full genome sequencing on the same day and at the field, rather than laboratory sites, thus allowing same-day management and breeding decision-making on animals without the need for additional cattle handling expenses;
- The ability to accurately impute whole-genome sequence data from lower-density, lower-cost DNA marker panels (more correctly known as Single Nucleotide Polymorphism (SNP) panels) (Hayes & Goddard 2007; Meuwissen & Goddard 2010a; Meuwissen & Goddard 2010b; Schadt et al. 2010);
- The potential to use whole-genome sequence data to discover the mutations causing variation amongst animals and in turn, using that knowledge of functional mutations in genetic improvement programs to improve the accuracy of predictions (Heard et al. 2010);
- Resolution of the ‘missing heritability’ problem (Yang et al. 2010) proving that genomic selection approaches account for significant proportions of genetic variation for economically important complex traits;
- Vastly improved computational capacity that is now allowing cost-effective storage and processing of petabyte ( $10^{12}$  bytes) scales of data

- (Schadt et al. 2010);
- The ability to use pooled DNA samples from mobs of animals to identify the average genetic merit at low cost, thereby enabling the development of new, cost-effective management applications based on genomic information; and
  - The ability to capture essential individual animal performance data (phenotypes) through the use of automated or semi-automated electronic data capture methods.

Brief examples of the use of genomic information with the potential to significantly improve on-farm productivity in tropical and sub-tropical regions follow.

### ***Genomic selection***

Genomic selection is the use of genome-wide genetic markers to estimate the genetic merit of individual animals (Meuwissen et al. 2001). It is substantially different from marker-assisted selection based on genome-wide association studies (GWAS), where the aim is to identify associations between individual SNP and economically important traits. A few early GWAS were successful in identifying such associations: for example, the DGAT1 gene affecting fat content and milk volume in dairy cattle (Grisart et al. 2001) and the Calpastatin and Calpain genes associated with beef tenderness (Barendse 2002). However based on current GWAS and genomic selection results (Meuwissen et al. 2016), it is clear that most economically important traits in livestock are affected by 2,000 to 10,000 genes, with the number of genes believed to affect these traits increasing 100-fold over the last 15 years *i.e.* the complex traits turned out to be much more complex than expected 10-20 years ago. Hence genomic selection assumes all SNP might be linked to a gene affecting a complex trait and concentrates on estimating the combined effect, rather than testing the significance of individual SNP (Meuwissen et al. 2016). It uses information on hundreds of thousands of real and imputed SNP, with no attempt made to differentiate the effects of single genes on the trait under study.

Genomic selection is already transforming animal and plant breeding and human health diagnostics globally, with an economic benefit in the order of billions of dollars annually. Most value to date has been generated in the dairy, pig, and poultry industries in developed countries. Even though beef and dairy cattle share the same genome, the impact of genomic selection in the beef industry has been much lower than in the dairy industry for many reasons including the use of multiple breeds, the lack of phenotypes (measurements) for economically important but difficult-to-measure traits,

poor use of artificial insemination and because beef businesses tend to be slower to adopt due to long lag- times between measurement and realization of product value (Berry et al. 2016).

In both developed and developing countries, the main limitation to genomic selection in cattle is the difficulty and expense of measuring cattle in appropriate contemporary groups for the full range of economically important productive and adaptive traits. As discussed by Burrow & Henshall (2014), technology may in the future provide the means of measuring animals, but it cannot replace the statistical imperative that, for the measurements to be meaningful, contemporary groups of appropriate structure and sufficient size are required. If the design is inadequate in terms of contemporary group size and structure the measurements will not provide useful predictions of genetic merit. To overcome this constraint, reference populations that are specifically designed to accurately manage and record animals within contemporary groups and capture data for the traits of interest have been established to capture the opportunities provided by genomic selection. Examples of such populations in beef cattle are described by (Upton et al. 2001) for growth, feed efficiency, and carcass and beef quality and (Burrow et al. 2003) for the full range of productive and adaptive traits in the breeding objective. Similar populations have also been established for smallholder farmers in sub-Saharan Africa through the African Dairy Genetic Gains (ADGG) and similar programs for small ruminants and poultry (Mrode et al. 2019; Ojango et al. 2019; Marshall et al. 2019).

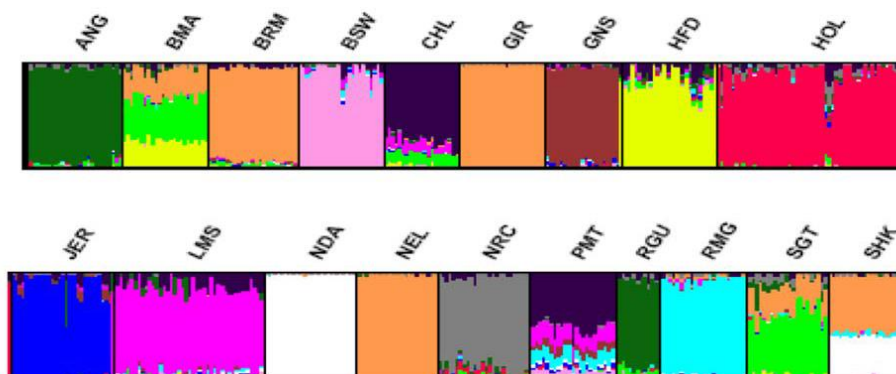
In the future, applications of genomic selection are anticipated (Meuwissen et al. 2016) to be within-breed, where the accuracy of selection is obtained by maintaining huge within-breed reference populations, or across-breed, where accuracy is obtained from across-breed reference populations and high-density genomic selection methods focusing on causative genomic regions discovered through programs such as the 1000 bull genome project (Hayes et al. 2014). Likely, future applications of genomic selection will increasingly turn towards across-breed genomic selection (Meuwissen 2016).

### ***Characterization of indigenous cattle breeds***

Many potentially very important cattle breeds for use in the tropics and sub-tropics are yet to be fully evaluated and there is a risk of extinction for many of them. In sub-Saharan Africa, 32% of identified cattle breeds were identified as being at risk of extinction and 13% had already become extinct in 1999 (Marshall et al. 2019). Yet many of those breeds have great potential to improve the productivity of cattle herds in tropical and sub-tropical regions

globally. Ensuring they are available for further livestock development requires their conservation and improvement. Hence there is an urgent need to systematically evaluate these breeds and, where relevant, implement conservation programs to ensure their ongoing availability for beef and milk production in tropical and sub-tropical environments. The availability of genomic technologies is assisting with the characterization of these breeds.

Two examples are used here to demonstrate the value of genomic technologies for this purpose. Figure 1 shows the genetic structure of several diverse cattle breeds based on genome-wide data (Gibbs et al. 2009). Of relevance here is the Ethiopian Sheko breed which has been promoted as a high priority breed for conservation due to its productivity in the presence of the tsetse fly and Trypanosomiasis, transmitted by the fly. Figure 1 shows the Sheko is an admixed breed based on crosses between the N'Dama, a breed known to be resistant to Trypanosomiasis, and *Bos indicus* which are generally susceptible to Trypanosomiasis. Hence it is probable the superior performance of the Sheko is due to heterosis and, all other considerations being equal, it appears the N'Dama would have a higher priority for conservation than the Sheko.



**Figure 1.** Genetic structure of diverse cattle breeds based on a genome-wide survey of SNP variation (source [40]; ANG - Angus; BMA - Beefmaster; BRM - Brahman; BSW - Brown Swiss; CHL - Charolais; GIR - Gir; GNS - Guernsey; HFD - Hereford; HOL - Holstein; JER - Jersey; LMS - Limousin; NDA - N'Dama; NEL - Nellore; NRC - Norwegian Red; PMT - Piedmontese; RGU - Romagnola; SGT - Santa Gertrudis; SHK - Sheko)

The second example relates to the Senepol breed which is now becoming popular in tropical and sub-tropical regions in the USA, South America, and Australia. Historically, the breed was believed to be derived from a cross between the Red Poll and the N'Dama, with recommendations for its use

being made based on knowledge of the performance of its nominal parental breeds. Genomic studies have now clearly demonstrated that: (a) there is no African taurine in the origin of the Senepol breed (Flori et al. 2012); and (b) the ancestral origins of the breed are from the Criollo breeds, specifically the Romosinuano and Carora from South America (Flori et al. 2012). The SLICK locus reported in the Senepol breed and associated with improved thermo-tolerance and improved productivity in tropical environments also derives from those Criollo breeds (Huson 2014).

### ***Using genomics to develop vaccines to control parasites and diseases***

Genomic information and biotechnology approaches are now being used to develop non-genetic, locally-relevant 'products' such as vaccines, drenches, slow-release compounds, and feed additives. To ensure application in developing countries, this research is often undertaken in collaboration with commercial companies prepared to ensure cost-effectiveness and local marketing. The development of a vaccine to control ticks in cattle is presented in this paper as an example of the use of genomic information to transform beef and dairy production through non-genetic applications.

Around 80% of the world's cattle are at risk from ticks and tick-borne diseases, with estimated losses of production of the order of US\$20-30 billion per annum (Lew-Tabor & Rodriguez 2016). Existing tick control methods such as the use of acaricides are losing efficacy due to the rapid evolution of resistance of the ticks to the chemicals.

A vaccine with efficacy against the cattle tick was developed, patented, and marketed in Australia under the name TickGARD (Willadsen et al. 1995) based on the Bm86 antigen. It was developed using conventional vaccine development methods in studies of the tick to identify vaccine candidates known as 'antigens' or 'peptides'. The process required more than 12 years of development and trials involving about 18,000 cattle. Whilst TickGARD was useful for Australian dairy farmers operating in tick-endemic areas, it was ultimately withdrawn from the market because the need for several booster vaccinations per year meant it was not viable for beef producers operating under extensive production systems, where cattle are often only handled once or twice a year.

Research is now targeting a next-generation tick vaccine, which could integrate the Bm86 antigen, as it is now no longer covered by a patent. Current research using genomics and proteomics approaches 'reverse vaccinology', (Guerrero et al. 2012; Lew-Tabor & Rodriguez 2016) has reduced the lengthy conventional development process from 12+ years to 1-2 years for discovery of vaccine candidates, though the animal trials required for vaccine

registration remain a lengthy part of the commercialization process. Up to 20 novel vaccine candidates were identified using a reverse vaccinology approach, where sequence information from the entire cattle tick genome was used to identify several proteins believed to be good targets to try to disrupt the cattle tick's function and impact its survival. Those candidates have been tested singly and in combination in animal trials in Australia and Brazil (Lew-Tabor, Rodriguez Valle, Bellgard, Guerrero, and Andreotti, unpublished data) and successful candidates will be commercialized for use in cattle in tick-endemic areas of the world.

### **Information and communication technologies**

As described by (Delaney 2016), information and communication technologies have enormous potential to reduce the costs and difficulties of production, improve precision, reduce labour costs, replace scarce or non-existent labour, improve animal management and improve compliance with market specifications. Where data apply to individual animals, they can also be used to improve the rates of genetic gain through genomic selection. Spatial technologies including precision satellite positioning, high-resolution satellite imaging, autonomous and robotic vehicles, unmanned aerial vehicles, and advanced databases are some of the fastest-growing technologies globally. Spatial technologies are developing at unprecedented rates. Over coming years, hundreds of new and higher-resolution imaging satellites and a near-doubling of the global and regional navigation satellite systems will be available. The opportunity to effectively store and analyze 'Big Data', including location information, will allow new levels of insight across entire value chains. New, more energy-efficient global satellite and terrestrial positioning systems mean that real-time tracking of assets such as animals and their products is now becoming a reality. The impact of these core spatial technologies will be significantly increased and value-added by other developments including high-speed networking capability and other terrestrial and satellite-based communication systems, mobile technologies and applications (apps), decision-support tools, autonomous aircraft and vehicles, a proliferation of sensor systems and much more.

These technologies are now transforming the horticulture, cropping, and intensive livestock industries in developed countries, but they are significantly under-developed in the global beef and dairy value chains, and particularly in LMICs. This is largely due to several previously intractable problems arising from extensive production systems and a lack of high-speed connectivity to national communication networks. However spatial technologies have a key role to play in improving beef and dairy value chains

and ongoing research will deliver solutions to existing problems over coming years, thereby providing the enormous potential to transform production systems in developed and developing countries. This will see enormous improvements in productivity at a whole-of industry, value chain, and individual enterprise levels (Delaney 2016) through:

- Improved compliance with quality market specifications;
- Remote monitoring and increased traceability and feedback from the farm through to domestic and international consumers;
- Increased transparency in the value chain and options for more direct market access for producers, leading to higher farm-gate value;
- Increased productivity and efficiency through the entire value chain;
- Increased communication and connectivity technology for producers;
- More efficient financial transactional capabilities; and
- Decision-support along the entire value chain.

### **Artificial reproductive technologies**

Artificial reproductive technologies such as AI, multiple ovulation and embryo transfer, juvenile in- vitro embryo transfer, sexed semen, cloning, and gene-editing will potentially contribute to improved productivity of beef and dairy herds in tropical and sub-tropical environments primarily through their role in increasing rates of genetic gain in elite nucleus breeding herds. Combining these technologies with genomic selection will create the potential for productivity gains over coming decades that would otherwise take centuries to achieve with traditional selection. However, it is unlikely these technologies will have a major impact on smallholder herds by 2050, particularly those in tropical and subtropical areas, due to their current high cost and poor success rates. The exception to this generalization could be significantly increased use of AI to expand the use of elite genetics to commercial and smallholder herds. The success of current research focused on fixed-time AI, storage of semen at ambient temperatures and improvements in sexing of semen would encourage much broader use of AI in tropical and sub- tropical regions.

### **THE NEED FOR COMMERCIAL INCENTIVES TO ENCOURAGE FARMERS' PRACTICE CHANGE**

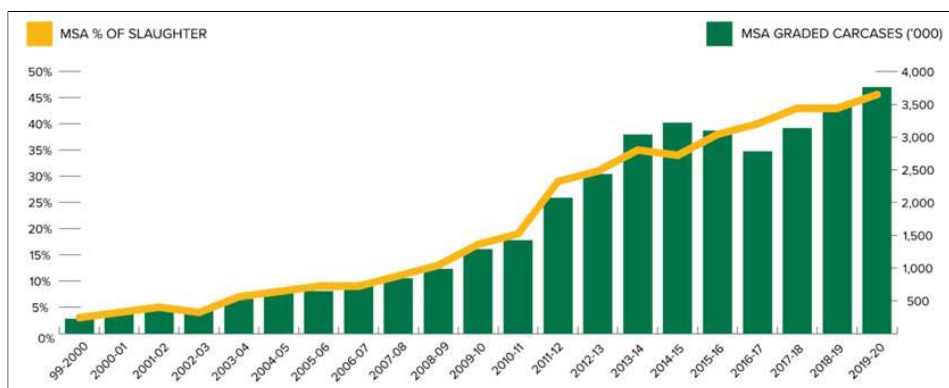
Achieving the productivity improvements needed to achieve food security in Africa and Asia by 2050 will not be easy, but using a combination of existing and new and emerging technologies, will be feasible based on the author's knowledge of a systematic change in the beef and dairy

industries in Australia and elsewhere. However, it will require that smallholder farmers take a whole-of-system, integrated approach to animal, rangeland, and pasture management to address a very wide range of issues including the environment, animal welfare, biosecurity, and even social/cultural issues (e.g. smallholder farmers in Africa often keep cattle for cultural reasons such as 'lobola' or bride price, or as a sign of wealth, with the farmer having the greatest number of animals regarded as the wealthiest, regardless of the condition of either the cattle or the land on which those cattle graze). It will also require genuine and strong collaborations across all sectors of product value chains.

In the author's experience, the strongest on-farm practice change has occurred when farmers are financially rewarded based on the quality of the products they deliver to markets. By way of example, Australia's experience with Meat Standards Australia (MSA 2021) provides compelling evidence that, if high-quality market specifications are to be met, then farmers must implement best practice animal, pasture, and rangeland management systems, not adopt technologies in isolation.

MSA is a voluntary beef grading system that describes and predicts the eating quality of individual cuts in the beef carcass prepared using different methods. It uses a Total Quality Management approach, whereby the system addresses all critical control points along the supply chain, from the genetics of the animals to the cooking method used by the consumer (MSA 2021). By itself, MSA offers little commercial advantage to the Australian beef industry, unless it is integrated into a business model that includes procurement, value-adding and retailing. It, therefore, uses a payment system to the wholesaler and producer based on a fixed proportion of retail value, thereby enabling partners in the supply chain to be rewarded for small increments in eating quality, as opposed to conventional marketing systems that provide little or no incentive for improvements in quality (MSA 2021).

Figure 2 shows the uptake of MSA by Australian beef producers since the inception of the scheme in 1999-2000 (MSA 2021). In 2019/20, the total value-add to beef producers in just that year was A\$172 million, with additional benefits accruing to meat processors and retailers (MSA 2021). Research currently underway in Indonesia in collaboration with researchers from Massey University in New Zealand and the University of New England in Australia (Dahlanuddin, Panjaitan et al. unpublished) is aiming to develop a 'Special Bali Beef' value chain modeled on the MSA scheme, to enable Indonesian smallholder farmers to implement whole-of-system technologies to deliver high-quality beef that financially benefits the entire value chain and simultaneously significantly improves both the productivity and profitability of Indonesian smallholder cattle farmers (Special Bali Beef 2021).



**Figure 2.** Adoption by beef producers of the Meat Standards Australia grading scheme which financially rewards farmers who deliver beef products that meet consumers' quality specifications (MSA 2021)

## CONCLUSION

As summarised here, several transformational technologies are now becoming available and have the potential to be applied by smallholder farmers globally. In the author's opinion, the most promising of these is the use of genomic and associated technologies to increase rates of genetic gain, optimize management of individual animals or groups of cattle to maximize productivity and profitability and develop new non-genetic products aimed at increasing production and reducing costs. Novel information and communication technologies used in conjunction with genomic technologies will value-add that transformation. Further development of AI to enable fixed-time insemination, cost-effective sexing of semen, and storage of semen at ambient temperatures would ensure a much broader use of genetically elite sires in smallholder cattle herds in tropical and sub-tropical regions globally. But to achieve major productivity improvements in smallholder beef and dairy herds, commercial incentives are also needed to encourage smallholder farmers to implement whole-of-farm practice change. The most promising of these incentives appear to be the development of commercial value chains that reward farmers for the quality of the products they deliver.

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## **Dietary Mitigation of Enteric Methane Emissions and Animal Production from Ruminants: Plant Tannins Mitigation Options**

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### **SUMMARY**

Our population global population will be registered about 910 billion by 2050 so to meet the food demand for this increasing population, it was estimated that milk and meat production must increase by 63 to 76%, respectively. Therefore, the livestock industry will play a central role in global economy in the next decade. However, increasing food production will likely result in increased greenhouse gas (GHG) emission including enteric methane production from animals, manure, crop production and fertilization. Many mitigation practices have been discovered and are implemented.

Beef cattle and dairy cattle are the major global GHG emission contributors and buffalo, sheep, goat and other livestock are minor contributors. How is enteric CH<sub>4</sub> produced? Methanogenesis in the rumen during feed digestion especially including bacteria, protozoa fungi and methanogens, they produce CH<sub>4</sub> gas during digestion and also this entering methane emission in ruminant which is produced by fermentation of feed by methanogens archaea, it represents a loss of 2-12% of gross energy or feed which is contributed to the global GHG emission.

Most of gas production from forage diet especially winter wheat forage diet for cattle, would cause frothy bloat as well as produce a bunch of gas that including CO<sub>2</sub> about 45%, CH<sub>4</sub> about 30% and rest of them are volatile various gas productions. It is generally known that ruminant produce CH<sub>4</sub> gas differently between two different diets. Forage diet produced high CH<sub>4</sub> gas productions, however, grain-based diet that lowering CH<sub>4</sub> gas emission depending on microbial population in the rumen.

### **Measurement enteric methane emissions**

There are 3 different measurements in this world. Respiration chambers bring feed systems and SF<sub>6</sub> system and in vitro gas production system.

What kind of factors affecting enteric methane emissions especially dry matter (DM) intake which is the major contributor for CH<sub>4</sub> emission. This is the average meta-analysis measuring using direct chambers SF<sub>6</sub> and green

feed system. All of these measurements done in dairy and beef cattle. Each one kilogram increase in dry matter intake increased  $\text{CH}_4$  production by average about 20.5 grams per kilogram of dry matter intake in beef and dairy cattle industry, so dry matter intake is the major contributor for methane gas emission also losing energy diet.

What kind of thing actually in the dairy cattle, milk production also slightly important associated with methane gas production? However, most of average daily gain in beef cattle actually increasing methane gas production especially forage based diet.

Other rumen fermentation associated with methane gas production especially acetic acid is highly affected with methane gas inhibition however negatively impact for the propionic acid and butyric acid associated with methane gas productions.

### **Mitigation option for tannin dietary supplementations**

Manipulation rumen fermentation using condensed tannin. The condensed tannin containing forages such as the mimosa trees. In Indonesia country, there may have a lot of mimosa trees, acacia and cocoa but in temperate zone, we have perennial grass that contain high level of condensed tannins, also some fruits and berries like apples, grapes, strawberries, blackberries contain some little amount of condensed tannin, pine bark, peanut skin contain high level of condensed tannin.

### **Mitigation of methane gas and sustainable livestock productions**

Firstly, let's see that mode of action of condensed tannins. Condensed tannin actually in complex form at the neutral pH in the rumen about 6.6 to 7 and during the chewing and rumination. Then, the condensed tannin protein complex at acid condition will dissociate in the abomasum. Therefore, condensed tannins is able to make bypass protein and potentially is able to reduce methane gas and ammonia production in the rumen.

It is evidence that reduce inhibition of proteolysis of white global flexion on proteins, In this figure, there are large and small subunits of protein fractions without condensed tannins. Most of protein levels were digested within two or four hours of incubation. However, additional one percent of condensed tannin (extracted tannin), actually most of large protein will be protected from the proteolysis and but some of small proteins would disappear. This figure also showed that an additional condensed tannin in the rumen able to protect and increase bypass proteins. Additional tannin in general 1,1.5%, 2% in yellow line so increasing tannin concentration in the

rumen actually declined gas products with different time basis and also we added the different chemical compound for examples monensin which is one of popular feed additives for beef cattle. With increasing concentration and time actually increase gas production, however additional tannin and mimosa and monensin decline methane gas productions.

### **Methanogen bacteria and other microbial population**

We added using as a model for pine bark diet 15-30% in meat type goat diet. Actually methanogen bacteria population is declined, also CH<sub>4</sub> production is significantly reduced about 33%. Methanogenic bacteria reduced indicating that tannin in each diet can reduce methane emission in ruminant. Most of microbial population at phylum level show that some microbial phylum especially bacteroides and other bacteria decline, however especially phylum of firmicutes significantly increased indicated that tannin in each diet potentially manipulate over rumen bacterial community. This figure shows that acetic acid and propionic acid ratio versus firmicutes and bacteroides phylum ratio and the bottom line is average daily gain in meat goat. Increasing firmicutes population in the rumen, significantly increased average daily gain however acetic acid:propionate ratio declined, actually increasing average daily gain. Control of rumen fermentation and rumen microbial population is highly associated with the average daily gain in meat goat.

### **Mitigation of methane production in field experiments**

Increasing tannin concentration in the diet using peanut skin, lotus, pine bark decreased methane and methanogen population in the rumen. These figures also show that series of results, a high level of tannin in the forages compared to crabgrass. We measure 12 animals for each treatment using open circular respiration chamber. Addition of feed supplementation over series of results significantly decline of CH<sub>4</sub> and ammonia gas which are consistent evidence for the CH<sub>4</sub> gas inhibition in ruminants.

## **Recent Advances on Our Understanding of the Pathogenesis of Brucellosis**

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### **SUMMARY**

Brucellosis is one of the most frequent zoonotic infections in the world. Bacteria of the genus *Brucella* can infect a wide range of wild and domestic animals. It is best known as an infection of livestock. It is passed on to man by either contact with infected animals or consuming infected animal products, especially dairy products. In animals, it causes a substantial economic problem throughout the world. It has considerable problems in the livestock trade and livestock movement and eradicating the disease in the final stages. Infected animals are killed, so there is a high cost there. It is regularly transferred to humans and causes a disease known as a multi-fever, a flu-like illness. It can become chronic, very long-term infections.

In small and large ruminants, brucellosis is a debilitating disease that can cause abortion, decreased milk production, infertility, and lameness. Infected animals can shed *Brucella* in their milk, which is a major source of infection for men that presents as an influenza-like infection. If not properly diagnosed and treated, it can become chronic, localizing in many body sites. *Brucella* is a facultative intracellular pathogen; it enters, survives, and multiplies within host cells. To do this, it uses a range of virulence factors.

Over the last 30 years, the number of species has suddenly rapidly increased. First of all, strains here start from cetaceans from sea mammals, seals, and foxes. Then over the last ten years, we have had an enormous number of new atypical *Brucella* which have been isolated. These atypical strains can be identified by genome sequence analysis. This classical *Brucella* can be distinguished into seven distinct branches; each of them strangely has a specific animal host. The genome sequencing has been shown by biochemical assays confirming that *Brucella* is composed of clonal images resulting from their co-evolution with their host.

The vertical transmission is either intrauterine infection, infection of the young by lactation and the infection of humans, mechanical transmission from one animal to another, and sexual transmission. However, in most cases, this is an epidemiological dead end. The human who has got infected with *Brucella* is not contagious. They started many millions of years ago as soil

organisms, and the closest related ones are called the okra bacterium, for example. They went through a period of genome reduction and then the acquisition of virulence factors. The bacteria cause very highly pathogenic, acute infections and atypical bacteria.

The major virulence factor is the VirB Type IV secretion system. This molecular syringe injects "effector proteins" modulating the biology of the infected cell, turning it into a niche permissive for the bacterium to survive and replicate. The bacteria have to adapt and live within a host, so they begin first contact with host cells and, in this first contact to invade the cells. They have to establish within the host to avoid host defenses by changing their gene expression. They grow, multiply and extend their niche and eventually get out. Either move in the environment within the animal's blood, or it can restart the cycle outside the animal. The major virulence factor of *Brucella* is the VirB Type IV secretion system. A type IV secretion system is a multi-protein complex found in the envelope in the inner and outer membranes of the bacteria. Transferring DNA from one bacterium to another will transmit one protein to another and kill other bacteria.

*Agrobacterium* transfers DNA of other bacteria, such as *Helicobacter*, that sit on the surface of the back of the bacterium and inject the toxin and KGA protein into the cells. The bacteria secrete effector proteins, a set of proteins that affect the biology of the host cell. They affect vesicular transport and stop the bacteria from being killed by lysosomes and endosomes. Then they allow the bacteria to create the niche in which it is going to grow and live. They do this by capturing the cyst with the bacteria in capturing vesicles transported from the Golgi to the endoplasmic reticulum. The bacteria live and grow in a compartment that resembles the endoplasmic reticulum, and then this matures cells, further called an ortho-phagosome. There are a large number of proteins that affect the biology of the Golgi. There is three strain of *Brucella*: BO1, BO2, and BO3 *Brucella*. The BO1 was discovered in an abscess or a breast implant, which is very different from the classical presentation of brucellosis. The BO2 was found in chronic disruptive pneumonia, and the BO3 originated from multiple abscesses of pulmonary condensations. *Brucella* is encoded by a set of genes found in all the *Brucella*.

Control of human brucellosis depends on controlling its source: animal disease and requires a 'One Health' approach with actors in both animal and human health working together. Control measures depend on the incidence of animal brucellosis; surveillance of disease in both the animal and human populations is essential. In endemic situations, mass vaccination is required, followed by vaccination of selected heard and finally test and slaughter as incidence decreases. Three *brucella* species, *B. melitensis*, *B. abortus*, and *B. suis* are generally associated with human disease. Over recent years several new

*Brucella* strains have been identified, from a wide range of mammalian hosts, including cetaceans, seals, monkeys, foxes, and small rodents. More recently, atypical strains have been found in amphibians, reptiles, and fish. At present, little is known about the zoonotic potential of these atypical strains; however, a small number of human infections have been reported. It is possible that they are more common but are not identified as brucellosis.

Brucellosis is one of the most common laboratories acquired infections. They are entirely different; this means that human diseases cannot be detected using the standard serological assays used in laboratories throughout the world. Generally, the isolate is not correctly identified and is manipulated without the correct safety procedures. Incorrect manipulation and because the machines to place them are less effective and not working very well, so at the moment, MALDI-TOF MS spectroscopy is revolutionizing bacteriology; therefore, diagnosis of *Brucella* can be done within 15 minutes, rather than three days. Another important thing in terms of safety for mass spectroscopy is simple. This equipment is vital to save the health and safety of laboratory workers.

Moreover, this multi-spot allows samples to be stored for several days (four degrees) and can be done in small laboratories. They can be safely sent to laboratories with the machines and be identified. With the availability of new databases, *Brucella*, even atypical strains can be rapidly and accurately be identified by MALDI-TOF MS. Access to MALDI-TOF MS may be difficult in rural areas and underdeveloped countries. A recently described solvent inactivation protocol allows samples to be sent safely to a central laboratory for testing. PCR can perform identification of the species level.

## **Dorper Sheep Development in Indonesia**

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### **ABSTRACT**

Sheep production in Indonesia has not met the demands for both domestic and international markets which tends to continually increase every year. There is a significant gap between the upstream and the downstream sectors in the sheep business process resulting in a stagnant supply of live animal, meat and co-products. The sheep productivity in most traditional farmers is still relatively low. The local sheep are categorized as medium type in size and have several advantageous characteristics such as adaptive to the environment, resistance to ectoparasites, adaptive to low-quality feed, and prolific births. It is necessary to improve farming management and the genetic quality of the local sheep to increase productivity and better meat quality. Garut sheep is one of the local breeds in West Java. Genetic improvement of Garut sheep can be done through crossbreeding with superior types of meat sheep. The crossbreds are expected to have better performance than those of their parents. Dorper is a superior type of meat sheep spread out all over the world with excellent adaptability and productivity. HPDKI (Indonesian Sheep and Goats Farmers Association) introduced Dorper sheep (Black Head) from Australia with 8 males and 26 females to be used for improving sheep production. During quarantine, Dorper sheep showed good adaptability with no incidence of disease. Dorper sheep were grouped into 3 groups of 8-10 females each with 1 male. The mating system was done naturally. They were fed a complete ration met the nutritional requirement of both macro and micronutrients. On the farms, Dorper sheep performed of litter size 1.18, birth weight 3.50 kg (singles) and 2.94 kg (twins); weaning weight of 21.43 kg (singles) and 15.50 kg (kg); weaning age of 90.05 day; average daily gain (ADG) during lactation period of 177.31 gram/head/day; lambing interval of 245.91 days; gestation period of 155.91 days; rebreeding rate of 0.12%, male lamb 42.50%, female lamb 57.50%, lamb mortality 13.04%. The the body shape of Dorper sheep was deep, wide, long, and dense, no horns, with combination of fur, of thin and short wool. The hair colour of the head was black (20%) and the body was white (80%). their performance was uniform, stable and. They are good in utilizing low-quality feed, resistance to heat stress, and resistance to ectoparasites. It can be

concluded that Dorper sheep are well adaptive to Indonesia's tropical environment.

**Keywords:** Dorper sheep, Quantitative traits, Qualitative traits, Productivity, Adaptability

## INTRODUCTION

### Background

Sheep is one of the small ruminants that is very popular in Indonesian society. They are easy to raised, and can be used as savings due to easily sold. Sudarmono & Sugeng (2005) stated that there are various types of Indonesian sheep. Indonesian native sheep or also known as Domba Kampong or local sheep with characteristics such as small body, late maturity, various coat color, and low carcass percentage. Priangan Sheep or known as Garut sheep is a crossbreeding of Native sheep, Merino sheep, and Fat-Tailed sheep from South Africa. Garut sheep is widely found in Garut as fighting sheep with the following characteristics: having a large and wide-body weight (60 kg for males and 35 kg for females); the male has horns and arches behind the slender auricles; coat colour combinations of white, black and brown or mixed colours. Fat-tailed sheep are abundant in East Java, Madura, Sulawesi, and Lombok and have characteristics: large body shape (50 kg for males and 40 kg for females), horns in males, and a long tail (a lot of fat at the tail base and small at the tip).

Indonesia has several types of sheep with high ability in adapting to the environment, resistant to ectoparasites, and low-quality feed. Garut sheep is famous for the best skin quality. The rearing sheep in Indonesia is still a subsystem and does not take into account the cost and the quality factors in its maintenance. The scale of rearing is still small and medium. The results showed that the weight of adult ewes in the North Coast area ranged of 12.5-22.5 kg and 17.5-32.5 kg for males. It is very rare to find sheep that have a bodyweight of up to 35 kg (Sondi et al. 2007). In the South Coast area, the bodyweight of adult ewes ranged of 20-27 kg, and 25-35 kg for males. (Nurachma 2003). According to Diwyanto (1982), the potential weight of male Priangan Sheep ranges of 60-80 kg, and, 30 to 40 kg for females. There was a decrease in the bodyweight performance of adult sheep compared to the previous body weight.

The weight gain of local sheep is relatively low. Nuraliah et al. (2014) reported that thin-tailed sheep fed a complete diet with a protein source of soybean meal protected by tannins ranged of 42.70-55.70 grams/head/day. Another study showed that the daily body weight gain of thin-tailed sheep

reached 92.5 grams/head/day (Mathius et al. 2001). Bandiati (2010) reported that the pre-weaning weight gain in SPTD Trijaya was 96.64 g/day for male and 89.33 g/day for female.

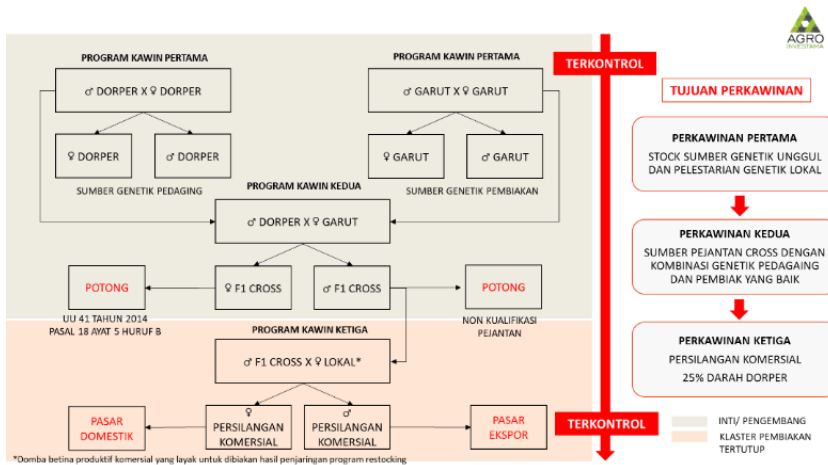
The largest market segmentation for sheep and goats is still dominated by the Aqiqah and Qurban markets. The Indonesian Aqiqah Entrepreneurs Association in the National Work Meeting on February 26, 2019, in Cikole Lembang, stated that in 2017-2018, The market was still growing ranged of 10-12%, while for 2019 it was predicted to decline in the first quarter due to a decrease in supply compared to the previous period. The meat consumption, can not be definitively explain in terms of quantity, but in terms of price, there has been a price increase of more than 20% in the past year. Meanwhile, in the Qurban market, the current position is decreasing for the amount of supply; and there is a 20% price increase from last year's.

In general, the sheep and goat farms has been developing. It can can open opportunity, both for domestic markets and export markets in the ASEAN region, with positive impact on sheep farmers to to be developed. In addition, sheep farming has encouraged policies and programs through the National Sheep Program, the Silatnas Program (national gathering), and the Presidential Cup livestock contest for the last three years. and goats have had a significant impact on developing sheep farming.

The domestic and foreign market are not accompanied by readiness for integration of upstream-downstream development and policy supports causing a significant supply declining. The sheep breeders are still concentrated in the downstream and middle (fattening) which forms a corporation, at the commercial breeding level is still small. The development of commercial sheep breeding requires the introduction of exotic breed of meat sheep with good production and adaptive to environmental conditions in Indonesia. The development of the Dorper Sheep is expected to be an alternative solution to increase productive ewes and superior rams in developing breeding to produce better offspring centre.

## **Development plan**

The Dorper sheep development plan in the future is carried out through 3 stages of development, namely:



**Figure 1.** Dorper's sheep development scheme

1. First development (first mating)  
 The first mating development system aims to produce meat-type genetic sources through Dorper sheep and Garut sheep. At this stage, a Dorper sheep and Garut sheep was maintained purebred.
2. Second development (second mating)  
 The second mating development system aims to produce rams with a good performance of meat type and genetically. At this stage, the Dorper sheep purebred is mated with Garut sheep purebred. The crossbred male offsprings are used as rams to produce commercial crossbreeds in the farm level with closed breeding system.
3. Third mating (third development)  
 The third mating development system aims to produce commercial crossbred sheep. At this stage, the crossbred rams of Dorper and Garut sheep mated with a local ewes, the male offsprings selected for meat type will be used for the Aqiqah and regular markets.

## METHODS AND HOW TO GET DORPER SHEEPS

### Dorper sheep introduction

The introduction of Dorper sheep was carried out in 2 (two) stages, namely the development of Dorper sheep purebreed from Australia and then bred in Malangbong Garut with 8 males and 26 females. The following is a timeline for the Dorper sheep stages.



**Figure 2.** The timeline of Dorper Sheep introduction

The import process for Dorper sheep arrived in Indonesia on February 15, 2018, through Soekarno Hatta Cengkareng Airport with SPI Number No. 04.PI-54.17.0233 and recommendation number No. 11547/kpts/PI.500/F/11/2017. The Dorper sheep imported from Australia were then subjected to an animal quarantine process in Malangbong Garut After quarantine process. the Dorper sheep was released on March 9, 2018, by the Animal Quarantine Agency. Dorper sheep were then distributed for further breeding on March 13, 2018, at the PT Agro Investama farm. The first mating of the Dorper Sheep was carried out on May 16, 2018, and the birth of first lamb on September 28, 2018. The following is the pedigree of the Dorper sheep and a list of farm and breeder, and the pedigree is contained in the certificate of Dorper sheep breed attached.

**Table 1.** Dorper sheep pedigree

Tag number	Sex	Date of birth	Age today (month)	Male parent	Female parent
170230	F	02-Jun-17	28.01	BOK 150045 D 520 150045	WHYNOT 130124 D250 130124
170195	F	03-Jun-17	27.98	BOK 150066 D 520 150066	KAYA 060728 (ET) D030 060728
170129	F	01-Jun-17	28.04	BOK 150066 D 520 150066	WHYNOT 140081 D250 140081
170194	F	04-Jun-17	27.94	BOK 150066 D 520 150066	KAYA 060728 (ET) D030 060728
170095	F	05-Jun-17	27.91	KAYA 150865 (ET) D 030 150865	KAYA 150693 D030 150693
170121	F	01-Jun-17	28.04	KAYA 150865 (ET) D 030 150865	KAYA 060905 (ET) D030 060905
170209	F	04-Jun-17	27.94	KAYA 150865 (ET) D 030 150865	WHYNOT 150281 D250 150281
177574	F	15-May-17	28.60	LYNKM SPRINGS 130022 D 822 130022	BILGAMMA 133836 D246 133846
177573	F	02-Jun-17	28.01	LYNKM SPRINGS 130022 D 822 130022	BILGAMMA 155184 (ET) D246 155184
177664	F	06-Jun-17	27.88	TORCHWOOD REBEL D840 155112	BILGAMMA 166411 D246 166411
177634	F	28-May-17	28.17	TORCHWOOD REBEL D840 155112	BILGAMMA 122562 (ET) D246 122562
177633	F	03-Jun-17	27.98	TORCHWOOD REBEL D840 155112	BILGAMMA 166352 D246 166352
177657	F	04-Jun-17	27.94	TORCHWOOD REBEL D840 155112	BILGAMMA 166471 D246 166471
177646	F	08-Jun-17	27.81	TORCHWOOD REBEL D840 155112	BILGAMMA 155092 (AI) D246 155092
170147	F	05-Jun-17	27.91	WHYNOT 130186 D250 130186	MUNBILLA 10-509 D170 100509
170167	F	02-Jun-17	28.01	WHYNOT 150015 D520 150015	WHYNOT 120176 (AI) D250 120176
170198	F	06-Jun-17	27.88	WHYNOT 150015 D520 150015	WHYNOT 120016 (AI) D250 120016
170046	F	07-Jun-17	27.84	WHYNOT 150015 D520 150015	WHYNOT 099040 D250 099040
170061	F	08-Jun-17	27.81	BOK 150045 D 520 150045	WHYNOT 130043 D250 130043

Tag number	Sex	Date of birth	Age today (month)	Male parent	Female parent
170053	F	10-Jun-17	27.74	KAYA 150865 (ET) D 030 150865	KAYA 060094 D030 060094
170122	F	01-Jun-17	28.04	KAYA 150865 (ET) D 030 150865	KAYA 060095 (ET) D030 060095
177520	F	02-Jun-17	28.01	LYNKM SPRINGS 130022 D 822 130022	BILGAMMA 111490 D246 111490
177614	F	01-Jun-17	28.04	LYNKM SPRINGS 130022 D 822 130022	BILGAMMA 155056 (AI) D246 155056
177726	F	02-Jun-17	28.01	TORCHWOOD REBEL D840 155112	BILGAMMA 122694 D246 122694
177652	F	09-Jun-17	27.78	TORCHWOOD REBEL D840 155112	BILGAMMA 166367 D246 166367
177686	F	07-Jun-17	27.84	TORCHWOOD REBEL D840 155112	BILGAMMA 166313 D246 166313
170060	M	07-Jun-17	27.84	BOK 150045 D 520 150045	WHYNOT 130043 D250 130043
170253	M	09-Jun-17	27.78	BOK 150066 D 520 150066	WHYNOT 140072 D250 140072
170149	M	09-Jun-17	27.78	BOK 150066 D 520 150066	WHYNOT 140169 D250 140169
170200	M	10-Jun-17	27.74	BOK 150066 D 520 150066	WHYNOT 140071 D250 140071
170052	M	10-Jun-17	27.74	KAYA 150865 (ET) D 030 150865	WHYNOT 150093 D250 150093
177591	M	02-May-17	29.03	LYNKM SPRINGS 130022 D 822 130022	BILGAMMA 122617 D246 122617
170249	M	11-Jun-17	27.71	WHYNOT 130186 D250 130186	WHYNOT 120175 D250 120175
170244	M	10-Jun-17	27.74	WHYNOT 150015 D520 150015	WHYNOT 099065 D250 099065

## Breeding method

### *Mating system*

The breeding methods of Dorper sheep are grouped into three purebreed of Dorper sheep first imported from Australia. The ratio of mating system for each group is 1 male mated to 8-10 females. The following is a mating grouping system to produce Dorper sheep, namely:

**Table 2.** Mating group 1 red ear-tag

Tag	Sex	Male	Date of birth	Sex	Number	Weight
<b>170060</b>	<b>Male</b>	<b>BOK 150045 D 520 150045</b>				
170195	Female	BOK 150066 D 520 150066	14-Oct-18	Male	019 M	4.8
170129	Female	BOK 150066 D 520 150066	07-Oct-18	Female	003 M	3.2
170194	Female	BOK 150066 D 520 150066	29-Sep-18	Male	018 M	3.7
				Male	015 M	2.4
170095	Female	KAYA 150865 (ET) D 030 150865	30-Oct-18	Male	017 M	3.4
170053	Female	KAYA 150865 (ET) D 030 150865				
170122	Female	KAYA 150865 (ET) D 030 150865	15-Feb-19	Male	008 M	3.1
170121	Female	KAYA 150865 (ET) D 030 150865	12-Oct-18	Male	016 M	4.1
170209	Female	KAYA 150865 (ET) D 030 150865	25-Oct-18	Female	006 M	3.3

**Table 3.** Mating group 2 yellow ear-tag

Tag	Sex	Bucks	Date of birth	Sex	Number	Weight
<b>170253</b>	<b>Male</b>	<b>BOK 150066 D 520 150066</b>				
170061	Female	BOK 150045 D 520 150045	01-Jul-19	Female	007 K	4.6
170230	Female	BOK 150045 D 520 150045	02-Nov-18	Female	003 K (L)	3.9
177574	Female	LYNKM SPRINGS 130022 D 822 130022	10-Nov-18	Female	005 K	1.7
177520	Female	LYNKM SPRINGS 130022 D 822 130022				
177614	Female	LYNKM SPRINGS 130022 D 822 130022	26-Aug-19	Male	009 K	2.5
				Female	010 K	2.1
177573	Female	LYNKM SPRINGS 130022 D 822 130022	13-Oct-18	Female	002 K (L)	3.5
177726	Female	TORCHWOOD REBEL D840 155112	04-May-19	Female	006 K	1.5

Tag	Sex	Bucks	Date of birth	Sex	Number	Weight
177634	Female	TORCHWOOD REBEL D840 155112	04-Oct-18	Male	001 K (L)	4.8
177664	Female	TORCHWOOD REBEL D840 155112	20-Oct-18	Female	004 K (L)	4.3

**Table 4.** Mating group 3 green ear-tag

Tag	Sex	Bucks	Date of birth	Sex	Nomer	Weight
<b>170149</b>	<b>Male</b>	<b>BOK 150066 D 520 150066</b>				
177633	Female	TORCHWOOD REBEL D840 155112	29-Sep-18	Female	006 H (L)	2.7
177657	Female	TORCHWOOD REBEL D840 155112	08-Oct-18	Female	007 H (L)	4.2
177652	Female	TORCHWOOD REBEL D840 155112				
177686	Female	TORCHWOOD REBEL D840 155112	06-Jun-19	Male	016 H	2.7
177646	Female	TORCHWOOD REBEL D840 155112	15-Oct-18	Female	009 H (L)	4.6
170147	Female	WHYNOT 130186 D250 130186	30-Sep-18	Male	003 H (L)	3.3
				Female	005 H (L)	2.8
170167	Female	WHYNOT 150015 D520 150015	01-Oct-18	Male	002 H (L)	4.2
170198	Female	WHYNOT 150015 D520 150015	30-Sep-18	Male	001 H (L)	3.5
170046	Female	WHYNOT 150015 D520 150015	30-Sep-18	Male	004 H (L)	3.5
				Female	008 H (L)	3.3

### *Operational management*

The production system of the Dorper sheep breeding aims to produce superior lamb breeds and rams and ewes that will be used in the production system. Following is the process of rearing system to produce Dorper sheep.



handling and control. After 3-5 days of lambing, the docking process is carried out on the tail.

#### **5. Nursering**

The postnatal grouping of sheep and lambs for nursering was carried out a, ewes are given a health treatment such as shaving, deworming, and vitamins before mating to ensure that the ewe and lamb are healthy and their nutritional needs are met after lambing.

#### **6. Weaning lamb**

Separating lambs was carried out from their ewes to be introduced to feed, deworming lambs, recording lambs, and separating male and female lambs to ensure that the lambs are separated from their ewes and not suckling.

#### **7. Preparation of selected replacement stock**

Selection was carried out through performance tests on sheep to ensure the availability of sheep for the next production process. The rams are prepared for the males, while the ewes are prepared for the selected parents.

## **RESULTS AND DISCUSSION**

### **Australian Dorper sheep characteristics**

Dorper sheep is a composite breed originating from South Africa resulted from crossing the Black-Headed Persian sheep (Black-Headed Persian) with Dorset sheep (Dorset Horn) (Cloete et al. 2000). Dorper sheep were first developed in Australia in 1996 brought from South Africa. The Dorper sheep breed was developed in Australia due to good reproductive and production. Dorper sheep are also considered strong and suitable for grazing conditions in Australia similar environmental conditions in South Africa.

There are two main breeds of Dorper sheep, which are Dorper, and White Dorper. Most Dorpers are woolly sheep, some of which produce wool. The Dorper sheep has a white body with a black head (Figure 4a), much like the Persian Black Head, while the White Dorper is completely white (Figure 4b). This different colors of Dorper sheep are chosen only because of preference (Milne 2000). Therefore, the term "Indonesian Dorper" relates to the black-headed Dorper as it has been incorporated into Indonesia. There are also differences in the types of coat in Dorpers that are hair and wool type. However, since these species do not differ in economic important traits as suggested by Snyman & Olivier (2002), they will be considered the same in this literature review. Although there is a slight variation in the breed within the Dorper, these differences are of no economic importance and so will not be discussed further.

## ***Qualitative traits***

### **a. Feather colour**

The fur colour pattern of the black head type of Dorper sheep has a characteristic colour of 20% black on the head to the bottom of the neck and 80% white on the whole body up to the feet. As for the type of white Dorper, the whole body is white.



Dorper male (image courtesy of Jilakin Downs Dorper Stud)

Male white Dorper (picture courtesy of Jilakin Downs Dorper Stud)

**Figure 4.** Fur Color Black Head Dorper sheep and White Dorper (**Source:** Megan Chadwick et al. 2013)

### **b. Body shape**

No horns, body shape deep, wide, long, and dense, a mixture of fur and wool hair, thin and short fur.

## Quantitative traits

### a. Production traits

**Table 5.** Dorper sheep production performance from various mating systems and feeding systems

Litter size	Birth weight (kg)	Weaning percentage (%)	Growth from birth to weaning (g/head/day)	Mating system	Feed system	Reference
137	2.8	80	-	Accelerated	Complete diet	Elias et al. (1985)
141	4.1	91	273	Every year (Des/Jan)	Natural pature	Cloete & de Villers (1987)
112	4.2	88	188	Accelerated	Pasture with additional feed	Manyunchi et al. (1991)
121	4.2	94	270	Accelerated	Irrigated pasture	Schoeman & Burger (1992)
83	5	-	190	Accelerated	Complete pellets	Schoeman et al. 1993a., Schoeman et al. (1995)
105	4.2	87	269	Accelerated	Natural pasture	Schoeman et al. (1990)
150	4.1	96	246	Annual (April)	Natural pasture	Snyman & oliver (2002)
135		80	282	Annual (April)	Intensive complete diet	Snyman & Herselman (2005)
157		91		Accelerated	Intensive complete diet	Basson et al. (1969)

Litter size	Birth weight (kg)	Weaning percentage (%)	Growth from birth to weaning (g/head/day)	Mating system	Feed system	Reference
102 (until weaning)		81	229 (until slaughtered)	Annual (Feb/Maret)	Pasture with additional feed	Kilminster (2010) (Australian study)
Average from literature						
124%	4.1	87.50%	243	21.80 kg (weaning 3 month)		
1-2 heads	2.8-5	80-96%	188-282	16.92-25.30 kg (weaning 3 month)		

**Source:** Megan Chadwick et al. (2013)

**Table 6.** Average daily gain (ADG) of Dorper sheep and their crosses from various feed nutrition systems

ADG (g/day)	Diet	Pure or cross breed	Sheep growth rate breed comparison	Reference
187	Summer grazing	Dorper male × Dorset cross- female	Dorset = Dorper sired lambs	Notter 2004
142	Dry lot (14% CP, 72% TDN)	Dorper male × Dorset cross female	Dorper = Suffolk = Columbia sires	Notter 2004
239	Feedlot pellets (18% CP, 71% TDN)	Dorper male × Columbia female	Hair Dorpers = Wool Dorpers	Snowder & Duckett 2003
105 (3-9 bln umur)	Extensive, low quality pasture	Pure hair Dorper	Dorper>Katahdin and Pelibuey > Blackbelly	Snyman & oliver 2002
238	Housed (14.5% CP, 72% TND, 2.8 Mcal/kg DE)	Pure	Dorper > Katahdin and Pelibuey > Blackbelly	Lopez- Carlos et al. 2010
65	Natural pasture in semi-arid Kenya	Pure		Kariuki et al. 2010
261	Feedlot (14% CP, 2.6 Mcal/kg ME)	Pelibuey × Dorper	Dorper> Damara cross and Pelibuey	Salinas- Chavira et al. 2010
246	Feedlot (16.9% CP) moderate formulation for growth	Dorper × St. Croix	Dorper × St Coix > Katahdin and St Croix	Burke et al. 2003
260	Finishing pellet (16% CP, 20% Fibre, 2% fat)	Dorper × St. Croix	Dorper × St. Croix > St Croix and wool types	Bunch et al. 2004
147	Grazing + 680 g/head/day corn/soybean meal	¾ dan 7/8 Dorper	Dorper > Katahdin> St Croix	Burke & apple 2007

**Source:** Megan Chadwick et al. (2013)

**Table 7.** Feeding efficiency of Dorper sheep and their crosses in fattening programs

Feed Efficiency*	Diet	Pure or cross	Notes	Reference
7.1	Housed (14.5% CP, 10.9 MJ/Kg ME)	Pure	Katahdin is more efficient in the use of feed	Lopez – Carlos et al. 2010
4.6	Feedlot (14% CP, 10.9 MJ/kg ME)	Pelibuey x Dorper	There is no difference in feed efficiency between dorper and damara cross	Salinas-Chavira et al. 2010
6.85	Finishing pellet (16% CP, 20% Fibre, 2% fat)	Dorper x St Croix	No difference	Bunch et al. 2004
8,3**	Feedlot pellets (18% CP, 10.7 MJ/kg ME)	Dorper sire x Columbia ewe	There is no difference between Dorper, Suffolk and Columbia sire lambs	Snowder & Duckett 2003
5	15-19% CP, 2.2 Mcal/kg DM (9.2 ME)	Dorper x Pelibuey	There is no difference between dorper, white dorper, katahdin/pelibuey cross pelibuey lambs	Canton et al. 2009
5.4	15-19% CP, 2.2 Mcal/kg DM (9.2 ME)	White Dorper x Pelibuey		
10.7	Pellets, 10% CP, 10.5 MJ ME/kg DM	Pure	There is no difference between dorper and finn composite	Schoeman et al. 1993

\* Feed Efficiency (%) = Total feed intake / body weight gain

\*\* Converted dari 0.12 feed efficiency rasio

Source: Megan Chadwick et al. (2013)

## b. Reproductive traits

**Table 8.** The mating system pattern and reproductive ability of the Dorper sheep in Australia

Mating month	Mating time	Reproductive rate per year (Number of lambs born to parents per year)	Reference
January/February/ May/June/ September/October	1 month	1.46	Schoeman 1990
March/April/ November/ December/ July/August		2.36	Basson et al. 1969
May/ June/ September/ October/ January/ February	1 month	1.21	Schoeman & burger 1992
April/May/ November/December/ July/August	5 weeks	2.06	Elias et al. 1985
8-9 month March/April December/January August/September May/June	35 day	1.17	Manyunchi et al. 1991
April/May December/January August/September		1.24	Schoeman et al. 1993

*Source: Megan Chadwick et al. (2013)*

**Table 9.** Dorper sheep reproductive performance compared to Merino sheep

Item	Dorper	Merino
Age of first mating	8 month	18 month
Average number of children born per mother	1.57	1.25
Mating range	8 month	1 year
Raising time	6	6
Number of mating in life	8	4
Number of children born during life	12	5
Average weight	30.8	21.7
Total weight of children born to each parent during life	370	108

*Source: Megan Chadwick et al. (2013)*

## **Characteristics of the introduced Dorper sheep**

### ***Qualitative traits***

#### **a. Feather colour**

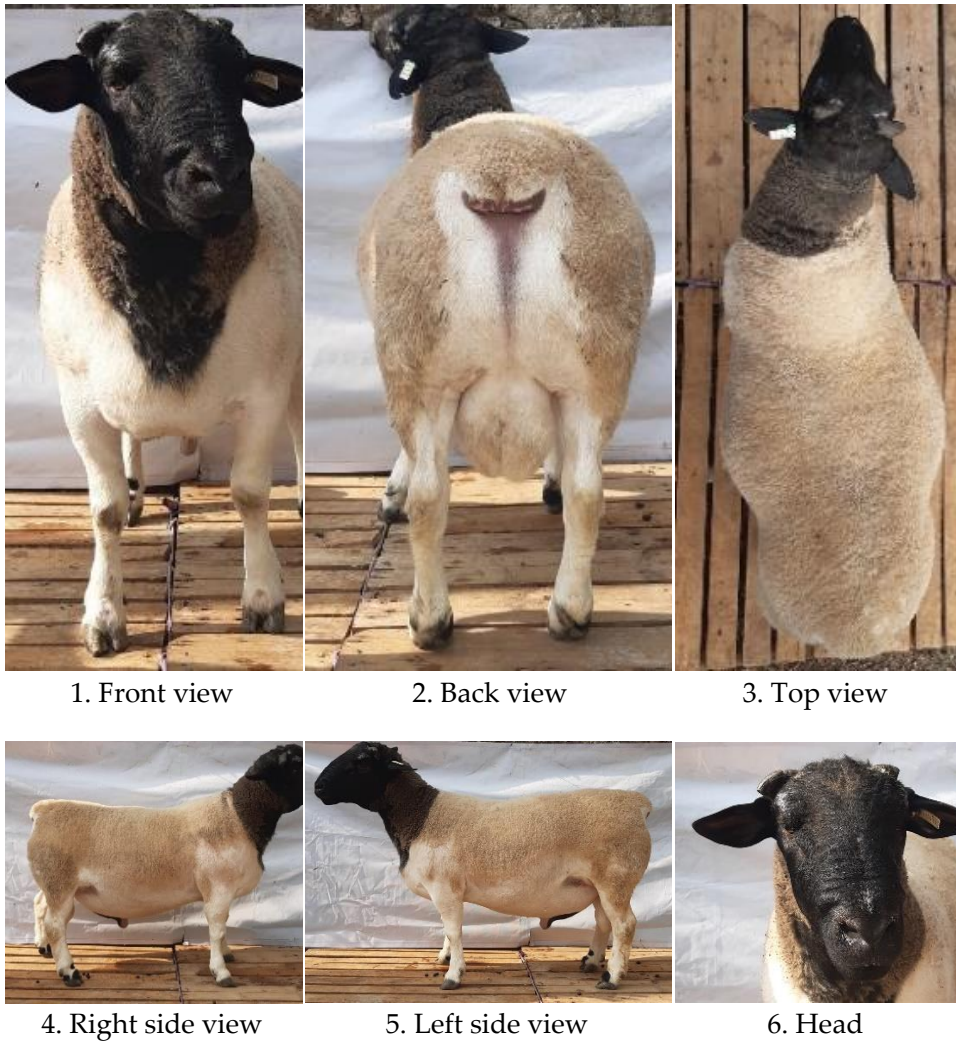
The black head type of Dorper sheep's coat colour pattern has a characteristic colour of 20% black on the head to the base of the neck and 80% white on the whole body up to the feet. The following is the body colour pattern of the black head type Dorper sheep.



**Figure 5.** The colour pattern of the Dorper sheep is black and the head to the base of the neck and white on the whole body and legs

#### **b. Body shape**

No horns, body shape deep, wide, long, and dense, a mixture of fur and wool hair, thin and short fur (figure 6).



**Figure 6.** Dorper sheep male (front, back, top, right side, left side, and head specific)

### *Quantitative traits*

#### **a. Body size (Table 10)**

**Table 10.** Dorper sheep body size

Parameters	Male				Female			
	Age $\pm$ 1 year		Age $\pm$ 2 year		Age $\pm$ 1 year		Age $\pm$ 2 year	
	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
Age (month)	12.00	-	28.00	-	11.86	0.35	28.00	-
Body weight (kg)	62.50	6.89	89.10	16.27	54.38	7.76	66.17	11.20
Shoulder height $\times$ 1 (cm)	66.57	3.74	71.89	3.81	61.64	3.64	64.89	3.83
Hip height $\times$ 2 (cm)	66.57	3.33	72.44	3.34	63.07	3.59	65.67	3.40
Body length $\times$ 3 (cm)	82.43	7.11	86.11	4.36	74.57	6.91	86.67	6.89
Chest width $\times$ 4 (cm)	28.29	4.33	32.89	3.84	25.43	3.60	29.67	4.14
Chest depth $\times$ 5 (cm)	35.29	2.25	36.44	6.47	27.36	3.58	32.00	3.90
Hip width $\times$ 6 (cm)	28.86	3.52	32.33	5.12	26.79	2.11	28.00	3.28
Chest round $\times$ 7 (cm)	93.71	7.40	104.67	8.10	88.79	4.59	97.89	9.22
Round of cannon $\times$ 8 (cm)	16.57	1.29	16.56	2.31	13.64	1.29	14.33	1.53
Hip width $\times$ 9 (cm)	27.29	2.49	30.11	4.46	21.50	3.04	24.56	2.50

*Dorper sheep aged  $\pm$  1 year are Dorper sheep born in Indonesia from breeding from their parental family which were introduced from Australia.*

*Dorper sheep aged  $\pm$  2 years are Dorper sheep born in the country of origin, namely Australia which were introduced to Indonesia*

## b. Performance traits

**Table 11.** Dorper's sheep production traits

Production trait	Average	Standard deviation
Litter size (head)	1.18	0.38
Birth weight of single (kg)	3.50	0.84
Birth weight of twin (kg)	2.94	0.45
Weaning weight 1of singles (kg)	21.43	2.95
Weaning weight 1of twins (kg)	15.50	3.62
Age of weaning (day)	90.05	1.07
ADG pre-weaning (3 month)	177.31	40.74

*This performance trait data is an introduced female Dorper sheep which was developed in Indonesia*

## c. Reproductive traits

**Table 12.** Dorper sheep reproductive traits

Reproductive characteristics	Average	Standard deviation
Lambing interval (day)	245.91	26.15
Pregnancy length (day)	155.91	26.94
Percentage of failed pregnancies	0.12	0.01
Percentage of male births (%)	42.50	
Percentage of female births (%)	57.50	
Percentage of lamb mortality (%)	13.04	

*This production trait data is an introduced female Dorper sheep which was developed in Indonesia*

**Table 13.** Dorper sheep male reproductive traits

Characteristics	Average	Standard deviation
1. Physical characteristics of the testis		
a. Testicular diameter		
Left testicle (cm)	16.88	1.47
Right testicle (cm)	17.14	1.75
b. Testicular length		
Left testicle (cm)	11.66	1.00
Right testicle (cm)	11.85	0.95
c. Scrotal circumference (cm)	28.71	2.89

Characteristics	Average	Standard deviation
2. Fresh semen characteristics		
a. Volume	0.88	0.24
b. Colour	PS	
c. Consistency	K	
d. pH	7.30	0.33
e. Spermatozoa motility (%)	86.10	3.59
f. Mass movement	2.70	0.46
g. Total concentration ( $\times 10^7$ )	233.00	99.15
h. Abnormality (%)	4.00	1.26

*This production trait data is an introduced female Dorper sheep which was developed in Indonesia*

## **New, unique, uniform and stable**

### ***New***

According to the clause in the Regulation of the Minister of Agriculture Number 117 of 2014 Article 9 Paragraph (4) letter (a) that a herd of livestock is said to be new if, at the time of receipt of the application for release, the breed or line has never been traded/circulated in Indonesia or has been traded/circulated for less than 5 (five) years. The Dorper sheep breed is a new breed that was first developed through the introduction of Dorper sheep from Australia on February 15, 2018, through Soekarno Hatta Airport Cengkareng with SPI Number No. 04.PI-54.17.0233 and recommendation number No. 11547/kpts/PI. 500/F/11/2017. Then, the breeding system and development is carried out in Malangbong Garut. The first mating system for the Dorper sheep took place on May 16, 2018, and the first lambing of the Dorper sheep was on September 28, 2019.

### ***Unique***

According to Article 9, paragraph 4 letters (b) of the Regulation of the Minister of Agriculture Number 117 of 2014, that a breed or line is considered unique if the breed or line can be clearly distinguished from the breed/line whose existence was known to the public at the time of receipt of the application for release of the breed or line.

Dorper sheep have a unique coat color with 20% black on the head to bottom neck and 80% white on the whole body to the feet. The characteristics of the Dorper sheep were no horns, deep, wide, long, and compact, a combination of fur hair and thin and short wool hair. It can be distinguished

from the Garut Sheep by its relatively spread color, its black spots appear on the head, nose, eye circles, legs and are more asymmetrical. The Garut Sheep has horns, a combination of breed or *ngadaun hiris* ears with a *bagong* tail or a *beurit* tail (Heriyadi 2011) (Ministry of Agriculture Decree 2011).



**Figure 7.** Comparison of the unique characteristics of Dorper sheep and Garut sheep

### ***Uniform***

According to Article 9, paragraph 4 letters (c) of the Regulation of the Minister of Agriculture Number 117 of 2014 that a breed or line is considered uniform if the main or important traits in the breed or line are proven to be uniform. The results of Dorper sheep development through introduction and breeding show distinctive and consistent characteristics both in terms of colour and body shape.

### ***Stable***

According to Article 9, paragraph 4 letters (d) of the Regulation of the Minister of Agriculture Number 117 of 2014, that a breed or line is considered stable if the nature or line does not change after being propagated or bred. The results of comparisons and field observations, Dorper sheep purebred from Australia showed that after being bred, they produced stable both qualitative and quantitative traits.

## GENETIC INFORMATION

The genetic information in this review is limited on the adaptability of the Dorper sheep to the Indonesian environment and disease. In terms of genetic makeup, the development of Dorper in Indonesia does not have adequate data to present because the offspring produced are still the first generation. Therefore, this review also adds the results of the study from the origin of the elders who were imported, namely from Australia.

### *Adaptation to feed (ability to utilize low quality food)*

It is becoming more important to produce adaptive sheep to changes in the quality and quantity of feed sources. In Indonesia, a tropical country, Dorper sheep can adapt well, overcoming periods of limited food availability and low nutrition during the dry season. The Dorper sheep were palatable for various types of feed that is a distinct advantage over other local livestock (Garut, Priangan, or DEG crosses). Such as forage stalks of corn/Taiwan grass are usually unpalatable for local sheep, but not Dorper sheep are. In a review, Brand (2000) showed Dorper's grazing behavior compared to Merino breeds:

- Dorper is a less selective sheep compared to the Merino breed
- Dorpers fed more bushes and less grass
- Dorpers fed more different plant species
- Dorpers walk less to choose food and grazing locations
- The Dorper sheep trample grass less than Merino.

In a study in Western Australia, pelleted feed (9.3 MJ ME/kg and 11.5% CP based on DM) was given to Dorper sheep so that they gained 100 g/d/d or lost 100 g/h/d according to their individual requirement. (SCARM 1990). Dorper sheep gained 88% more weight and lost 81% less than expected of their body mass. In addition to having lower metabolic requirements, the adapted Dorpers survive in harsh environments through increased digestive efficiency. This will allow them to get more protein and energy from the same amount of food.

### *Response to heat stress and water intake*

The hot and dry environment in many parts of Australia may favor breeds that can better cope with these conditions. Hair type breeds are thought to be better adapted to heat stress than wool type breeds (Wildeus 1997). The Dorper's water intake appears to be less efficient than that of its breeding ewes. The Persian Blackhead was 53% more efficient at utilizing water intake for weight gain (water intake/kg weight gain) than the Dorper and 77% more efficient than the SAMM (Schoeman & Visser 1995b).

The weekly water consumption of the Dorper and SAMM (South African Meat Merino) breeds doubles per 1°C increase in ambient temperature compared with the Persian Black-headed (Schoeman & Visser 1995a). It could be that the adaptation to the heat stress that Black head has may not been fully transmitted to Dorper due to the Dorset blood, a medium breed that would not have this adaptation. This may explain the Dorper may be less tolerant of heat stress than other hair type breeds such as the Damara.

Dorper does have the ability to consume water quickly after experiencing water restriction. When water becomes available after being held for 4 days, Dorpers can immediately consume more than the amount of body mass they have lost due to dehydration (Degen & Kam 1992). Few other animals can do this, even camels can only consume 58% of their lost body mass immediately after dehydration (Degen & Kam 1992). This water intake strategy is similar to their strategy of dealing with limited food availability by gaining weight rapidly when food is available to replenish the body's reserves. This "boom and bust" method of dealing with fluctuations in food and water scarcity is likely the reason that the Dorper can cope well in arid and harsh environments.

### ***Resistance to parasites***

In addition to adaptations to overcome the scarcity of feed ingredients, other adaptations such as resistance to worms, and grazing behavior can also have an economic impact on farmers. In various literatures, it has been stated that Dorper's resistance to worms is similar to that of Black Head Somali and higher than Romney Marsh (Mugambi et al. 1997). With a low to moderate worm load, all three hair types Dorper, Katahdin and St. Criox had similar resistance to infection. However, under high infection rates, Dorper sheep are less resistant to worms than other hair breeds. Under low to moderate worm infection, all breeds had greater worm resistance than Suffolk sheep (Burke & Miller 2004). When the number of faecal eggs was similar between Dorper sheep and Suffolk sheep, the packed cell volume (anemia measure) was greater for Dorper sheep showing greater tolerance or resistance to helminths (haemonchosis) (Burke & Miller 2004). Under low worm load in Western Australia there was no difference in the number of worm eggs between Dorper, Merino, and Damara (Kilminster 2010). The Dorper does not have the extreme resistance to worms that the Red Maasai does, but under the low to moderate worm load commonly found in Australia, this resistance is comparable to that of another hair breed, the Merino and perhaps tougher than the Suffolk.

The only other study to find Dorper's disease resistance was in footrot. Burke & Parker (2007) examined response to exposure and response to treatment in Dorset, Dorper, Dorper, Gulf Coast Native, Katahdin, and St. Croix. Under footrot exposure, Dorper sheep has more infected areas and Dorper have a greater percentage of smelly sheep when infected than other breeds. However, foot scores were similar among breeds and all breeds responded similarly to treatment. Dorper sheep may have a slight disadvantage when exposed to footrot, but respond to treatment as well as other breeds.

## Population

According to the Ministry of Agriculture number 117/Permentan/SR.120/10/2014 concerning Establishment and Release of Animal Clumps or Lines, it is stipulated that the minimum number of released sheep available at the institution/company proposing a minimum of 50 broods and 20 adult males. The following is the stock population of Dorper sheep in Malangbong Garut.

**Table 14.** Stock of Dorper sheep population at PT Agro Investama, Malangbong Garut

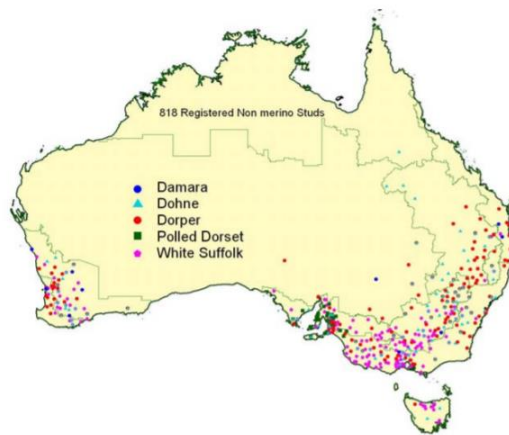
Dorper sheep group	Stock	Sat.
Female	55	heads
Parents Dorper mating process	14	heads
Dorper ewes pregnant	8	heads
Dorper ewes feeding	11	heads
Female Dorper lambs (>3-10 month)	14	heads
Female Dorper lambs (0-3 month)	8	heads
Male	32	heads
Dorper rams	21	heads
Male Dorper lambs (>3-10 month)	6	heads
Male Dorper lambs (0-3 month)	5	heads
Total	87	heads

*\* Stock data as of April 20, 2020*

Since 1947, the number of Dorpers in South Africa has steadily increased and in 2000, the estimated number of Dorpers in South Africa was 7 million (Milne 2000). In numbers, the Dorper is the second-largest sheep breed in South Africa, behind the Merino (de Waal & Combrinck 2000).

So far, there is no more detailed official data information related to the specific population structure of the Dorper from the country of origin, namely

Australia. However, if the estimation is calculated based on the MLA and AWI Wool and sheep meat Report in February 2018 which conducted a survey report in 2018 related to the number of sheep population in Australia. It is estimated that the number of adult ewes including Dorper sheep is not more than 0.5% or about 234,521 heads of the total Australian national ewes population of 42,638,123 heads. Based on these data, the estimated number of adult male Dorper sheep in Australia can be estimated at 23,452 heads. Meanwhile, based on a report submitted in 2014 by the weekly times, there are more than 550 registered ram Dorper and White Dorper breeds in Australia. This has developed significantly as the report of the Royal Agricultural & Horticultural Society Data in (2005) reported that there were 220 Dorper rams in Australia spread across several regions. The following is data on the distribution of Dorper sheep in the area of origin.



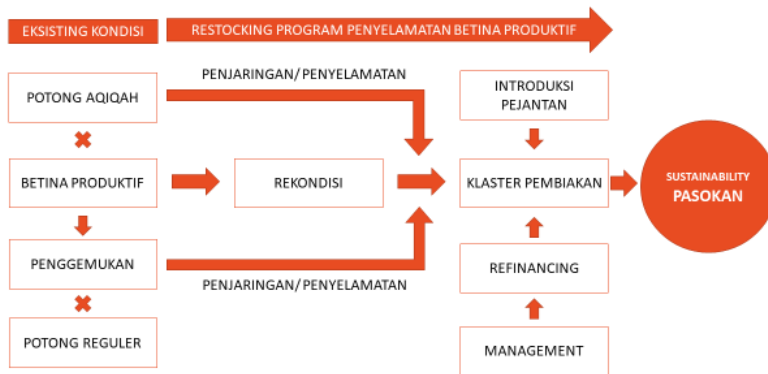
Source: Royal Agricultural & Horticultural Society Data (2005).

**Figure 8.** Distribution of non Merino sheep rams in Australia

## CONCLUSION

This Dorper sheep is the result of the introduction and breeding carried out by PT Agro Investama in Malangbong Garut. The introduction of Dorper Sheep to Indonesia is expected to increase the genetic improvement of sheep as to support the availability of quality seedstock. The production characteristics of Dorper Sheep can also be developed as meat type breeds and carry out further development to produce commercial crossbreeds with local sheep, especially Garut Sheep, which have good production performance. The development of Dorper Sheep and their future crosses can be carried out through a closed nucleus system through a national breeding program for productive females. Therefore, the increase in genetic quality shown by good

meat productivity in Dorper Sheep should be directed at the formation of cross-breed sheep as a source of supply for domestic and foreign markets by utilizing the Dorper Sheep breedstock that have been produced. The following is a strategy development for the Dorper Sheep.



**Figure 8.** Productive female breeding program scheme through the introduction of Dorper sheep crosses and nucleus closed breeding systems

1. Accelerating production in breeding Dorper sheep as a superior genetic source for meat in the development area.
2. Introducing Dorper sheep and their crosses in the local productive female breeding program.
3. Mass production of commercial crossbred sheep derived from Dorper sheep in the community through closed nucleus breeding system developed by HPDKI and PT Agro Investama to meet domestic and foreign (export) market supply sources as stated in Minister of Agriculture No. 2 of 2018.

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## Dairy Production in New Zealand

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### ABSTRACT

The dairy industry in New Zealand is an important contributor to the national economy and the aim of this paper is to discuss differences and perceived strengths of its pastoral-based production system and co-operative industry structure compared to other dairy systems. New Zealand produced 3% of the world's milk, totalling 21.1 billion litres of milk containing 1.90 billion kg milk solids (MS) during the 2019/20 season with 95% of the milk produced exported to 150 different markets. The value of dairy exports has risen from \$6.3 billion in 2001 to \$17.1 billion in 2020. In 2019/20 there was 4.92 Million dairy cows in 11,179 herds and the average herd size was 440 cows. The average milk MS produced per hectare was 1,096 kg and the average MS produced per cow was 385 kg, consisting of 215 kg milk fat and 170 kg milk protein. The New Zealand dairy industry is an integrated system, encompassing the production, manufacturing, and marketing of milk products. Historically, and still to date, the industry is based on co-operative, farmer owned structures whereby milk processors (e.g. Fonterra) focus on maximising financial return to shareholder farmers. Genetic selection and herd recording are carried out by breeding companies, the largest being Livestock Improvement Corporation (a farmer-owned co-operative), along with research and extension from DairyNZ, which is paid for by an industry levy on each kg MS produced. This integrated structure enables the continued progression of the New Zealand dairy industry.

**Keywords:** Dairy industry, Integrated, Co-operative,

### INTRODUCTION

Dairy production in New Zealand is pasture-based, distinctive from dairying in other parts of the world. Optimising pasture grown and then matching stocking rate (cow and herd feed demand) as closely as possible with the supply of pasture grown so that pasture supply (that eaten by the grazing herd) is a high proportion of the annual pasture grown. Supplementary feeds and crops are only used when available pasture is less than cow (herd) demands usually in the summer and winter periods. The New Zealand dairy herd is comprised of 33% Holstein- Friesian, 9% Jersey and the

greatest proportion are Holstein Friesian/Jersey crossbreeds, which farmers feel are best suited to New Zealand farm systems.

Pasture production in New Zealand continues throughout the year and on average, around 8 to 16 tonnes DM/ha pasture is produced annually. Spring (September to November) is the optimum season for pasture growth while winter (Jun to Aug) has the lower pasture production. Summer (Dec to Mar) has variable pasture production, dependent on rainfall and temperature. Climate and rainfall together with many management factors such as stocking rate, calving and dry off dates, fertilizer use, intensity of grazing, drainage and fencing also influence pasture production.

Manging rotation length and grazing residuals is crucial to optimise pasture grown and harvested and is key to good management of a pasture-based system. As pasture growth is much greater in spring than in winter, the majority of herds in New Zealand calve in late winter/early spring (July to September) to produce milk during spring, summer, and autumn. The dry-off date (when cows are not milked any more) is also chosen to allow cows enough time to gain weight and regain body condition before calving again. The average lactation length, or days that a cow is milked is 270 days and milk solids per cow in 2019/20 was 385 kg and the average milk solids production per hectare was 1096 kg. Milk solids per hectare is a key performance indicator as land is the major limiting resource for dairying in New Zealand and farmers like to compare amongst themselves re milk solids per hectare.

Farmers become adept at allocating feed accurately via the amount allocated for grazing by the herd each day. They monitor how they are progressing through the use a feed budget and comparisons with pasture available on-farm. When pasture is growing fast (as in spring) the rotation length (the time it takes to move through and graze all the paddocks on the farm is short (less than 21 days) to utilise pasture effectively. If too much pasture is growing the farmer will remove paddocks from the grazing rotation and make silage in those paddocks.

A feature of the New Zealand dairy industry is the integrated system, of production, manufacturing, and marketing of milk products. Historically and still to date the industry is based on co-operative, farmer owned structures whereby milk processors (e.g. Fonterra) focus on maximising financial return to shareholder farmers. Research and extension is carried out by DairyNZ, which is funded by an industry levy on each kg MS produced. Genetic selection and herd recording are carried out by breeding companies, with the largest being the Livestock Improvement Corporation (a farmer-owned co-operative). There are other service industries that operate through a cooperative structure, with companies such as Ravensdown and Ballance Agri Nutrients, who provide fertiliser products and technology to farmers.

Veterinary services and supplies are provided by a network of veterinary companies in most rural towns throughout the country. Agricultural supplies and services across the supply chain are provided through rural supply companies such as PGG Wrightson. This co-operative based structure has also been shown to operate successfully in dairy industries in other countries such as Ireland.

The integrated structure previously described enables the continued progression of the New Zealand dairy industry to compete in the world market as 95% of milk products are exported. Two of most important co-operatives (Fonterra and the Livestock Improvement Corporation) in the dairy sector are now discussed in more detail.

### **FONTERRA COOPERATIVE**

Fonterra is a co-operative which means it is owned by farmers who buy into the co-operative and agree to sell their milk to Fonterra. In return Fonterra collects their milk from their farms and pays them for the milk collected (measured in kilograms MS) plus an annual dividend. The Fonterra Co-operative is governed by a board of directors who are responsible for leadership and direction of the Co-operative. The views of the farmers are represented by the Fonterra Co-operative Council, a body of elected farmer shareholders who represent the views of all dairy farmers. Currently Fonterra control around 80% of the raw cow's milk produced in New Zealand and is the major dairy processor. The co-operative exports milk products to over 150 countries and is the world's largest exporter of dairy products. The Fonterra Co-operative has led the growth and development of functional foods, such as low fat, high calcium and high protein milk, biomedical and health products such as hyper-immune milk products within New Zealand's dairy industry.

### **Livestock improvement cooperation**

The Livestock Improvement Corporation (LIC) is a herd improvement and Agri-technology co-operative that delivers genetics and technology to farmers. It is a farmer-owned co-operative which, for more than 100 years, has provided genetics expertise, information, and technology to the New Zealand dairy sector. LIC originated in 1909, when the first organised routine herd testing service was conducted by and for New Zealand dairy farmers and has since grown to become a major enabler and contributor for the New Zealand dairy sector, with over 75% of all dairy cows within New Zealand being sired by LIC-owned bulls.

Herd tests (where individual milk samples are taken from individual cows and analysed) play a vital role in improving the quality of the national herd. Data from herd tests is used to identify productive animals that can be selectively bred to provide genetic gains for the dairy industry. One of the strengths of the LIC co-operative has been the collective ability to use data to drive improvements in genetic gain and farm performance. Data has been collected for more than 30 years, Farmers through LIC have the ability to verify ancestry and parentage for current and future herd members, while also keeping track of their matings and calvings. Herd testing has enabled farmers to identify high producers for breeding, poor producers that need to be dried-off or culled, and animals with mastitis that need treatment, drying-off or culling.

## **ISSUES THAT ARE OF PARTICULAR CONCERN TO DAIRY FARMERS TODAY**

### **Water quality**

There is a growing concern in New Zealand about the environmental impacts of dairying on surface and groundwater quality. The main causes of dairying's adverse impact on water quality is nitrogen leaching and phosphorous run-off. Approximately 20% of the nitrogen consumed in forages on a dairy farm is partitioned into meat and milk, with the remaining 80% being excreted as dung and urine (Ref). Farmers improve grass growth by applying nitrogenous fertilizer and this further increases the nitrogen content of grass and in water run-off. There is now starting to be limits on how much nitrogen fertiliser can be applied on dairy farms in New Zealand.

### **Green house gasses (GHG)**

The dairy sector is a major contributor to biogenic methane output in New Zealand and recently the Climate Change Commission, a government appointed advisory group has proposed methane gas output reduces by 13.2% by 2030, relative to 2017 levels. This 13.2% reduction in biogenic methane by 2030 is perhaps potentially achievable with currently available farm management practices and technologies. The commission has suggested changes can be made to reduce emissions while maintaining or improving farm productivity. The industry advisory service within DairyNZ is undertaking further assessment of the farm systems, science and economic assumptions made by the Commission. The Commission has highlighted the need for long-term investment in Research & Development for agriculture to

develop solutions such as inhibitors, vaccines, and research into selective breeding of dairy cows to reduce methane emissions. They have suggested that to achieve the targets there will be a potential 15% reduction in the numbers of dairy cows in New Zealand, by 2030 while maintaining current production levels. It is also envisaged that there will be direct land use change from dairy farming to horticulture of around 2000 hectares per year from 2025 to 2030

## **Animal welfare**

Animal welfare is at the heart of any good farming business. The responsibility for the wellbeing of stock starts at birth and continues, not only while they are in the farmers care, but also beyond the farm gate on the way to market. New Zealand farmers take animal welfare seriously and there are legal requirements to ensure on-farm standards that encourage every animal being treated with care and respect. The dairy industry understands consumer and market expectations in animal welfare and DairyNZ provides training to build skills and develop resources to support the best standards of care. The Animal Welfare Act 1999 of New Zealand sets out how people should take care of, and act towards animals. The Act sets out the obligations of animal owners or people in charge of animals. There are welfare codes that outline the industry agreed standards of animal care and good stockmanship practices. It is important that farmers know about Codes of Welfare because they set out the 'Minimum Standards' required to meet the welfare requirements of different animals. Failure to meet a Minimum Standard can lead to prosecution.

## **CONCLUSION**

The New Zealand dairy industry has evolved over the last 100 years into an integrated system from farm to consumer. Dairy farmers face many challenges including running a high producing farm while meeting environmental standards. There is increasing scrutiny from the public about how their food is produced in terms of animal welfare. The New Zealand dairy industry is meeting these challenges and is constantly evolving and will continue to evolve to meet these challenges with new solutions. It provides a potential blueprint for Indonesia to consider while developing an intergraded dairy industry. It will be a challenge for the Indonesian Dairy industry to link farmers along the value chain, however Indonesia has a history of industries driven by cooperatives so this knowledge should be applied to the dairy industry. However, the spread-out nature of the Indonesian dairy industry,

its smallness and exclusive supply to the local market and the low consumption of milk products by Indonesians will all be challenges to overcome.

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## Moving into More Profitable Beef Production Systems

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### ABSTRACT

Profitable beef production is a pathway out of poverty for smallholder cattle farmers. Current systems of production are often not profitable and growth rates of bulls under village production systems are low with low income over food costs (IOFC). An analysis of daily IOFC of several production systems suggests that growth rates can be increased and a system for formulating rations to supply a certain amount of nutrients or target a specific growth rate on a least-cost basis would increase IOFC. Growth rate can only increase if metabolisable energy (ME) intake of the ration can be increased. A least-cost ration (LCR) formulation system provides a way of formulating rations to increase the ME content at the least cost and can modify rations based on the local availability and prices of ingredients. Such an approach has been shown to increase IOFC. The choice of breed is also important. Potential for growth declines from EuroxOngole to Ongole to Bali cattle but the IOFC for bulls of different breeds may not differ very much. The bigger breeds may not be able to express their potential for growth if inadequately fed and IOFC is reduced. A LCR process which accommodates feed price fluctuations can be used to formulate rations which promote high growth rates to increase IOFC. Examples are given of the approach and the application of the LCR system to devise new feeding systems.

**Keywords:** Beef production system, Feed cost, Diet formulation

### INTRODUCTION

Indonesia aims to increase beef production to meet increasing demand. Smallholder farmers account for most of the cattle production within Indonesia and their participation in supply and value chains offer a pathway out of poverty (Priyanti et al. 2012). There are a wide variety of production systems across Indonesia and the price and availability of ingredients varies markedly with regions. Recommendations for improving current feeding

systems thus needs to take this into account and to offer a means for adjusting rations based on cost and availability. A least cost ration system (LCR) provides a means by which farmers, advisors and agribusiness can adjust rations to meet minimum requirements for metabolizable energy (ME), crude protein (CP) and neutral detergent fibre (NDF). In this paper we examine the current feeding systems, the opportunities to improve them, the implications for national production of beef and the application of a LCR formulation approach to developing more profitable beef cattle production systems. We draw largely from work funded by the Australian Centre for International Agricultural Research (ACIAR) in collaboration with various Indonesian agencies so as to provide a summary of the work to date.

### **Current production systems**

Within the suite of projects funded by ACIAR to study cattle production in Indonesia, several current feeding scenarios were documented. These projects looked at cow-calf systems (*e.g.* Mayberry et al. 2016) and fattening systems (*e.g.* Quigley & Poppi 2009; Mayberry et al. 2016). This paper will only examine the bull fattening systems. Production is usually characterised by live weight gain (LWG, kg/day) or feed for gain (FFG, kg feed DM intake/kg live weight gain) yet it is profit which will determine the livelihood of farmers. This is best calculated as daily Income Over Food Cost (IOFC) which provides a means of accounting for profit and uses daily production indices to describe profit and return on investment for an individual farmer (Priyanti et al. 2012; Cowley et al. 2020). The investment capital for a fattening period (usually around 50-365 days depending on region) can also be calculated as this may provide a barrier for a farmer to adopt a recommended strategy.

The full range of current feeding systems cannot be captured here as Indonesia has many such systems dictated by regional breeds, feed availability, land and access to by-products and markets. Table 1 outlines some of these production systems and some parameters of production and IOFC. It is not an exhaustive list but seeks to capture the broader systems typology. Breed types vary across regions with various regulations restricting breed types to some regions. Bali, Ongole PO and European × Ongole (Limousin and Simmental predominantly) bulls are the main breed types with some interest in Brahman and Brahman X bulls and steers.

**Table 1.** Some examples of live weight gain (LWG) and daily Income Over Food Cost (IOFC) in current production systems and some selected improved systems of production

Region	Breed	Feeding system	LWG (kg/day)	IOFC IDR/bull.day
East Java	Ongole (Cowley et al. 2020)	Elephant grass	0.23	(-) 2,830
		Onggok, protein meal, total mixed ration (TMR)	1.00	25,758
	Ongole (Antari from Mayberry et al. 2014)	Corn, protein meal, TMR	1.3	41,900
	EuroX (Retnaningrum et al. 2021)	Gaplek (40%), protein meal, TMR	1.35	42,453
		Gaplek (70%), protein meal, TMR	0.30	(-) 6,394
	EuroX (Ratnawati et al. 2015)	Village forage and by-products	0.52	15,774
		Village forage and by-products plus 1.4%W/d supplement ongkok and protein meal	0.82	24,182
	Euro X (Setiadi et al. 2020)	Village forage and by-products	0.84	25,400
		Village forage and by-products plus 2%W/d supplement of gaplek and protein meal	1.08	26,525
	Euro X (Priyanti et al. 2012)	Village forage and by-products	0.26-0.43	1,458-5,936
	Ongole (Priyanti et al. 2012)	Village forage and by-products	0.14-0.21	1,444-2,217
Yogyakarta	Ongole (Winarti et al. 2021 a,b)	Village forage and by-products	0.31-0.39	10,201-15,881
		Village forage and by-products plus 1%W/d supplement gaplek, soybean hulls, copra meal	0.59-0.75	18,779-23,396
	Ongole (Shihabudin et al. 2021)	TMR of concentrate ingredients	0.99	30,421

Region	Breed	Feeding system	LWG (kg/day)	IOFC IDR/bull.day
Central Sulawesi	Ongole (Marsetyo et al. 2021)	Corn stover	0.30	7,802
		Corn stover plus 1.6%W/d supplement cassava tuber and gliricidia	0.69	13,949
	Bali (Marsetyo et al. 2021)	Elephant grass	0.20	7,687
		Elephant grass plus 1.6%W/d supplement cassava tuber and gliricidia	0.46	12,543
NTB	Bali (Panjaitan et al. 2014, Dahlanuddin et al. 2014, 2017)	100% leucaena	0.47-0.60	19,086-21,707
		Leucaena 50% plus corn grain 50%	0.66	17,882
		Sesbania 100%	0.43	17,103
NTT Sumba	Ongole (Mayberry et al. 2021)	Grass and rice straw	0.16	6,443
		Grass and rice straw plus 2%W/d of Clitoria legume	0.46	12,543

Table 1 outlines how LWG varies with production systems and breed types but IOFC also varies markedly across regions and systems. Priyanti et al. (2012) have outlined how smallholder characteristics based on animal numbers (small scale, medium scale number of bulls being fattened) and breed types also affect IOFC with small- and large-scale bull fattening operations similar in IOFC but EuroX breeds having a higher IOFC. Small scale (1-2 bulls) systems have a higher IOFC because they expended less on external feed and used more home-grown feed which was not assigned a cost. If a cost is assigned to home grown feed, then there is little difference between the systems as LWG is similarly low and there is a large opportunity to increase LWG but at the right cost for a ration. Table 1 indicates that LWG in most systems is low with a low IOFC (using market price for feed) and this relates to the use of low-cost ingredients, *e.g.* rice straw and other crop residues, native grass, home grown grass and cheap by-products when available, all of which are low in ME and CP content. However, the cost of some of these ingredients can vary markedly depending on the inclusion of labour or

opportunity cost. Farmers do not generally put a value on their labour to gather grass or feed cattle but they are costs to the system. In the analysis of various systems and strategies we have included a labour cost by assigning a value of the feed with a labour or market value if buying and in some cases the cost of production if home grown. This inevitably lowers the IOFC calculation (Priyanti et al. 2012). With these provisos Table 1 shows that current systems are usually low in production (daily LWG) and IOFC and that systems that target higher LWG with regard to the cost of the ration (least cost) meeting minimum ME, CP and NDF contents have higher IOFC and produce more annual LWG than current systems. However, these systems come at a cost of capital outlay for feed and animals and not all farmers have that investment capital with its associated risks. Some examples of very profitable systems are the cassava based rations in East Java (Ratnawati et al. 2015; Setiadi et al. 2020; Retnaningrum et al. 2021), the gliricidia, soybean hulls and cassava based supplements in Yogyakarta and Central Sulawesi (Marsetyo et al. 2021; Winarti et al. 2021a,b), the concentrate based rations in Yogyakarta (Shihabudin et al. 2021), the leucaena and sesbania based systems in NTB (Dahlanuddin et al. 2014, Panjaitan et al. 2014; Dahlanuddin et al. 2017; Dahlanuddin et al. 2018) and the herbaceous legume based systems in NTT (Mayberry et al. 2021). There are no doubt many others limited only by the imagination of formulations and the availability and cost of ingredients.

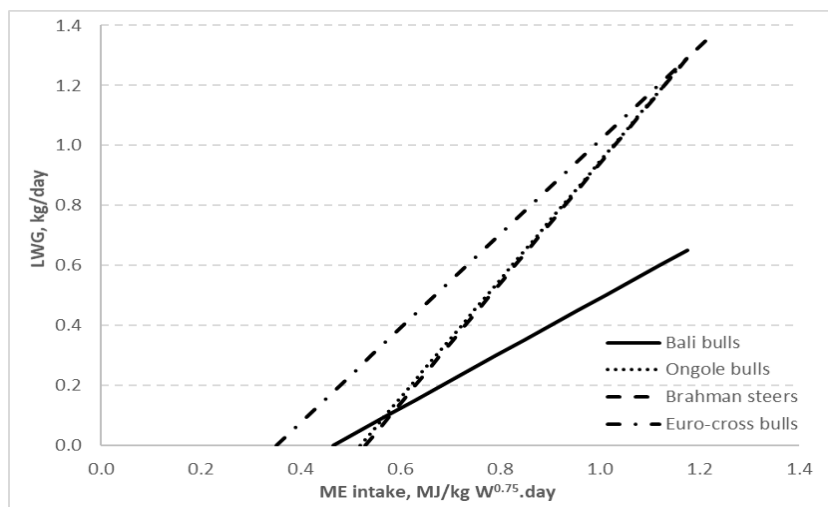
Thus, there is a large opportunity to increase beef cattle productivity (annual LWG) with a resultant increase in IOFC for the farmer. There are two strategies 1) continue with low input systems but improve the availability of feeds and supplementing them with low amounts of ingredients with a high ME content or 2) shift to feeding high amounts of LCR formulated rations which will promote high LWG. The latter approach has the most potential to markedly increase IOFC and to increase meat production nationally. If there is to be a large investment into feed costs then the nutritional principles of feeding and breed effects should be known with established response relationships to ME intake. Farmers often feed for daily cost or availability and the level of feeding may be lower than required to meet production targets and there is a production and financial penalty for this approach.

### **Biological principles of growth of various breeds**

To better understand the consequences of various management strategies (level of feeding, choice of ingredients and breed) it is important to understand how animals respond to nutrient intake. The basis for this is the response to ME intake in various feeding standards around the world including Indonesia. Table 2 outlines for the first time the regression equations for the

LWG response to ME intake for various breeds when ME intake is expressed as MJ ME/kg W<sup>0.75</sup>.day. Figure 1 illustrates the response over the range of data points and especially outlines the maximum LWG recorded for various breed types. Some conclusions from this data set are:

- The regression equations for Bali cattle and Brahman X steers are published equations from large data sets. There is a lot of variability in the Bali regression data reflecting the wide variety of feed types used in the experiments. The equations for Ongoles are only from the ACIAR experiments so as to have a standardised procedure for experimentation which is important in these regressions. The equations for Euro x Ongole (specifically Limousin × Ongole) come from more limited data from the University of Brawijaya using cassava-based rations and more data in this format need to be collected. These limitations in the equations are acknowledged but the  $r^2$  values are significant and good.
- There is no difference in the response relationship between Ongole bulls and BrahmanX steers. This might be expected given the similar *Bos indicus* background.
- The maximum LWG for Bali cattle is approximately 0.65 kg/day. The maximum LWG recorded for Ongoles and BrahmanX is 1.3 and 1.2 kg/d respectively and for EuroX 1.35kg/day from the data sets. This agrees with Haryanto and Pamungkas (2010) but the one high value for Ongoles is based on one dataset from Grati using corn grain and a protein meal (Antari in Mayberry et al. 2014) with the average of the high LWG results approximating 1.1 kg/day. Based on collated data on feedlot animals from Australia (Schutt et al. 2009) we have assumed a safer maximum LWG of 1.06 kg/day for Ongoles and Brahman X and 1.35 kg/day for EuroX under most high-quality rations. There is no doubt much genetic variation in Ongoles and opportunities to select for animals with higher LWG potential but for the purposes of ration formulation it is safer to assume these values at present.
- The ME intake required by Bali bulls to reach a maximum LWG (0.65 kg/d) will result in a higher LWG for the three other breed types of approximately 1.1 kg/day.



**Figure 1.** Regression relationship of live weight gain response to metabolisable energy intake with maximum LWG of cattle based on breeds

The ME intake required by all breeds to achieve 0.3 kg/day, a value commonly seen in most village systems, increases from EuroX (0.5MJ/kg  $W^{0.75}$ .day) to Ongole and Brahman X (0.7 MJ/kg  $W^{0.75}$ .day) to Bali bulls (0.8 MJ/kg  $W^{0.75}$ .day). Marsetyo et al. (2021) have shown that Bali bulls and Ongole bulls respond similarly to a high ME content supplement with a similar IOFC. Priyanti et al. (2012) and Antari et al (2016) have shown that in village-based systems with low LWG that LWG decreases from EuroX to Ongole an observation in agreement with the calculations based on ME requirement. Data for Euro × Ongole bulls from Retnaningrum et al. (2021), Kusmartono et al (2021 unpublished data), Ongole bulls from Mayberry et al. (2014), Cowley et al. (2020), Shihabudin et al. (2021), Bali bulls from Quigley et al. (2014) and Brahman X steers from McLennan & Poppi (2005). Number of observations (n) for each equation is given.

**Table 2.** Regression equations for live weight gain (LWG, kg/day) response to metabolizable energy intake (MEI, MJME/kg $W^{0.75}$ .day) for Euro × Ongole, Ongole and Bali bulls and Brahman X steers

Euro × Ongole bulls	$LWG = 1.568MEI - 0.551$	$r^2 = 0.90$ n = 10
Ongole bulls	$LWG = 1.970MEI - 1.024$	$r^2 = 0.72$ n = 84
Bali bulls	$LWG = 0.916MEI - 0.426$	$r^2 = 0.66$ n = 42
Brahman X steers	$LWG = 2.00MEI - 1.060$	$r^2 = 0.80$ n = 62

The practical consequences of these relationships are outlined in Table 3 using EuroX, Ongole and Bali bulls as examples. The conclusions from these calculations are:

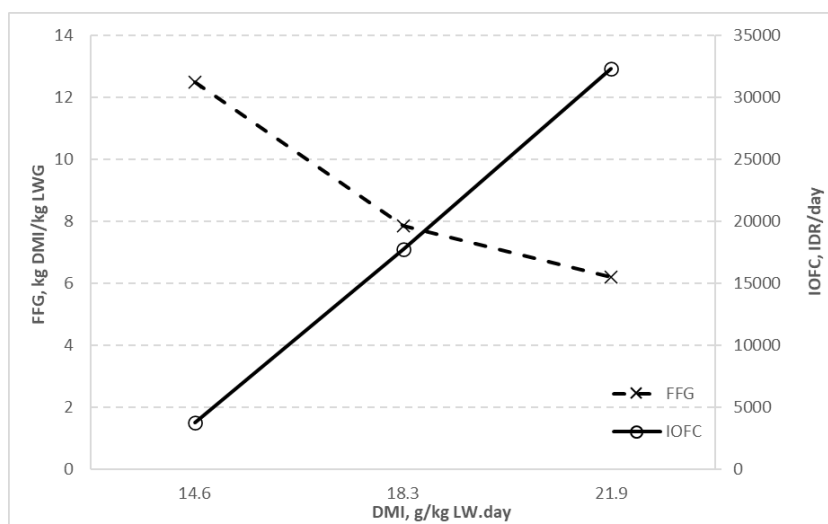
- Live weight of the bull has a marked effect on Feed for Gain (FFG) and the daily requirement of DM intake both of which markedly increase with live weight of the bull reflecting the higher maintenance requirement of the heavier animals. The consequence is that IOFC decreases markedly. It is more profitable to grow and fatten younger animals.
- The feed DM required is similar for all breed types at any given live weight given the specification of differences in maximum LWG for the breeds.
- IOFC increases at any live weight from Bali to Ongole to EuroX bulls, largely determined by the maximum LWG possible if all animals are given a very high-quality ration. This may not be the case if bulls are fed a low-quality ration or fed a restricted amount of feed resulting in a lower LWG.
- IOFC is generally much higher across all breeds when feeding a high-quality ration compared to current systems (Tables 1 and 3).
- The feed for gain (FFG, kg feed DM/kg live weight gain LWG), feed DM required (kg/day) and daily income over food cost (IOFC, IDR/day) for EuroX bulls, Ongole bulls and Bali bulls of 100, 200, 300 and 400 kg live weight at maximum LWG of 1.35, 1.06 and 0.65 kg/d respectively when fed a high quality ration (11.5 MJME/kg DM, 12% CP and 32% NDF) at a cost of IDR3140/kg DM and sale price of bulls of IDR50,0000/kg live weight. Values derived from regression equations (Table 2).

**Table 3.** Performance of cattle based on breed and live weight

Live weight (kg)	Breed	100 kg	200 kg	300 kg	400 kg
Feed for gain	EuroX	2.46	4.15	5.62	6.97
	Ongole	2.73	4.58	6.21	7.70
	Bali	4.95	8.32	11.29	14.00
Feed DM required (kg/day)	EuroX	3.32	5.60	7.59	9.41
	Ongole	2.89	4.86	6.58	8.16
	Bali	3.22	5.40	7.34	9.10
Income over food cost (IDR/day)	EuroX	57,076	49,916	43,668	37,953
	Ongole	43,926	37,700	30,339	27,378
	Bali	22,390	15,544	9,453	3,926

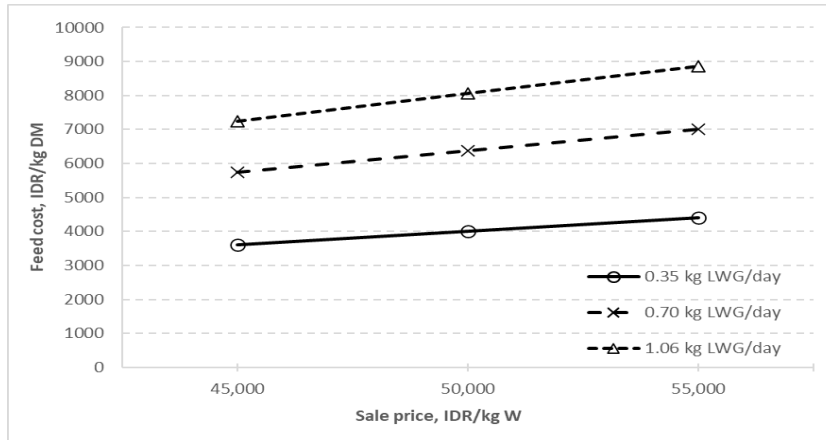
Bulls are usually restricted in ME intake either by feeding a low ME content (digestibility) feed or by physically not feeding to *ad libitum* either due to limitations in feed availability or labour. The consequence of this has a marked effect on LWG (see Fig 1), FFG and IOFC (Fig 2). Fig 2 shows that the

FFG declines markedly as intake increases up to *ad libitum* and that IOFC markedly increases. If a high-quality ration is fed then it is important to feed *ad libitum* if the advantages are to be captured in terms of IOFC.



**Figure 2.** The effect of decreasing the feed intake of a high quality ration on the feed for live weight gain and daily income over feed cost

The maximum feed cost (IDR/kg DM) for a high-quality ration (formulated according to the specifications listed) which will result in a zero IOFC is depicted in Fig 3 in response to the variable sale price of Ongole bulls and variable LWG. Some observations are obvious but the detail of the values is important. The higher the sale price and the higher the LWG, then the higher the break even feed cost. As seen later (Table 4), most rations using external feed can be formulated for approximately IDR2,800-3200/kg DM if all ingredients are bought in the market place, less if home grown. Thus, IOFC approaches zero when LWG is low (approximately 0.35 kg/day) and sale price is IDR45,000/kg LW or less. These situations often occur within a village system if farmers are not able to purchase or provide *ad libitum* amounts of the ration and this is a barrier to adoption of this strategy of feeding high quality rations at high levels to maximise IOFC. Cowley et al. (2020) have looked at the sensitivity of IOFC to these price variations. Farmers are practical and understand this quickly as they lose money.



**Figure 3.** The feed cost for break even return at LWG of a 300kg Ongole bull

**Table 4.** Least cost ration formulations based on ingredient prices from three regions of Indonesia

Region	Ingredient formulation	ME	CP	NDF	IDR/kg DM
East Java	40% gaplek, 34.2% rice bran, 25% PKC, 0.8% urea	11.5	12.0	31.5	3142
	21.2% gaplek, 50% rice bran, 25% PKC, 3.8% CM	11.2	12.0	38.3	3167
	40% gaplek, 29.2% rice bran, 30% PKC, 0.8% urea	11.6	12.0	33.4	3109
	19.7% gaplek, 50% rice bran, 30% PKC, 0.3%CM	11.1	12.0	40.0	3119
Sumbawa	100% leucaena	11.0	23.3	40.9	1672
	87% leucaena, 13% cassava tuber	11.2	20.6	36.6	1662
	59.2% leucaena, 40% cassava tuber, 0.8% corn grain	11.5	14.9	27.4	1671
	58.9% leucaena, 40% cassava tuber, 1.1% CM	11.5	15.0	27.8	1708
Central Sulawesi	100% gliricidia	11.5	22.3	49.7	1581
	60% gliricidia, 40% cassava tuber	11.8	14.4	32.9	1586
	50% gliricidia, 40% cassava tuber, 10%PKC	11.8	13.9	35.3	1604
	50% gliricidia, 25% rice bran, 25% PKC	11.2	18.5	51.7	1736

PKC palm kernel cake; CM copra meal.

Metabolisable energy (ME MJ/kg DM), crude protein (%), Neutral detergent fibre (NDF %), ration cost (IDR/kg DM)

Least cost ration formulations based on May 2021 ingredient prices from three regions of Indonesia by application of the ACIAR LCR system (Table 4). Rations were formulated to meet minimum ME (11.0 MJME/kg DM), CP (12%) and NDF (20%). Upper limits of 50% rice bran, 40% gaplek or onggok, and 25% or 30% palm kernel cake or copra meal were set 100% for all other ingredients except where evaluation of 100% Leucaena.

### Using a LCR system to formulate rations

The discussion to date has outlined the biological principles of growth of various breeds and how this impacts IOFC. The *ad libitum* ME intake is determined by the digestibility or ME content of a ration or forage with a well-established positive relationship, albeit quite variable, between intake and digestibility or ME content (eg collated in Minson 1990). There may be a high IOFC even with low quality rations or forages and low LWG (eg Priyanti et al. 2012) but this depends very much on how feed is costed (market price vs home grown with or without opportunity labour costs). Cowley et al. (2020), Marsetyo et al. (2021), Retnaningrum et al. (2021), Setiadi et al. (2020), and Winarti et al. (2021a,b) have shown positive IOFC associated with current feeding systems but that feeding a high quality supplement up to levels of 1-1.6%W/day results in a large increase in IOFC. Using a ration formulated for minimum ME, CP and NDF and fed *ad libitum* results in higher LWG and IOFC (Cowley et al 2020, Retnaningrum et al. 2021) and similar results have been found for herbaceous and tree legumes (Dahlanuddin et al. 2014, Panjaitan et al. 2014; Mayberry et al. 2016) (Table 1).

There are two approaches in developing rations. The most common is to formulate a ration according to accepted nutritional principles for ME and CP content, with a minimum NDF content for rumen health, measure the LWG response and then to recommend a recipe to farmers for application. This approach is appropriate and can give excellent results. The problem is that it may have no relationship to cost of ingredients as the scientist seeks to test response to a by-product or processing method or some regionally available feed. There may be a cheaper alternative but the recipe approach does not encourage this. Also, when cost or availability of an ingredient changes markedly the recipe approach cannot quickly adjust to the changing circumstance. An alternative approach used by large commercial feedlots is to use a LCR system. In this approach rations are formulated according to minimum ME, CP and NDF content, based on the cost of ingredients and their composition, to devise the least cost combination. In a commercial feedlot these rations are adjusted regularly. In a smallholder village system, the same approach can be used by utilising extension staff, co-operatives or agri-

business to advise individual farmers or village groups. A recipe is still developed and recommended but it can change quickly whilst still adhering to meeting minimum nutritional content eg cassava tips and peels can become available at very cheap prices at certain times of the year within various regions and can be utilised quickly to develop a new ration recipe for those farmers. There are many international commercial LCR packages but they are expensive and often require nutritional information which is not available.

A recent ACIAR project has developed an ACIAR LCR system that is free and available for use within Indonesia (K.J. Harper pers. comm.) and various Indonesian groups have trialled and modified this simple LCR system. These groups (Indonesian authors of this paper) are available for advice and training on the use of the package. An App version is available (Beefup) free for android phones which has less features but can be used quickly and simply to devise some rations. Table 4 outlines some examples of rations formulated by the ACIAR LCR approach in various regions and Table 5 outlines some consequences. Some conclusions are:

- The LCR ration outputs vary across regions reflecting the cost and availability of ingredients. Thus, a recipe for Central Sulawesi could be very different to a recipe for East Java.
- The cost of ingredients varies markedly according to region and whether they are bought in the market place or home grown and, for home grown and collected, how labour is accounted for by the farmer.
- Altering the ingredient input may have little effect in some cases eg not using urea to avoid potential toxicity problems results in a small amount of copra meal being substituted in one example (Table 4) with little change in cost. The LCR system allows these sorts of scenarios to be investigated.
- Decreasing the minimal ME content allows more ingredient combinations but the effect on LWG and IOFC is not known eg decreasing the minimal ME content from 11.5 to 11.0 MJME/kg DM is unlikely to have a significant practical effect on LWG.
- Total forage systems, eg leucaena, can meet the nutritional requirements for high LWG and the LCR will show this. If this is limited in supply or the farmer wants to carry more animals /ha on leucaena land then an upper inclusion rate can be set and other ingredients added to meet the total mixed ration (TMR) requirements.
- Currently it is recommended that a TMR should be formulated to meet minimal levels of ME (11.0-11.5 MJME/kg DM, CP (12%) and NDF (20%).
- Estimated IOFC was based on ration cost (Table 4) and 300 kg Ongole bull growing at 1.06kg/d or 200 kg Bali bull growing at 0.6kg/d with income estimated at IDR50,000/kg live weight and DM intake required from Table 3 and specified ration cost from Table 4 presented in Table 5.

**Table 5.** Estimated income over food cost IOFC based on ration cost and 300 kg Ongole bull DM intake required and specified ration cost

Site	Breed	Ration cost IDR/kg DM	IOFC IDR/day
East Java	Ongole	3,109	32,543
Central Sulawesi	Ongole	1,586	42,565
Sumbawa	Ongole	1,662	42,065
Sumbawa	Bali	1,662	21,161

There are some limitations and unknowns in the application of the system which require further testing. These include:

- The minimum ME content could be set at 11 MJME/kg DM with little effect on LWG. This has yet to be tested but would enable a wider source of ingredients to be used. Currently cassava and corn grain are the only two readily available ingredients with high enough ME content to raise the total mixed ration (TMR) ME content to 11.5 MJME/kg DM.
- Limits are currently placed on upper inclusion rates of some ingredients to avoid digestive issues such as acidosis, HCN intake or palatability. Currently these are 50% rice bran, 40% for cassava powder (gaplek), whole cassava tuber or cassava bagasse (onggok), 25% for copra meal and palm kernel cake, and 2% urea.
- The values for ME, CP and NDF content vary depending on the source of information. The ACIAR LCR uses Feedipedia values or local values where confident of analysis. Various systems around the world have feed tables and the nutrient content can vary markedly between systems. This has a marked effect on the output based on which value is used for nutrient composition. A standardised approach and collation for Indonesia would be useful. Such proximate tables do exist in Indonesia but they require upgrading to reflect changing processing methods eg in the production of rice bran.
- Formulating various rations and testing the LWG response would give confidence in the application of the LCR. If two rations are formulated for the same ME and CP content but using different ingredients then it is expected that the LWG response will be the same but this has not been tested locally with Indonesian ingredients although it is an assumption for all international feeding standards. The nutritional principles underpinning the LCR system suggest that they should be the same but this requires testing under Indonesian conditions and ingredients. If LWG differs, then it might suggest that the nutrient composition allocated to the ingredients are not correct or that the mix of ingredients affects intake or the efficiency of use of ME for growth. This can be seen in the results of Cowley et al. (2020;

2021) and Retnaningrum et al. (2021) where the level of gaplek or onggok was increased and protein meals decreased and inclusion levels of cassava products greater than 40% resulted in marked depression in intake and LWG which would not be expected if simply looking at ME content.

The message is that the ACIAR LCR system is flexible and allows rations to be changed to meet changing circumstances of availability and cost but there needs to be testing of the various combinations to confidently rely on the outputs. The ACIAR LCR system is simple and can be used by extension staff, farm co-operatives and small-scale feed manufacturers and agri-business to formulate high quality rations for farmers based on meeting minimal nutritional values at lowest cost. Market price ingredients can be used and usually formulate rations for IDR2,500-3,200/kg DM but similar nutrient content rations can be devised using home grown cassava tubers and tree or herbaceous legumes for IDR1500-2000/kg DM. If the same LWG response occurs, then the marked increase in IOFC is apparent.

### **Potential production systems**

The application of the ACIAR LCR system allows novel systems to be devised and evaluated (Tables 1, 4, 5).

To increase LWG and IOFC, rations need to be formulated to meet the minimum ME (11MJME/kg DM), CP (12%) and NDF (20%). Tables 4 and 5 outline some new combinations and using a standardised approach of a fattening 300kg Ongole bull with potential outputs in Table 5. Leucaena alone has become a very profitable system in NTB (Dahlanuddin et al. 2014, Panjaitan et al. 2014) with average LWG of 0.6-0.65 kg/d achieved by Bali bulls. The use of a limited amount of leucaena can be extended when fed in combination with a high energy source eg cassava or corn (Harper et al. 2019). Corn grain is perhaps best used for poultry and human rations rather than ruminants. Cassava has a high cost in the market place depending on type of cassava and processing eg chips, powder etc. A simpler system is for farmers to grow their own cassava with minimal processing such as whole tuber with chipping and sun drying to remove HCN. The cost will vary from market prices of IDR1,000/kg fresh tuber for the farmer but with a cost of production of approximately IDR500/kg fresh tuber. The farmer could value add this product by feeding to bulls. A ration of approximately 40% cassava tuber and 60% leucaena meets the nutritional specifications for ME, CP and NDF (Table 4). T. Panjaitan and Dahlanuddin (pers. comm.) have proposed that cassava could be grown within the rows of leucaena and this would increase the production/ha of the current leucaena only system or with grass grown in the inter-row area. The current DM yield of leucaena in rows in Sumbawa is

approximately 24 t DM/ha.year and it is estimated that cassava could yield 3.5 t DM/ha.year within the rows increasing the total yield/ha to 27.5 t DM/ha.year. A ration formulated on 87% leucaena and 13% cassava tuber to match production would give a very high-quality product (Tables 4,5). The IOFC would be similar to 100% leucaena using Ongole bulls as a comparison or lower for Bali bulls (IDR 42,005-42,565 vs 21,161 respectively, Tables 4,5) The live weight production for Ongole bulls would be 2920-3869 kg LWG /ha.year for leucaena alone (for LWG of 0.8 kg/d or 1.06 kg/d) vs 3708-4913 kg live weight/ha.year for a Leucaena-Cassava system. The equivalent values for Bali bulls would be 2207-2938 kg LWG/ha.year for leucaena alone. The production/ha would be the highest values recorded in the world eg Petty et al. (1998) recorded a value of 1570-2110 kg LWG/ha.year for Brahman steers grazing Leucaena and supplemented with corn grain in the Ord River region of NW Western Australia. There is no reason that this model could not be applied to other regions of Indonesia where land is not such a limitation and could use *Gliricidia* (Marsetyo et al. 2012; Marsetyo et al. 2021), herbaceous legumes such as *Clitoria ternatea* or *Stylosanthes* (Mayberry et al. 2021) or any other high yielding annual that could grow at the end of the wet season and be conserved. The LWG yields/ha depend solely on the DM/ha of leucaena, other tree legumes or herbaceous legumes combined with a high ME content source such as cassava. Cassava yields under commercial dry land conditions in the dryland tropics are approximately 6.5t DM/ha.year (Fukai & Hammer 1987) which would increase the LWG/ha.year to 3358-4913 kg LWG/ha/year depending on daily LWG.

In regions where tree legumes or cropping with herbaceous legumes are not possible or not practical due to soil type or land availability (eg Java) then intensive use of crop by-products or feed ingredient processing by-products (eg wheat pollard, soybean hulls) can be devised but formulated better than at present. Some examples are seen in Tables 1, 4 and 5. In Central Sulawesi the use of *gliricidia* with rice bran (which on farm can be much cheaper than in the market place) offers an opportunity. In Yogyakarta, soybean hulls and *gliricidia* can be used to improve LWG and IOFC. In East Java, the use of whole cassava tuber chips, cassava tips and cassava peels with palm kernel cake and/or copra meal offers various combinations at low cost especially if some by-products are purchased at times of low cost by entrepreneurial small scale feed manufacturers or co-operatives. The cost for these various options are provided in Table 4 and range from IDR1,500-3,000/kg DM and for our standardised 300kg Ongole bull this potentially results in an IOFC of IDR32,000-42,000 (Table 5). This may be compared to current cut and carry systems which have IOFC based on market bought feeds resulting in a IDR loss or positive IDR8,000 (Table 1). Priyanti et al. (2012) have calculated daily

IOFC in East Java of IDR1,444-5,936 if no cost is assigned to home grown feeds. In the calculations in this paper a cost is assigned to all feeds based on market price or cost of production.

The same principles apply to the breeding herd but the target production indices are different ie weight maintenance, target body condition score (Syahniar et al. 2012). Low cost and lower quality feed ingredients can be directed towards the cow leaving higher quality ingredients for the fattening bulls. Increased turn-off of heavier and younger bulls will require an increased supply of healthy weaned calves for fattening operations.

## CONCLUSION

The collated regression equations of LWG in response to ME intake provide a basis for comparing breeds and calculating the ME requirements of fattening bulls. For any given intake, they show that EuroX bulls will have a higher LWG than all other breeds but, as intake approaches the highest values achieved, when high quality rations are fed, then there is little difference between EuroX, Ongole and Brahman X animals which reach higher LWG than Bali bulls. Bali bulls are smaller so the outlay for feed and daily expenditure is lower with less risk, and at restricted ME intake (either through diet quality or level of available feed) there is little difference between Bali and Ongole bulls and Brahman steers. The daily food cost associated with higher live weight breeds is higher and this has a big impact on capital outlay and risk, important considerations to cash poor farmers. The higher expenditure is less of a risk to medium scale village feedlot systems. The highest IOFC are usually achieved at the highest ME intake and LWG but this depends very much on the cost of external feed and home grown or harvested feed. Reasonable IOFC can be achieved under low input and low production in village-based systems but higher IOFC can be achieved if rations are fed which will enable higher LWG approaching the maximum seen for each breed type.

The formulation of a low-cost ration to meet specifications for ME, CP and NDF content is best served by the application of a LCR system such as the ACIAR LCR. This enables local recipes to be devised and changed in response to changing feed prices and bull sale prices. It also enables new systems to be evaluated such as the use of tree legumes, herbaceous legumes or cassava-based rations. Better combinations of existing feed ingredients especially in systems where all feed needs to be purchased externally provide another way to decrease cost and increase LWG. Many other opportunities no doubt are possible. The outputs of the ACIAR LCR need to be tested especially in the combinations of by-products and forages and to identify upper limits of

inclusion of specific ingredients unique to Indonesia. The experimental testing of combinations devised by a LCR system is recommended.

Better formulation will enable higher growth rates and higher IOFC to be achieved which has the dual outcome of enabling farmers to increase their income and participate in the value chain but also nationally enable an increase in beef production which will go some way to meeting increasing domestic demand for beef.

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## **Understanding Genomic Resources of Indigenous Chickens for the Rapid Improvement of Their Production Efficiency**

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### **SUMMARY**

After the domestication, tremendous chicken genetic resources were developed across the world. Thus they have become both the most numerous farmed animals and the most widely preferred animal-sourced protein. The distribution of chicken population in the world southeast Asia down to Indonesia is a place where say the chicken product is very important to produce animal source food in cheaper cost. There is more than 1500 chicken breed or types in the world.

We are running a global chicken genome project to sequence or analyze the indigenous and traditional breeds of chicken worldwide. The first genome was sequenced for domestic animals in 2004. The result was used as an animal model for various biological research. Chickens are used to produce meat and egg and as a critical role in human society for a cultural activity or some for cockfighting. The total population of chickens is around 24 billion today.

Over the last 200 years of human selection, many unique genetic resources were built and recognized by the phenotypes or the morphology we can see today. There is a lot of different types of chickens already. Indonesia has documented more than 30 indigenous chicken breeds already, while there is still a lot of the Ayam kampung, what is so-called undescribed local chickens everywhere in Indonesia.

This global chicken genomic project is to identify how the chicken was domesticated and spread across the world. We know jungle fowl species follow this zoological taxonomy, and the red jungle fowl species could be the ancestor of the domestic chicken. There are five subspecies under the red jungle fowl; they are all distributed in south and southeast Asia. Indonesia has two/three subspecies of red jungle fowl like *G. g. spadiceus* in North Sumatra and a unique green jungle fowl, *G. g. bankiva* in Java, Bali, and Lombok.

Large-scale whole-genome sequencing initiatives were conducted on a worldwide sampling of chickens and all wild Junglefowl species/subspecies to understand the origin and the domestication of different chicken breeds. The study was conducted through an intensive international collaboration involving scientists from 60- 70 countries (Asia 30 countries, Africa 25 countries and Europe 15 countries). This project has participated major partners of LIPI Indonesia. We have been analyzing a DNA variation from mitochondria moving into the whole genome sequences. The first part of our result shows mitochondrial phylogeny. Then we can classify all the mitochondria into different clades. These clades are presenting today domesticated chicken in different frequencies. There are only six-seven maternal clades or lineage of mitochondria which suggests all the domestic chickens are originated from six or seven maternal lines in the past.

When we put all our data on the geographic map, there is a strong geographic pattern that may suggest that chicken could have originated from South Asia to Southeast Asia. More importantly, there could be a uniqueness of all domesticated in Indonesia. The 863 genomes reveal the origin and domestication of chicken was published last year. This study becomes the landmark for the chicken origin and chicken domestication. We covered all of our sampling for the four species and the five subspecies of red geography in south southeast Asia.

These latest efforts suggest that domestic chickens were derived from the Red Junglefowl subspecies *Gallus gallus spadiceus* distributed in southwestern China, northern Thailand, and Myanmar. Chickens were first transferred into Southeast and South Asia before they were subsequently taken to Europe and Africa. On their journeys out of the domestication centre, chickens were further and continuously interbred with other Red Junglefowl subspecies and Junglefowl species, which have certainly facilitated their rapid adaptation and diversification in terms of unique adaptive, behavioural, and morphological attributes. These complex dispersal processes have enriched the genetic diversity of modern-day chickens relative to their ancestors after the initial bottleneck occurred along with their domestication. This bottleneck led to the accumulation of deleterious mutations across the chicken genomes. Because most of such mutations are inherited in heterozygous states and masked as recessive alleles, the power of recent selective breeding to eradicate these genetic loads is questioned. Therefore, it is recommended to develop reliable genomic markers for monitoring the impact and efficiency of genetic improvement for indigenous chickens.



# **Livestock Production**



## The Reproductive Performance of Dairy Cattle in Smallholder Farmers

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### ABSTRACT

The reproductive performance of dairy cattle can be determined by the level of reproductive efficiency. This activity aims to obtain information on the reproductive performance of dairy cattle in 5 dairy cooperation unit (KUD) locations (Bogor, Cisarua, Cianjur, Garut and Pangalengan), conducted in January 2018 - May 2019. Data was collected through direct observation in the field and information from farmers. The parameters observed were: Body Weight (BW), Body Condition Score (BCS), parity, Calving Interval (CI), Service per Conception (S/C), Age of first calving (AFC). Data were analyzed with the GLM model from SAS (2004). The mean BW of cattle showed a difference between KUD ( $P < 0.05$ ), the highest was in Pangalengan ( $502.36 \pm 5.96$  kg) and the lowest was in Cisarua ( $415.19 \pm 9.21$  kg). The total BCS of KUD was  $2.76 \pm 0.30$ . The mean overall age of KUD was  $56.20 \pm 25.71$  months, varying based on KUD location ( $P < 0.05$ ). The mean parity of all KUDs was  $2.87 \pm 1.65$ . The overall AFC average for KUD was  $26.80 \pm 2.27$  months, differently based on the lowest KUD in Bogor and the highest in Cisarua. The mean CI in this study was still quite good, around  $13.14 \pm 1.73$  months. The highest CI was in Bogor ( $13.53 \pm 0.17$  months), while the lowest was in Garut (12.04 months). The overall average S/C of KUD was  $1.25 \pm 0.30$ . The S/C in KUD Cianjur was higher than in the other 4 KUDs. The reproductive performance of cows in overall KUD farmers is still in good condition. The existence of differences in the reproductive performance of cows between locations may be differences in mating management.

**Keywords:** Dairy cattle, Reproduction, KUD

### INTRODUCTION

The dairy business in Indonesia plays an important role in supporting the fulfillment of domestic milk consumption, which is currently only about 30% fulfilled (Ditjen PKH 2019). Dairy cattle are generally developed in Java and are raised by farmers with small-scale businesses in rural areas. Reproductive performance in dairy cattle is an important key in determining the success of

a business. The reproductive performance of dairy cattle can be determined by the level of reproductive efficiency. To evaluate the reproductive efficiency of dairy cattle, it can be done by observing Days Open (DO), Service per Conception (S/C) and Calving Interval (CI) (Atabany et al. 2011).

The reproductive performance of dairy cattle raised by farmers in each observation location is suspected of having differences, given the variety of supporting factors that can affect the efficiency of cattle reproduction. The diversity of agroecosystems and climates can also affect the diversity of feed sources and availability throughout the year, AI officers and animal health facilities and maintenance management, all of which have an impact on the reproductive performance of dairy cattle kept by farmers in each KUD location (Sulaiman 2016; Butler 2000; Bindari, et al. 2013; Lanyasunya et al. 2005). Other factors that affect the S/C value are semen quality at the farmer level, poor reception conditions due to genetic or physiological factors and lack of feed, improper detection of heat and breeder neglect and inseminator skills (Ihsan & Wahjuningsih 2011). Another thing that needs attention is the influence of body weight (BW) and body condition score (BCS) on the reproductive performance of dairy cattle (Budiawan et al. 2015; Roche et al. 2007). Age of cows can also affect the reproductive performance of dairy cattle (Zainudin et al. 2013).

This activity aimed to obtain information about the reproductive performance of dairy cattle in 5 KUD locations which can be used as a basis for consideration of improving the reproductive performance of dairy cattle in each KUD.

## **MATERIALS AND METHODS**

The research was conducted in 5 Dairy Cooperatives (KUD) in West Java (Bogor, Cisarua, Cianjur, Garut and Pangalengan). Observations were done from January 2018 - May 2019.

The parameters observed were Body Weight (BW), Body Condition Score (BCS), Calving Interval (CI), Service per Conception (S/C), Age of First Calf (AFC) and Parity. The data collected apart from direct observation in the field was also based on interviews and records of cattle reproduction from farmers.

Generally, the feed given to cows was very dependent on the availability of forage and concentrate at the location. For highland areas such as Cikajang Garut and Pangalengan, besides being given grass, horticultural waste such as carrots, cabbage, *etc.* was widely available on site. Apart from grass and agricultural waste, dairy cattle in smallholder farms were given concentrate in varying amounts for each farmer with an average per head for production dairy cattle about 6 kg of concentrate.

Measurement of BW using a measuring tape that has a BW conversion. To determine the effect of BW on the observed parameters, BW is grouped as group 1 = <400; 2 = 400 - <450; 3 = 450 – 500; 4 = >500 kg. BCS observations by observing directly the condition of the cattle's body with a score of 1 to 5, are: 1 = very thin and 5 = very fat (Dairy Australia 2013). To determine the effect of BCS on the observed parameters, BCS is grouped To determine the effect of BW on the observed parameters, BCS is grouped into 3 groups, namely: BCS <2.5, BCS 2.5-3.0 and BCS >3.0

To find out the differences between BW, BCS, and age of cows in several KUD locations, the GLM model was applied (SAS 2013)

$$Y = \mu + K + F + \varepsilon_{ijklm}$$

K = KUD; F = farmer

To determine the effect of several factors on CI, S / C, AFC, the GLM model was used:

$$Y = \mu + K + B + C + \varepsilon_{ijklm}$$

K = KUD; B = BW group; C = BCS group

## RESULTS AND DISCUSSION

### Body weight (BW), Body condition score (BCS), Age and Parity

The Least square means (LSM) and standard error (SE) of body weight (BW), body condition score (BCS), age and parity of cows at 5 KUD were presented in Table 1. The observed locations (KUD) showed differences in the mean BW ( $P < 0.05$ ). The average body weight of cows for all KUDs was  $460.92 \pm 84.71$  kg. The average BW of cows in Pangalengan was the highest compared to the other 4 KUDs. The average body weight of cows in KUD Bogor was no different from KUD Cisarua, likewise, KUD Cianjur was no different from KUD Garut. The results of previous research in KUD Cikajang Garut, the average body weight of FH dairy cows was  $307.68 \pm 21.85$  kg (Gumelar & Aryanto 2011). The difference in the average body weight at each location was possible because in addition to the diversity of types and amounts of feed given, management, as well as a different average age for each KUD where BW in the older cattle was higher. The higher average BW in Pangalengan might also be related to the average age of the cattle. The mean age of cows observed for all KUDs was  $56.20 \pm 25.71$ . The mean age of cows varied based on KUD location ( $P < 0.05$ ). The highest mean age of cows was in Pangalengan and the lowest was in Cisarua. While the average age of cows in 3 KUD locations (Bogor, Cianjur and Garut) did not show a significant difference ( $P > 0.05$ ). The age of the cows showed a pattern almost the same as body

weight, where the higher average BW in Pangalengan was also in line with the higher average age of cattle.

The mean BCS of cows from all KUDs was  $2.76 \pm 0.30$ . The BCS of cattle in KUD Bogor was the highest, followed by Pangalengan which was almost the same as Cianjur, then Garut and the lowest in Cisarua. However, in general, these results did not differ much, ranging from 2.5-3.0. The ideal BCS for calves and dry cows is between 2.75-3.25 (Dairy Australia 2013). The physiological status of cows that was observed varied greatly, therefore the BCS average of 2.76 still did not meet the optimal conditions. BCS in dairy cows is very important, generally, dairy cows at the beginning of lactation will experience a decrease in BCS due to reduced energy. Likewise, BCS should not be so high when giving birth because it will also interfere with birth (Bewley & Schutz 2008; Bastin & Gengler 2013). However, the difference in BCS in cows with different locations is very likely considering that in this analysis no correction was made to the reproductive status of cows, which for locations with a large number of cows that have just calving will show a lower BCS. The decrease in BCS and BW mostly occurs during the period between calving and milk production (Roche et al. 2007).

For all KUD, the average parity of cattle among farmers was  $2.87 \pm 1.65$ . From these data, it showed that the cows that were raised by farmers in all KUDs still gave birth on average about 3 times. Parity showed a significant difference ( $P < 0.05$ ) between KUD. Pangalengan and Bogor KUDs had a higher parity than the other 3 KUDs. Furthermore, Cianjur and Cisarua had higher parity than Garut. The amount of parity in dairy cows can also determine the productivity performance of the cows (Wasike et al. 2014). Therefore, these results can be used as a basis for evaluation to measure the productivity of cattle in each location.

**Table 1.** Least square means (LSM) and standard error (SE) of body weight, body condition score, age and parity of dairy cattle at 5 KUD

KUD	Body weight (kg)		Body condition score		Age (month)		Parity	
	N	LSM $\pm$ SE	N	LSM $\pm$ SE	N	LSM $\pm$ SE	N	LSM $\pm$ SE
Bogor	238	429.04 $\pm$ 5.17 <sup>a</sup>	253	2.94 $\pm$ 0.02 <sup>a</sup>	238	52.01 $\pm$ 1.65 <sup>a</sup>	244	3.36 $\pm$ 0.10
Cianjur	162	474.57 $\pm$ 6.26 <sup>b</sup>	176	2.72 $\pm$ 0.02 <sup>b</sup>	177	52.97 $\pm$ 1.92 <sup>a</sup>	272	2.49 $\pm$ 0.10
Cisarua	75	415.19 $\pm$ 9.21 <sup>a</sup>	75	2.53 $\pm$ 0.03 <sup>c</sup>	75	50.32 $\pm$ 2.95 <sup>a</sup>	206	2.44 $\pm$ 0.11
Garut	224	467.12 $\pm$ 5.33 <sup>b</sup>	225	2.65 $\pm$ 0.02 <sup>d</sup>	225	56.60 $\pm$ 1.70 <sup>a</sup>	265	2.79 $\pm$ 0.10
Pangalengan	179	502.36 $\pm$ 5.96 <sup>c</sup>	179	2.74 $\pm$ 0.02 <sup>b</sup>	179	65.49 $\pm$ 1.91 <sup>c</sup>	294	3.18 $\pm$ 0.09

*Different superscripts in the same column are significant differences ( $P < 0.05$ )*

### **Age of first calving**

The mean of age of first calving (AFC) for all KUDs was  $26.80 \pm 2.27$  months. This was much lower than the results of research in West Sumatra which were  $31.63 \pm 6.37$ . The age at first calving was different based on the KUD (Table 2). The lowest was in Bogor and the highest was in Cisarua. Likewise, different ages were based on the group of body weight tendencies with increasing body weight showing a decrease in the age at first birth. Body weights above 500 kg indicated that the first calving age was faster than those less than 400 kg. However, the age at first calving did not differ based on the body condition score. An AFC average of fewer than 23 months is recommended to obtain optimal production and reproduction with appropriate maintenance (Krpáľková et al. 2014). However, the objective of the maintenance period leading to AFC between 22.5-23.5 months of age, proved to be a more suitable option for obtaining a better lifetime profit (Do et al. 2013). The difference in the age of the first calving, indicates a difference in the time the cows are first bred. Age at first mating in cattle is influenced by various factors, including late estrus, detection of estrus, lack of body weight, and environmental factors (Pirlo et al. 2000) 2000; Heinrichs et al. 2005). The first age of mating in dairy cows will influence subsequent performance so that some farmers delay the mating of cows to achieve optimal body weight. Some research results state that the age at first mating should be adjusted to the ideal body weight conditions, for the existing FH breed cows are usually bred after reaching a body weight of around 285-350 kg at the age of 15-18 months (Maulana et al. 2021). Thus, it is very possible that differences in the reproductive management of dairy cattle show differences in the AFC of cattle between locations.

**Table 2.** Least square means (LSM) and standard error (SE) of age of first calving, calving interval (CI) and Service per conception of dairy cows at 5 KUD

	Age of first calving (month)		Caving interval (month)		Service per conception	
	N	LSM±SE	N	LSM±SE	N	LSM±SE
KUD						
Bogor	151	25.07±0.24 <sup>a</sup>	93	13.53±0.17 <sup>a</sup>	29	1.24±0.12 <sup>a</sup>
Cianjur	131	26.29±0.31 <sup>b</sup>	100	13.52±0.20 <sup>a</sup>	81	1.77±0.12 <sup>b</sup>
Cisarua	56	30.70±0.39 <sup>c</sup>	42	12.98±0.26 <sup>b</sup>	41	1.15±0.13 <sup>a</sup>
Garut	210	26.57±0.26 <sup>b</sup>	163	12.09±0.17 <sup>c</sup>	129	1.11±0.11 <sup>a</sup>
Pangalengan	179	25.36±0.25 <sup>a</sup>	174	13.33±0.15 <sup>a</sup>	6	1.00±0.27 <sup>a</sup>
Body weight (kg)						
<400	79	26.73±0.34 <sup>a</sup>	36	12.98±0.27 <sup>ab</sup>	40	1.45±0.14 <sup>a</sup>
400-450	159	26.30±0.27 <sup>ab</sup>	123	12.95±0.17 <sup>a</sup>	61	1.34±0.13 <sup>ab</sup>
>450-500	203	26.29±0.23 <sup>ab</sup>	155	13.10±0.15 <sup>ab</sup>	86	1.22±0.12 <sup>b</sup>
>500	286	26.00±0.22 <sup>b</sup>	258	13.33±0.14 <sup>b</sup>	99	1.31±0.11 <sup>ab</sup>
Body condition score						
<2.5	92	27.08±0.29 <sup>a</sup>	83	13.31±0.17 <sup>a</sup>	37	1.32±0.12 <sup>a</sup>
2.5-3.0	565	26.80±0.14 <sup>a</sup>	442	12.97±0.11 <sup>a</sup>	243	1.26±0.06 <sup>a</sup>
>3.0	70	26.53±0.38 <sup>a</sup>	47	12.98±0.25 <sup>a</sup>	6	1.03±0.17 <sup>a</sup>

*Different superscripts in the same column are significant differences (P<0.05)*

## Calving interval

The mean of calving interval (CI) in this study was short, around  $13.14 \pm 1.73$  months. The highest CI was in Bogor (13.53 months), while the lowest was in Garut (12.09 months) (Table 2). The difference in CI between KUD locations may also be influenced by differences in management by farmers in each KUD, including the failure of estrus detection and the availability of AI implementers. The calving interval of dairy cattle on smallholder farms is influenced by, among others, the length of the day open, the dry period time, and the length of lactation (Al-amin et al. 2017). However, the CI average for all KUDs was still short when compared to the results of other studies in Malang Regency, which is around 15-22 months (Zainudin et al. 2013). Several other research results show that the CI duration of dairy cows on smallholder farms ranges from 12.2-14.5 months (Mahmud et al. 2018; Pamungkas et al. 2016; Makin 2012; Rasad 2009; Pramono et al. 2012). Thus, overall, the CI for smallholder dairy cows in the 5 research KUDs still showed good conditions. If the average pregnancy was around 9 months, it means that the cows observed have an average day open for about 4.14 months. The almost is the same as the previous study in West Java with a day of open of about 4 months (Makin 2012). Differences in CI in dairy cows in several KUDs also showed differences in S/C, where with increasing CI, S/C tended to decrease. This is in line with the observations of Rusadi & Hartono (2015).

Longer CIs were found in cows with higher body weight. Cows with mean BW ( $>500$  kg) had a significantly longer CI than BW (400-450 kg) ( $P < 0.01$ ). However, the BW below 450 kg did not show a significant difference. The results of the regression analysis of BW showed a positive relationship with CI ( $P < 0.01$ ), with the equation:  $Y = 11.54 + 0.003 (X)$ , with  $R^2 = 1.24\%$  which indicates that the relationship is very weak. In line with research results (Budiawan et al. 2015) in PO cattle. It is suspected that the BW of cattle in this study is still in the optimal range so that the CI is still relatively short.

BCS did not show a significant effect on CI. However, the results of the BCS regression analysis on CI were significant ( $P < 0.05$ ), with the equation  $Y = 11.60 + 0.53 (X)$  and  $R^2 = 0.89\%$ . Based on these results, there was a very weak relationship, both BW and BCS, to CI. Similar results were reported for PO cattle (Budiawan et al. 2015).

## Service per conception

The average service per conception (S/C) of cattle raised by farmers for each KUD is shown in Table 3. The average S/C of dairy cows for all KUDs was  $1.25 \pm 0.30$ . is still low compared to the results of the same study, which is

around 1.5 (Nita Opi 2016) and 1-6-2.0 (Sulistiyowati et al. 2015). The S/C of dairy cows in KUD Cianjur was higher than in the other 4 KUDs which had S/C that was not significantly different. There are several factors that affect S/C, namely lactation period, lactation duration, day open (Rusadi & Hartono 2015). In this study, these factors were not corrected in the analysis model, so it is possible that variations in S/C values for each farmer or location may occur.

Cows with body weights of 400 and above have almost the same S/C, while those with body weights <400 kg have higher S/C than those with body weights > 400-500 kg and are not different from those >500 kg. This shows that to get the ideal S/C, cows should not be too thin nor too fat.

Cows with low body weight tend to have difficulty returning to estrus after giving birth, it is possible that the nutritional intake of feed is not optimal for the ideal reproductive cycle. Likewise, if cows are too fat, it is possible that there will be fat in the reproductive organs so that reproductive disorders can occur. Nevertheless, the difference between the BCS groups did not show a significant difference to S/C. It is possible that in general the observed cows kept by farmers in almost all KUD locations had good BCS conditions, namely  $2.76 \pm 0.30$ . This is in line with what was stated (Carvalho et al. 2014), the reproductive performance of dairy cows with BCS 2.75 is better than that of BCS 2.5.

## **CONCLUSION**

In general, the reproductive performance of the dairy cows that are kept by farmers in 5 KUDs is still in normal condition. Differences in reproductive performance between KUDs may be due to differences in rearing management. The results of this research can also be used as a reference in providing technology assistance to farmers for each KUD

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## **AUTHOR CONTRIBUTIONS**

Romjali E, Talib C, and Puastuti W, as the main contributors, while the others as member contributors

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## Birth Weight and Body Measurements of Crossbred Belgian Blue Calves

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### ABSTRACT

The Belgian Blue (BB) cattle breed has been well-known for double muscling. The importation of BB cattle is mainly to increase beef production. A study was done to analyze birth weight and body measurements of newborn crossbred BB calves. Data were collected from Cattle Breeding Centre (Baturaden, Padangmangatas and Sembawa) and Livestock Embryo Centre (Cipelang). The crossbred BB calves were produced by crossing of BB bull and some breed cows (Ongole grade, Friesian Holstein, Simmental, Brahman, and Limousine) through artificial insemination. A total of 286 crossbred BB calves from five different crossbreeds born in 2018-2019 were used in this study. Data of birth weight (BW), chest girth (CG), body length (BL) and body height (BH) were analyzed using a linear model with genotype, calf sex, and their interaction as sources of variation. Results showed that BW and body measurements were significantly affected by genotypes of the calf ( $P < 0.05$ ). The BB  $\times$  Friesian Holstein (FH) calves had heavier BW and bigger body sizes. The BB  $\times$  Ongloe Grade (PO) showed lighter BW and smaller body size. The means of BW, BH CG, and BL of crossbred BB calves were 37.18 kg, 71.94 cm, 69.13 cm, and 65.54 cm, respectively. In conclusion, there were differences in birth weight and body size among crossbred Belgian Blue calves affected by genotypes. Crossbreeding Belgian Blue with Friesian Holstein breed cattle resulted in heavier birth weight and bigger body size. This study was preliminary information that can be used as a recommendation in developing Belgian Blue cattle.

**Keywords:** Birth weight, Body measurements, Belgian Blue crossbreeds

### INTRODUCTION

Belgian Blue cattle, original cattle breed from Belgium, have been known as double-muscled due to mutation of myostatin gene. In the last decade, the Belgian Blue developed for increasing meat production by doing crosses with local cattle breeds in many countries has been reported (Leroy et al. 2016; Tagliapietra et al. 2018). The introduction of Belgian Blue to Indonesia was

done by the Ministry of Agriculture by importing frozen semen and frozen embryos to improve beef cattle production (Purwantara et al. 2018).

Crossbreeding was applied to increase the production of local cattle. Productivity of crossbred in beef cattle is generally superior to the mean of the parent breeds because of heterosis that improves both performance and carcass traits (Favero et al. 2019). The Belgian Blue cattle were used in many crossbreeding programs for beef improvements by combining its superiority and well adaptation of local cattle. Myostatin gene was found in the Belgian Blue F1 generation and heterozygous individuals (Agung et al. 2016; Aiello et al. 2018). Therefore, any Belgian Blue crossbreds were expected to perform double-musled. Crossbred cattle with the Belgian Blue perform higher body weight, meat yield, meat tenderness and feed efficiency than the dam breed (Leroy et al. 2016; Adi et al. 2019; Jakaria et al. 2020). Besides, crossing BB x local calf has less problem of dystocia than those of BB purebred that needs cesarean section (Kolkman et al. 2012; Aiello et al. 2018).

Body measurements are one of the crucial means for describing cattle breeds. Linear body measurements were related to production and reproduction performance in cattle. Some studies showed there was considerable variation influenced by breed, age, sex, nutritional condition and environmental factors on body measurements (Hartatik et al. 2019, 2020; Praharani et al. 2019, 2020).

It is known that the birth weight and body size of the calf have an important role in contributing to calving easily. The calf size provided the obstetrician accurate information in predicting the probability of natural calving, especially in BB blood cattle (Kolkman et al. 2012). While Fiems & Ampe (2015) showed no significant association between birth weight and heart girth of calf and dystocia.

The objective of this study was to analyze birth weight and body measurements of crossbred Belgian Blue calves. This study was preliminary information used for recommendation in developing BB to improve the productivity of beef cattle.

## **MATERIALS AND METHODS**

The study used data collected from Cattle Breeding Centre (Dairy Cattle-Baturaden; Beef Cattle-Sembawa and Padangmangatas) and Livestock Embryo Centre (Cipelang) under the Directorate of Livestock and Veterinary Services, Ministry of Agriculture. A total of 286 F1 crossbred BB calves were used in this study born in 2018-2019. The crossbred calves consisted of 123 BB x Brahman, 76 BB x Friesian Holstein (FH), 10 BB x Limousine, 40 BB x Ongole Grade (PO) and 37 BB x Simmental calves. All crossbred calves produced by

crossing of BB bull purebred with Ongole Grade (PO), Simmental, Limousine, Brahman and Friesian Holstein cows through artificial insemination (AI).

The traits evaluated were birth weight (BW), body height (BH), body length (BL) and chest girth (CG). After calves born, they were weighed and measured. Chest girth was measured circle the chest cavity behind the shoulder joint (os scapula), in units of cm using tailor tape. Body length was measured in a straight line from the elbow (humerus) to the lump of the filter bone (tuber ischii). Body height was measured starting from the highest point of the shoulder (os vertebrae thoracalis) upright to the ground surface, in units of cm. Body length and height were measured using a measuring stick.

The data generated were subjected to analysis of variance using a linear model (SAS 2003). Genotype, sex of calves, location, and their interactions were included in the model as the source of variation. Effects were considered significant at 0.05 level using P-DIFF (p-values for differences) test.

The model employed for analyses of traits measured was:

$$Y_{ijkl} = \mu + G_{i(k)} + S_j + L_k + e_{ijkl}, \text{ where:}$$

$Y_{ijkl}$  = the observed  $l$  (BW, BH, BL, CG) in the  $i$ th Genotype,  $j$ th Sex of calf and  $k$ th Location,

$M$  = Overall mean,

$G_i$  = the effect of  $j$ th Genotype group ( $i=1-5$ ) nested on the location ( $k$ )

$S_j$  = the effect of  $i$ th Sex of calves ( $j:1-2$ ),

$L_k$  = the effect of  $k$ th Location ( $k=1-4$ ),

$E_{ijkl}$  = random residual error.

## RESULTS AND DISCUSSION

### Birth weight

Results, in Table 1, showed that birth weight (BW) was significantly affected by the genotype of calves ( $P < 0.05$ ). Many studies had reported that BW and body measurements were affected by breed composition and genotypes of calves resulted from crossbreeding with BB bull (Tagliapietra et al. 2018; Jakaria et al. 2019; Hartatik et al. 2020; Praharani et al. 2019 2020). However, the sex of calves in this study did not affect BW and body measurement similar to some studies that found that the BW of BB male calves was not significantly different from the females (Praharani et al. 2019 2020). A study by Maylinda & Wahyuni (2020) stated that the sex of calves had no effects on birth weight.

**Table 1.** Least square means ( $\pm$  standard error) and P-value of birth weight (kg) and body measurements

Variable	N	Birth weight (kg)	Body height (cm)	Chest girth (cm)	Body length (cm)
LSMean	286	37.18 $\pm$ 0.72	71.94 $\pm$ 0.50	69.13 $\pm$ 0.70	65.54 $\pm$ 0.30
Genotype	P-value	<0.0001	<0.0001	<0.0001	0.0010
BB $\times$ Brahman	123	31.03 $\pm$ 1.68 <sup>a</sup>	69.36 $\pm$ 1.14 <sup>a</sup>	67.83 $\pm$ 1.63 <sup>a</sup>	64.02 $\pm$ 0.83 <sup>a</sup>
BB $\times$ FH	76	42.53 $\pm$ 0.82 <sup>b</sup>	74.78 $\pm$ 0.56 <sup>b</sup>	74.37 $\pm$ 0.80 <sup>b</sup>	70.19 $\pm$ 1.49 <sup>b</sup>
BB $\times$ Limousine	10	36.14 $\pm$ 1.85 <sup>ac</sup>	70.55 $\pm$ 1.26 <sup>a</sup>	68.46 $\pm$ 1.00 <sup>a</sup>	66.72 $\pm$ 0.66 <sup>b</sup>
BB $\times$ PO	40	30.91 $\pm$ 1.03 <sup>ad</sup>	69.36 $\pm$ 1.14 <sup>a</sup>	64.73 $\pm$ 1.80 <sup>a</sup>	61.36 $\pm$ 1.35 <sup>a</sup>
BB $\times$ Simmental	37	42.58 $\pm$ 1.57 <sup>b</sup>	70.59 $\pm$ 1.07 <sup>a</sup>	71.33 $\pm$ 1.53 <sup>ab</sup>	66.25 $\pm$ 1.27 <sup>b</sup>
Sex of calf	P-value	0.0606	0.9760	0.7341	0.3801
Location	P-value	0.0473	0.0003	0.0294	0.0380

<sup>ab</sup>within the same column, values with different letters are significantly different at  $P < 0.05$ )

The variation of birth weight in this study was influenced by breed composition in the crossing program. The overall mean of BW of BB  $\times$  local cattle was 37.18 $\pm$ 0.72 kg. The crossbred calves born after AI of the local breed with BB semen have various birth weights ranging between 15.0 and 55.0 kg with an average of 34.5 kg (Purwantara et al. 2018). While Casas et al. (2011) showed that the mean of BW calf sired by BB was 42.3 kg, which was higher than this present study due to different dam breeds of the calf.

The data show from highest to lowest BW (in kg). The BB  $\times$  FH and BB  $\times$  Simmental had heavier BW ( $P < 0.05$ ). While the BB  $\times$  PO (Ongole Grade) and BB  $\times$  Brahman had lower BW ( $P < 0.05$ ). Praharani et al. (2019) showed that the BW of BB  $\times$  FH raised in the Research Institute for Animal Production was 42.20 $\pm$ 1.47 kg, similar to this study. Although, this study used data from a different source. While the study of Jakaria et al. (2019) showed BW of F1 BB  $\times$  PO was 26.83 $\pm$ 5.1 kg that was lower than our study due to a different number of animals and environments used. However, the result of this study was closed to those of Aji et al. (2017) that BW of BB  $\times$  Brahman was 32.44 $\pm$ 5.50 kg.

Some studies reported the BW of the breeding of the dam used in this study. The BW of Limousin and Simmental born in Breeding Centre of Padangmangatas were 38.23 kg and 40.15 kg (Ningsih 2017). Whereas, the BW of and Brahman crossbreds and PO were 25.69 kg (Maylinda & Wahyuni 2020) and 25.38 kg (Jakaria et al. 2019). While Permatasari et al. (2021) reported that the BW of FH was 37.5 kg. In general, the BW of BB crossbred calves of this study were higher than those of the breed of the dam.

This present study indicated that crossbreeding of BB bull with local cows can increase the BW of calves. This crossbreeding program using BB breeding

can be applied to increase the production performance of local cattle. Crossbreeding is a way of realizing quicker genetic improvement and benefiting from complementarity combining different characteristics of genetically different animals in the crossbred (Casas et al. 2011; Favero et al. 2019). The crossbred offspring have the tendency to be superior in some quantitative traits referred to as hybrid vigor (Tagliapietra et al. 2018). This program can be applied to increase the production performance of local cattle.

## **Body measurements**

Morphological characterization is one of the crucial means for describing the cattle breeds. Body measurements are important tools for phenotypic description. The main measurements were body height, chest girth, and body length. Table 1 presented the least square means ( $\pm$  s.e) of body measurements of BB crossbred calves based on genotypes, sex of calves. All body measurements (BH, BL, CG) were significantly affected by the genotype of calves ( $P < 0.05$ ). Some studies had reported that body measurements were affected by breed composition and genotypes of calves resulted from crossbreeding with BB breeding cattle (Praharani et al. 2019). The sex of calves in this study did not affect BW and body measurement similar to the study of Praharani et al. (2019) found those body measurements of BB male calf were not significantly different from the females.

The mean of BH, CG and BL of BB crossbred calves were closed to Hartatik et al. (2019) that found in BB crossbred that the mean of BH, CG and BL were 73.64, 75.93, 58.43 cm, respectively. The BB  $\times$  FH showed bigger body sizes ( $P < 0.05$ ). While the BB  $\times$  PO performed smaller body size ( $P < 0.05$ ). Praharani et al. (2019) found body size of BB  $\times$  FH was BH, CG, and BL 75.80, 81.10 cm 66.66 cm, respectively, closed to this present study because of a similar management system, though a bigger number of animals. While the BH, CG and BL of BB  $\times$  PO calves were 68.33, 71.68 and 64.66 cm, respectively, reported by Aji et al. (2017) closed to the present study.

The results of body measurement of the present study were generally in agreement with a study of Belgian Blue calves reported by Praharani et al. (2019). However, the small differences among body measurements were due to different environments and the dam of calves. Kamal et al. (2014) found the CG, BH and BL of FH newborn calves of FH purebred were 81.9 cm, 76.2 cm, 70.9 cm. The crossbreds of BB  $\times$  FH of this present study had lower body sizes than those of FH purebred. The Ongole size at birth was 69.4, 56.35 and 66 cm for BH, BL and CG, respectively (Said et al. 2016). While the BH, BL and CG for the Brahman cross were 69.25, 63.7 and 72.00 cm, respectively (Hartatik et al. 2019).

In general, this present study showed that the body size of BB crossbred calves was higher than the breed of the dam due to BB blood as crossbreeding effect. Introduction of BB Favero et al. (2019) stated productivity of crossbred in beef cattle is generally superior to the mean of the parent breeds because of heterosis that improved both performance and carcass traits.

## CONCLUSION

Birth weight and body size of crossbred Belgian Blue calves were affected by genotypes. Crossbreeding Belgian Blue with Friesian Holstein breed cattle resulted in heavier birth weight and bigger body size. This study was preliminary information that can be used as a recommendation in developing BB breed cattle.

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## Organoleptic Quality of Processed Chicken Nuggets with the Addition of Chicken Liver, Mayonnaise, and *Indigofera* sp. Leaves

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### ABSTRACT

Chicken liver is commonly used as substitute for meat in nugget production. However, chicken liver will give fishy smell, bitter taste, and less protein content. This research is aimed to find additional ingredients to reduce the fishy smell and bitter taste of the liver and also to increase the protein content. The material used is chicken meat, chicken liver, mayonnaise, and *Indigofera* sp. leaves. This research is experimental study with organoleptic test involving 31 panelists. The parameter were color, aroma, flavor, and texture. The experimental design was a complete randomi by testing 4 nugget variants: P0: chicken meat + liver (nugget) without mayonnaise and *Indigofera* sp. leaves; P1: Nugget + mayonnaise 5% + *Indigofera* sp. leaves 5%; P2: Nugget + mayonnaise 7.5% + *Indigofera* sp. leaves 7.5 %; P3: Nugget + mayonnaise 10% + *Indigofera* sp. leaves 10 %. The data from the organoleptic test analyzed with ANOVA to find out any significant differences. The result shows that addition of mayonnaise and *Indigofera* sp. leaves has significant effect on the color but not on aroma, flavor, and texture. Both mayonnaise and *Indigofera* sp. leaves does not have strong aroma and flavor so they cannot replace the strong aroma and flavor of chicken liver. However, *Indigofera* sp. leaves have strong color so it can change the color. Based on the result of organoleptic test and statistical analysis, it was concluded that the addition of mayonnaise and *Indigofera* sp. leaves does not improve the organoleptic quality of the product.

**Keywords:** Chicken nugget, Chicken liver, Mayonnaise, *Indigofera* sp. leaves, Organoleptic test

### INTRODUCTION

material with certain ingredients (Yuliana et al. 2013). Chicken meat is the standard primary raw material for nuggets sold in the market. Chicken liver is commonly used as a substitute for meat to reduce the cost of producing the nugget. However, this substitution generates another problem: the chicken liver will give a fishy smell and bitter taste. Also, the protein content of the liver is more petite than meat (Wijayanti et al. 2013). Nevertheless, on the other

hand, chicken liver is a good source of vitamin A and iron (Krismaputri et al. 2013).

One proposed to overcome this problem is by adding other ingredients such as mayonnaise. Added mayonnaise is used for reducing the fishy smell of the nuggets and improve the taste of the produced nuggets since the distinctive aroma and taste of sour mayonnaise can also increase consumer palatability (Kartikasari et al. 2019).

Besides mayonnaise, other additional materials are still needed to improve the protein content and as additional filler. The function of the filler in the manufacture of nuggets is to improve water binding capacity, emulsion stability, flavour formation, reduce shrinkage during cooking and reduce production costs (Toana et al. 2018). Some of the fillers that can be used in making nuggets usually come from vegetables and flour.

*Indigofera* sp. is a plant that is usually used as forage for livestock. This plant has several advantages; for example, it has a high protein content, around 21.54-26.22% (Setiyaningrum et al. 2018). *Indigofera* sp., apart from being used as forage, can also be used as herbal medicine. Certain species of *Indigofera* sp. can be used as antipyretic, laxative, anti-cancer, and diuretic drugs (Suharlina 2014).

This study is aimed to find the best composition of additional ingredients to reduce the fishy smell and bitter taste of the liver and on the other hand, to increase the protein content. This study hypothesizes that the addition of mayonnaise and *Indigofera* sp. leaves can reduce unpleasant smells and tastes and increase the protein content of the nugget.

## MATERIALS AND METHODS

This research was conducted in the Poultry Product Processing laboratory within the Poultry Product Processing Study Program Community College State of Putra Sang Fajar Blitar. This research is an experimental study carried out by applying the organoleptic test to 31 panellists. The experimental design was completely random by testing four nugget variants: (1) P0: chicken meat + liver (nugget) without mayonnaise and *Indigofera* sp. leaves; (2) P1: Nugget + mayonnaise 5% + *Indigofera* sp. leaves 5%; (3) P2: Nugget + mayonnaise 7.5% + *Indigofera* sp. leaves 7.5%; (4) P3: Nugget + mayonnaise 10% + *Indigofera* sp. leaves 10 %.

All nuggets variants made with chicken meat and chicken liver as main ingredients with 1:1 composition. The differentiating variabel from each product is the content of mayonnaise and *Indigofera* sp. leaves.

## Research tools and materials

The materials used and its composition in this research are described in Table 1.

**Table 1.** The materials used and its composition in this research

Material item	Material volume (gram)			
	P0	P1	P2	P3
Chicken meat	1000.00	1000.00	1000.00	1000.00
Chicken liver	1000.00	1000.00	1000.00	1000.00
Mayonaise	0.00	100.00	150.00	200.00
<i>Indigofera</i> sp. leaves	0.00	100.00	150.00	200.00
Tapioca starch	63.75	63.75	63.75.00	63.75
Corn starch	85.0.0	85.00	85.00	85.00
Salt	29.83	29.83	29.83	29.83
Sugar	21.00	21.00	21.00	21.00
Powdered chicken broth	14.23	14.23	14.23	14.23
Pepper	10.00	10.00	10.00	10.00
Onion	150.00	150.00	150.00	150.00
Garlic	75.00	75.00	75.00	75.00
Egg	250.00	250.00	250.00	250.00

*P0: Control Treatment; P1: Treatment 1; P2: Treatment 2; P3: Treatment 3. The tools used in this research are digital scale, plastic hand glove, knife, food processor, mixer, saucepan, gas stove, pan, and LPG gas*

## Research procedure

In general, the research was carried out in 3 stages. The first is making the nuggets, then test the product with organoleptic tests, and finally, analyzing the data from organoleptic test results using analysis of variance (ANOVA). Making nuggets is done by mixing ingredients, which the composition has been determined in research design. The ingredients are mixed using a food processor and mixer before being shaped, steamed, and stored in the freezer. The product is then allowed to freeze for  $1 \times 24$  hours before being presented to the panellists for organoleptic testing.

The nugget product is then fried until cooked to be presented to the panellists for organoleptic testing. The parameters used in this organoleptic test are colour, aroma, flavour, and texture. The initial plan was 30 panellists but in the process, this research finally has 31 panellists.

The feedback from each panellist was compiled in a commonly used data processing application. After this, the compiled data are analyzed with analysis of variance (ANOVA) to determine any significant differences.

## Test procedure

### Organoleptic test

The organoleptic test was carried out in several stages. In the first stage, the panellists were given information and guidance about an organoleptic test and its procedure. Then the panellists were given a piece of a survey paper. Furthermore, the panellists tasted product samples individually and assessed each product based on four parameters, namely colour, aroma, taste, and texture. The procedure for collecting organoleptic data was carried out using the hedonic test by giving a score of 1-5, 1 is least favourable, and five is most favourable. An organoleptic test was carried out using 31 untested panellists aged 19 to 45 years.

### Data analysis

The organoleptic test results were analyzed using one-way ANOVA (analysis of variance) statistical analysis to find any significant differences. If there is a significant difference in the data being tested, it will be analyzed further with the Duncan test.

## RESULTS AND DISCUSSION

The result of organoleptic test data analysis of this research is presented in Table 2.

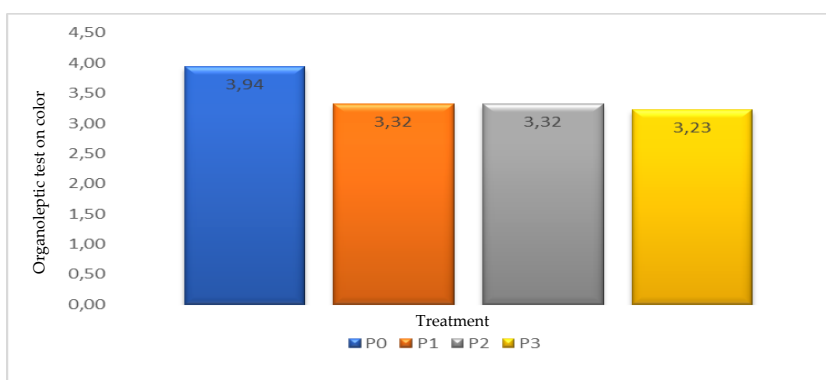
**Table 2.** Organoleptic test result

Parameter	P0	P1	P2	P3
Color	3.94±0.929	3.32±0.909	3.32±0.909	3.23±1.053
Aroma	3.87±1.024	3.48±0.996	3.65±0.950	3.55±1.060
Flavor	4.06±0.929	3.55±0.888	3.45±1.028	3.45±1.179
Texture	4.23±0.950	3.26±0.930	3.55±0.768	3.68±0.748

P0: chicken meat + liver (nugget) without mayonnaise and Indigofera sp. leaves; P1: Nugget + mayonnaise 5% + Indigofera sp. leaves 5%; P2: Nugget + mayonnaise 7.5% + Indigofera sp. leaves 7.5%; P3: Nugget + mayonnaise 10% + Indigofera sp. leaves 10 %

## Color

The analysis of variance showed that the addition of *Indigofera* sp. leaves and mayonnaise in nugget production had a significant effect ( $P < 0.05$ ) on nugget colour. The results of the hedonic value for colour are range from 3.23 to 3.94. In general, the colour of the nugget made in this research varies from golden yellow to greenish-yellow. The highest value was obtained in the control treatment P0 (without *Indigofera* sp. leaves and mayonnaise) with an average value of 3.94, while the lowest value is the P3 treatment with an average value of 3.23.



P0: chicken meat + liver (nugget) without mayonnaise and *Indigofera* sp. leaves; P1: Nugget + mayonnaise 5% + *Indigofera* sp. leaves 5%; P2: Nugget + mayonnaise 7.5% + *Indigofera* sp. leaves 7.5%; P3: Nugget + mayonnaise 10% + *Indigofera* sp. leaves 10 %

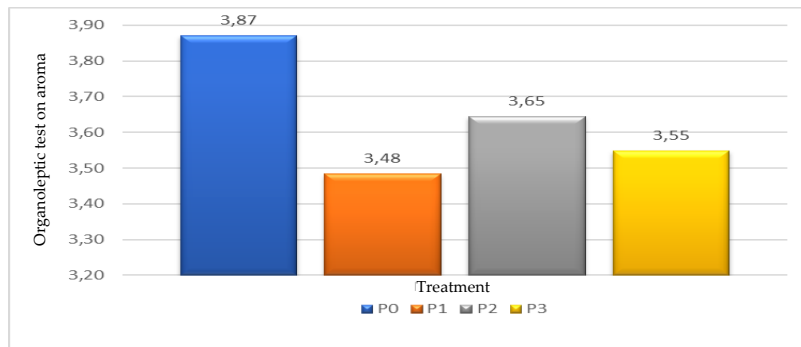
**Figure 1.** Average score of organoleptic test on color

The addition of *Indigofera* sp. leaves in the manufacture of nuggets affects the colour of the nuggets produced. The more concentration of *Indigofera* sp. leaves added, the nugget colour will be greener. The chlorophyll content in *Indigofera* sp. leaves causes nuggets to be green and causes the level of preference for the colour of nuggets added by *Indigofera* leaves to be lower. The results of the panellist's assessment of the colour of the nuggets preferred the nuggets with the control treatment (P0), which was brownish-yellow. The previous study stated that consumers' colour characteristic of chicken nuggets favoured is a golden yellow (Khatimah et al. 2018).

## Aroma

The aroma of nuggets is influenced by additional ingredients such as flavour enhancers (Khatimah et al. 2018). Aroma testing is essential to be carried out because, without the aroma, the other four flavours (bitter, sweet,

sour, or salty) will be too dominant (Sinta et al. 2019). Therefore, the addition of *Indigofera* sp. leaves and mayonnaise is expected to give better results in the aroma. However, the results of the variance analysis show that the addition of *Indigofera* sp. leaves and mayonnaise in the nuggets production process did not have a significant effect ( $p>0.05$ ) on the organoleptic aroma test. Based on the results of hedonic values from 31 panellists, the highest preference value for the aroma of nuggets was in the control treatment P0 with an average score of 3.87, followed by P2 with an average score of 3.65, P3 with an average score of 3.55, and the lowest hedonic value for the aroma of nuggets is P1 with average score 3.48.



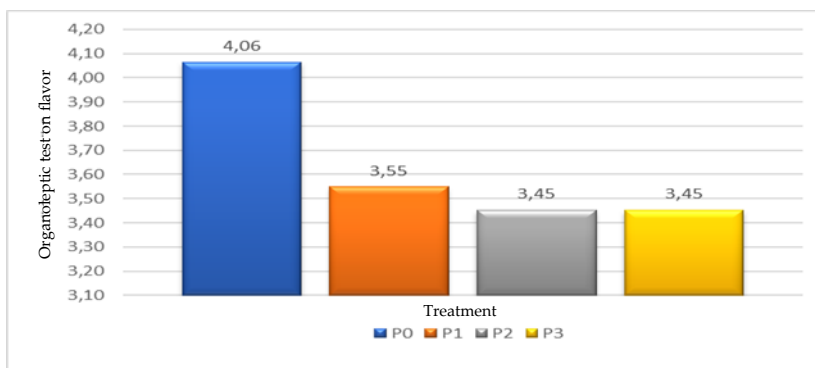
P0: chicken meat + liver (nugget) without mayonnaise and *Indigofera* sp. leaves; P1: Nugget + mayonnaise 5% + *Indigofera* sp. leaves 5%; P2: Nugget + mayonnaise 7.5% + *Indigofera* sp. leaves 7.5%; P3: Nugget + mayonnaise 10% + *Indigofera* sp. leaves 10 %

**Figure 2.** Average score of organoleptic test on aroma

The addition of Mayonnaise and *Indigofera* sp. leaves to a level of 10% did not change in aroma produced by the nuggets because the constituent raw materials and additives influence the aroma of nuggets. In this study, the raw materials used were meat and chicken liver which have a powerful aroma, so mayonnaise up to 10% has not been able to change the distinctive aroma caused by the raw material for nuggets.

## Flavor

The analysis of variance showed that the addition of mayonnaise and *Indigofera* sp. leaves had no significant effect ( $P>0.05$ ) on the organoleptic test of chicken nuggets flavour. The results of the organoleptic test from 31 panellists show that the highest average hedonic value is in the P0 treatment with a score of 4.06. Followed by P1 with an average score of 3.55, while P2 and P3 are least favourable with an average score of 3.45.



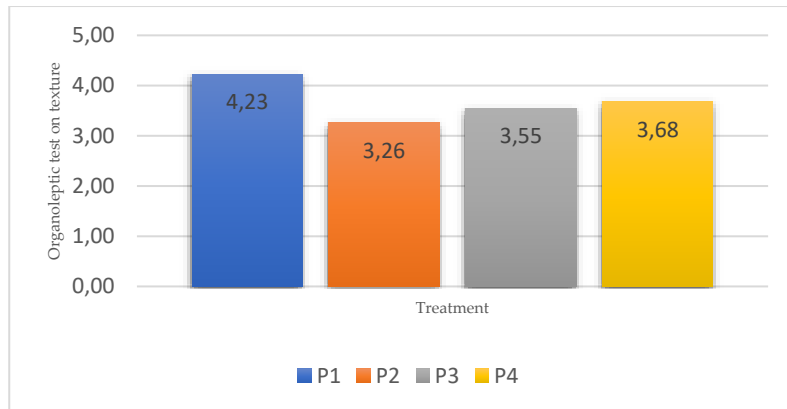
P0: chicken meat + liver (nugget) without mayonnaise and *Indigofera* sp. leaves; P1: Nugget + mayonnaise 5% + *Indigofera* sp. leaves 5%; P2: Nugget + mayonnaise 7.5% + *Indigofera* sp. leaves 7.5%; P3: Nugget + mayonnaise 10% + *Indigofera* sp. leaves 10 %

**Figure 3.** Average score of organoleptic test on flavor

The addition of Mayonnaise and *Indigofera* sp. leaves to a level of 10% did not affect the quality of the taste of the nuggets due to the use of *Indigofera* sp. leaves is to increase protein levels. Instead, the more *Indigofera* sp. leaves are added, the taste becomes too leafy. Therefore, the P2 and P3 become least favourable. The nuggets taste is influenced by additional ingredients such as salt and other seasonings such as flavouring, shallots, and garlic, following Khatimah et al. (2018), which states that the use of certain flavouring ingredients in making nuggets can increase the taste according to consumer tastes.

## Textures

Observation of nugget texture is done by determining the level of preference based on the sensation of pressure in the mouth when bitten, chewed, swallowed, and palpated using fingers (Khatimah et al. 2018). The texture is one aspect that affects consumer choices for a food product (Thalib 2011). Therefore, the addition of mayonnaise and *Indigofera* sp. leaves is expected to improve the texture of the resulting product. However, the analysis of variance results showed that the addition of mayonnaise and *Indigofera* sp. leaves had no significant effect ( $P > 0.05$ ) on the organoleptic aspect. The hedonic value of the organoleptic test of chicken nugget texture showed the highest average value in treatment P0 (without mayonnaise and *Indigofera* leaves), which was 4.23, followed by P3 with 3.68, P2 with 3.55, and the lowest value is P1 with 3.26.



P0: chicken meat + liver (nugget) without mayonnaise and *Indigofera* sp. leaves; P1: Nugget + mayonnaise 5% + *Indigofera* sp. leaves 5%; P2: Nugget + mayonnaise 7.5% + *Indigofera* sp. leaves 7.5%; P3: Nugget + mayonnaise 10% + *Indigofera* sp. leaves 10 %

**Figure 4.** Average score of organoleptic score on texture

The changes in nugget texture are influenced mainly by cooking, frying, and flour composition. The occurrence of water evaporation and temperature increase during frying can also affect changes in the texture of nuggets, following the research of Sinta et al. (2019), which stated that the texture produced in the process was influenced by the binder used, such as eggs and flour. Meanwhile, the addition of mayonnaise and *Indigofera* sp. leaves tends to increase changes in nuggets' taste quality and protein content.

## CONCLUSION

Based on the result of the organoleptic test and statistical analysis, it was concluded that the addition of mayonnaise and *Indigofera* sp. leaves does not improve the organoleptic quality of the product. Therefore, further research with other ingredients is needed to determine what kind of ingredients are required to reduce and diminish the strong taste and aroma of the chicken liver.

## AUTHOR CONTRIBUTIONS

Utama ASW; Anang Widigdyo; and David Kurniawan were contributed equally to this work

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## Carcass Composition of Bangkok Chickens in Different Sex

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### ABSTRACT

This study aims to determine the extent of the difference in the percentage of Bangkok chicken carcasses in male and female sexes. The total number of chickens used was 24 with 12 males and 12 females. The study used a student t-test to test the significance of both sexes. The parameters observed were slaughter weight, the percentage of carcass parts consisting of the percentage of thighs, chest, back, and wings. Based on the results of the discussion, it can be concluded that there are significant differences between the sex of Bangkok chickens. In this case, sex significantly affects slaughter weight, carcass percentage, breast and back percentage of Bangkok chickens. The percentage of thighs and wings did not differ in both sexes.

**Keywords:** Percentage of carcass, Bangkok chicken, Sex

### INTRODUCTION

Humans need nutrition to support their basic needs. These nutrients can be obtained through the consumption of meat as a source of animal protein. Meat is widely used because it has a good taste and high nutritional content. One of the most widely consumed sources of meat by the Indonesian people is chicken, such as native chicken and broilers (Hafid & Patriani 2021).

Nowadays consumers are more selective in choosing meat, especially urban communities, who tend to choose meat with the criteria of yellow carcass color, bright red meat color, no weird smell, low-fat content, lots of meat, and low levels of meat contamination. Therefore, it is necessary to strive for the production of good quality meat which also would suit consumer's tastes (Hafid 2011). Chicken meat is usually obtained from chicken slaughter which is distributed through the trading system from chicken carcass producers to retailers. Chicken meat has special characteristics, including whitish or pale red in color, has smooth and long meat fibers, and no fat in the meat fibers one indicator of livestock productivity that can be measured is carcass production (Hafid et al. 2019). The percentage of broiler carcass, when compared to Native chickens and Bangkok chickens, is different due to

differences in body weight growth. Broiler chickens have a faster growth compared to other chickens. Broiler chickens are able to produce a bodyweight of 1.5-1.9 kg/head at the age of 5-6 weeks (Amrullah 2004). Native chickens reach adult body weight at the age of 12 months, namely 1.6 kg in males and 1.0 kg in females (Hafid et al. 2017). The difference in the growth of each breed of chicken certainly has an influence on the difference in the percentage of carcasses produced. Another factor that affects the percentage of carcasses is sex (Soeparno 2009). Different sexes can affect the growth pattern of meat and fat, where young chickens develop a higher meat growth rate in male chickens compared to female chickens.

Consumers' need for meat consumption is increasing every year, in line with the increase in population. Chicken meat is seen as an alternative product to beef, and one breed most seen in the local markets are Bangkok chickens. Looking at the appearance of chicken carcasses would be best supported by information about carcass production. This research is aimed to find the carcass percentage of Bangkok chickens of different sex.

## **MATERIALS AND METHODS**

This research was conducted at the Animal Products Technology Unit, Department of Animal Husbandry, Faculty of Animal Science, Halu Oleo University, Kendari.

### **Research materials**

This study uses Bangkok chicken breeds. A total of 24 birds (12 males and 12 females) were studied. The equipment used was a cutting knife, cutter, sacks, label stickers, chicken scales, digital scales, plastic carcass storage containers, stationery, and a camera.

### **Research procedure**

Prior to slaughter, feed fasting was carried out for 6 hours to obtain the accuracy of the slaughter weight due to variations in the contents of the digestive tract, while drinking water was still prepared. Before slaughtering, the chicken was weighed to ensure the accuracy of the slaughter weight.

Slaughter Procedure refers to Hafid (2011) and Hafid et al. (2017; 2018), where chickens were slaughtered in a halal manner according to Islamic law, namely by cutting the esophagus, trachea, jugular vein, and common carotid artery in the neck. After that, the chicken was left hanging by the leg for 1-3 minutes until the blood stops dripping. Next, the chicken was weighed and

then immersed in hot water at a temperature of approximately 80°C for 10 minutes until the feathers are easily removed. The feathers were removed manually, and when the chicken was featherless, it was then weighed again. Then the head and both legs up to the knees are cut and the contents of the chicken and fat that are still attached to the abdominal cavity of the chicken were removed, then the carcass was weighed.

## Research design

This study uses a student t-test to test the differences between the sexes in all parameters (Gaspersz 2010). The treatment consisted of two variables: sex of chicken (A), consists of two levels, namely: A1 = Male and A2 = Female.

## Observed parameters

The parameters observed in this study refer to the instructions (Hafid 2011): (1) Slaughter weight (g) is the result of weighing chickens immediately before slaughter after being fasted from feed for 6 hours (water is still given); (2) Carcass weight (g) is the result of weighing the carcass after removing the non-carcass parts such as blood, feathers, neck to head, legs, crop, gizzard, intestine, liver, heart and spleen; (3) carcass percentage; (4) percentage of carcass parts consisting of thighs, chest, back and wings as the following formulas.

$$\begin{aligned}\text{Carcass percentage (\%)} &= \frac{\text{Carcass weight (g)}}{\text{slaughter weight (g)}} \times 100\% \\ \text{Thigh percentage (\%)} &= \frac{\text{Thigh Weight (g)}}{\text{Carcass Weight (g)}} \times 100\% \\ \text{Breast percentage (\%)} &= \frac{\text{Breast Weight (g)}}{\text{Carcass Weight (g)}} \times 100\% \\ \text{Back percentage (\%)} &= \frac{\text{Back Weight (g)}}{\text{Carcass Weight (g)}} \times 100\% \\ \text{Wing percentage (\%)} &= \frac{\text{Wing Weight (g)}}{\text{Carcass Weight (g)}} \times 100\%\end{aligned}$$

## RESULTS AND DISCUSSION

### Slaughter weight

Slaughter weight was the live weight of the chicken that was weighed before slaughtering after the chicken has been fasted for 6 hours. The slaughter weights of Bangkok chickens by sexes are presented in Table 1.

Based on the results of the variance analysis, it was shown that different breeds of chickens had significant difference ( $P < 0.05$ ) on the slaughter weight. This indicated that the three breeds of chicken had similar slaughter weight.

The ages of the three breeds of chicken used in this study were different, with Bangkok and Native chickens around 1-2 years old, while Broiler chickens were 40 days old. North (1984) argued that the live weight achieved at the same age between various strains would be different and this was caused from differences in genetic quality, as well as by environmental factors that support the genetic potential. The increase in body weight of an animal was resulted from an interaction between genetic and environmental factors (Suharsino, 1976). Further, North (1984) added, the contribution of genetic factors to growth was smaller than environmental factors, meaning that environmental factors had more dominant influence on growth.

**Table 1.** Slaughter weight, carcass percentage, thigh percentage, breast percentage, back percentage, and wing percentage of Bangkok chicken by sex

Variable	Sex	
	Male	Female
Slaughter weight (g)	1800.00±100.00 <sup>a</sup>	1566.67±57.74 <sup>b</sup>
Carcass percentage	74.10±2.72 <sup>a</sup>	71.34±2.06 <sup>b</sup>
Thigh percentage	25.72±1.21	24.04±0.31
Breast percentage	19.85±2.62 <sup>a</sup>	16.52±0.99 <sup>b</sup>
Back percentage	19.11±1.66 <sup>a</sup>	17.26±0.96 <sup>b</sup>
Wing percentage	11.85±0.23	11.10±1.63

*Different superscripts on the same row show significantly different treatment (P<0.05)*

Based on the results of the analysis of variance, sex had a significant difference (P<0.05) on the slaughter weight. The male chickens had a heavier weight than the females. The difference in the mean value of slaughter weight in male and female chickens was due to genetic and hormonal factors. Livestock growth was influenced by breed, sex, age, ration, and the environment. According to Noor (2010), sex hormones had a prominent influence on the body weight gain of livestock, which at the same time provided differences in weight and carcass percentage. The hormones that had the most prominent effect on body weight gain were estrogen and testosterone. Estrogen could inhibit bone growth, so that females would have a smaller body frame than males, and stimulate the growth of body fat, causing females to accumulate more fat in their bodies compared to males. Meanwhile, testosterone could stimulate bone growth and suppress the growth of body fat.

The results of the analysis of variance showed that different sexes resulted in a significant difference (P<0.05) in the slaughter weight. According to

Soeparno (2009) and Hafid et al. (2010; 2017; 2018), sex differences could cause differences in growth rates where males usually grow faster and are heavier than females of the same age. In line with Soeparno (2009), Yaman (2010) stated that generally, the body weight gain of male chickens is greater than that of female chickens due to differences in genetic abilities and the fact that males tend to consume more rations compared to females.

### **Carcass percentage**

Carcass weight was obtained from weighing chickens without blood, feathers, head to the base of the neck, legs to the knee, and internal organs (Hafid & Patriani 2021). The carcass percentage of Bangkok chickens can be seen in Table 1.

The results of the analysis of variance showed that the sex of different chickens has a significant effect ( $P < 0.05$ ) in the percentage of carcasses. The percentage of carcass produced is strongly influenced by the composition of the carcass which includes (the proportion of bone, muscle, fat, and skin). The mean percentage of carcasses of male and female chickens each produced a mean, that was not much different. It was assumed that the proportion of bone and the proportion of fat contained in the carcass was also the same. Carcass composition usually varies depending on the body weight of the animal. The ratio between meat and bone and the ratio between meat and fat can show the proportion of lean meat at the same fat level (Hafid et al. 2018).

### **Thighs percentage**

The thighs were separated in the acetabulum, the pelvic muscles were included, while the pelvic bones are not included in the thighs (Swatland 1984). The thighs percentage of Bangkok chickens can be seen in Table 1.

The results of the analysis of variance showed that different sexes did not show a significant effect ( $P > 0.05$ ) in the percentage of the thighs. Statistically, the percentage of male thighs had no difference with that of females. The percentage of the carcass was influenced by the age of slaughter Hafid (1998) suggested the proportion of bone tissue, meat and fat will be influenced by age, breed, body weight, sex, and food.

### **Breast percentage**

The breast was the part of the carcass that was cut from the last rib to the junction of the coracoid and clavical and cervical vertebrae. The percentages of breasts of Bangkok chicken by sex are presented in Table 1.

The results of the analysis of variance showed that different sexes did not show a significant difference ( $P>0.05$ ) in the percentage of the breast. Slaughter weight could affect the overall breast weight so that in addition to weight, the percentage value of breast meat also increases. Hafid (1998) stated breastbone growth will decrease while muscle growth (meat) increases.

### **Back percentage**

The back was cut from between the 6th and 7th ribs to the last thoracic vertebrae. The percentage of Bangkok chicken by sex is presented in Table 1.

The results of the analysis of variance showed that different breeds and sex resulted in a significant difference ( $P<0.05$ ) in the percentage of the back. The back percentage of female Bangkok chickens showed a significant difference against each breed and sex, except in male Bangkok chickens. Breeds and sexes can equally affect the percentage of backs. Hafid (2011) states that the proportion of bone tissue, meat, and fat will be influenced by age, breed, body weight, sex, and food.

### **Wing percentage**

The wing was obtained by cutting the joint between the upper arm with a scapula. The percentages of wings of Bangkok chickens by sexes are presented in Table 1.

The results of the analysis of variance showed that different sexes did not show a significant effect ( $P>0.05$ ) in the wings percentage. The percentage of carcasses in males and females was not significant, because the wings were not part of the main meat producing components. With increasing age, the percentage would also decrease. Wings were more dominated by bone, and the fat deposition on the wings was also low, so that during this growth period the results were not significant. According to Pribady (2008), wing pieces could be said to be growing moderately or in line with growth in general. In addition, bones and skin were the components of the more dominant wing pieces.

## **CONCLUSION**

It can be concluded that there are significant differences between the sex of Bangkok chickens. In this case, sex significantly affects the slaughter weight, carcass, breast, and back percentage of Bangkok chickens. Meanwhile, the percentage of thighs and wings did not differ between of sex.

## **AUTHORS CONTRIBUTION**

Harafin Hafid designed the experiment, supervised, and participated in the preparation and execution of the experiment, and in writing of the manuscript. Ugi Ardiyansyah conducted sample preparation Arby'in Pratiwi and Michala Michala participated in writing the manuscript, La Malesi and Arby'in Pratiwi conducted the data analysis, manuscript structure reviewing and language editing. All authors read and approved the final manuscript

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## **Association of Prolactin gene with Laying Traits in Merawang and KUB-2 Chicken**

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### **ABSTRACT**

Prolactin gene (PRL) is one of the physiological candidate genes for egg production which can be used as a marker-assisted selection. It was a peptide hormone that has a wide spectrum of regulated functions. In chicken, PRL leads to in reproductive cycle such as brooding behavior which causes a decrease in egg-laying intensity. The study aims to identify Single Nucleotide Polymorphism (SNP) in exons 2 and 5 of the PRL gene and its association with egg production, and (age, body weight, egg weight) at the first egg in Merawang and KUB-2 chicken. A total of 40 Merawang and KUB-2 chickens were used in the study. SNPs in exon 2 and 5 of the PRL gene were identified with direct sequencing and analysis using Bioedit. The association of genotype with laying traits was analyzed using the Generalized Linear Model (GLM) procedure of SAS. The result found 2 and 4 SNPs at exon 2 and 5 of the PRL gene in Merawang and KUB-2 chicken. Based on the chicken breed there are found the same SNP at Merawang and KUB-2 chicken, were 1) g.3805C>G and g.3838C>T at exon 2 and 2) g.7927C>G, g.7955C>G, g.8030A>T, and g.8052T>C at exon 5. In exon 2 the mutation site at SNP g.3838C>T, while in exon 5 at SNP g.8052 T>C were polymorphic. The SNP g.3838C>T at exon-2 and g.8052 T>C at exon-5 were significantly ( $P<0,05$ ) associated with egg production, age at first egg, and body weight at first egg

**Keywords:** Merawang chicken, KUB chicken, Prolactin gene, SNP

### **INTRODUCTION**

Merawang and KUB-2 chickens are Indonesian local chicken breeds. These chickens have a dual purpose for both egg and meat productions (Iskandar & Sartika 2014). Merawang chicken has special value for China society in Bangka Belitung Province since it is usually used for religious ceremonies. KUB-2 chicken is an improved local chicken that resulted from a selection program on KUB-1 chicken (Sartika & Iskandar 2019; Pratiwi et al. 2020). The productivity of egg number of Merawang chicken was 165 eggs/hen/year or 45.21% (Darwati et al. 2017) and the KUB-2 chicken was 103,3 and 101.9 eggs/hen/24 weeks or 61.5% and 60.7% (Sartika & Iskandar 2019).

The productivity of the local chicken is relatively lower than the commercial-laying chicken.

The low productivity of local chicken was influenced by several factors such as genetics background, feeding management or environment, and broodiness trait (Fadillah 2016). The broodiness traits have a negative correlation to egg production, cause the cessation of egg-laying (Li et al. 2013). Therefore, the study of molecular markers to support genetic information related to egg production or broodiness trait could be done using functional genes such as the prolactin gene. Prolactin (PRL) was a polypeptide hormone secreted by the anterior pituitary gland that has a wide spectrum of biological functions. In chicken, PRL has a crucial role to induce broodiness behavior which causes a decrease in egg-laying intensity (Mohamed et al. 2017). The prolactin gene is located on chromosome 2, which consists of 5 exons, 4 introns with a total length equal to ~6.14 kbp (Kulibaba 2015). The prolactin gene is a polypeptide with a molecular weight of 21700-26000 daltons (Alipanah et al. 2011). The sequence length of the five exons are 28 bp, 182 bp, 108 bp, 180 bp, and 192 bp for exons 1-5, and for introns, they are 1,520 bp, 408 bp, 1,348 bp, and 1,909 bp for intron 1-4 (Au & Leung 2002). Association prolactin gene in exon 2 with age at sexual maturity and body weight at hatch have been carried out by Rashidi et al. (2012). Meanwhile, prolactin genes associated with egg production also have been done (Cui et al. 2006; Jiang et al. 2009; Chen et al. 2011)

Previous research identification of the PRL gene has been done at local chickens such as IPB-D1 chicken (Rohmah et al. 2020), Shek-ki chicken (Wang et al. 2010), and Blue-shell chicken (Jiang et al. 2005). Based on previous studies, research on the PRL gene has been held widely in the world. Therefore, the study of the PRL gene in Merawang and KUB-2 chicken is specially related to laying traits: egg production, age at the first egg/sexual maturity, bodyweight at first egg, and egg weight at the first egg needed to be known.

## **MATERIALS AND METHODS**

### **Animals**

A total of 40 birds samples of Merawang and KUB-2 chicken consisted of 20 hen Merawang and 20 hen KUB-2 chickens. Merawang chicken was from BPTP Bangka Belitung Province and KUB-2 chicken from Balai Penelitian Ternak. Data were collected at 12 months of age. Blood samples had taken from the brachial (wing) vein using 3 ml syringes. The samples were taken into 1,5 ml tubes containing EDTA as an anticoagulant, stored at -20°C until DNA extraction. The procedure has fulfilled the requirement clearance ethic according to (Registration No. Balitbangtan/Balitnak/A/03/2020).

## DNA Extraction

DNA was extracted from blood samples using Quick-DNA miniprep plus kit Zymo Research that consisted of sample preparation, lysis cell, DNA binding, washing, and DNA elution. The DNA was extracted according to the manufacture's protocol. The quality of total genome extractions was performed by 1% agarose gel electrophoresis and was checked by spectrophotometry.

## Amplification

Amplification fragment of PRL gene two pairs of primers, one sense (F) and one antisense (R) primer for each of the two exons (exon 2, exon 5) of the chicken prolactin gene was designed by the web-based software Primer3Plus from the published DNA sequence of chicken prolactin gene (cPRL) deposited in the gene bank under accession number (GenBank: AF288765; Au & Leung 2002). The primers sequences and the product size were shown in Table 1. PCR reaction was carried out in 20 µl consisted DNA template (2 µl), My Taq™ Red Mix (15 µl), Nuclease Free Water (1 µl), primer forward (1 µl) and reverse (1 µl). Amplification condition consisted of predenaturation at 95°C (1 min), 35 cycles: denaturation at 95°C (15 sec), annealing 61.7°C (15 sec) and extension 72°C (10 sec) and final extension 72°C (5 min). PCR products were electrophoresed using 1.5% agarose gel.

**Table 1.** The primers sequences and the product size

Exon	Primer name	Nucleotide sequence length	(bp)	Product size
Exon 2	PRL 2F	5' CTG CCT CTG ACA GCT ATT TCC A 3'	22	294
	PRL 2R	5' CAT GTT CTC ACT CCC AGG AAA A 3'	22	
Exon 5	PRL 5F	5' CTG TTC TAC ACC CAG ACA GAT TGA 3'	24	609
	PRL 5R	5' AAG GTA TAA GCC ATC CCA GCT ATT 3'	24	

## Sequences analysis

PCR products of the PRL gene in Merawang and KUB-2 chicken were sequenced by services from MacroGen companies in Korea. Alignment sequences using BioEdit program.

## **Data analysis**

The PRL gene and its association with laying traits: egg production, age at the first egg/sexual maturity, bodyweight at first egg, and egg weight at the first egg in Merawang and KUB-2 chicken using a general linear model (GLM) with Duncan's Multiple Range test in SAS 9.4. The mathematical model follows:

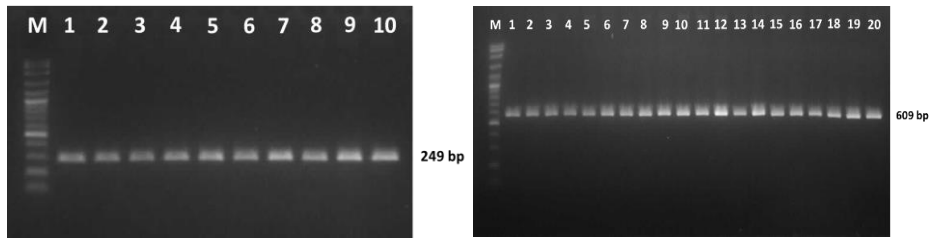
$$Y_{ij} = \mu + G_i + B_j + e_{ij}$$

Where  $Y_{ij}$  is the dependent variable;  $\mu$  is the mean square value;  $G_i$  is the effect of genotype;  $B_j$  is the effect of the breed, and  $e_{ij}$  is the random error.

## **RESULTS AND DISCUSSION**

### **SNP detection in PRL gene**

Alignment of DNA sequence for PRL gene in exon 2 and 5 in Merawang and KUB-2 chicken was amplified using PCR method with the length of PCR product 294 and 609 bp (Figure 1 and 2). Sequencing analysis in exon 2 and 5 of PRL gene in Merawang and KUB-2 chicken resulted in two SNPs in exon 2 and four SNPs in exon 5 compared to a reference gene (GenBank: AF288765). Furthermore, we only found one potential SNP in exon 2 (g.3838C>T) and exon 5 (g.8052T>C) based on Merawang and KUB-2 population (Table 2 and 3) the other SNPs were monomorphic. In exon 2 (g.3838C>T) founded mutation site, transition mutation pyrimidine to pyrimidine, one synonymous polymorphic SNP at ACC>ACT codon in exon 2, also in exon 5 (g.8052T>C) founded transition mutation polymorphic SNP at TTG>TCG, therefore, this SNP could be used as selection marker. (Mohamed et al. 2017) also found non synonymous mutation in SNP exon 2 (g.3838C>T) change amino acid from Leucin to Phenylalanin. Rohmah et al. (2020) found 5 mutation site prolactin genes in exon 5 of IPB-D1 chicken and two non-synonymous markers could be used as MAS/marker-assisted selection. Based on mutation sites in Merawang and KUB-2 chicken founded 3 diplotype variations of mutation site in exon 2 and exon 5 respectively which differed from the reference (GenBank: AF288765).



**Figure 1.** Amplification PRL gene Exon 2 (line 1-10; 249 bp) and 5 (line 1-20; 609 bp); M: 100bp ladder

**Table 2.** Nucleotide variation of Exon 2 in Merawang and Kampung Unggul Balitbangtan (KUB-2)

Population	N	Exon 2		Diploptype
		3805	3838	
Merawang	11	G	T	4
	7	G	C	3
	2	G	Y	2
KUB-2	2	G	T	4
	15	G	C	3
	3	G	Y	2
References chicken	1	C	C	1

**Table 3.** Nucleotide variation of exon 5 in Merawang and Kampung Unggul Balitbangtan (KUB-2)

Population	n	Exon 5				Diploptype
		7927	7955	8030	8052	
Merawang	13	G	G	T	T	4
	5	G	G	T	C	3
	2	G	G	T	Y	2
KUB-2	17	G	G	T	T	4
		G	G	T	C	3
	3	G	G	T	Y	2
References chicken	1	C	C	A	T	1

## Genetic variability based on allele frequencies

Allele frequency was needed to measure genetic variability in a population calculated use heterozygosity and homozygosity (Rohmah et al. 2020). In exon 2, the highest allele frequency in the KUB-2 population was the C allele (0.8250). On the other hand, the T allele (0.6000) was found with the highest frequency in the Merawang population (Table 4). In exon 5, the T allele was found dominantly in Merawang and KUB-2 populations. Homozygosity in Merawang and KUB-2 was very high (0.8500-0.9000) (Table 5 and 6), because of the two breed was include the same clade 2 of the Indonesian native chicken breed (Sulandari et al. 2008). High homozygosity is a sign that the population is not in Hardy-Weinberg equilibrium. KUB-2 is a chicken selected based on egg production so that high homozygosity can be considered a natural thing. However, for Merawang chickens, inbreeding or small sampling is possible for high homozygosity. Rohmah et al. (2020) mention that Hardy-Weinberg equilibrium was affected by mutations, migration, directed marriages, selection, and large sample sizes.

**Table 4.** Frequency allele of Exon 2 and Exon 5 in Merawang and Kampung Unggul Balitbangtan (KUB-2)

Population	g.3838C>T (Exon 2)		g.8052T>C (Exon 5)	
	Allele C	Allele T	Allele C	Allele T
KUB-2	0.8250	0.1750	0.0750	0.9250
Merawang	0.4000	0.6000	0.3000	0.7000

**Table 5.** Homozygosity and heterozygosity of KUB-2

Locus	Obs_Hom	Obs_Het	Exp_Hom	Exp_Het
E2-3838	0.8500	0.1500	0.7038	0.2888
E5-8052	0.8500	0.1500	0.8577	0.1423
Mean	0.9500	0.0500	0.9269	0.0712
SD	0.0775	0.0775	0.1232	0.1201

**Table 6.** Homozygosity and heterozygosity of Merawang

Locus	Obs_Hom	Obs_Het	Exp_Hom	Exp_Het
E2-3838	0.9000	0.1000	0.5077	0.4923
E5-8052	0.9000	0.1000	0.5692	0.4308
Mean	0.9667	0.0333	0.8462	0.1538
SD	0.0516	0.0516	0.2391	0.2391

## Association of the SNPs and laying traits

Using GLM, we found C allele (g.3838C>T) in exon 2 was significantly associated with egg production, age at the first egg, and body weight at the first age (Table 7). This result according to Fu Wey et al. (2012) found a significant association between SNPs in exon 2 and egg production in Wenshang Barred Chicken. Also Rashidi et al. (2012) found a significant association between SNPs in exon 2 and age at the first egg, and body weight at the first age. In commercial chicken, the C allele (g.3838C>T) was found in Hubbard, while the T allele was found in Lohman, Cobb 500, and Avian 48 (Mohamed et al. 2017). In This research, the T allele (g.8052T>C) in exon 5 was significantly associated with egg production, age at the first egg, and body weight at the first age (Table 8). According to Li et al. (2013), g.8052T>C was significantly associated with age at first age and total egg number at 300 days of age in Qingyuan partridge chicken and Recessive White chicken.

**Table 7.** Association of PRL exon 2 SNPs with laying traits, age at first egg, bodyweight at first egg, initial egg weight in Merawang and Kampung Unggul Balitbangtan (KUB-2) Chicken

SNPs	Genotype	N	Egg production (eggs)	Age at first egg (days)	Body weight at first egg (gram)	Initial egg weight (gram)
g.3838C>T	TT	13	85.00±24.54 <sup>b</sup>	169.46±15.83 <sup>b</sup>	1423.46±291.20 <sup>b</sup>	29.53±7.63
	CC	20	117.50±14.64 <sup>a</sup>	149.95±14.37 <sup>a</sup>	1604.70±223.75 <sup>a</sup>	27.85±3.61
	CT	4	114.25±11.14 <sup>a</sup>	144.00±4.32 <sup>a</sup>	1695.75±383.57 <sup>a</sup>	29.00±1.41

**Table 8.** Association of PRL exon 5 SNPs with layer traits, age at first egg, bodyweight at first egg, initial egg weight in Merawang and Kampung Unggul Balitbangtan (KUB-2) Chicken

SNPs	Genotype	N	Egg production (eggs)	Age at first egg (days)	Body weight at first egg (gram)	Initial egg weight (gram)
g.8052T>C	TT	29	109.58±20.95 <sup>a</sup>	154.13±14.73 <sup>a</sup>	1579.28±290.91 <sup>a</sup>	28.72±5.84
	CC	3	73.66±23.67 <sup>b</sup>	182.66±21.73 <sup>b</sup>	1288.67±34.01 <sup>b</sup>	27.33±1.15
	TC	5	102.60±28.92 <sup>a</sup>	152.00±18.10 <sup>a</sup>	1543.40±200.76 <sup>a</sup>	28.40±1.81

## **CONCLUSION**

At Merawang and, KUB chicken resulted in three diplotype PRL gene exon 2 and 5 respectively which differed from the reference. PRL gene exon 3 (g.3838C>T) and exon 5 (g.8052T>C) was significantly associated with egg production, age of the first egg, and body weight at the first egg. However, more sample size is needed to confirm these findings.

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## **AUTHOR CONTRIBUTIONS**

Sartika T : conceived the study, contributed to the discussion and correction the final manuscript,  
Soewandi, BDP: contributed to the method and discussion,  
Angga : collected the data and the method,  
Saputra F : contributed analyses the data and discussion,  
Pratiwi N : wrote the draft of manuscript and discussion,  
Komarudin : contributed analyses the data,  
Hidayat Z : contributed to collect the data. All authors read and approved the final manuscript.

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## **Body Weight of Kampung Unggul Balitbangtan Chicken Reared in Two Farmers Groups in the Brebes Regency**

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### **ABSTRACT**

Millennial agriculture assistance activities aim to promote the millennial generation's interest in agriculture, one of which is to encourage an interest in livestock farming. This study aimed to determine the body weight at 50 days old of Kampung Unggul Balitbangtan (KUB) chicken was reared in two millennial farmer groups in Brebes Regency. This activity was carried out by conducting a pilot of KUB chicken farming in several young farmers. A total of 500 DOC of KUB chicken were reared in 2 group members (A and B) using a limited free-range area with 6x5 m<sup>2</sup>. In the first month, the chickens are given the same feed (commercial). The following month, the Central Java Assessment Institute for Agricultural Technology introduced the alternative feed with a crude protein content of 18.05%. Animals in group A were fed commercial feed, while group B was fed alternative feed. Data were analyzed by using a t-test. The study results showed that the average body weight of the KUB chicken at the age of 50 days old in groups A and B was 617.38±23.26 vs 441.64±28.56, respectively. The mortality percentage in groups A and B was 12.67 and 17.87%, respectively. The study results showed that the growth of KUB chickens raised in two groups' millennial farmers had fairly good body weight gain.

**Keywords:** Body weight, KUB chicken, Millennial farmer

### **INTRODUCTION**

Indonesia was an agricultural country, which important sectors to support food security. Central Java is expected supporting national food security. However, the number of employees in the agricultural sector continues to decline every year. The millennial generation is expected to be one of the generations that can further accelerate disseminating technology to support increased agricultural productivity (BPPSDMP 2019; Susilowati 2016). One of the aims of the government is to develop the interest of millennials to engage in agriculture by technology pilot assistance according to the interests of farmers. The millennial farmers are currently interested in local chicken development.

There is no doubt about the potential of local chicken as a food source. One of the local chickens that can be developed is KUB chicken (Kampung Unggul Balitbangtan). KUB chickens are superior egg-producing of native chickens; it is generated from six generations of local chicken selection. This chicken was released in 2014 through the Decree of the Ministry of Agriculture No. 274/Kpts/SR.120/2/2014. Some of the advantages of KUB chickens include various feather colours, adult weight 1,200-1600 grams, egg weight 35-45 grams, first egg-laying age 20-22 weeks, egg production of 160-180 eggs/head/year. The average egg henday production is 50%, with a peak egg production of 65% and more resistance to disease (Humaedah et al. 2018).

The environment influences the growth of KUB chicken. Different rearing systems, cage sizes, and feeding procedures can affect the growth of chickens. Urfa et al. (2017) stated that efforts to increase local chicken productivity are not enough just by improving the feed quality and maintenance management, including maintaining livestock health, needs to be continuously pursued. Under these conditions, the growth of chickens in different rearing was evaluated to obtain information on the productivity and mortality of KUB chickens at the field farmer level.

## MATERIALS AND METHODS

A 500 DOC KUB chickens were reared by two groups of young farmers, A and B. Each group was consist of 250 DOC KUB chickens. Chickens were placed in each cage equipped with a feeder, drinking place, and heating lamp and using a limited free-range area of 6×5 m<sup>2</sup>. In the first 30 days, the chickens in groups A and B were given the same commercial feed for the starter period.

**Table 1.** Feed formulation used in alternative feeding

Feedstuffs	Percentage (%)
Corn	24.71
Rice bran	16.41
Bakery waste	20.04
Palm kernel meal	15.03
Soybean meal	1.00
Poultry meal	5.03
Crab meal	5.02
Lime	6.69
Premix	1.03
Fish meal	5.03

In the next 30 days, animals in group A still given commercial feed while animals in group B were fed with AIAT feed formulation. The energy and protein balance in the two feed treatments is still within the standard for the growth of native chickens, according to Sartika (2016). The AIAT feed formula has an 18.05% crude protein content. Data were analyzed by using a t-test. Chicken body weight was weighed to determine the growth of chickens at 50 days. Data of mortality were analyzed descriptively. Feed formulation was depicted in Table 1.

## RESULTS AND DISCUSSION

The bodyweight of KUB chickens at 50 days in both groups is shown in Table 2. In group A, the bodyweight of KUB chickens at 50 days ( $617.38 \pm 28.56$ ) was higher ( $P < 0.05$ ) than group B ( $441.64 \pm 23.26$ ), but the body weight in the two groups achieved was still within the standard at that age. The average body weight of these chickens exceeds the study results by Urfa et al. (2017). KUB chickens with different feeds such as corn and rice bran with 17% CP was produced an average body weight of 49 days old of  $402.04 \pm 27.69$  grams.

Other literature was documented that the bodyweight of KUB chickens aged seven weeks was 521.3 g (Takdir et al. 2019) while in females and males was 417.66 g and 477.78 g, respectively (Sartika 2016). This difference is presumably due to the difference in feed consumption. The data on feed consumption in this study was not collected. However, ration consumption was not affected by the protein level in the ration given at the time of growth (Urfa et al. 2017).

**Table 2.** Body weight of KUB chicken in group A and B

Group	Chicken body weight 50 days (g)
A (250 DOC; 5 replicates)	$617.38 \pm 23.26^a$
B (250 DOC; 5 replicates)	$441.64 \pm 28.56^b$

*The same superscript in the same column was not significantly different ( $P > 0.05$ )*

The different feed formula causes the different result of this study. Commercial feed verse alternative feeding has a different average of KUB chicken's body weight and animals' adaptability. Rajab (2019) reported that adaptability to the environment indicates the influence of the environment on growth performance. The chicken will grow from birth to sexual maturity. Castellini et al. (2016) reported that the slow-growing chickens show better welfare status and adaptability to the organic system followed by medium-growing ones. Although fast-growing chickens have the best productive performance, they appeared not adapted to the outdoor environment, as

demonstrated by the high number of culled birds and mortality. Sidadolog (2011) reported that the growth rate of chickens until the age of four weeks is more than 100%, and after that, it only decreases to below 100%. The percentage of body weight gain is high until the age of six weeks; even at the age of two weeks, it reaches 129.24%, and then it decreases after the age of six weeks and decreases until it stops (Rahmawati et al. 2017).

Wardi et al. (2019) and Kurnia (2011) reported that genetics, environment, and feed affect growth. The performance of an animal is determined by its genetic ability and adapts ability to the environment. The difference result in the genetic potential of each offspring and the ability to adapt to different environments are in each individual (Zaenal & Triwardani 2014).

The percentage of KUB chicken mortality until 50 days of age in each group is as shown in Table 3. Generally, chicken deaths are caused by physically pinched and pecked at each other even though it was reared in 10chickens/m<sup>2</sup>. Severe feather pecking is related to feeding and foraging behaviour (Linares et al. 2018). Tong et al. (2012) suggest that increasing the stocking density advantageously affected feed/gain and decreased the final body weight. In contrast, no evidence was found that stocking density caused changes in any measured immune parameters. Native chicken mortality of 5 to 8 weeks under an improved management system was 7.4 to 8.5% (Priyanti et al. 2016).

Meanwhile, the first week in broiler chickens is a sensitive period where many of the chicks 'systems and organs are considered immature. During this period, many factors can negatively influence chick morphs-physiology affecting welfare. A decrease in early life chick welfare could be reflected in first-week mortality (Yerpes et al. 2020).

**Table 3.** Mortalities of KUB chicken at the age of 50 days

Group	Mortality (%)
A	12.67
B	17.87

## CONCLUSION

The bodyweight of KUB chickens in the millennial farmers was higher in group A than in group B. The average bodyweight of the KUB chicken at the age of 50 days in groups A and B was 617,38±23,26 VS 441,64±28,56 respectively, while the mortality percentage of group A chicken was lower than group B. The mortality percentage in groups A and B was 12,67 and 17,87%, respectively. This study showed that the growth of KUB chickens

raised in millennial farmers had reasonably good growth in terms of body weight gain.

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## **Studies in Physical, Chemical and Microbiological Characteristics of Spray Dried Kefir with Skim Milk Filler**

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### **ABSTRACT**

Kefir is a popular fermented milk that easily found in a form of a drink product and still less available in a form of powder. Drying process of kefir drink into kefir powder can be proceed by spray drying method. The aim of this research was to evaluate the changes of physical, chemical, and microbiology characteristics of the kefir powder using skim milk filler with spray drying method. The Experiment was carried out by complitelly randomized design with 5 treatments of skim milk filler composition (w/v) (0%; 2.5%; 5%; 7.5 %; 10%) respectively. The result showed kefir treated with different concentration of skimmed milk filler has significant effect on its physical, chemical, and microbiological characteristics. It was concluded that supplementation of 10% skimmed milk was able to maintain the quality standard of kefir during the spray drying process.

**Keywords:** Milk, Kefir, Powder, Spray dry

### **INTRODUCTION**

Kefir is fermented milk that has similarities of color, flavor, and consistency as yogurt and a particular yeasty aroma (Setiawati and Yunianta 2019). Kefir contains energy, carbohydrate, protein, fat, sodium, calcium, vitamin A and vitamin D (Atalar & Dervisoglu 2015). The contains of carbohydrates, protein, and fat has become one of the causes of kefir drink easily deteriorate. Alteration of kefir drink which is a liquid to powder can proceed as an effective alternative to prevent the obstructions of kefir drink.

A spray dryer is common equipment used to produce dried particles by transforming fluid into fine particles using a gaseous hot drying medium to evaporate the water contained within the product (Teijeiro et al. 2018). This drying method has several advantages such as maintaining flavor and substances sensitive to heat due to the brief time of drying compared to the conventional drying method but the spray drying method requires filler to be

added to the product and is quite uneconomical for a small-scale production (Selvamuthukumar 2019).

This research was aimed to provide a physical, chemical, and microbiological characteristic alteration of kefir powder manufactured by spray-drying method with different filler concentrations due to maintain several quality standards of the dried kefir powder.

## **MATERIALS AND METHODS**

### **Kefir powder production**

Fresh cow milk was purchased from the local dairy farm located in Semarang, Central Java, Indonesia. Kefir grain from the Laboratory of Food Chemistry and Nutrition (Faculty of Animal and Agricultural Sciences - University of Diponegoro, Semarang, Indonesia) collection were used as fermentation starters. Kefir production refers to the method of (Lindawati et al. 2015) with some modification. Fresh milk is pasteurized at 85°C for 30 minutes, then cooled to a room temperature of 27°C, 5% kefir grains (w/v) are added to the milk and then stirred until evenly spread, inoculated in a sterile jar, and incubated at room temperature ( $25 \pm 1^\circ\text{C}$ ) for 20 hours. Milk will form kefir. Kefir is filtered to separate kefir grains and kefir substrates. The filtered substrate is stored in a sterile jar container and stored at 5°C for continuous treatment.

The production of kefir powder with spray drying method was carried out according to (Teijeiro et al. 2018) method by samples prepared according to the treatment of skim milk filler concentration 2.5%;5%;7.5%;10% (w/v) and dissolved into the kefir treatment based on the treatment concentration using a homogenizer at 10,000 rpm temperature 4°C for 15 minutes. Samples are dried with a laboratory-scale spray dryer (BUCHI Mini Spray Dryer B-290) at a constant inlet air temperature of 135°C, feeding flow at 10ml/min. The airflow on the nozzle is 473 L/h, the dryer airflow is 30 m<sup>3</sup>/h, the outlet temperature is between 66°C to 69°C. Suspense cells will be atomized and sprayed into the drying chamber with a two-fluid nozzle. The resulting powder products are stabilized at room temperature (25°C) and stirred evenly before being stored in an airtight container and stored in a sterile air-tight container at 4°C.

### **Analysis**

Kefir powder yield production was calculated by comparing the total weight of kefir powder products and the weight of the initial kefir drink

proceed into the spray dryer (Dewi & Satibi 2015). The moisture content analysis was performed according to the standard method reported previously by Ulfindrayani & A'yuni (2018). The amount of 2 g sample was placed in a porcelain cup which. The sample-filled cup was heated in the oven at 100°C until the sample weight was constant. The value  $a_w$  was determined using cleaned and calibrated  $a_w$  according to the method described by Saenab et al. (2010). The colorimeter Hunter method was obtained to determine the color scale (Purbasari 2019).

Total acid was conducted with a 10 ml of dissolved sample and then added PP indicator 1% as much as 2 drops and titration was done using 0.1 N NaOH as a buffer until appeared a constant pink color on the sample solution (Harjiyanti et al. 2013). The pH was determined using a pH meter (Hanna HI2209) according to SNI 06-6989.11 (2004). Total Lactic Acid Bacteria (BAL) was determined by using the pour-plating method. 10 g of kefir powder was diluted with 90 ml of 0.85% sterilized NaCl solution, then 1 ml of sample was diluted to 9 ml NaCl until dilution  $10^{-6}$ . Every 1 ml of sample diluted from  $10^{-4}$  until  $10^{-6}$  were transferred into the de Man Rogosa and Sharper (MRS) agar media and was incubated at a temperature of 37°C for 48 hours in anaerobic conditions. The calculation result corresponds to the amount on the media and is expressed as a log of the amount of dilution and CFU/g as a unit of measurement. The number of colonies is between 30-300 CFU/g (Papapostolou et al. 2008). Potato Dextrose Agar (PDA) was used as the medium for yeast calculation. The media is incubated at room temperature for 48 hours then the number of colonies was calculated and presented in CFU/g (Sinurat et al. 2018).

### **Statistical analysis**

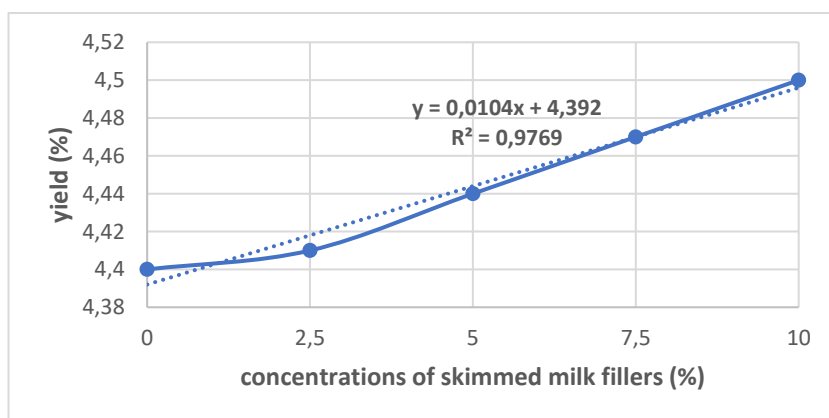
Data from the results of yield, water content,  $a_w$  value, color, total acid, pH value, total lactic acid bacteria, and total yeast was tabulated by calculating an average, standard deviation then presented as scatter chart with trend line.

## **RESULTS AND DISCUSSION**

### **Physical characteristics of kefir powder with skim milk filler concentration variation yield**

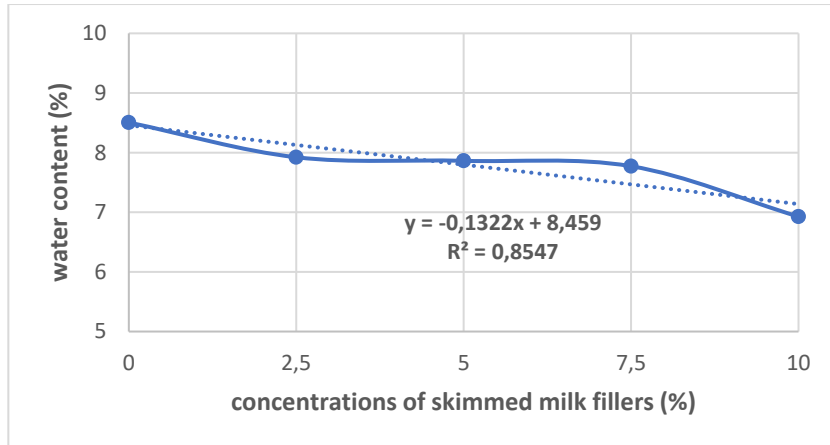
The yield, water content and water activity ( $a_w$ ) result of kefir powder with variations of concentration skimmed milk as fillers can be seen in Figure 1-3.

Based on Figure 1, the yield has a coefficient of determination ( $R^2$ ) of 0.9769, therefore the change in yield depends on the concentration of skim milk fillers added. The use of fillers was used to speed up the drying process, prevent damage to the flavor of the product, as well as increase the yield of both the total solids and the volume produced adjusted to the type of filler used (Kusuma et al. 2019). The trend line that tends to rise explains that the more concentrations of skimmed milk fillers added, the higher the yield produced. The increase in yield was due to the use of fillers that contains a lot of total solids and low water content, containing protein 3.5%, carbohydrates 5.1%, and water content 5% (Gafar & Maurina 2019). It was also in accordance with the Selvamuthukumaran (2019), which stated that the absence or low concentration of fillers would cause more surfaces to be exposed to hot steam from the drying process and would ultimately decrease the yield of the product.



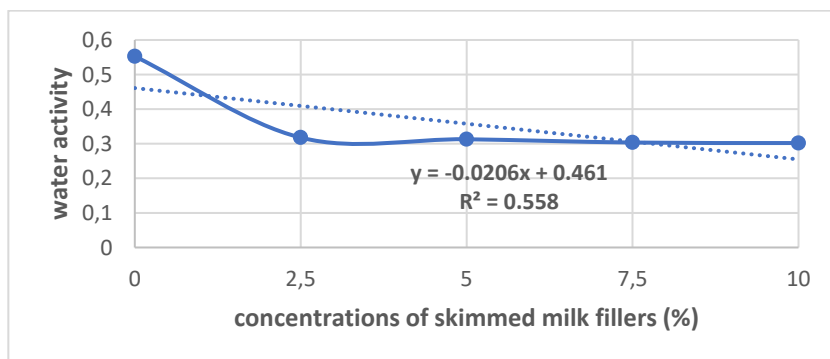
**Figure 1.** Changes of yield value on kefir powder with variation concentration of skimmed milk filler

Figure 2 showed that the water content has a coefficient of determination ( $R^2$ ) value of 0.8547 and the change in water content depends on the concentration of skim milk fillers added. In addition to the drying method, the type of filler or carrier used in the drying process will also affect the water content that will be produced in the drying process. The water content analyzed was 8.5%-6.92% whereas SNI 01-2907-2006 requirement of water content was less than 5% (Tampubolon et al. 2016).



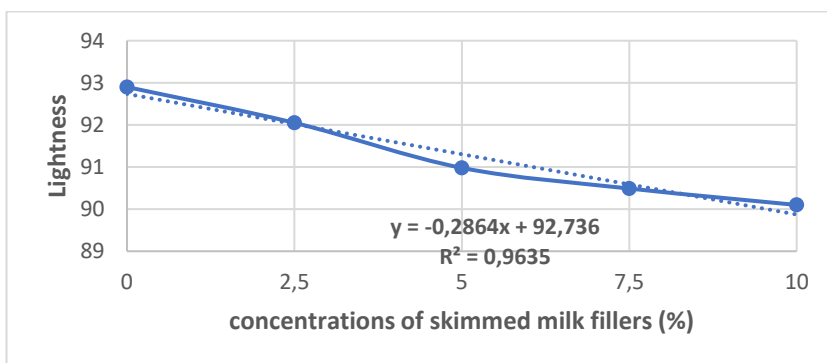
**Figure 2.** Changes of water content on kefir powder with variation concentration of skimmed milk filler

Figure 3 showed that the  $a_w$  value has a coefficient of determination ( $R^2$ ) of 0.558 meaning that the change in  $a_w$  value depends on the concentration of skim milk fillers added. In general, the  $a_w$  value is also influenced by the spray drying method used to produce kefir powder. Dried powder products by spray drying method will be determined stable if the water content was less than 5% and  $a_w$  value less than 0.6 (Nale et al. 2018). The  $a_w$  value in the illustration tends to decrease because the casein micellar content in skim milk is affected by the microencapsulation performance in the spray drying process. Since casein micelles are amphiphilic, a layer is formed on the kefir powder particles which can bind water and form good microencapsulation (Chen et al. 2019). The more fillers used, the more encapsulated of kefir powder particles. This could bind the amount of free water in the kefir powder particles wrapped in skimmed milk and prevent free water from evaporating due to the spray drying process.

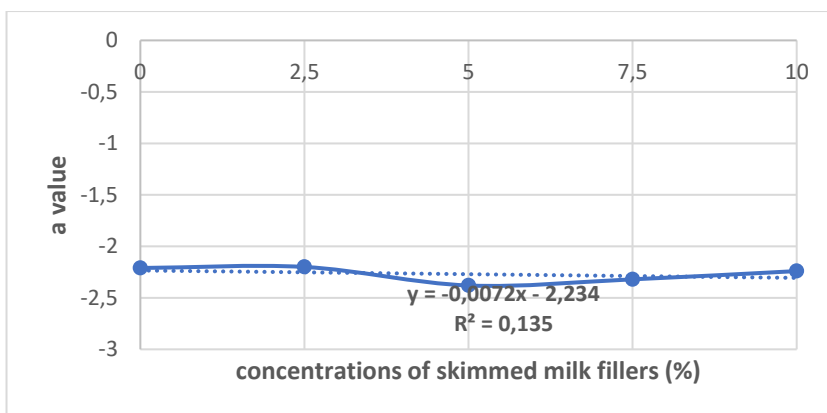


**Figure 3.** Changes of water activity ( $a_w$ ) on kefir powder with variation concentration of skimmed milk filler

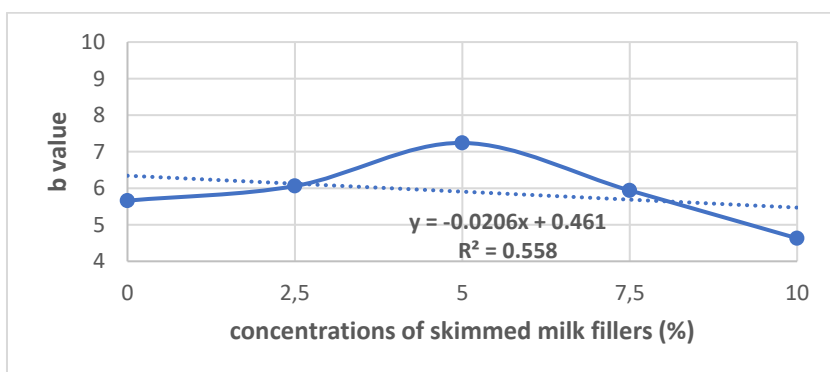
The color result of kefir powder with variations of concentration skimmed milk as fillers can be seen in Figures 4, 5, and 6.



**Figure 4.** Changes of L\* value on kefir powder with variation concentration of skimmed milk filler



**Figure 5.** Changes of a\* value on kefir powder with variation concentration of skimmed milk filler



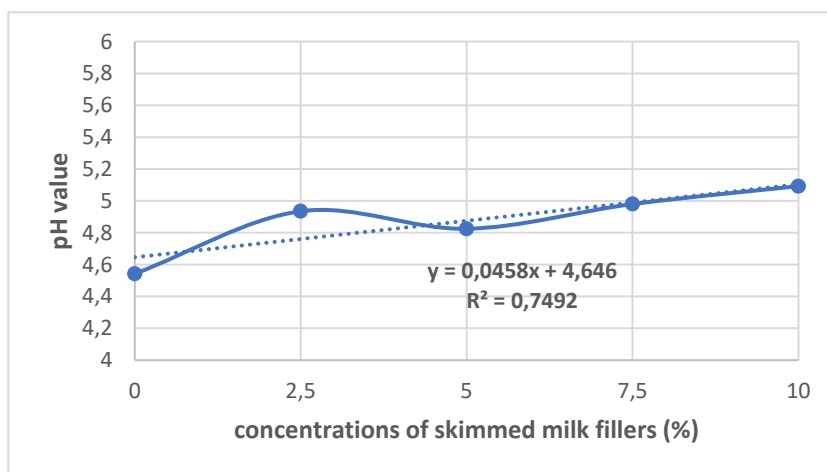
**Figure 6.** Changes of b\* value on kefir powder with variation concentration of skimmed milk filler

Figure 4, 5, and 6 showed that the color changes indicated by the values  $L^*$ ,  $a^*$ , and  $b^*$  has a coefficient of determination value ( $R^2$ ) indicating values ranging from 0.135-0.99635. This means that changes in  $L^*$ ,  $a^*$ , and  $b^*$  values depend on the concentration of added skimmed milk fillers. Color testing on kefir powder was done by Hunter color notation method (chromameter) where the higher the  $L^*$  value, the higher the degree of whiteness, the value  $a^*$  indicates green to red color, and the  $b^*$  value for yellow to blue degrees (Sutardi et al. 2010). The decrease in lightness level in kefir powder products was caused by the occurrence of browning reactions or non-enzymatic browning reactions known as Maillard reactions.

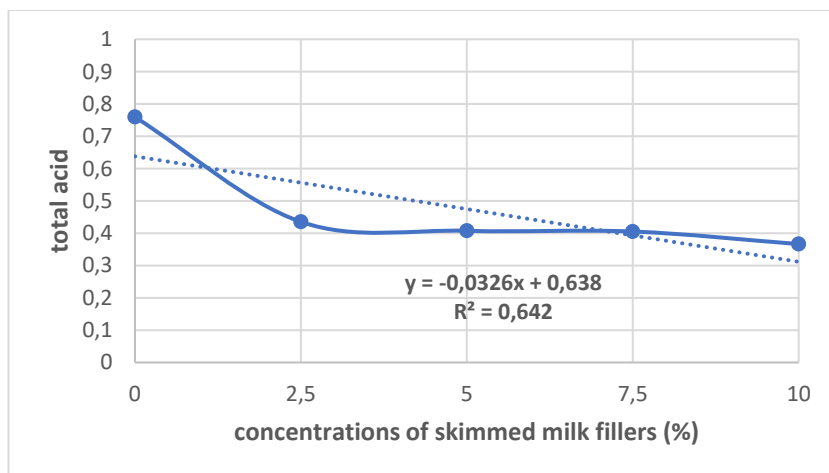
Non-enzymatic browning reaction or Maillard reaction was a reaction between reducing sugar (milk lactose) and compounds that have  $NH_2$  groups such as amino acids, proteins, peptides, and ammonium which is then given heat treatment or dehydrated (Edam 2017). Lactose in skimmed milk will react with  $NH_2$  components contained in kefir products during the spray drying process that causes browning reactions and decreased the brightness of the product. The heating of carbohydrate and protein components in milk will produce many compounds, affecting the color of the products (Nugraha et al. 2014).

### Chemical characteristics of kefir powder with skim milk filler concentration variation yield

The pH and total acid values of kefir powder with variations of concentration skimmed milk as fillers can be seen in Figure 7 and 8.



**Figure 7.** Changes of pH value on kefir powder with variation concentration of skimmed milk filler



**Figure 8.** Changes of total acid value on kefir powder with variation concentration of skimmed milk filler

In this study, the coefficient of determination ( $R^2$ ) was 0.7492, meaning that the change in pH value depends on the concentration of skim milk fillers added. The pH value tested has a range of 4.5-5.09. In general, the fermentation process of milk into kefir will change the pH value of milk to acid which is about 4.6 (Sulmiyati et al. 2018). The pH value contained in kefir powder was caused by the fermentation process carried out by microorganisms contained in kefir. Microorganisms in kefir would perform a fermentation process, converted carbohydrates in milk, whereas lactose converted into lactic acid. Shortly, the potential of producing higher lactic acid would occur (Rohmah et al. 2018)

The increase of pH value on the illustration was caused by the concentration of skimmed milk fillers added to kefir powder products. A low pH value at a 0% concentration of skimmed milk fillers indicates microorganism activity characterized by low pH values (Marwati et al. 2020). The increased concentration of skimmed milk fillers in kefir powder can also affect the increase in the pH value of kefir powder resulting from the amount of lactose contained in skimmed milk fillers. Although there was an addition of lactose substrates the number of concentrations of starter bacteria was not increased because the bacteria required more time to produce lactic acid (Septiani et al. 2013).

Figure 8 showed that the total acid has a coefficient of determination value ( $R^2$ ) of 0.642, meaning that the total acid change depends on the concentration of skim milk fillers added. The total value of acid contained in the sample was 0.75-0.36%. The decrease in the total value of acid in kefir powder dried by spray drying method was caused only by the concentration of fillers given to increase the total solids. Based on Codex Alimentarius (Fermented Milk

Standards), the total acid contained in kefir was at least 0.6% (Atalar & Dervisoglu 2015). The lower total acid along with the addition of skimmed milk fillers in kefir powder products was caused by the increased concentrations of lactose added to kefir products while the same fermentation time when producing kefir. The addition of lactose to kefir powder causes the addition of substrates to microorganisms and the fermentation process stopped due to kefir was stored at low-temperature storage (Ningsih & Bintoro 2018).

### Microbiological characteristics of kefir powder with skim milk filler concentration variation yield

The results of total Lactic Acid Bacteria and yeast in kefir powder with variations in the concentration of skimmed milk fillers can be seen in Table 1.

**Table 1.** Total calculation of yeast and lactic bacteria in kefir powder with different concentrations of skimmed milk fillers

Parameter	Percentage of skim milk powder in kefir production (%)				
	0	2.5	5	7.5	10
Lactic acid bacteria (CFU/ml)	$6.58 \times 10^5$	$1.27 \times 10^6$	$1.58 \times 10^6$	$1.74 \times 10^6$	$1.94 \times 10^6$
Yeast (CFU/ml)	$2.34 \times 10^5$	$2.54 \times 10^5$	$2.92 \times 10^5$	$2.46 \times 10^7$	$2.64 \times 10^7$

Table 1 showed the total calculation of lactic acid bacteria that increased along with the addition of skim milk filler concentrations added based on Codex Alimentarius Commission (2018) the minimum total lactic acid bacteria in kefir was  $10^7$  CFU/ml. Total Lactic Acid Bacteria contained in liquid kefir was  $2.87 \times 10^8$  CFU/ml. This indicated that skimmed milk was able to protect the viability of lactic acid bacteria in kefir. The viability of kefir microorganisms with spray drying process was influenced by several factors such as starter type, filler, drying temperature, drying duration,  $a_w$  value, and storage, and skimmed milk was suitable and an efficient filler for probiotic products dried by spray drying method (Golowczyc et al. 2011). Proteins from skimmed milk were exposed to heat during the spray drying process and were denatured and able to increase Lactic Acid Bacterial resistance from heat exposure due to the drying process (Zheng et al. 2015). The addition of skimmed milk fillers in the spray drying process decreased the number of Lactic Acid Bacteria's by as much as 1 log resulting in a quite unstable micro-encapsulated material.

The selection of micro-encapsulated materials was not easy because required hat can support the encapsulation process, including materials that are safe and registered in local regulations, stable during the digestion process

before the intestine, and unstable in the intestines so that microorganisms can be detached (Cassani et al. 2020).

Skimmed milk was a type of polymer that can be used as a micro-encapsulant because it consists of polysaccharides and proteins (Cassani et al. 2020). Amino acids will bind through peptide bonds and will form polymer compounds namely proteins (Chen et al. 2019). Lactose is a carbohydrate composed of 2 types of polysaccharides namely glucose and galactose polymers (Novianti et al. 2017). The addition of skimmed milk as a filler in the kefir spray drying process had advantages of low cost that produce products on a large scale (Maciel et al. 2013). Microencapsulation was able to improve the viability of probiotics during product storage as well as during digestive processes in the body (Liao et al. 2017). The viability of lactic acid bacteria in kefir powder can be seen from the total acid produced which can indicate the amount of lactic acid in kefir powder.

The total yeast calculation result increased in line with the concentration of skimmed milk fillers added and based on CODEX STAN (2003) the minimum total yeast in kefir is  $10^4$  CFU/ml. Total yeast content in liquid kefir is  $2.57 \times 10^7$  CFU/ml. The increase in total yeast occurs due to the increase in the number of substrates in kefir resulting from the metabolism of lactic acid bacteria, namely simple sugars. Simple sugar in kefir will be converted into pyruvate acid which will be decarboxylated into acetaldehyde which will be reduced to ethanol by yeast to produce alcohol and carbon dioxide (Yusriyah & Agustini 2014).

## **CONCLUSION**

Various changes occurred in the yield, water content,  $a_w$  value, color, total acid, pH value, total Lactic Acid Bacteria, and total yeast of kefir powder products after performing the spray drying method, along with the addition of skimmed milk fillers in the spray drying process. The addition of 10% skimmed milk filler was able to maintain the quality standard of kefir during the spray drying process.

## **AUTHOR CONTRIBUTIONS**

All authors were contributed equally to this work

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## **Nerve Growth Factor Levels in Seminal Plasma and Their Correlation with Madura Cattle Semen Analysis**

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### **ABSTRACT**

Nerve Growth Factor (NGF) is one of the Neurotrophin protein families. There is an evidence that NGF not only plays an important role in the nervous system but also plays an important role in the reproductive system. The objective of this study is to determine NGF levels in seminal plasma and their correlation with Madura cattle semen quality. Ten candidate of Madura bulls belonged to the Livestock Breeding and Animal Health Unit Pamekasan Madura were selected with the classification 3-5 years of age, healthy, normal genitalia, and good libido. Semen evaluation included sperm concentration, sperm motility, sperm viability, and sperm plasma membrane integrity. The NGF levels in the samples of seminal plasma were analyzed using indirect enzyme-linked immunosorbent assay (ELISA). The results of the study showed that the average of NGF levels in seminal plasma of ten Madura cattle were  $0.29 \pm 0.15$  ng/ml and it had a significant correlation with sperm motility ( $P < 0.05$ ) but did not have a significant correlation with sperm concentration, sperm viability, and plasma membrane integrity ( $P > 0.05$ ). In conclusion, NGF levels in seminal plasma had a positive correlation with sperm motility, which are related to sperm fertility.

**Keywords:** NGF, Seminal plasma, Semen, Madura cattle

### **INTRODUCTION**

The population of beef cattle in Indonesia continues to increase every year. The population of beef cattle in 2020, especially in East Java province, reached 4,815,330 heads (DGLAHS 2020). Madura cattle is one of the beef cattle commodities that contribute to meat self-sufficiency program in Indonesia. Madura cattle have the advantages of being able to adapt well to environmental stress, high adaptation to low feed quality, and resistance to disease (Siregar 2008). The quality of the semen of Madura cattle from the genetic aspect is better than Bali and Ongole cattle (Salim et al. 2012).

Improving reproductive quality is an important effort to increase livestock productivity. The reproductive system is not only influenced by hormones, but also influenced by a peptide growth factor that known as growth factor (Hafez & Hafez 2000). Growth factors are substances like hormones that function in regulating the growth and development of organs, tissues, and cells. One of the growth factors that affect the growth and development of the reproductive system is Nerve Growth Factor.

Nerve Growth Factor (NGF) is one of the neurotrophic protein (NT) families that function in regulating growth and maintaining nerve cells. There is an evidence that NGF not only plays an important role in the nervous system but also plays an important role in the reproductive system of several animal species and human. The presence of NGF has been found in the prostate gland secretions of guinea pig (Harper et al. 1979), rabbit, and bull (Harper & Thoenen 1980). The addition of exogenous NGF as semen extender has been shown to increase the viability of bovine spermatozoa (Li et al. 2010a). Previous studies have also found NGF proteins in the reproductive tract of llamas, mice, bull, bison, and deer (Bogle et al. 2018). NGF also plays an important role in the reproductive system of female animals. Purification of seminal plasma NGF from *Bos taurus* cows injected into female cows have been shown to affect the increase of progesterone levels in blood plasma on day 10 after artificial insemination (Stewart et al. 2018).

Accessory glands are the source of secretion of seminal plasma NGF. The most abundant secretion is in the vesicular glands, followed by the ampullary glands, and the least secretion is in the prostate and bulbourethral glands (Lima et al. 2020). The accessory glands are the sites for the secretion of seminal plasma, where the liquid part in the semen plays a very important role as a medium for spermatozoa from the male reproductive tract into the female reproductive tract and contains specific biochemical components to regulate spermatozoa. The NGF protein was detected in the head and tail of the spermatozoa while the trkA receptor protein was detected in the acrosome cap, nucleus, and tail of the spermatozoa (Li et al. 2010a). The presence of NGF in seminal plasma and spermatozoa indicates that NGF may also play a role in improving the quality of spermatozoa.

The research on the role of NGF in the reproductive system has not been widely reported in Indonesia, especially in Madura cattle. The purpose of this study was to determine the correlation of seminal plasma NGF levels to the quality of Madura cattle spermatozoa.

## **MATERIALS AND METHODS**

Total sample of ten Madura bulls candidate at the Pamekasan Madura Animal Health and Breeding Unit which have been selected with the criteria of 3-5 years of age, healthy, normal genital organs, and have a good libido.

### **Semen collection dan sample preparation**

Semen collection in Madura cattle is carried out in the morning with an artificial vagina. The collection of semen in this study was carried out 3 times with an interval of once a month. The collected samples were then subjected to macroscopic and microscopic examination including volume, color, odor, pH, motility, viability, membrane integrity, and concentration of spermatozoa were adopted according to Susilowati et al. (2010). Semen was centrifuged to separate spermatozoa and seminal plasma. Centrifugation was carried out for 5 minutes at a speed of 2,720 G at 4°C, then seminal plasma was inserted to a microtube, labeled according to the eartag code then stored in a freezer at -20°C for examination of NGF levels using indirect ELISA.

### **Sperm quality assessment**

The variables observed in this study included motility, viability, plasma membrane integrity, and concentration of spermatozoa.

#### ***Sperm motility test***

One drop of semen was dropped on to a glass object and then observed by microscopy at 400× magnification. The motility assessment is expressed in the percentage of spermatozoa that progressively motile to the total number of spermatozoa and the individual motility.

#### ***Sperm viability test***

The viability of spermatozoa was carried out by eosin-negrosin staining with a ratio of 1 spermatozoa : 3 eosin negrosin, then smeared on a glass object and fixed on a Bunsen. Observation is under a microscope at 400× magnification. The assessment was based on the ratio between the number of live spermatozoa marked with colorless heads and the total spermatozoa observed and then expressed in percentages (%).

### ***Plasma membrane integrity test***

The measurement of plasma membrane integrity used hypoosmotic swelling test. A total of 0.1 ml of semen was mixed with 1 ml of a hypoosmotic solution (150 mOsmol fructose and 150 mOsmol NaCl). The solution was then incubated in a water bath for 30 minutes at 37°C. After that, the staining was carried out using the eosin-negrosin and then observed under a microscope at 400x magnification in ten fields of view. The functional of plasma membranes integrity showed a coiled tail, whereas the incomplete plasma membranes integrity showed straight tails.

### ***Sperm concentration test***

Sperm concentration indicates the number of spermatozoa per mm<sup>3</sup> or cm<sup>3</sup> in semen. The assessment of sperm concentration using a spectrophotometer (Photometer SDM 1, Minitube, Germany).

### **Measurements of NGF**

A total of 10 seminal plasma samples were examined for NGF levels using the Bovine Nerve Growth Factor (BNGF) ELISA kit (MyBioSource, USA). Reagents, standard solutions, and samples were prepared for testing. A total of 40 µl of seminal plasma was added to the sample well and then 10 µl of anti-NGF antibody was added. 50 µl of streptavidin-HRP was added to the standard well and the sample well, then shaken with a micromixer. Incubation was carried out for 60 minutes with the plate is covered. The plate was washed with buffer wash 5 times and then dried with absorbent material. After adding 50µl of substrate solution A then adding 50µl of substrate solution B into each plate, then incubated for 10 minutes. Adding 50 µl stop solution to each plate until the blue color changes to yellow. Samples were read using an ELISA-reader at 450 nm within 30 minutes after the addition of the stop solution.

### **Data analysis**

The data was analyzed using correlation of spearman analysis between seminal plasma NGF levels and the spermatozoa quality of Madura cattle include motility, viability, plasma membrane integrity, and concentration of spermatozoa.

## RESULTS AND DISCUSSION

### Seminal plasma NGF levels

Seminal plasma NGF levels of 10 Madura cattles were obtained on average  $0.29 \pm 0.15$  ng/ml. According to Harper et al. (1982), the purification level of NGF protein in bull is 0.7 mg/ml semen. Seminal plasma NGF levels of Madura bull were lower than in Jersey (5.2 g/ml) and Frisian Holstein (5 g/ml) dairy cattle (Stewart et al. 2018), while the Indian Murrah buffalo was 65 ng/ml (Namagiri & Laxmi 2015). The low levels of seminal plasma NGF may be due to more NGF binding to specific receptors in spermatozoa than non binding NGF in seminal plasma. NGF in ejaculated sperm modulate pro-survival factors by selectively binding to trkA (Saeednia et al. 2015). The trkA receptor is a member of the tropomyosin receptor kinase (trk) family.

NGF exploits the trkA receptor-mediated effects by activating various signaling pathways to form the NGF-trkA complex (Petruska & Mendell 2009). The NGF-trkA complex was found in hamster spermatozoa (Jin et al. 2010), bovine spermatozoa (Li et al. 2010a), and llama spermatozoa (Sari et al. 2018). Madura cattle are small-type of beef cattle compared to other breeds, with a body weight range from 250 to 300 kg. However, this breed has many advantages among others such as, high resistance to environmental stress and disease, and high adaptability to low feed quality. The presence of NGF and receptor trkA in ejaculate bovine sperm increased leptin secretion and cell viability, that the effect of NGF could be species specific (Li et al. 2010). NGF and its receptors were also detected in Sertoli cells and Leydig cells that play a role in the growth of testis and spermatogenesis (Li et al. 2010). NGF synthesis by the non neuronal cells is growth dependent, suggesting that the expression of some genes relevant to cell growth is associated with upregulation of NGF synthesis (Furukawa & Furukawa 1990).

**Table 1.** Average and standard deviation of seminal plasma NGF levels and sperm quality of Madura cattle

Variables	Average and standard deviation
NGF levels (ng/ml)	$0.29 \pm 0.15$
Motility (%)	$53 \pm 23.12$
Viability (%)	$60.1 \pm 23.94$
Plasma membrane integrity (%)	$38.3 \pm 16.13$
Sperm concentration ( $1 \times 10^6/\text{mm}^3$ )	$967 \pm 504.47$

**Table 2.** The coefficient correlation (r) between seminal plasma NGF levels and sperm quality of Madura cattle

Variables	Coefficient correlation (r)			
	Motility	Viability	Plasma membrane integrity	Sperm concentration
NGF Levels	0.681*	0.593	0.612	0.171

\* Significant correlation at the 0.05 level

### Correlation of NGF levels with quality of spermatozoa

The results of this study showed that seminal plasma NGF levels were correlated with sperm motility ( $P < 0.05$ ), but did not correlate with viability, plasma membrane integrity, and concentration of Madura cattle sperm ( $P > 0.05$ ). The average of sperm motility of 53% was correlated with seminal plasma NGF levels of Madura cattle with an average of 0.29 ng/ml. The results of spermatozoa quality of madura cattle can be seen in Table 1. Motility is one of the important factors that contribute to the fertility of spermatozoa where is regulated by several hormones and growth factors, such as testosterone, nerve growth factor, neurotrophin-4, epidermal growth factor, and fibroblast growth factor (Saeednia et al. 2015).

NGF acts on the target reproductive organs with activated by two membrane receptors, namely the TrkA receptor which works in cell survival and the p75 receptor which works in the process of apoptosis (Castellini et al. 2019). NGF protein was found in the head and tail of the spermatozoa while the trkA receptor protein was detected in the membranes of the acrosomal cap, nucleus, and tail of the spermatozoa (Li et al. 2010). According to Li et al. (2010), indicates that NGF may also play a role in increasing sperm motility and viability. The correlation coefficient (r) between NGF levels and sperm motility showed a strong affinity ( $r \geq 0.5-0.75$ ) compared to sperm viability that showed to moderate affinity ( $r \geq 0.4-0.55$ ). The results of the correlation coefficient can be seen in table 2. The tail of the spermatozoa contains an axoneme center which is enclosed by a plasma membrane and responsible for the motility of spermatozoa. The axoneme itself is composed of 9 pairs of microtubules radially around 2 central filaments. In the middle piece, the microtubule arrangement is surrounded by an outer layer of dense and coarse fibers which are associated with 9 pairs of axonemes. The axoneme associated with the dense fiber will be closed with several mitochondria perperally, where the mitochondria as an energy source for the motility of spermatozoa (Hafez & Hafez 2000).

Seminal plasma NGF levels do not correlate with viability, plasma membrane integrity, and concentration of Madura cattle spermatozoa, this is because NGF is not the only protein that works in influencing spermatozoa quality are not only NGF. Seminal plasma contains many proteins, including bovine seminal plasma proteins (BSP)-A1, BSP-A2, BSP A-3, heparin bindin protein (HBP) (Gwathmey et al. 2006), osteopontin (Erikson et al. 2017), clusterin, albumin, phopholipase A2 (PLA2), nucleobindin, prostaglandin-D synthase (Moura et al. 2007), spermadhesin Z13, Tissue Inhibitor of Metalloproteinase (TIMP-2) (Odhiambo & Dalley 2011). The high percentage of sperm viability 60.1% and concentration  $967 \times 10^6/\text{mm}^3$  may also be influenced by other proteins present in the seminal plasma.

The integrity of the plasma membrane is needed by spermatozoa, because sperm damage of plasma membrane will affect the metabolic process that relate to sperm motility and viability. The plasma membrane integrity of spermatozoa will retain hypoosmotic fluid in the cell with coiled or bent tail, while spermatozoa with straight tails indicate damage to the plasma membrane, due to their inability to hold water. If the plasma membrane has been damaged, it will result in anisomotic conditions that cause intracellular leakage, which will affect the reshuffling of ATP, thus affecting the motility of spermatozoa (Bohlooli et al. 2012). The integrity of the plasma membrane of spermatozoa can be damaged in the presence of toxic substances derived from dead spermatozoa or from other substances that can cause high levels of free radicals. NGF has a role as an antioxidant to prevent the high level of free radicals in the semen cryopreservation process (Saeednia et al. 2015).

Sperm concentration is an important criterion in determining the quality of spermatozoa and the degree of extender for the purpose of making frozen semen. The results of concentration measurement of Madura cattle spermatozoa were  $967 \times 10^6/\text{mm}^3$ . According to Garner and Hafez (2000), the normal concentration of spermatozoa ranges from  $800\text{--}2000 \times 10^6/\text{mm}^3$ . Based on this reference, it shows that the spermatozoa concentration of Madura cattle is still in the normal range, even though Madura cattle in the Livestock Breeding Unit and Animal Health Pamekasan Madura have never carried out routine semen collection. NGF expressed in testis of mouse, rat and guinea pig that plays an important role in promoting the development of testis and the differentiation, maturation and movement of spermatozoa (MacGrogan et al. 1991). NGF and its receptors play a specific role in regulating steroid hormone synthesis in Leydig cells, indicating that NGF may regulate spermatogenesis through autocrine and paracrine functions (Wang et al. 2011). The spermatogenesis cycle takes 60 days starting from the initial spermatogenesis process in germ cells until it is ejaculated in the form of mature spermatozoa (Srivasta 2014). Sperm concentration was calculated based on the number of

spermatozoa from the male reproductive tract that had been ejaculated. The high concentration of spermatozoa does not necessarily affect the quality of spermatozoa such as motility and viability because the condition of spermatozoa in each individual is different. Spermatozoa that have been damaged or died probably originate from the ampulla of the ductus vas deferens as a temporary reservoir for spermatozoa before being ejaculated.

## **CONCLUSION**

Seminal plasma NGF levels in Madura cattle are quite low compared to other breed of cattle, this is because NGF binds more to specific receptors in target organs than in seminal plasma. In addition, genetic factors also influence to the low levels of NGF in the seminal plasma of Madura cattle. Seminal plasma NGF levels positively correlated with sperm motility, which indicated a role in sperm fertility.

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## **AUTHOR CONTRIBUTIONS**

Nurul Azizah, Rimayanti, Pudji Surianto, Trilas Sardjito where contributed equally to this work

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## Haplotype Block Analysis of FSHR gene in Sragen and Jabres Cattle

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### ABSTRACT

The Follicle Stimulating Hormone (FSHR) is an anterior pituitary gonadotropin belonging to the family of glycoprotein hormones. The gene controls growth and fecundity in cattle. Blood samples of Sragen cattle (10 samples) and Jabres cattle (19 samples) were used for DNA extraction to define the single nucleotide polymorphism (SNP). Three SNPs have been detected, namely SNP g.175A/G (SNP1), SNP g.227T/C (SNP2) and SNP g.275A/C (SNP3). Three blocks (SNPs combination) have been analyzed for haplotype block using haploview. As a result, all the blocks have high D' (D prime: 1.0). The highest LOD and r-squared were detected in block 1 (SNP1&2 combination), 9.23 and 1.0, respectively. Two of the blocks having similar LOD (1.23) and r-squared (0.28). Two haplotypes frequencies are defined in SNP1&2 combinations/block 1 (GT/79.3% ; CC/20.7%). Three haplotypes have been detected in SNP2&3 combinations (block 2), namely TC, TA and CA, with genotype frequency are 51.7%, 27.6% and 20.7%, respectively. Similarly, SNP1&3 combinations (block 3) indicate three haplotypes with the same frequency but different genotypes; GC, GA and CA, respectively. In conclusion, the haplotype from block 1 (SNP1&2 combination) could be used for further association analysis with reproduction traits in Sragen and Jabres cattle.

**Keywords:** FSHR gene, Haplotype, Sragen cattle, Jabres Cattle

### INTRODUCTION

SNPs (single nucleotide polymorphisms) are the most prevalent genetic variant used in association research (Wu et al. 2014). However, as we know, genetic inheritance is regulated by segments of closely linked nucleotides. Hence, the use of phased multi-marker elements (*i.e.*, haplotypes) has the potential to improve current models for understanding domestication, population diversity, inbreeding, admixture, demographic trajectories, identification of loci associated with economically important traits, and accurate prediction of breeding values (Mészáros et al. 2021). A single SNP association study with phenotypic characteristics is likely quicker and more economical in breeding programs. Still, haplotype analysis helps evaluate the effect of genes on phenotypic traits. Linkage disequilibrium (LD) is the non-

random assortment of alleles at different loci (Al-Thuwaini et al. 2020). Haploview software generates marker quality data, LD information, haplotype blocks, population haplotype frequencies, and single-marker association statistics for haplotype analysis. The haplotype and LD patterns have been widely employed in studies of the relationship between DNA polymorphisms and disease (Geesaman et al. 2003; Stoll et al. 2004). We can select one or more block definitions in the haploview application to designate the region of SNPs with weak or strong LD (Wang et al. 2002). A selection of markers group can illustrate the LD relationship by manually specified using different colour schemes.

FSH is a glycoprotein hormone required for gonadal development and maturation, and gamete production in animals during puberty. The FSHR gene is found on chromosome 11 and has ten exons and eleven introns, with the first nine exons enclosing the extracellular domain and exon ten enclosing the transmembrane environment (Ishak 2012). The follicle-stimulating hormone receptor (FSHR) gene plays a crucial role in proper reproductive function and oocyte maturation in cattle (Yang et al. 2010). Sragen cattle are believed to be descended from the crossbreeding of Aberdeen Angus, American Brahman, and Ongole grade cattle breeds with body shape and colour close to the Angus cattle (Subiharta et al. 2021). The Jabres cattle is one of the Indonesian cattle breeds and is well-known to outperform in the tropical environment compared to other breeds; these cattle is known as a descendent from Madurese, Bali, and Ongole cattle (Haq et al. 2019). SNPs within the FSHR gene and the linkage among the SNPs have not been explored in numerous Indonesian cattle breeds, especially in Sragen and Jabres cattle. Therefore, this study aimed to identify the polymorphism of the FSHR gene and analyze the linkage among the SNPs.

## MATERIALS AND METHODS

### **Ethics statement**

The Institutional Animal Care and Use Committee (IACUC) of the Indonesian Agency for Agricultural Research and Development approved the animal study proposal, with the permit number: Balitbangtan/Lolitsapi/Rm/09/2020.

### **Sample collection and DNA amplification**

Three millilitres of 29 blood samples were collected from the jugular vein of Sragen (n = 10) and Jabres (n = 19) cattle using a venoject and an EDTA

vacutainer tube. The blood samples were then taken to the laboratory in an icebox for DNA extraction using the gSYNC DNA extraction kit (Geneaid, Taiwan). The DNA extraction kit consists of proteinase K and GSB buffer for cell lysis and protein degradation, W1 and wash buffer for removing contaminants, and elution buffer to elute the purified genomic DNA. Amplification of the FSHR gene was conducted in a 25 µl volume containing 2 µl genomic DNA, 0.5 µl of forward. It reversed primers mentioned by Hernandez-Cruz et al. (2009) as shown in Table 1, 9.5 µl ddH<sub>2</sub>O and 12.5 µl of MyTaq HS Red Mix (Bioline, UK). The amplification was performed in Thermocycler (Sensoquest Labcycler, Germany). It consisted of 94 °C for 5 min pre-denaturation, followed by 35 cycles of 94 °C for 45 sec (denaturation), 60 °C for 45 sec (annealing), and 72 °C for 1 min (extension) with a final extension at 72 °C for 5 min. The PCR products could be seen on a 1.5% standard agarose gel stained with ethidium bromide and documented digitally. Each amplicon was sequenced using Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific, USA) in Universitas Gadjah Mada Central Laboratory (LPPT-UGM).

**Table 1.** Primer sequences and PCR condition of FSHR gene

Primer	Sequence	Product size (bp)
Forward (PF)	CTGCCTCCCTCAAGGTGCCCTC	306
Reverse (PR)	AGTTCTTGGCTAAATGTCTTAGGGGG	

### SNP identification and haplotype analysis

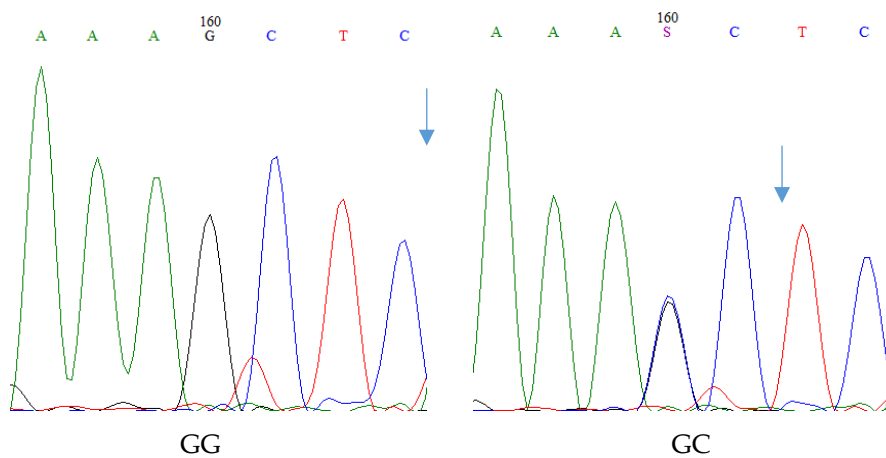
Raw sequence data were edited using BioEdit software. Sequence alignments were performed with Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) to identify single nucleotide polymorphism (SNPs) of FSHR gene and to genotype the samples. Manual examination of electropherograms was used to confirm the polymorphic site found by sequence comparison. Analyze the SNPs for LD and haplotype block analysis was done using haploview software. In addition, the haplotype block established, and the D'prime; LD; r-square (r<sup>2</sup>), and haplotype frequency were also calculated.

## RESULTS AND DISCUSSION

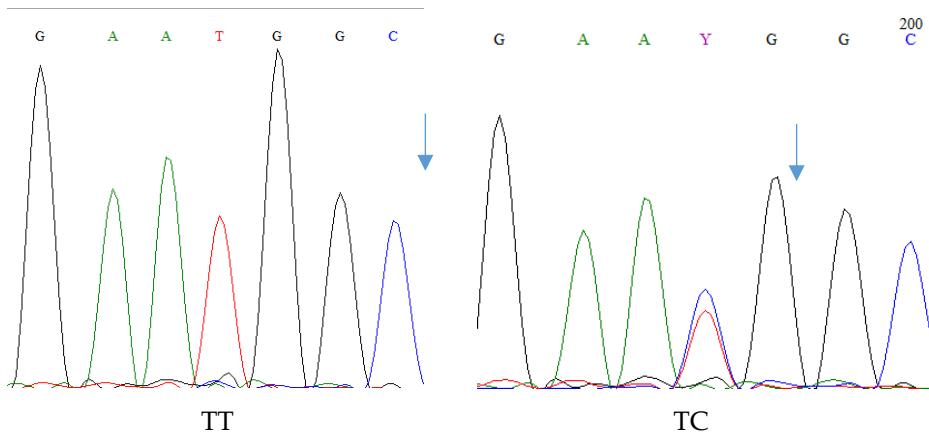
### Polymorphism of FSHR gene

The polymorphic sites, namely SNP g.193G>C (AAAGCTC to AAACCTC), g.227T>C (GAATGGC to GAACGGC), and g.275A>C

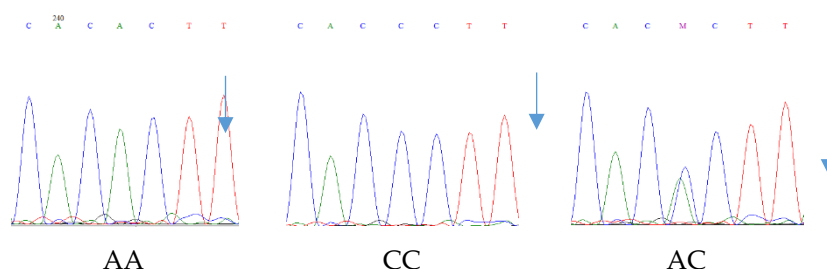
(CACACTT to CACCCTT), were detected by PCR and DNA sequencing methods. All three SNPs were located in intron 1 of the FSHR gene (Genebank acc no. NC\_032660). In SNP g.193G>C and g.227T>C, the CC genotype was not found in the samples (Figure 1 and 2). However, for SNP g.2775A>C, all three genotypes had a distinct peak in the electropherogram (Figure 3). Even though the SNPs are located in a non-coding sequence, it could still affect the phenotype since the non-coding region RNAs (intron) control transcriptional and post-transcriptional gene expression (Nakaya et al. 2007). Yang et al. (2010) previously identified two SNPs in the non-coding region (5'UTR), SNP -320 A>T and -278 G>A. The GG genotype in Chinese Holstein cows had a considerably larger number of degenerate embryos (NDE), the number of transferable embryos (NTE), and the total number of ova (TNO) based on SNP -278 G>A.



**Figure 1.** Electropherogram of SNP g.193G>C



**Figure 2.** Electropherogram of SNP g.227T>C



**Figure 3.** Electrophoregram of SNP g.275A>C

### Haplotype block analysis

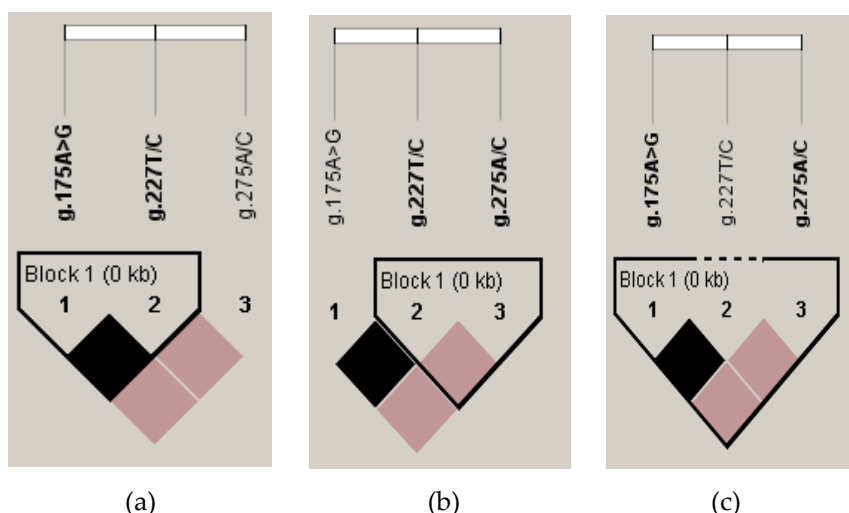
A haplotype block is a group of closely related markers on a chromosome that share a significant LD and inherit together. This method used to test one SNP within each block for significant association with a trait could indicate association with all SNPs in that block to reduce the number of SNPs required to be tested in association studies (Qanbari 2020).  $r^2$  is a commonly used measure of LD in animal breeding and genome-wide association mapping. In various ways,  $r^2$ 's reliance on allele frequencies influences the findings and outcomes of population genetics investigations. Population characteristics such as effective population size and recombination landscape pattern, for example, are linked to the estimated value of LD; this means that effective size estimations or recombination maps based on expected  $r^2$  values are frequency-dependent (Ober et al. 2013).

In this study, the SNPs identified (SNP g.175A/G (SNP1), SNP g.227T/C (SNP2) and SNP g.275A/C (SNP3)) were analyzed for LD and haplotype block analysis using haploview software. Three types of haplotype block have been created to indicate the  $D'$ prime, LD,  $r$ -square ( $r^2$ ) and haplotype frequency. Each block in this findings had a different SNPs combination, as shown in Table 2 and Figure 4. All block has a high  $D'$  ( $D'$  prime: 1.0). Block 1 (SNP1&2 combination) had the greatest LOD and  $r$ -squared, 9.23 and 1.0, respectively. Two blocks with the same LOD (1.23) and  $r$ -squared (0.28). In SNP1&2 combinations/block 1, two haplotype frequencies are defined (GT/79.3 percent; CC/20.7 percent). In SNP2&3 combinations (block 2), three haplotypes, TC, TA, and CA, were discovered, with genotype frequencies of 51.7 per cent, 27.6%, and 20.7 per cent, respectively. SNP1&3 pairings (block 3) suggest three haplotypes with the same frequency but different genotypes: GC, GA, and CA.

**Table 2.** The LD and haplotype block based on SNPs combination

Linkage between locus	D'	r <sup>2</sup>	LOD	Haplotype allele	Haplotype frequency
SNP 1 – SNP 2	1.0	1.0	9.23	GT	0.79
				CC	0.21
SNP 2 – SNP 3	1.0	0.28	1.23	TC	0.52
				TA	0.28
				CA	0.21
SNP 1 – SNP 3	1.0	0.28	1.23	GC	0.52
				GA	0.28
				CA	0.21

In this result, the  $r^2$  and  $D'$  behave differently, with  $D'$  remaining equal to 1, but  $r^2$  smaller. It shows that mutations occur in different lineages, but there is no recombination between them (between SNP2 and SNP3 and between SNP1 and SNP3). Mészáros et al. (2021) stated that if the minor allele frequencies of an SNP marker and a causative variant differ, the power to detect an effect at the marker may be low because high values of  $r^2$  are not realized. The LOD score is a statistical test commonly used in human, animal, and plant populations for linkage research (Yan et al. 2017). A positive LOD score shows the presence of linkages. Evidence of linkage is considered if the LOD score is greater than 3. In this study, all LOD scores were positive, but only for LOD between SNP 1 and SNP 2, greater than 3; this demonstrated the linkage among these SNPs. The power and precision of association mapping investigations are also determined by the degree of LD between linked markers, which directly impacts our capacity to locate genes and or loci responsible for agricultural economic traits. Given that SNPs are in LD with one another, if a common SNP affects a characteristic, it is likely that an SNP in LD will be genotyped and linked with the trait of interest (Qanbari 2020). Hence, we could recommend the haplotype block 1 (combination of SNP1&2) for further association analysis since it has a high LOD and  $r^2$  value.



**Figure 4.** The block of SNP1&2 (a), SNP2&3 (b), and SNP1&3 (c) combinations

## CONCLUSION

Three SNPs have been detected, namely SNP g.175A/G (SNP1), SNP g.227T/C (SNP2) and SNP g.275A/C (SNP3). Three types of haplotype block, A, B, and C, were performed, and all blocks indicated having high D'prime (=1.0). Based on the haplotype analysis, the haplotype block 1 (combination of SNP1&2) could be recommended for further association study between the FSHR gene and reproduction traits in Sragen and Jabres cattle.

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## AUTHOR CONTRIBUTIONS

Prihandini PW, Primasari A, Luthfi M, Pamungkas D, and Dina TB participated in designing the research and completed the entire field work and molecular assays. Sari APZNLS carried out the haplotype analysis and drafted the manuscript. Maharani D provided critical insight into the manuscript. All authors read and approve the final manuscript

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## **Peranakan Ettawa Goat Production System Condition in the Breeding Stock Area in Banjarnegara, Central Java**

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### **ABSTRACT**

Banjarmangu Subdistrict, Banjarnegara District, Central Java Province, has been designated a breeding stock area for Peranakan Ettawa (PE) goats. The purpose of the study was to determine the performance of PE goats in the breeding stock area. The study was conducted using a survey method, interviews with goat breeders about reproductive characteristics, number of ownerships, livestock management, and observations of the breeder's location. The respondents interviewed were 24 people. Criteria for determining respondents are livestock farmers who have pure PE goats. The results showed that the current population averages based on the sex ratio of adult males and females are not ideal, which is 2:3; meanwhile, the sex ratio of the male and female kids is 1:1. Hand mating management is still carried out here, which is that female animals are being lured together with superior males. The selection system is carried out by farmers based on the quality of the kids it produces. The animal maintenance system is carried out intensively. It was concluded that the performance of PE goats in the breeding source area needs to be improved in quality, namely by introducing reproduction and management technology.

**Keywords:** PE goats, Performance, Breeding stock, Central Java

### **INTRODUCTION**

Breeding Source Area was an agroecosystem area that was not bounded by government administrative areas and could develop a breeding stock of certain types or breeds of livestock (Kementerian Pertanian Republik Indonesia 2011). The aim of establishing an area as a breeding source area was to map an area with a particular agroecosystem as a breeding source area. The map of the breeding source area was to establish a particular area for breed purifying of local/native livestock breeds in Indonesia to be sustainable. This government facilitation is an embodiment while guaranteeing the availability of livestock breeds both in quantity and quality.

The PE goat was a superior commodity in Central Java Province. However, the pure breed of PE goats in Banjarnegara Regency, Central Java, was endangered because many pure PE goats breeding stock were sold outside the region. The Central Java province has appointed Banjarmangu Sub-District as a breeding source area for PE goats to overcome this problem. Banjarmangu Sub-District was chosen because raising PE goats in this area has practised the farming system so that the forage fulfilment will not be a problem. Based on this potency, in 2015, Banjarmangu District was established as a breeding source area for PE goats by Ministry of Agriculture Decree number 353/Kpts/PK.040/6/2015.

Based on the above consideration, a study on the breeding practice of PE goats in the breeding source area in Banjarmangu Sub-District, Banjarnegara District, Central Java Province, was done. The research aimed to determine the condition of the production system involved in the PE goats breeds in the breeding source area.

## **MATERIALS AND METHODS**

Material and method used in this study are as follow:

### **Material**

The material used in this research is 24 livestock farmers of pure PE goat located in Banjarmangu Sub-District, Banjarnegara Regency, Central Java Province as response. Only the farmer who owns pure PE breed was chosen as a response in this study.

### **Methods**

#### ***Location***

The research was conducted in breeding source area of PE goat, at Banjarmangu Sub District, Banjarnegara District, Central Java Province.

#### ***Data collection technique***

The research was conducted by the on-the-spot survey on the research location, interview with the farmers about reproduction characteristics, ownership amount, and livestock rearing management, followed by direct observation of farming location. The collected data consisted of primer data obtained from the farmers by filling out the questionnaire while interviewing. Data collected are farmers' age, formal and non-formal education, family

member, and the income from livestock rearing and other businesses during the year. The production data collected are: (1) the population dynamic within one year period, including the number of goats sold, based on age and sex structure; (2) reproductive characteristics includes the number of pregnant goats, gestation length, lactation period, dry period and unpregnant, first estrus age, mating age, weaning age, litter size, kidding interval, birth weight and also sex ratio of kid. In addition, direct observation of the farming location was conducted to get the data on animal housing, farming management, animal feed, and other supporting data on the PE goat rearing business.

### ***Data analysis***

The data were analyzed using GLM analysis of the SAS program and if there were differences, it was tested using Duncan's Test.

## **RESULTS AND DISCUSSION**

This study resulted several outcomes described as follow:

### **General Condition of Research Location**

Based on the geographical conditions, Banjarnegara district was a district in Central Java with 106,970.997 Ha area. Banjarnegara district was located at 7°12'-7°31' South latitude and 109° 29'-109° 45'50" East longitude. Banjarnegara district was bordered by Pekalongan district and Batang district in the north, the Wonosobo district in the east, the Kebumen district in the south, and the Banyumas district and Purbalingga district in the west; it was consist of 20 sub-districts with 12 political villages and 266 villages. Banjarnegara district has an average rainfall of 3.000 mm per year and an average temperature of 20-26°C (BPS 2017). Based on this condition, Banjarnegara districts were suitable for PE goats breeding, so that in 2015 Banjarnegara district was established as one of the breeding source areas in the Banjarmangu sub-district for PE goats as mentioned in Ministry of Agriculture decree number 353/Kpts/PK.040/6/2015.

The Banjarmangu sub-district was one of the sub-districts within the Banjarnegara district, which could be developed as a Pondoh snake fruit plantation area. At this time became the centre for Pondoh snake fruit producer, with 106,339,200 kg production within 2016 (BPS 2017) and combined with PE goat farming. The PE goat is a dual function breed, as milk and meat producers also for breeding stock.

## **Characteristics of farmers**

Based on data in Table 1, the PE goat farmers' age was relatively old on average. The farmers' age was significant in a farming business; it was closely related to the physical abilities and intelligence of the farmers. Working productivity was rationally influenced by physical strength and intelligence. Productive age was likely to increase the knowledge and skills in receiving new appropriate technologies to support business and increase livestock productivity (Mastuti & Hidayat 2009). The productive age was still good strengths, response to action was quite good too, according to Matatula (2008) statement, which states that the age factor was usually more identical with working productivity. If someone was still classified as productive age, then the tendency of productivity was also high.

Most of the respondents in the Banjarmangu sub-district were accomplished their primary education in elementary school, almost the same reported by Rumiyaniti & Hamdani (2007). The low level of farmers' education showed the respondents' lack of knowledge and skill, which may influence the way farmers think and work in developing their farming business. The farmers with formal education experience were expected to be more open to innovation and increase the productivity of their business. Mandaka & Hutagaol (2005) stated that the education level can affect the ability of the farmers in farming techniques and science adoption. Some farmers with a low education level were already increasing their knowledge by following farming courses and training.

The respondent's main job was land-farming; meanwhile, the livestock rearing was only their side job, and they only spent less than 5% of their time doing it. The same result was also reported by Febrina & Liana (2008) that livestock rearing was still considered a side job. The time spent was only 30% of farmers' time, which is also influenced by the slow development of the livestock rearing business.

Someone's experience in doing their business can be seen from the length they doing the business. In this case, the business experience was the time length the farmer used to rear PE goat from the start until now. Farming experience was almost the same reported by Rumiyaniti & Hamdani (2017) at Ternak Sehati Jaya group, Sungai Langka village, Gedong Tataan sub-district, Pesawaran district, Lampung province. The farming experience was affected by the farmers' knowledge and experience in managing their business (Mandaka & Hutagaol 2005). The experience was affected farmers much better in the technology adoption, push the knowledge, behaviour, and decision making (Eddy et al. 2012). The farmers still traditionally carry out PE goat maintenance, using family labour as the primary labour source. The resources

**Table 1.** Farmer's characteristic as the respondents of study

Description	Value
Farmers' average age (year)	50.54±10.64
Main job:	
Livestock rearing (%)	4.17%
Farming (%)	66.67%
Trader (%)	12.50%
Official (%)	8.33%
Other (%)	8.33%
Formal education	
Not attending school education (%)	4.17%
Elementary school (%)	50.00%
Junior high school (%)	12.50%
Senior high school (%)	20.83%
University (%)	12.50%
Informal education	
Ever (%)	29.17%
Never (%)	70.83%
Livestock rearing experiment (year)	8.20±6.91
Livestock owning status	
Owner (%)	86.96%
Worker (%)	8.70%
Owner and also worker (%)	4.35%
Labor source	
The farmers themselves (%)	82.61%
Family members (%)	4.35%
Hiring others (%)	13.04%
Net income per month from livestock	
<1 million rupiah	50.00%
1-2,5 million rupiah	14.29%
2,5-5 million rupiah	0.00%
>5 million rupiah	14.29%
Uncertain	21.43%

utilization has not been maximized, so that the level of profit has not been adequate. Most PE goat breeders act as owners and workers, although some

farmers do not make this livestock rearing business their main job or just a side job. Livestock was used as savings to fulfil their family needs.

### **Livestock ownership**

Developing local livestock in rural areas needs to be known to estimate population rising time per unit. The information about changing values in population and the development program in the future can be obtained (Sukendar et al. 2005). Livestock ownership was based on each farmer as a respondent. At the beginning of the farmer's group establishment, the average PE goats owned by the farmers for each sex were two males and five females, as seen in Table 2.

The number of livestock ownership was quite a lot in the research location, considering that subsistence farmers in Indonesia have an average of 1-3 goats per household (Nugroho 2010). Initial livestock was obtained from government assistance. Meanwhile, the current population average sex ratio of an adult male and female PE goat was not ideal, that is 2:3 in comparison. In the meantime, the sex ratio of young male and female PE goats was 1:1.

During raising the livestock, the obstacle that often arises for PE goat farmers is that they do not want to suckle directly to their mother when a kid is born. It is probably due to the lack of mothering ability of PE goat.

To encourage the success of the farms that we manage must be supported by cages and cage equipment. The interview results proved that the availability of cages and cages equipment enclosures were the most critical constraints mentioned by the farmers. According to Kurniasih et al. (2013), several determinants influence the development of the livestock population, including matting, handling of newborn kids, availability and quality of feed, and health care.

### **Reproduction characteristics**

The matting system of PE goat, as shown in Table 3, was still hand matting system, that an estrus period female goat was put together with the superior males. The reason for this practice is because they have their males.

The matting system was determined by farmers using their males or from groups. Farmer group chief lends the breeders a buck to those who do not have their male mate with their female estrus goat. In order to get superior offspring, even the PE goat breeders hired a male goat from others breeders to be mated to their female estrus goat. The criteria for males being considered by PE goat breeders was by seeing the conformation of the body and body weight.

**Table 2.** Status of livestock raising

Description	Value
Livestock ownership model	
Adult male (head)	1.86±1.29
Young male (head)	1.71±1.11
Adult female (head)	3.38±3.47
Young female (head)	1.67±1.03
Male kid (head)	2.18±1.66
Female kid (head)	1.83±1.47
Initial number of livestock raising	
Male (head)	1.92±2.27
Female (head)	4.50±2.07
Recent amount	
Male (head)	1.86±1.29
Pregnant female (head)	2.60±3.10
Lactation female (head)	2.36±1.57
Dry period female (head)	2.00±1.41
Young pregnant female (head)	1.00±0.00
Post weaning male (head)	1.78±1.30
Post weaning female (head)	1.67±1.03
Male kid (head)	2.11±1.69
Female kid (head)	2.20±1.55
Obstacles in livestock rearing	
Lack of animal feed stock (%)	12.50%
Lack of capital stock (%)	4.17%
Disease (%)	12.50%
Lack of animal health services (%)	4.17%
Others (%) (no obstacles, direct suckling by the kid to their mother)	66.67%
Carrying capacity of livestock raising business	
Cages availability (%)	100.00%
Cages equipment (%)	100.00%
Others (%) (Government assistance)	4.17%

**Table 3.** Matting and reproduction aspects

Description	Value
Livestock matting technique	
Only by AI (%)	0.00
Only by natural matting (%)	100.00
Both AI and natural matting (%)	0.00
Action when estrus occurred	
Reported to the inseminator (%)	0.00
Matted naturally (%)	100.00
Male goat for natural matting source	
One's own (%)	44.00
Belongs to the group (%)	32.00
Rent (%)	4.00
Belongs to others' (%)	20.00
The other (%)	0.00
Expected criteria for bucks	
Body conformation (%)	51.72
Bucks's age (%)	13.79
Bodyweight (%)	24.14
The other (%) (based on the condition of bucks's candidates)	10.34
The reason to do natural matting	
More practical (%)	22.22
More successful in getting the pregnancy (%)	18.52
Cheaper fee (%)	0.00
Habits (%)	25.93
The other (%) (the bucks can be seen before, own male, having the better progeny)	33.33
Natural matting obstacle	
The high maintenance cost of a male (%)	17.39
The high rental cost of a bucks (%)	4.35
Others (%) (no obstacle)	78.26
For the good bucks, will be continued to be matted to the progeny?	
Yes (%)	43.48
No (%)	56.52

Description	Value
If No, the reason:	
The kid will not have a good performance (%)	15.38
Abnormal kid (%)	0.00
Low body posture and body weight (%)	7.69
The other (%) (in order to get the new bucks, worries about abnormalities that might occur)	76.92
Estrus observation	
Morning (%)	43.48
Noon (%)	0.00
Evening (%)	56.52
Frequency of estrus observation	
One time (%)	76.19
Twice (%)	0.00
Three-time (%)	0.00
Irregular (%)	23.81
Length of estrus (hours)	14.71±11.59
Age of first estrus (month)	9.25±2.46
Age of first matting (month)	12.47±1.94
The way to know the pregnancy	
Observing the subsequent estrus (%)	55.56
The other (%) (bigger tummy, delicate feather, noisy, unwigged tail, partus)	44.44
Abortion	
Often (%)	0.00
Seldom (%)	6.25
The other (%) (never)	93.75
The age of kid to be weaned (month)	4.20±0.41

The matting system was determined by farmers using their buck or from groups. Farmer group chief lends the breeders a buck to those who do not have their male mate with their female estrus goat. In order to get superior offspring, even the PE goat breeders hired a bucks from others breeders to be matted to their female estrus goat. The criteria for bucks being considered by PE goat breeders was by seeing the conformation of the body and body weight.

First mating on PE goat. The estrus observation was usually done in the evening with uncertain frequency. The average age of first mating was following Kementerian Pertanian Republik Indonesia (2011) that stated that the first mating age was at 10-12 months. The purpose of arranging the mating age of female cattle is to maintain productivity, and it was recommended that when the goat was mated, they were nearing adulthood. The management was carried out so that the goat pregnancy rate immediately reached the optimum level after mating. According to Budi (2005), improper mating time can have an impact on pregnancy failure. Based on data in Table 3, breeders do not directly mate their livestock but wait for their animals to reach body maturity before mated. According to the breeders, the purpose of delaying the age of the first mating was to provide opportunities for the goat to achieve sufficient condition and weight to maintain pregnancy and subsequent production and reproductive performance.

The farmers' knowledge about the success or failure of their livestock was pregnant after mating was by observing the next estrus cycle. If the pregnancy were occurring, the female goat would not show signs of estrus. This method was quite successful, where PE goats have never experienced an abortion.

### **Livestock breeding pattern**

Livestock breeding was a long-term effort. A significant challenge was to estimate what kind of livestock will be in demand in the future and plan to produce the expected animals (Warwick et al. 1990). The role of breeding in livestock production activities was significant, among others, to produce livestock that was efficient and adaptable to the environment.

The initial livestock, as mentioned above, were obtained from government assistance (Table 4). Because the pattern of mating adopted by the breeders was natural mating, then the determination of the bucks to be mated with the estrus female was determined by the breeders. The farmers themselves carried out maintenance of the prospective future breed. the breeders carried out the PE goat selection system based on the quality of the progeny produced. According to the PE goat breeder, the purpose of the selection was to get a kid with a high birth weight.

Factors considered in selecting a bucks and female goat for the future breed was based on the body shape. Because the eyes can directly see the body shape of the farmers. Also, by seeing the body shape, it can immediately recognize the originality of the PE goat.

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shape of the farmers. Also, by seeing the body shape, it can immediately recognize the originality of the PE goat.

**Table 4.** Livestock breeding pattern

Description	Value (%)
Breed source from	
The government assistance	50.00
Deposit from other groups/breeders	0.00
From the animal market	8.33
The others (the results of the contest, buy from other farmers)	41.67
How to determine the prospective bucks for natural mating	
By the head of the group	0.00
By the farmer	95.83
Not programmed	0.00
The other (the results of the contest)	4.17
The selection system was done by considering	
The progeny of the male	17.39
The progeny of the female	4.35
The progeny of both the bucks and the female	21.74
The progeny quality	43.48
The other (the bucks resulted from the contest)	13.04
Breed source maintenance was done by	
The farmer	92.00
The farmer group	8.00
The other	0.00
Selection goal	
To get a kid with high birth weight	56.52
To get a kid with breeding source criteria	34.78
To get the milk production	4.35
The other (%) (to get a better result)	4.35

Description	Value (%)
Factors to consider in breed selection	
Bucks:	
Body shape	40.35
Horn	8.77
Feather color	8.77
Ear shape	10.53
Tail shape	3.51
Character/temperament	5.26
Growth rate	3.51
Fertility	14.04
Disease resistant	3.51
The other (head shape)	17.56
Female :	
Body shape	37.93
Horn	8.62
Feather color	8.62
Ear shape	13.79
Tail shape	3.45
Character / temperament	5.17
Growth rate	3.45
Fertility	12.07
Disease resistant	1.72
The other (udder shape, head shape)	5.17

### **Livestock production management**

The PE goat rearing system was carried out intensively. That is, the goat was kept in the cage all the time. For PE, goats are occasionally carried out of the cages to be bathed and sun-dried. The type of cage used on PE goats was often caged in old houses with the ground floor. The cage material used is made of wood and bamboo. Such maintenance systems cause farmers to

provide feed for their animals, where the feed was given, i.e., natural/field grass. Almost most of the farmers do not have the land for planting forage cultivation. However, some farmers grew forage on their own, such as *Pennisetum purpureum*, *Gliricidia*, and *Calliandra* in between the Pondoh snake fruit tree.

Forages for PE goat were given varies from one to two times per day. Sources of forage given to PE goats were agricultural byproducts, natural grass, cultivated grass (*Pennisetum purpureum*), and being released at the grassland. Forage given as animal feed was obtained from their farm, buying or renting the land from the local Agriculture Service. Almost precisely as reported (Hadi et al. 2011; Nurlaha et al. 2015), forage given by farmers as feed material derived from plants consisting of leaves mixed with stems, branches and flowers that come from grass, legume, an agricultural waste product, or forage from other plants. The amount of forage given by the farmers is sufficient to live, produce, and reproduction as seen in Tables 2 and 3.

**Table 5.** Rearing management

Description	Value
Rearing management system	
Intensive (%)	73.91
Semi intensive (%)	21.74
Extensive (%)	4.35
Source of animal feed	
Agricultural byproduct (%)	10.53
Natural grass (%)	47.37
Cultivated grass (%)	39.47
Grassland (%)	2.63
Grass:	
Feeding amount (kg)	48.26±16.82
Feeding frequency (time):	
1 time (%)	73.91
2 time (%)	26.09
3 time (%)	0.00
Feeding time:	
Morning (%)	17.24
Noon (%)	6.90
Evening (%)	72.41
Night (%)	3.45

## CONCLUSION

The ownership of PE goats in the Banjarmangu Sub-District was quite a lot (>4 heads per household) of livestock raising intensively, so the mating system was still done naturally. This decision was made because the farmers already had superior male and knowledge about the well-breeding system. Thus, the forage feed sustainability was always available forage sources such as *Pennisetum purpureum*, *gliricidia*, *calliandra* and agricultural waste products. The production and reproduction of PE goats in the Banjarmangu Sub-District was not disrupted.

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## **External Quality, Fertility, and Hatchability of Egg from Brown and Black Plumage Quail**

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### **ABSTRACT**

Besides its role as laboratory animals and research models in poultry species, quail is characterized by fast growth, high reproductive performance, short generation intervals, and lower feed requirements. Research has been carried out to evaluate the characteristics of brown and black plumage quail eggs in Balitnak. A total of 99 quail eggs was obtained from Slamet Quail Farm, Sukabumi, consisting of 50 brown plumage quail eggs and 49 black plumage quail eggs. Eggs were incubated in 1 batch in a portable incubator. The variables observed were the weight of the incubated eggs, length and width of egg, shape index of egg, fertility, hatching ability of fertile, and hatchability of total hatching eggs. All data collected were tested using independent samples t-test to compare variants of plumage color. The results showed that all variables observed between brown and black plumage quails were significantly different ( $P < 0.05$ ). It was concluded that the black plumage quail external quality and reproductive efficiency were higher than the brown plumage quails.

**Keywords:** Egg, Fertility, Hatchability, Plumage color, Quail

### **INTRODUCTION**

The quail has become popular as a source of meat and eggs in various parts of the world. This is in addition to its role as laboratory animals and research models on poultry species (Raji et al. 2009). The quail is characterized by fast growth, small body size, high reproductive performance, short generation interval, low feed need, good meat taste, and shorter hatching time compared to other poultry species (Arora & Samples 2011; Khosro et al. 2011).

The key to the success of the hatching process is influenced by many factors, including egg quality, egg weight, and hatchability (Widiyaningrum et al. 2016). Egg quality will describe its true potential in producing a decent DOQ, while egg weight is positively correlated with hatching weight (Mbajorgu & Ramaphala 2014). Hatchability is influenced by egg quality and fertility (Ayman 2011). Thus, high egg weight and hatchability can be obtained when we select hatching eggs.

Egg quality and hatchability, have been reported to vary within and between breeds or genotypes (Alkan et al. 2016). These two traits are the main reproductive parameters in poultry. In addition, egg quality depends on age and plumage color (Sari et al. 2012). Yilmaz et al. (2011) have reported the effect of plumage color on the external and internal quality of quail eggs. In addition, plumage color correlates with quantitative traits (Delmore et al. 2016).

The continuous evaluation of egg quality, fertility, and hatchability is essential. Therefore, this study was conducted to evaluate and compare egg quality, fertility, and hatchability of two color variants of quail (brown and black plumage) in Balitnak.

## **MATERIALS AND METHODS**

The research used 99 hatching quail eggs from Slamet Quail Farm, Sukabumi, consisting of 50 brown plumage quail eggs and 49 black plumage quail eggs. The eggs are selected supported shape and free from cracked shells.

The selected eggs were then cleaned employing a soft cloth that has been moistened with 70% alcohol slowly then put into a portable incubator machine. On the 15th day of incubation, candling was performed and both infertile and fertile eggs were recorded. Then the fertile eggs are transferred from the incubator tray to the hatching tray on an equivalent machine. The entire number of eggs hatched was recorded on the 18th day of incubation.

In the research, the observed variable was the load of the eggs incubated, namely by weighing the eggs one by one using a digital scale, then calculating the mean and standard deviation. Measuring the length and width of the eggs employing a caliper. The variables of the egg shape index (Duman et al. 2016), fertility (Ahmed & Al-Barzinji 2020), hatchability of fertile eggs (Ahmed & Al-Barzinji 2020), and hatchability of total hatching eggs (Ahmed & Al-Barzinji 2020), using the formula:

$$\text{Egg shape index} = \frac{\text{Egg width}}{\text{Egg length}} \times 100\%$$

$$\text{Fertility} = \frac{\text{Number of fertile eggs}}{\text{Total egg set}} \times 100\%$$

$$\text{Hatchability of fertile eggs} = \frac{\text{Number of eggs hatched}}{\text{Total fertile eggs}} \times 100\%$$

$$\text{Hatchability on the base of total eggs set} = \frac{\text{Number of eggs hatched}}{\text{Total eggs set}} \times 100\%$$

All data collected was first entered into an Excel table, then analyzed using SPSS version 25 software. The test used in this research was the independent samples t-test to compare the plumage color variants.

## RESULTS AND DISCUSSION

### Egg external quality

The average of some external characteristics of quail eggs based on differences in plumage color is shown in Table 1. It is clear that differences in plumage color significantly affect some external characteristics of eggs ( $P < 0.05$ ) because egg characteristics have a significant effect on plumage color (Minvielle et al. 1999). Black plumage quail eggs were found heavier than brown plumage quail. Likewise, the average length and width of black plumage quail eggs are longer and wider than brown plumage quail eggs. In such a way that the highest egg shape index was observed in black plumage quail.

Based on the data obtained, it was shown that plumage color had a significant effect on egg weight ( $P < 0.05$ ) (Table 1). This difference in quail plumage color was also found in previous studies (Sari et al. 2012; Islam et al. 2014; Chimezie et al. 2017; Lydie et al. 2019). The weight of incubated eggs is the weight of the fertilized fertile eggs used for hatching. The average weight of brown plumage quail eggs in this study was about 1 g heavier than that reported by Lydie et al. (2019). However, the average weight of brown plumage eggs was the same as the results obtained by Sari et al. (2012) and Ahmed & Al-Barzinji (2020). While the average weight of black plumage quail eggs is higher than the results of research by Islam et al. (2014) which obtained an egg weight of  $9.78 \pm 0.65$  g.

**Table 1.** The appearance of some external characteristics of quail eggs of variants two colour

Variable	Brown plumage quail	Black plumage quail	Statistic test
Number of eggs	50	49	-
Incubated egg weight (g)	$11.65 \pm 0.86$	$12.63 \pm 0.73$	Significant
Egg length (mm)	$32.46 \pm 1.09$	$33.29 \pm 1.07$	Significant
Egg width (mm)	$25.43 \pm 1.82$	$26.73 \pm 0.54$	Significant
Egg shape index	$78.43 \pm 6.04$	$80.35 \pm 2.27$	Significant

Larger egg sizes in black plumage quail can be an advantage in the quail breeding industry. Meanwhile, from the consumer's point of view, egg weight is considered the most important quality trait (Genchev 2012), so the possibility of egg preference from black plumage is higher than brown plumage quail.

Plumage color showed a significant effect ( $P < 0.05$ ) for the variable length and width of the eggs. The average brown plumage quail egg length was recorded to be lower than the black plumage quail population. The same range was observed for the variable width of brown and black plumage quail eggs. Furthermore, the average value of the length and width of brown plumage quail eggs in the study was higher than the results of Taha et al. (2019) and Lydie et al. (2019).

The egg shape index of quail in the research depends on the color of the plumage. It can be seen from the average value of the egg shape index obtained, the brown plumage color in this research is similar to the value reported by Sari et al. (2012) and higher than reported by Taha et al. (2019) and Lydie et al. (2019). The egg shape index value depends on the results of measuring the length and width of the egg, as seen in this study, the longer and wider the egg, the greater the egg shape index value. In line with the formula for calculating the egg shape index (Duman et al. 2016).

### **Fertility and hatchability**

The reproductive characteristics of brown and black plumage quails are presented in Table 2. Fertility percentages showed significant differences ( $P < 0.05$ ) for brown and black plumage quail eggs. The fertility of black plumage quail is higher than that of brown plumage quail, this is in accordance with the results of Matsuzaki et al. (2021) that the sperm of black plumage quail has a flagellum and a longer midsection, allowing the sperm to swim faster, so fertilization can be successful. This result is in a line with the research conducted by Nwachukwu et al. (2015) who reported that the percentage of fertility can be significantly influenced by differences in color variability of a plumage quail. Likewise, for a black plumage quail, it was higher than the results of Chimezie et al. (2018) who get a fertility percentage of 93.88%. Fertility is one of the main factors that determine success in hatching efforts because only fertile eggs can produce the DOQ.

Egg hatchability is a number that indicates the level of the egg's ability to hatch. Hatchability can be seen from the fertile eggs and the total eggs that enter the incubation machine. The research found the average hatchability of black plumage quail eggs showed a higher percentage ( $P < 0.05$ ) than brown plumage quail (Table 2). The results of the study are in line with the findings of

**Table 2.** The appearance of the reproductive characteristics of quail

Variable	Brown plumage quail	Black plumage quail	Statistic test
Fertility (%)	84.00	97.96	Significant
Hatchability of fertile eggs (%)	88.10	93.75	Significant
Hatchability of total hatching eggs (%)	74.00	91.84	Significant

Chimezie et al. (2018) who reported that black plumage quail had a higher percentage of fertile egg hatchability compared to other plumage quail variants. In contrast, Islam et al. (2014) reported the lowest hatchability of eggs hatched by black plumage quail (31.03%). The possible cause of the difference in hatchability of black plumage quail at this time may be influenced by different incubation factors.

Almost the same as the hatchability of fertile eggs, the average hatchability of the total eggs entering the incubation machine for black plumage quail showed a higher percentage ( $P < 0.05$ ) than brown plumage quail (Table 2). Because it is calculated from the total eggs entering the incubator machine, the average hatchability value of the total eggs entering the incubator machine will be smaller than the average hatchability value of fertile eggs. Chimezie et al. (2018) gave the same result that black plumage quail gave a higher percentage than other plumage variants.

Differences in fertility percentages and hatchability can be attributed to differences in bucks and female fertility rates arising from genetic and/or management factors. A low fertility rate will naturally result in a low hatchability percentage as well as observed in this study.

## CONCLUSION

The black plumage quail external quality and reproductive efficiency were higher than the brown plumage quail. We hope that the results obtained in this study are expected to help researchers and breeders to understand these traits.

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## AUTHORS CONTRIBUTION

Tatan Kostaman: Tabulation of experimental data, writing, and editing; Soni Sopiya: Literatur collection

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## **Simmental Identification of SNP BTA10 to Twinning Birth Trait of Simmental - Ongole Grade Crossing**

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### **ABSTRACT**

Increasing of beef cattle population can be done by utilizing the genetic potential of twinning birth, which can be identified through exploration of quantitative trait loci markers (QTL) at locus chromosome 10 (BTA10). The aim of this research was to identify single nucleotide polymorphisms (SNPs) of BTA10 molecular markers on the trait of twinning birth. Ongole Grade (OG) cattle 22 heads and SIMPO (Simmental – OG crossing) cattle 86 heads, they were cows and its twin or single calves, taken its blood as samples research. The sequencing method was used to detect SNPs on BTA10 and SNAP to demonstrate validation markers of the twinning birth traits. The results showed that SNP verification in BTA 10 was not related to genetic of the twinning birth trait in OG or SIMPO cattle, but SNP A/T position of 75 bp at *consensus* area in its clamp sequence of locus Hapmap22923-BTA-129564 showed significant association with the twinning birth trait only in SIMPO cattle. It was concluded, SNP in BTA 10 had not been proven to be related to genetic traits of twinning birth in OC cattle, but SNP A/T in 75 bp position was significantly associated with SIMPO cattle.

**Keywords:** SNP, BTA10, Twinning, OG, SIMPO

### **INTRODUCTION**

Ongole Grade are one of the local beef cattle that are widely developed in East Java, Central Java, Sumatra, and East Nusa Tenggara and South Sulawesi, but crossbreed Simmental with Ongole Grade is mostly done, with aim to get more meat production (Tiesnamurti 2019). Increased livestock production can also be done through increasing twinning birth in cows (Praharani 2019).

Although the possibility of twinning births in cows can be found naturally, this cased rate is very low, which is 1-5% only (Garrick & Ruvinsky 2015). Several methods have been carried out to increase twinning birth in cattle, such as super ovulation or multiple ovulation (hormonal induction), artificial insemination (AI), embryo transfer (TE) (Dahlen et al. 2012; Sengupta et al. 2011), increased consumption of amino acids, adding forage sources of phytohormone (Marley et al. 2011), selection and mating arrangements (Triwulanningsih et al. 2011).

Twinning birth in cattle can provide several advantages compared to single calf, such as an increase in total weaning weight that can be increased productivity and efficiency of dam production (Hashiyada 2017), increase in farmer profits by increasing the total number of weans by 50-65%, weaning weight 50-75%, yearling weight and final weight, giving a 74% higher profit than single births (Gaafar et al. 2010; Hennesy et al. 2005). Twinning birth cattle cannot be separated from the effect of genetic, environmental and interaction factors. Increasing twinning births through selection and mating arrangements requires a longer time, so that currently several technologies have been able to increase twinning birth through manipulation environmental factor such as hormonal induction to affect the rate of ovulation (Echternkamp et al. 1990; Van Vleck & Gregory 1996; Gregory et al. 1997). The biomolecular selection process can be increased the frequency of twinning births (Lien et al. 2000), from 3.4% to 28.5% (Meuwissen et al. 2002). Selection based on molecular technology can identify the potential genes controlling fertility traits in cows (ovulation, conception and twinning birth rates), so can resolve long generation intervals and low heritability in genetically twinned cattle (Anggraeni et al. 2018). The development of reproductive technology has now been able to identify the genetic potential of twinning birth cattle. SNP variants BTA10 on genome bovine, that was developed by the Center of Agricultural Biotechnology and Genetic Resources to identify cattle that have genetic single or twin traits.

## **MATERIALS AND METHODS**

### **Time and location**

OG and SIMPO cattle blood samples were collected from several areas in Central Java, South Sumatra and West Sumatra, that were reported most widely twinning birth in cattle. DNA analysis was carried out at the Center of Agricultural Biotechnology and Genetic Resources.

### **Materials**

This research used 108 blood samples, consisting of 5 Ongole Grade dams with 11 calves (twinning birth), 3 Ongole Grade dams with 3 calves (single birth), 31 SIMPO dams with 43 twin calves (calved twin), and 6 SIMPO dams with 6 calves (calved single). Blood samples 2 ml were collected from the jugular vein using a vacutainer tube containing EDTA as anticoagulant. Blood samples were isolated using a DNA extraction kit (Qiagen), whose concentration and

purity were determined by absorbance at 260/280 and 260/230 using Nano Droop 1000. The resulting DNA was stored at -20°C before use.

## PCR amplificaton

Ongole Grade and SIMPO DNA were amplified using primer in Table 1. PCR reaction was performed in total volume 20 µl consist of 5 µl DNA (10-100 ng), 5 µl primer forward and reverse, 10 µl PCR mix (Bioline). Incubated on PCR machine with conditions were pre-denaturation 94°C for 5 min, denaturation 94°C for 30 s, annealing 50-70°C for 30 s, extension 72°C for 1 min, final extension 72°C for 10 s. PCR product visualized on 1% agarose gels, after that PCR product sent to sequencing services company.

**Table 1.** Design result of primer sequence on hapmap22923-BTA-129564 locus

Prime name	Sequence (5'.... 3')
Bvtwina-F	AAGGAGTGGCTGAAAGGTCA
Bvtwina-R	GGGTGTGGAGATGGACAGAG

## Sequencing

PCR product were purification and sequenced by direct sequencing on sequencing fragmens service company, after that sequence result was compared with SNP data from BTA10 on genome bovine, consistency of polymorphism verified on single and twin cattle and filogeny analysis to clarified clustering. Tassel program used for asosiation analysis between SNP target and sample. Sequencing done by once to look chromatogram quality, if the result not good, sequencing will be repeated from DNA amplification.

## Snap analysis

SNAP analysis was done by PCR approach on SNP that was asosiated with twinning trait from previous research. PCR reaction was performed total volume 20 µl (10-100 ng DNA), 5 µl primer forward reverse, and 10 µl Biolink PCR kit after that incubated on PCR machine with condition were pre-denaturation 94°C for 30s, annealing and extension 64°C for 1 min and final extension 72°C for 10s. Amplification result visualized on 1.5% agarose gel.

Each band visible on the gel was considered as one allele and was assigned score 1 (one), while the band that was not visible was assigned score 0 (zero). The genuine allele according to the SNP was identified according to the allele pattern in locus BTA10 on single and twin cattle. The scoring data with DNA markers changes to filogeny method to determine the difference

between single and twin cattles. Association for allele profile data were analysis in single and twin cattle.

## RESULTS AND DISCUSSION

The results of the analysis based on BTA10 markers in the bovine genome, A/G SNPs at locus 10 associated with twin traits in SIMPO cattle, were verified in Ongole Grade cattle at genome positions 200, 235, 363, 402 and 410 bp (Table 2). The results showed that SNPs not association with twin trait in Ongole Grade cattle.

**Table 2.** Polymorphism SNP pattern between Ongole Grade and SIMPO (birth twin and single) based on locus in BTA10

Cattle	bp				
	200	235	363	402	410
Ongole Grade Dam (birth twin calf)	G	A	G	G	G
Twin calf	G	A	G	A	G
Twin calf	G	A	G	G	A
Ongole Grade Dam (birth twin calf)	G	G	G	G	G
Twin calf	G	G	G	G	A
Twin calf	C	G	G	G	A
Ongole Grade Dam (birth single calf)	G	G	A	G	A
Single calf	G	G	A	G	A
Ongole Grade Dam (birth single calf)	G	G	G	A	G
Single calf	G	G	G	A	G
SIMPO Dam (birth twin calf)	C	A	A	A	A
Twin calf	C	A	G	A	G
Twin calf	C	A	A	G	A
SIMPO Dam (birth twin calf)	G	G	G	G	G
Twin calf	G	G	G	G	G
Twin calf	G	G	G	G	G
SIMPO Dam (birth twin calf)	G	G	G	G	G
Twin calf	G	G	G	G	G
Twin calf	G	G	G	G	G
SIMPO Dam (birth single calf)	C	G	A	G	A
Single calf	G	G	A	G	A
SIMPO Dam (birth single calf)	G	A	A	A	A
Single calf	G	G	G	G	G

A SNP was significantly association with twin trait, find on locus Hapmap22923-BTA-129564 (Moioli et al. 2017). SNP T/G located in chromosom 24 exactly in 40.020.911 bp with p-value 6.47 e-06. SNP T/G clamp sequence have been identified (Figure 1), and based for sequencing STS primer design with amplicon product 468 bp. The result of primer design shown in Table 1.

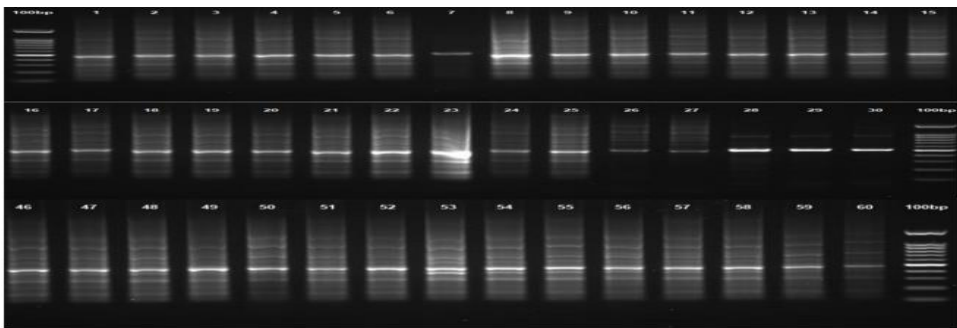
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AACTTTTATATTTTCATTTGTCATGTGACCATAATAACAATAAAAGGAG
TGGCTGAAAGGTCAGTGGTTTACATTTTCAATCAAACCGAGAGTCTA
AGATGGATAGTCACTGAATCCCCAAATGTTTCATATTTTTGTGTAATTT
CTTTCCTGTTTTGAGTCTGTCACTAACTGAATCCAAATCACTCACCTTT
TATTTCTTGGTGTCTAACACACATCAGGCAGTCACTGGGTTTTGGAAA
AATGCAGTGAAGTGAAGCAAGCCCTCCCACCAAGTTTTCTCCTTAAAA
ATAACCTACATCCAGCAGGTCGCACACTGGAAAAACAGTCATAGCA
ACTGACATTACAAAGTCCACTTCACCCCAGCCTTCCTCCATCTATGAA
CATTCACCCAGCTTCTCAT[T/G]CGAATATTCAGGGAAGTAATCTTAC
CCTCCCATCCCTAAGCAATTCTGTAAATTCTGCCCCGGGAAAGCACCC
TAGAAGTCCCTCCATCTCTGTCCATCTCCACACCCACCTCCCATCACC
TGGACCCCGTGATGGCATCCTATGGGGTCTACCACCTGCACAGTATT
CTAGAGACAGCAGCCCACAGGGGCCTTACCCATCACAAAGCAGATC
CTGTACGCCTGCTCACACTCTGCAAAGCCTGCTTGTGGCAAGAAAA
AGAGAATGCATGACCCTCAGTGTGGCCTTCCTGGGCTTCTGGCTCCCA
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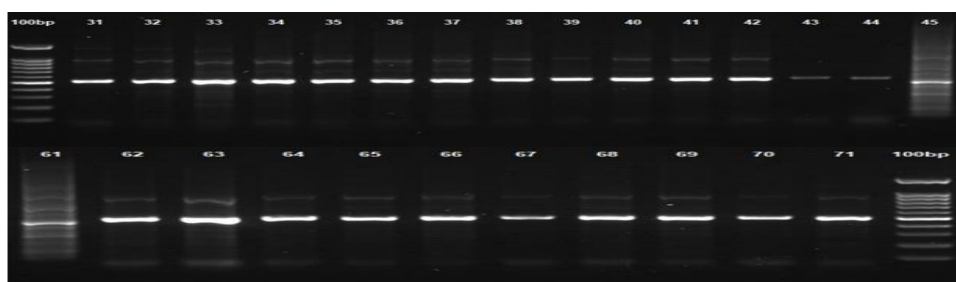
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**Figure 1.** SNP T/G clamp sequence in locus Hapmap22923-BTA-129564 which is the basis primer design for sequencing

SIMPO cattle DNA samples were sequenced using 2 primers, namely BvtWina and BTA 410 A/G. The PCR products of the two primers with a target size of about 500 bp are shown in Figure 2 (BvtWina) and Figure 3 (BTA410 A/G.). Association analysis showed that BTA10 A/G did not show a significant association with the character for twinning birth in SIMPO cattle.



**Figure 2.** Electrophoresis result of twin SIMPO Cross PO birth twin/twin which were amplified using Bvt Wina primer on 1% gel agarose



**Figure 3.** Electrophoresis result of twin SIMPO Cross PO birth twin/twin which were amplified using BTA 410-A/G primer on 1% gel agarose

At locus Hapmap22923-BTA, which showed that significant association at 75 bp consensus sequence position, namely A/T (Table 3). For the position of 40.020.911 bp with SNP T/G didn't show association as reported by Moioli et al. (2017). Considering genes that control of twin trait are complex and related to other characters such as growth, exploration of loci/genes, so needs to be done more comprehensively. Crosses cattle that carrier gene for twin trait and giving birth twins are needed for tracing genes that control twin traits.

**Table 3.** SNP polymorphism pattern between Ongole Grade and SIMPO birth twin/twins and single based on locus Hapmap22923-BTA-129564

Sapi Induk dan anaknya	Selection SNP (bp)		
	75	216	338
Ongole Grade dam (single birth)	T	T	T
Ongole Grade calf (single)	T	T	T
Ongole Grade dam (twin birth)	T	G	T
Ongole Grade calf (twin)	T	G	T
SIMPO dam (single birth)	A	T	T
SIMPO calf (single)	T	T	T
	A	T	G
	A	T	G
SIMPO dam (twin birth)	T	T	T
	A	T	T
	T	G	T
	A	G	T
	T	T	G
SIMPO calf (twin birth)	T	T	T
	T	G	T
	G	G	T
	T	T	G
	T	G	G
	T	G	G

## CONCLUSION

SNP in BTA 10 has not been shown to be associated with twinning trait in Ongole Grade or SIMPO cattle, but the SNP A/T at 75 bp in *consensus* region at the clamp sequence of the Hapmap22923-BTA-129564 locus showed a significant association with the twin trait of SIMPO cattle.

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## AUTHORS CONTRIBUTION

Sulistiyoungtiyas I and Aryogi as the main contributors, Peni Wahyu Prihandini as the member contributor

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## **Germline Chimera Production: Inspection Donor Primordial Germ Cells Transferred to Recipient Embryos**

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### **ABSTRACT**

Produce germ line chimeras in chickens has been possible by transferring donor Primordial Germ Cells (PGCs) to recipient embryos. The success of germ line chimera formation is one of the factors determining the presence and development of donor PGCs in recipient embryos. The aim of this study was to see be of donor PGCs in recipient embryos through staining and examination of embryo development. One hundred and fifty fertile eggs (100 KUB eggs and 50 WL eggs) incubated is in a portable incubator at 38°C temperature and 60% humidity. PGC-circulating collection of KUB chickens from 2.5-day old embryos (stages 15-16) purified by the ACK lysis buffer method. Staining performed is by visualizing donor cells using the PKH-26 Red Fluorescence Cell Linker Mini Kit. The PGCs are then transferred to the recipient embryo. The presence of PGCs and develop recipient embryos analyzed is descriptive. The results showed that through staining and examination, the presence of circulating PGCs into the recipient gonads was detected. It concluded is that germ line chimeras obtained, which indicated by the presence of PGCs in the recipient embryo.

**Keywords:** PGCs-circulation, Transfer, Stain, Germline chimera

### **INTRODUCTION**

Research on a transgenic animal is closely related to research on agriculture, biotechnology, medicine, and pharmaceutical. One type of livestock that is widely used in transgenic animal research is poultry. In poultry, chicken has been widely used and proved to be useful for transgenic research. Produce transgenic chicken has unlimited value in the field of animal biotechnology, which contributes greatly to produce bioactive ingredients for pharmaceuticals and food development (Han et al. 2002).

Make germ line chimera chicken is an advance develop transgenic animal research. A germ line chimera in biological terms is an organism that has genetically distinct tissues and lives together as a result of a graft, mutation, or other processes. In its development, the manufacture of germ line chimeras in poultry can use PGCs-circulation and PGCs-gonads as donors to be

transferred to other embryos as recipients. Using the migratory properties of PGCs, germ line chimeras have been produced in local chickens by transferring fresh or frozen PGCs (Tajima et al. 2003) or by transferring gonadal PGCs (Park et al. 2003) into the recipient embryo blood vessels (Nakajima et al. 2011). Primordial germ cells can be used as a genetic source and to produce transgenic chickens (Furuta 2012). Based on this, it can be said that until now, the transfer of PGCs donor to recipient embryo is the best alternative method to produce transgenic animals in poultry. This is under judge Han et al. (2002) that the use of PGCs in transgenic studies for the effective production of germ line chimeras is highly recommended.

Several research results have been reported, namely, make germ line chimeras from PGCs-circulation by Yashuda et al. (1992) and make germ line chimeras from gonads have been successfully carried out by Chang et al. (1997). A technique for producing germ line chimeras by transferring PGCs has been well-developed by Yashuda et al. (1992). In Indonesia, for the first time, make germ line chimeras has been successfully carried out in local Indonesian chickens, namely Gaok chickens, where Kostaman et al. (2014) have successfully transferred PGCs from Gaok chickens to recipient embryos of White Leghorn (WL) chickens. Research on the number of PGCs in KUB chickens has also been carried out with the results that the number of PGCs-circulation are 53 cells per embryo at the 15 HH stage (Sopiyana et al. 2016), while the PGCs-gonads are 143.5 cells per embryo at 7 days of embryonic age (Sopiyana et al. 2017). Furthermore, make germ line chimeras in KUB chickens has been reported by Sopiyana & Kostaman (2020), namely by transferring PGCs-gonads of KUB chicken donors to WL chicken recipient embryos.

Exist donor PGCs in the recipient embryos is one of the determinants of the success of germ line chimera formation, so it is important to study whether donor PGCs can induce the recipient embryos after transfer. This study attempts to decide to exist donor PGCs in recipient embryos so that it can be ensured that the germ line chimera formation process occurs perfectly.

## **MATERIALS AND METHODS**

The study was conducted at Genetics and Poultry Germ Plasm Laboratory, Indonesian Research Institute for Animal Production, Bogor.

### **Provision of donor and recipient embryo**

A total of about 100 fertile eggs of KUB chickens to providing donor embryos and 50 fertile eggs of WL chickens as recipient embryos are incubated at 38°C with 60% humidity and rotated 90° every 30 minutes using a portable

incubator (P-008B Biotype; Showa Furanki, Saitama, Japan). Fertile eggs of KUB chickens are incubated until they reached stages 15-16 (Hamburger & Hamilton 1951) and fertile eggs of WL chickens are incubated until they reached stages 14-16 (Hamburger & Hamilton 1951).

### **Isolation and collection of PGCs-circulation donor**

The isolation and collection stages of PGCs started from the eggshells that were broken from fertile eggs of KUB chickens that had reached embryonic development stages 15-16, and the contents of the eggs are transferred to a petri dish (90 x 15 mm). The donor embryo blood collection is collected from the dorsal aorta using a micropipette (50  $\mu$ m) under a microscope (Olympus SZX7). The collected blood is placed into a 1.5 ml eppendorf tube which had previously been filled with 100  $\mu$ l of PBS solution (-) then mixed with fetal bovine serum (FBS) in a ratio of 90%:10%. Subsequently, PGCs are purified with ACK lysis buffer (Yamamoto et al. 2007).

### **Transfer of primordial germ cells to recipient embryo**

Donor PGCs transfer method, in which the recipient embryo was transferred with a fresh circulating PGCs-donor. The criteria for the recipient embryo to be used are the embryo must be normal, the blood vessels are clearly visible, the yolk was large and not broken, and the eyes, heart, liver, ears, and amnion are present (Kostaman et al. 2014).

1. In the blunt part of the eggshell, a small hole is made with the aim of expelling air, so that it will be easier for the recipient embryo to be manipulated. Then the eggs are turned over.
2. A hole (diameter 1.5 to 2 cm) was then made in the pointy part of the shell so that the embryo was clearly visible in order to facilitate manipulation of the embryo because the pointy part of the eggshell is the place where the position of the embryo falls.
3. Then, under a microscope, all blood from the recipient embryo was taken through the dorsal aorta employing a micropipette. Then through an equivalent point, the prepared donor chicken PGC was injected into the recipient embryo.
4. The opening from the recipient's egg was covered with parafilm and glued using egg albumin.
5. Recipient embryo eggs were then incubated at 38°C with 60% humidity and rotated 90° every half-hour employing a portable incubator (P-008B Biotype; Showa Furanki, Saitama, Japan) for 21 days.

6. Especially for the control treatment, all blood from the recipient embryo was taken through the dorsal aorta employing a micropipette, then the embryo's blood was injected through an equivalent point.

### **Observation of recipient embryo development**

Kostaman et al. (2014) stated that the criteria used to determine donor embryos transferred to recipient embryos were said to be alive and developing normally under a microscope are as follows:

1. Embryo was 4 to 7 days old, by looking at its heart rate, and the development of organs such as the eyes, the beak already looks like a dark spot at the base of the eye, the brain, neck, and comb have started to form.
2. Embryo was 10 to 14 days old, by looking at the heartbeat, the beak begins to hard and the embryo's hair follicles begin to form, the back has been seen curled or curved. While the fur almost covers his entire body.
3. Embryos was 17 to 20 days old, by looking at the heart rate, the embryo is clearly visible like a chicken, will prepare to hatch. Toes, wings and feathers are well developed. This chicken embryo occupies almost the entire cavity in the egg.

### **Staining and examination of donor PGCs transferred to recipient embryos**

To determine the presence of donor PGCs in recipient embryos, the donor cells were visualized by staining using the PKH-26 Red Fluorescence Cell Linker Mini Kit (Sigma-Aldrich Inc., St. Louis, MO). PGCs staining refers to the method of Park et al. (2007) with the following stages:

1. The collected PGC cell suspension was stored in a 1.5 ml Eppendorf tube, then centrifuged at a speed of 1.070 rpm for 5 minutes.
2. The supernatant was discarded (reserving 30 µl with the pellet).
3. Wash the pellets by adding 1.000 µl of PBS (-) and centrifugation at 1.070 rpm for 5 minutes (first washing).
4. The supernatant was discarded (reserve about 30 µl with the pellet), then add 1 ml of diluent C (solution a).
5. Prepare a new 15 ml tube, add 1 ml of diluent C and 2.4 µl of PKH-26 dye (solution b).
6. Add solution a (diluent C + PGCs cells) into solution b (diluent C + PKH-26).
7. Incubate at 37°C for 5 minutes in an incubator.
8. Centrifugation at a speed of 1.070 rpm for 5 minutes.
9. Wash the pellets by adding 1 000 µl PBS (-) and centrifugation at 1.070 rpm for 5 minutes (second washing).

10. The labeled primordial germ cells are injected into the recipient embryo.
11. After the 6th day of incubation, the recipient embryos were taken for gonads, the injected PGCs were seen to detect PGC migration using a fluorescence microscope.

## RESULTS AND DISCUSSION

### Development of recipient embryos from transfer donor PGCs-circulation

Embryo development was observed at the incubation age of 4 to 21 days. The results showed that the observed embryo development showed a very good development because, until the incubation age of 4 days, the injected PGCs cells were still able to survive in the recipient embryo as many as 30 embryos (Table 1). This shows that the embryos for all treatments and controls have passed the first critical period (Sukra 2000).

On the 7th day of incubation, 10 eggs were taken to check to identify PGCs migration in the recipient embryo. Furthermore, developing embryos at the age of 7 days of incubation showed develop live and developing recipient embryos of 16 embryos, and 4 embryos died. The control treatment experienced the death of 2 embryos so that the embryos that were still alive and survived were 8 embryos. Mulyantini (2014) stated that embryonic death during the first week of incubation was thought to be due to physiological effects and temperature stress.

After 10 days of incubation, observation of the development of embryos treated with PGCs cell transfer and control showed that the recipient embryos were able to live and develop in 11 and 7 embryos, respectively. Sukra (2000) stated that the 10th day of the incubation period is the second critical period for embryo death because it has something to do with the disruption of the withdrawal of the yolk into the abdominal cavity. On the 10<sup>th</sup> day, the embryo's food comes mostly from albumen and a small part from the egg yolk, so if there is a disruption in the withdrawal of the yolk, the embryo will lack food because the embryo's nutrition is not enough just to rely on albumen.

**Table 1.** Development of WL recipient embryos from PGCs-circulation transfer of KUB chicken donors

Injection	Amount of sample (N)	Embryo development (days)						Hatch
		4	7	10	14	17	20	
		Amount						
PGCs	30	30	16	11	5	3	3	3
Control	10	10	8	7	7	6	5	5

On the 14<sup>th</sup> day, embryonic death occurred in the PGCs treatment, in which only 5 embryos survived, and the survival control was still good, namely 7 embryos. This period is a critical period for treatment, egg yolk is the main food for the embryo, and a small part comes from albumin. If there is a continuous disturbance in the process of withdrawing the yolk sac into the abdominal cavity, the embryo will die due to lack of food (Sukra 2000). Egg yolks contain protein in the form of LDL, HDL, phosphovitin, livetin, and other proteins. LDL is the major protein in egg yolk, which is 65% of the total protein present. The fats in egg yolks are triglycerides, phospholipids, sterols, and cerebrosides (Yuwanta 2010).

On the 17<sup>th</sup> day of development, the embryos treated with PGCs died and 3 embryos survived. Controls still survived at 60%. Mulyantini (2014) suggests that the last three days of the incubation period are the highest percentage of embryonic death, this has something to do with the time and place of the embryo, where the beak has not rotated into the air cavity so that the embryo will lack oxygen. Also mentioned Feng et al. (2006) that incubator embryo development in the late phase is more susceptible to changes in the external environment. This has to do with physiological processes during development. Furthermore, it was also stated that the critical period of the 20<sup>th</sup> day also occurs because the embryo undergoes a very rapid change to become a chick. Some organs of the body begin to grow perfectly, so they are quite sensitive to changes in outside air temperature. Vitellina begins to enter the embryonic cavity, the umbilical cord closes. There is a circular movement towards the air cavity because the embryo begins to breathe using oxygen. The hatching period experiences a critical period at the beginning of the incubation period when the circulatory system develops, while at the end of the incubation period there is a physiological change from the allantoic respiratory system to respiratory bubbles (air).

Overall, PGCs-circulating donors showed that the embryos that hatched after 21 days of incubation were 3 embryos and 5 control embryos. In the control treatment, the survival rate was 50%. This is because of the effect of treat embryo blood sampling and re-injection even though there is no PGCs donor transfer. In accordance with the judge, Chang et al. (1997) that the decrease in hatching success rate in manipulated embryos indicated that the injection treatment had an effect on embryonic development.

### **Staining and examining for presence of PGCs in recipient embryos**

Primordial Germ Cells staining is intended to detect migration and exist PGCs-circulation into the gonads of recipient embryos. Primordial Germ Cells

are usually stained more densely than the peripheral somatic cells (Yon & Akbulut 2015). This is under judge Park et al. (2003) that staining was carried out to check PGCs migration after transfer. Staining using PKH-26 Red Fluorescence Cell Linker Mini Kit. This staining was performed after the PGC donor was isolated, and after that, it was transferred to the recipient embryo. The examination was carried out on day 7 of the recipient embryo incubation period. After the stained donor PGCs have been transferred, the PGC donor population can be detected in the recipient gonads by fluorescence microscopy, as shown in Table 2 and Figure 1.

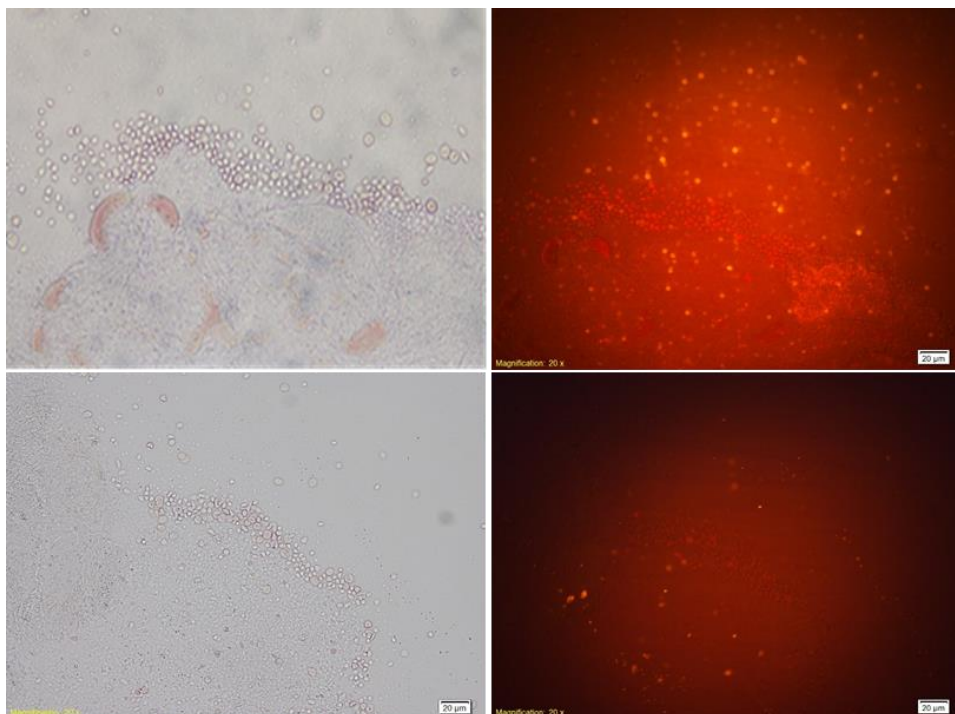
Based on Table 2, it can be seen that exist donor PGCs that have been labeled with PKH-26 dye and transferred to the recipient embryo's gonads have been detected. When the fluorescence signal was monitored at day 7 of embryonic age, 8 from 10 (80%) PGCs had a positive signal in their gonads. This means that there has been a migrated PGC-circulating donor into the gonads of the recipient embryo.

The large number of donor cells residing in the recipient gonad indicates that the transferred PGCs have high biological activity. The ability of PGC cells to migrate into the gonads and after differentiating into gametes has been reported (Park et al. 2003). However, the quantitative relationship between migratory ability and germ cell differentiation has not been evaluated further. The results showed that the ability of PGC cells to migrate to the gonads and their later differentiation would become intact gamete cells. It can be concluded that the migratory ability of PGCs is required to produce germ line chimeras.

**Table 2.** PGCs migration and presence in recipient embryos

Sample	Identification of PGCs migration	
	Bright microscope	Fluorescence microscope
1	V	V
2	V	V
3	V	V
4	V	X
5	V	V
6	V	V
7	V	V
8	V	X
9	V	V
10	V	V

*V: positive, X: negative*



**Figure 1.** Migration of donor PGCs chickens into the gonads of recipient chicken embryos after being injected at the 15-16 stage HH (2.5 days after incubation). Fluorescence staining with PKH-26 detects migration and localization of transplanted cells into embryonic gonads. Gonads were collected from recipient embryos at 7 days of incubation

## CONCLUSION

This study concluded that germline chimeras can be generated by transferring donor PGCs to recipient embryos. The presence of donor PGCs in recipient embryos through PGCs staining is expected to identify the success of germline chimera formation.

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## AOUTHOR CONTRIBUTIONS

All authors were contributed equally to this work.

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## **The Effect of GnRH to Litter Size, Colostrum and Milk Production in Nulliparous Sapera Goat Synchronized by PGF2 $\alpha$**

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### **ABSTRACT**

Estrous synchronization could become as an alternative to increase the efficiency of reproduction such as PGF2 $\alpha$  can be combined with GnRH to optimize reproduction. The objective of this study was to evaluate the effect of GnRH hormone to litter size, colostrum and milk production of Sapera goat synchronized by PGF2 $\alpha$  hormone. The study used 14 nulliparous Sapera does at the age of one yr. old after reaching sexual maturity. Estrous synchronization was treated by two intramuscular injections using PGF2 $\alpha$  (Lutalyse®) at a dose of 1 mL. The treatments were conducted for two different hormone regimens. Group one was injected twice with PGF2 $\alpha$  within an 11-d interval (n=6 heads). While group two was injected with GnRH (Ningbo Sansheng Pharmaceutical Co. Ltd) at a dose 0.25 ml on the 9<sup>th</sup> d. after the 1st injection of PGF2 $\alpha$  and before the 2<sup>nd</sup> injection of this hormone within 11 d. interval (n=8 heads). Then all goats were mated by natural mating. The research design was by Completely Randomized Design, in which the variables were observed for litter size, colostrum and milk production. The results showed that the does treated with GnRH compared to untreated GnRH resulted in the averages of litter size by  $1.33 \pm 0.51$  vs  $1.25 \pm 0.46$ , colostrum production by  $350.55 \pm 227.06$  ml vs  $528.33 \pm 209.20$  mL, and milk production by  $624.90 \pm 195.43$  ml vs.  $654.61 \pm 166.27$  ml, respectively. The conclusion was that there was no significant difference of both of the does treated with GnRH and without the addition of GnRH on litter size, colostrum, and milk production.

**Keywords:** Estrous synchronization, GnRH, Nulliparous Sapera goat, Litter size, Milk production

### **INTRODUCTION**

Dairy goat agribusiness has currently shown a good development, so that the dairy goat farmings, especially in Java Island, have been increased. Dairy goats produce milk which serves as a source of animal protein and also often is functioned as a therapy for several diseases. To support this intensive dairy goat development, the availability of breeding stocks for possessing high genetic potency of milk production and adaptive tropical climate is required

(Anggraeni et al. 2020). This is one of the other ways, attempted by crossing local female goats to bucks dairy goats from exotic breeds.

Crossbreeding of the local PE or Peranakan Etawah females to Saanen bucks was done by the Indonesian Research Institute for Animal Production (IRIAP). This cross mating resulted in a 'Sapera' goat with genetic composition of 50% PE, 50% Saanen. This Sapera goat was formed in order to use complementary effect of high milk production from the Saanen breed and a good tropical adaptation from the PE breed (Anggraeni et al. 2020). The mating program of Sapera goats was carried out naturally by the ratio of bucks to female (heads) within 1: 7-15. Before natural mating was carried out, female goats were synchronized to come into the same estrous period and to uniform the timing of kidding births for implementation of milk selection easier.

Estrous Synchronization controls the estrous cycle to make the estrous period coming simultaneously. Estrous synchronization could become an alternative to solve reproductive problems. One of the reproductive problems is hormonal balance disorders. This is generally related to the secretion of the gonadotropin hormone which plays a role in triggering estrous symptoms. This study used estrous synchronization in order to examine the signs of estrous signs clearly, so that does can be mated at the same time.

The treatment of estrous synchronization is usually by using several ways, such as prostaglandin (PGF2 $\alpha$ ), controlled internal drug release (CIDR), Sponge Progesteron (P4) and etc. For the treatment using the synchronization hormone only such as PGF2 $\alpha$  usually will be more effective if it is combined with other hormones to optimize the estrous signs, so that we use GnRH in this study. The previous study reported that the addition of 100  $\mu$ g GnRH at the time of estrous synchronization has been demonstrated to increase the pregnancy rate (Helguera et al. 2018). (Hafid et al. 2021) also reported that a combination of PGF2 $\alpha$  and GnRH caused more effectiveness to response, duration and onset of estrous in dairy does. Furthermore, the estrous synchronization using CIDR, GnRH and eCG were effective to promote follicular growth, estrous response, ovulation rate and pregnancy rate in anestrus does (Hameed et al. 2020).

The role of GnRH can increase follicular dynamics development. Follicular dynamics play an essential role in the maturation of follicles. So that in this study, it's hoped that the addition of GnRH at the time of estrous synchronization can improve the pregnancy and possibly increase the litter size on does. The objective of this study was to evaluate the effect of injecting GnRH hormone to litter size, colostrum and milk production of nulliparous sapera does synchronized by PGF2 $\alpha$  hormone.

## **MATERIALS AND METHODS**

### **Animals**

This research was conducted at a dairy goat station of IRIAP (Indonesian Research Institute for Animal Production), Ciawi, Bogor, West Java. Animals used in this study consisted of 14 nulliparous Sapera does at the age of one-year-old after reaching sexual maturity, with an average body weight of  $27.30 \pm 1.64$  kg. These nulliparous does were divided into two groups, namely the first group was the does synchronized with PGF2 $\alpha$  while the second group was the does synchronized with PGF2 $\alpha$  and combined with GnRH.

### **Estrous Synchronization Treatments**

Estrous synchronization treatments were done by intramuscular injection for two different regimen groups. The first group (n = 8 heads) as a control was injected twice of PGF2 $\alpha$  within 11 days interval. While the second group (n=6 heads) was injected by GnRH on the 9<sup>th</sup> day after the 1st injection of PGF2 $\alpha$  and before the 2nd injection of this hormone within 11 days intervals. The dosage of PGF2 $\alpha$  used was 1 ml (Lutalyse®), while GnRH (Ningbo Sansheng Pharmaceutical Co. Ltd) at a dose 0.25 ml. After these treatments, all goats were mated naturally.

Sapera does were kept under optimal management and fed based on physiological status. Forage sources consisted of king grass and other forages such as calliandra legume that was feed around 10% of body weight. A doe was feed fresh grasses at an average of 3-4 kg/head/d. The concentrate was given with protein content of 16-18% and TDN of 70-80%. Concentrate was feed around 0.8-1 kg/head/d. The animal was given additional feed in the form of tofu waste about 0.5 kg/head/d.

The estrous observation was evaluated every three hours for four days after the 2nd PGF2 $\alpha$  injection. Estrous characteristics were based on the behaviour and vulva condition of animals.

### **Variable**

Variables observed in this study were litter size, colostrum production and milk production. Litter size was observed by the number of total kids born, colostrum was collected twice in every day for the first three days after kidding, while milk production was collected twice every day for the first three months of lactation of each does. Further data of birth weight and weaning weight of the kids as well as mating weight and postpartum weight of does were weighed.

## Statistical analysis

The research design used was a completely randomized design. Litter size, colostrum production and milk production was analyzed using one-way analysis of variance (ANOVA). The model used was as the follows:  $Y_{ij} = \mu + T_i + \varepsilon_{ij}$ . Where:  $Y_{ij}$  was an observation,  $\mu$  was a population mean,  $T_i$  was the effect of GnRH administration, and  $\varepsilon_{ij}$  was a residual error. Mean differences were tested by the Tukey test and considered statistically significant at  $P < 0.05$ , and very significant at  $P < 0.01$ .

$Y_{ij}$  was an observation,  $\mu$  was a population mean,  $T_i$  was the effect of GnRH administration, and  $\varepsilon_{ij}$  was a residual error. Mean differences were tested by Tukey test and considered a statistically significant at  $P < 0.05$ , and a very significant at  $P < 0.01$ .

## RESULTS AND DISCUSSION

Research results from the two groups of nulliparous Sapera does provided the first group synchronized with PGF2 $\alpha$  and the second one synchronized with PGF2 $\alpha$  and GnRH for the effects on litter size, colostrum production, and milk production is presented in Table 1. Comparing the two synchronized treatments resulted that there was a slightly higher litter size (52%) in the does added with GnRH, whereas the does without GnRH resulted in more colostrum (60%) and milk production (51%). However, the differences in litter size, colostrum production, and milk production between the two groups of those nulliparous Sapera does were not statistically significant ( $P > 0.05$ ).

**Table 1.** The effect of GnRH and without GnRH on litter size, colostrum and milk production in Sapera Goat

Treatment	Litter Size (Average $\pm$ SD)	Colostrum (Average $\pm$ SD)	Average Milk Production (Average $\pm$ SD)
P-Value	NS	NS	NS
GnRH	1.3 $\pm$ 0.5	350.5 $\pm$ 227.0	624.9 $\pm$ 195.4
Non GnRH	1.2 $\pm$ 0.4	528.3 $\pm$ 209.2	654.6 $\pm$ 166.2

\*NS: Not Significant ( $P > 0.05$ )

GnRH is one of the hormones that plays a key role in follicular dynamics. GnRH surge center and tonic center in the hypothalamus act to stimulate the anterior pituitary to release follicle stimulating hormone (FSH) which helps to stimulate follicle development (Senger 2012). This study used nulliparous Sapera does at the age of one-year-old after reaching sexual maturity. Nulliparous does, compared to multiparous does, experience a low probability

gain of amplitude-frequency that might result in a low stimulation of the anterior pituitary to release follicle stimulating hormone (FSH). So that the development of follicles could not produce sufficient estradiol concentration thereupon reducing follicle production. 1279.5

Furthermore, the characteristic of nulliparous goats is predominantly monovular ovulating compared to multiparous ones which are predominantly polyovulatory (Simões et al. 2008). So this could be another possible reason why the litter size did not increase by the GnRH addition. A previous study also reported that prepubertal ewe still had low fertilization ability (Hafid et al. 2017). The follicular size of the weaning sows commonly has an average of about 3.7 mm (van Leeuwen et al. 2011), whereas good fertilization in ewes is usually of which the follicles reaching the antral follicle at a diameter size around 4-6 mm (Kochhar et al. 2002). This study is in according to the research results by Segabinazzi et al (2021) that reported by the addition of GnRH in mares did not change the luteal function and pregnancy rate of recipient mares.

During the lactation process occasionally several hormones participate in this process. GnRH takes a role in the regulation of milk production. GnRH is affected by several hormones in the processes of development and lactation of the mammary glands, such as prolactin, oxytocin, estrogen, progesterone and growth hormones. They are controlled by the hypothalamus and stimulated GnRH to release the hormones. During pregnancy, the development of mammary glands is affected by progesterone and estrogen levels. After parturition, P4 and E2 levels decrease which affects prolactin on milk synthesis (Kovacs 2016). In nulliparous rats and sheep, maternal behavior is expressed after parturition or alternatively when progesterone level reduces, estradiol level increases. At the same time of oxytocin in the hypothalamic paraventricular nucleus and supraoptic nucleus are strongly upregulated (Feldman & Bakermans-Kranenburg 2017). Milk production is regulated by prolactin which inhibits central GnRH and suppresses LH and FSH levels. This causes P4 and E2 levels to decrease. Based on the explanation of these hormonal mechanisms, therefore, it is understandable why the addition of GnRH did not have a significant effect in increasing milk production.

This study also reports that GnRH addition has no significant effects on birth weight, weaning weight, mating weight and postpartum weight. The results are presented in Table 2.

The results of this study showed the goat without GnRH having quite low litter size but slightly higher on the average birth weight to the goat with GnRH, although were not statistically significant ( $P>0.05$ ). As well as the weaning weight also shows slightly higher in goats without GnRH (the litter size is lower), although were not statistically significant ( $P>0.05$ ).

**Table 2.** The birth weight, weaning weight, mating weight and postpartum in Sapera goat added with GnRH and without GnRH

Treatment	Birth weight (average $\pm$ SD)	Weaning weight (average $\pm$ SD)	Mating weight (average $\pm$ SD)	Postpartum weight (average $\pm$ SD)
P-Value	NS	NS	NS	NS
GnRH	3.0 $\pm$ 0.5	8.7 $\pm$ 0.7	27.5 $\pm$ 0.9	25.4 $\pm$ 1.5
Non GnRH	3.1 $\pm$ 0.3	9.3 $\pm$ 1.3	27.1 $\pm$ 2.1	26.7 $\pm$ 2.6

\*NS: Not Significant ( $P>0.05$ )

(Anggraeni et al. 2020) reported a significant difference by birth type on body weight only evidenced at the birth instead of the at the age (30d to 120 d olds).

Several studies reported that birth weight and weaning weight in goat and lamb would decrease when litter size was higher. Birth weight, weaning weight and daily growth rate of both age in Barbari goats from the single kid was 1.94 $\pm$ 0.08 kg, 7.16 $\pm$ 0.44 kg, and 55.56 $\pm$ 4.80 gr/d, respectively. Being higher than twins were 1.83 $\pm$ 0.06 kg, 6.71 $\pm$ 0.40 kg, and 55.45 $\pm$ 4.41 gr/d, respectively (Rajendran 2009). Some previous studies also reported that single kid had benefits against the twins for birth weight by 6.01-37.62% and weaning weight by 3.58-25.37%; while those from single kids against triplets for birth weight by 25.37-37.62% and weaning weight by 25.37-31.35% (Dudhe et al. 2015) Supakorn & Pralomkarn 2012; Mohammed et al. 2018). Both of the treatments (with GnRH and without GnRH) did not affect mating weight and postpartum weight, probably because the GnRH treatment did not have a direct effect on mating weight and postpartum weight.

## CONCLUSION

Estrous synchronization using PGF2 $\alpha$  combined with GnRH responded insignificant to the increases in litter size, colostrum, and milk production in nulliparous Sapera does.

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## Functional Properties of Spray-Dried Chicken Egg with Various Fillers

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### ABSTRACT

The purpose of this study was to evaluate the effect of different filler type on the functional properties of powdered chicken egg product. Maltodextrin, CMC (Carboxymethyl Cellulose), and gum arabic are filler materials used as treatments. The functional properties of chicken egg powder, such as solubility, foam capacity, foam stability, and emulsifying properties are observed. This study used a Completely Randomized Design method with the addition of various types of fillers. The result showed that the addition of maltodextrin (0.5%) increased solubility, foam capacity, foam stability, and emulsifying properties of egg powder. The addition of CMC (0.5%) increased the solubility, foam capacity, foam stability of egg powder, and decrease the emulsifying properties. The addition of gum arabic (0.5%) as a filler reduced solubility, increase foaming capacity, foam stability, and emulsifying properties. The addition of fillers produces egg powder products with different functional properties.

**Keywords:** Chicken egg, Spray drying, Filler, CMC

### INTRODUCTION

Egg powder is one of the processed egg products with low water content in the form of powdered dry eggs (Nastiti et al. 2014). According to (Kumaravel et al. 2011), in the form of powder, egg products' shelf life would be longer and reduced its volume, thus can save the storage space, transportation costs, expand the marketing area, and more diverse utilization. The spray drying method is used for egg powder production. Spray drying is suitable for producing egg powder because of minimum heat contact, thereby minimizing the occurrence of protein denaturation (Pramitasari et al. 2011).

The principle of egg powder production using the spray drying method is atomizing liquid ingredients into smaller (particles droplets), mixing hot air with droplets, drying processes, separating the drying air from the product

and collecting powder in the product container (Kumalla et al. 2013). Several factors that affect the drying results are sample viscosity, average sample flow, temperature inlet/outlet, and gas flow velocity (Hasibuan et al. 2017).

The drying process using the spray drying method usually requires fillers to reduce heat damage, speed up the drying process, coat the flavor components and increase the yield and total solids formed (Rizqiati et al. 2020). According to (Nikoofar et al. 2013) types of fillers that can be added in the manufacture of food products include starch-based hydrolyses such as dextrans, maltodextrin, and CMC (Carboxymethyl Cellulose) as well as fillers fiber-based such as guar gum, locust bean gum, xanthan gum, gum arabic, pectin, and carrageenan. Maltodextrin, CMC (Carboxymethyl Cellulose), and gum arabic have different characteristics so that they will produce egg powder products with different functional properties. According to (Affandy & Widjanarko 2018), which states that the use of maltodextrin as a filler aims to coat components flavor, increase the total amount of solids, increase volume, accelerate the drying process and prevent heat damage. The application of CMC (Carboxymethyl Cellulose) in the food industry is usually used as a stabilizer, thickener, emulsifier (Bekti et al. 2017). Gum Arabic is used in the food industry to improve the viscosity, texture, shape of foodstuffs, stabilizers, emulsifiers, and can maintain the flavor of foodstuffs (Jumri et al. 2015). Based on this, it is necessary to know the optimization of each type of filler added so that the resulting egg powder has the desired functional characteristics. The purpose of this research is to find out the effect of adding of filler types on the functional properties of chicken egg powder.

## **MATERIALS AND METHODS**

### **Experimental egg powder production**

The ingredients in this study were eggs, maltodextrin, Carboxymethyl Cellulose (CMC), gum arabic, distilled water, coconut oil, and apple cider vinegar. The equipment used is a spray dryer (Mini Spray Dryer B-290), mixer (Cosmos), magnetic stirrer (Cimarec), Centro finger, oven, water bath, scale plastic container, tray, spatula, spoon, measuring cup, beaker, tube centrifuge, volume pipette, analytical scale, stopwatch, polipropilene plastic bag. The experiment used a completely randomized design (CRD) with variations in the type of filler, namely control/without the addition of filler (T0), maltodextrin 0.5% (T1), CMC 0.5% (T2), and 0.5% Arabic gum. (T5). The parameters observed included solubility, foam capacity, foam stability, and emulsifying properties. Each treatment was performed in seven replications.

Egg powder was produced by using spray drying method according to Medina-Torres et al. (2017) with a modification. The eggs were washed, then were pasteurized using a temperature of 60°C for 4 minutes. Subsequently, the eggs liquid was mixed with sterile distilled water with a ratio of egg volume: distilled water volume of 1:1. The filler was subsequently added to the mixed liquid egg according to the treatment (no filler/control (T0), addition of 0.5% maltodextrin (T1), addition of 0.5% CMC (T2), and the addition of 0.5% gum Arabic (T3). The mixture was then stirred until homogeneous. Calculation of 0.5% filler based on egg weight after being separated from the shell and before adding distilled water. The egg sample was then transferred in a beaker glass then stirred using a magnetic stirrer while flowing in a spray dryer with an inlet temperature setting of 110-120° C, outlet temperature around 60-70° C, aspirator 50%, pump 15%, nozzle 2, and Q flow 50. The obtained egg powder was then vacuum packed in a polypropylene plastic bag to keep the product dry and prevent external contamination. After packaging, egg powders were stored in the freezer temperature storage for analysis.

### **Solubility measurement**

Solubility test was carried out by weighing 0.5 g of egg powder and then putting it in a test tube and adding 10 ml of distilled water. Then heated sample in a beaker glass filled with water at 60 for 30 minutes. The sample was centrifuged at 1600 rpm for 15 minutes and 5 ml of the supernatant was taken and then dried using an oven at 105 to dry, after which it was weighed until it reached a constant weight. Solubility is calculated using the formula:

$$\text{Solubility} = \frac{\text{dissolved sample (g)}}{\text{initial sample (g)}} \times 100\%$$

### **Foaming capacity measurement**

A foam capacity test was carried out with 25 g of egg powder diluted with 75 ml of distilled water. The sample was shaken with a mixer in a plastic scale container for 90 seconds at medium speed and the next 90 seconds at high speed. The volume of foam was observed from the scale on the plastic measuring container. The foaming power is calculated using the formula:

$$\text{Foaming Capacity} = \frac{V2 - V1}{V1} \times 100\%$$

Where:

V1 = initial volume

V2 = volume of foam formed after shaking

## Foam stability

A foaming stability test was carried out with 25 g of egg powder diluted with 75 ml of distilled water. The sample was shaken with a mixer in a plastic container with a scale for 90 seconds at medium speed followed by another 90 seconds at high speed. After the shaking is complete, the foam is leveled and its volume is measured. The foam was allowed to stand for one hour, and the volume of leaks formed was measured. The percentage of foam drain is calculated by the formula:

$$\text{Drained foam} = \frac{\text{drained volume}}{\text{foam volume}} \times 100\%$$

$$\text{Foam Stability} = 100\% - \text{Drained foam (\%)}$$

## Emulsifying properties

The emulsifying properties test was carried out with 9 g of egg powder and 7.5 ml of apple cider vinegar homogenized using a mixer at maximum speed for 90 seconds, then 54 ml of coconut oil was added gradually at the same time to form an emulsion. The sample was put in a tube centrifuge and stored for 48 hours. After 48 hours, the samples were centrifuged for 15 minutes at 3000 rpm, then the volume of the separate oil was measured. The emulsifying properties can be calculated using the following formula:

$$\text{Emulsion Stability} = \frac{\text{sample volume} - \text{oil phase volume}}{\text{sample volume}} \times 100\%$$

## Statistical analysis

All parameters were performed in triplicates. The data were analyzed using Analysis of Variance (ANOVA) with a confidence level of 95%, followed by Duncan's Multi Range Test (DMRT) to determine differences between treatments.

## RESULTS AND DISCUSSION

The results of functional properties characteristics (solubility, foam capacity, foam stability, and emulsifying properties) of egg powder are presented in Table 1.

**Table 1.** Characteristic test results functional properties of egg powder

Parameters	Filler			
	Control (without filler)	Maltodextrin (0.5%)	CMC	Control (without filler)
Solubility (%)	95.85±0.59 <sup>a</sup>	96.16±0.28 <sup>a</sup>	95.65±0.32 <sup>a</sup>	94.75±0.36 <sup>b</sup>
Foam capacity (%)	110.00±10.00 <sup>c</sup>	144.00±5.47 <sup>a</sup>	136.00±5.47 <sup>ab</sup>	134.00±5.47 <sup>b</sup>
Stability foam (%)	24.35±2.29 <sup>d</sup>	41.97±1.96 <sup>b</sup>	100.00±0.00 <sup>a</sup>	37.43±2.29 <sup>c</sup>
Emulsifying roperties (%)	72.66±5.21 <sup>b</sup>	82.15±2.57 <sup>a</sup>	64.22±1.02 <sup>c</sup>	78.29±2.79 <sup>a</sup>

*Values are expressed as mean ± standard deviation*

*Data with different letters in the same row are significantly different (P<0.05)*

## Solubility

According to the data shown in Table 1, it was observed that filler type has a significant effect ( $P<0.05$ ) on the solubility of chicken egg powder. The addition of filler maltodextrin showed the highest solubility of egg powder because maltodextrin has a hydroxyl group that increases water binding faster. This highest solubility of maltodextrin filler could be due to a hydroxyl group of maltodextrin structure. Hydroxyl group has capability to interact with water molecules as well as small molecular weights and simple structures to increase the material solubility (Yuliawaty & Susanto 2015). In addition, the treatment without the addition of filter (control) produced similar high solubility because of the process of spray drying used high temperatures where high temperatures increased the solubility of powder product. The high inlet temperature in the process of spray drying reduced the moisture content, thus the material has a high enough hygroscopicity and results increased dissolved capability in water (Huda 2020). The treatment of adding filler CMC (Carboxymethyl Cellulose) shows high solubility and was not significantly different with the solubility of maltodextrin filler treatment. This could be due to similar characteristic of maltodextrin and CMC, which have higher hydrophilic properties that increase the solubility of egg powder. (Hasnelly et al. 2015) reported that the hygroscopic properties of CMC caused more water to be absorbed and bound so that increased the solubility. The solubility of egg powder with the addition of gum arabic resulted the lowest solubility due to the more complex chemical structure, as reported by Firdhausi et al. (2015) who mentioned that Arabic gum consists of the Arabinogalactan Protein (AGP) fraction which is hydrophobic amino acids on the outside and carbohydrate groups on the inside.

## **Foaming capacity**

The foaming capacity of the egg powder with different filler is presented in Table 1, it shows that filler treatments have a significant effect ( $P < 0.05$ ) on the foaming capacity of egg powder product. The highest foam capacity was shown by the maltodextrin treatment. Similar results have been reported by (Bovskova & Míková 2011) who observed that the supplementation of up to 2% increased the foaming capacity of the egg powder product. Maltodextrin has hydrophilic properties which probably might increase the foam complex formation by smaller air bubbles (Bovskova & Míková 2011). In addition, maltodextrin supplementation increased the amount of yield which was directly proportional to the increase in foam formation (Winarti et al. 2013). The foaming capacity of spray dried egg product with CMC (Carboxymethyl Cellulose) and gum arabic treatment were lower than that of maltodextrin treatment, could be due to characteristic of CMC and gum arabic which may play roles more as a stabilizer not as a foaming agent. The stabilizer plays a role in capturing air, increasing the strength of the foam body so that it can reduce the formation of leaks (Varhan et al. 2019). (Basito et al. 2018) reported that the supplementation of a stabilizer could serve to increase the amount of trapped air and strengthen the structure of the foam solids that are formed. Decreased foam capacity was caused by the heating process, the egg protein structure which has hydrophilic property was denatured, so that it affects the foam produced from the egg powder. (Nicorescu et al. 2011) reported that the heating process caused denaturation of egg protein, namely ovotransferrin occurring at 61°C, lysozyme at 65°C, and ovalbumin at 77°C so that it resulted in decreased foam produced by egg powder.

## **Foam stability**

Based on the data shown in Table 3, it is known that the addition of filler in the manufacture of egg powder has a significant effect ( $P < 0.05$ ) on the stability of the foam in chicken egg powder. The addition of filler CMC shows the highest foam stability because CMC can prevent water immobilization which can cause a decrease in foam stability. This is following the opinion of (Widiantoko & Yuniarta 2014) which states that the way CMC works to maintain foam stability is to bind a large amount of water and form a gel framework to prevent water molecules from moving freely. The foam stability with the addition of filler maltodextrin was greater than the addition of filler Arabic gum and without the addition of filler (control) because maltodextrin functions as a stabilizing property. (Pycia et al. 2016) stated that the stability of the foam can be increased by the addition of maltodextrin with low DE

because the viscosity will increase which is directly proportional to the stability of the foam. DE (Dextrose Equivalent) is a unit that shows the total reducing value of starch contained. The addition of filler gum arabic showed an increase in the stability of the foam because gum arabic is a hydrocolloid used to stabilize the emulsion. (Ali & El Said 2020) who state that gum arabic shows a real effect in stabilizing the emulsion so it can be used as an emulsifier. The treatment without the addition of filler (control) showed the lowest foam stability because the foam structure formed was not strong enough so it would cause water to come out and would form a leak. This is following the opinion of (Yu et al. 2012) which states that the addition of fillers in making egg powder will increase the viscosity so that it can form a stronger foam structure and will increase the stability of the foam that is formed. The foam stability decreases due to the transformation of ovalbumin into s-ovalbumin which causes a larger foam leakage. (Oliveira et al. 2020) stated that heating treatment of eggs caused changes in the protein structure of ovalbumin to s-ovalbumin which had a positive correlation with decreasing foam stability.

### **Emulsifying properties**

Based on the data shown in Table 4, it is known that the addition of filler in the manufacture of egg powder has a significant effect ( $P < 0.05$ ) on the emulsifying properties of chicken egg powder. The emulsifying properties of egg powder treatment with the addition of maltodextrin show the best results because maltodextrin can form a film layer that can stabilize the emulsion. This is following the opinion of (Assagaf et al. 2013) which states that maltodextrin stabilizes the emulsion by forming a film layer that prevents the formation of aggregates to prevent flocculation and coalescence which causes creaming of the emulsion. The addition of gum arabic increased the viscosity thus contributing to the enhancement of the emulsifier properties formed. The viscosity of the emulsion increases with the addition of gum arabic because the droplets in the emulsion system do not move, it prevents the separation of the dispersed phase and the dispersing phase and forms stable emulsifying properties (Wang et al. 2011). The mechanism of Arabic gum in stabilizing emulsions is related to its ability to form films to prevent the coalescence of oil globules. This is following the opinion of (Mandei 2019) which states that the gum arabic emulsion system forms a film to maintain equilibrium between the attractive and repulsive forces between particles to remain balanced to prevent coalescence of oil globules. The treatment without the addition of filler (control) showed a low emulsion because in the emulsion system there was destabilization which was marked by the formation of a floc. This is following the opinion of (Wibisana 2020) which states that to produce a stable

emulsion it is necessary to add an emulator/stabilizer to prevent the joining of several droplets of oil to form large droplets that disturb the stability of the emulsion. The addition of filler CMC (Carboxymethyl Cellulose) shows the lowest emulsifying properties because CMC is a stabilizer and cannot be used as an emulsifier. (Wulandari et al. 2017) stated that the addition of CMC at a concentration below 1% in the emulsion system can cause floc formation after one day of storage while the addition of 1% CMC can stabilize the emulsion until the seventh day of storage.

## **CONCLUSION**

The supplementation of different fillers produced egg powder products with different functional properties. The use of maltodextrin increased the solubility, froth, foam stability, and emulsifying properties of egg powder. The supplementation of CMC can increase the solubility of egg powder, the foaming power, and the stability of the foam in egg powder and decrease the emulsifying properties. The use of gum arabic can reduce solubility, increase foaming and foam stability, and emulsifying properties. The type of filler used for producing spray dried egg can be adjusted and combined according to the desired functional properties so that the egg powder produced is optimal and meet the required function for food application.

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## **AUTHOR CONTRIBUTIONS**

All authors were contributed equally to this work

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## **Morphometric Characteristics of Ettawa Grade Goats in Deli Serdang Regency of North Sumatra**

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### **ABSTRACT**

The Ettawa Grade Goat (PE) is a dual-purpose goat that is generally used as a meat and milk-producing goat. The study was purposed to investigate the morphometry performance of dairy goats belonged to the farmers at District of Deli Serdang, North Sumatra. The performance of an individual can be divided into quantitative and qualitative ones to investigate the ability of an individual's productivity such as body weight, milk production and body size. The parameters measured were age, sex, physiological stage, morphometry characteristics such as height at withers, height at rump, body length, heart girth, body weight, teat characteristics and coat color pattern. The data were analyzed using the General Linear Model (GLM) procedure from SAS.10 (2002). The results showed that age significantly influenced ( $P<0.05$ ) body weight and teat characteristics, whereas sex had a significant effect ( $P<0.05$ ) on body length, height at withers, height at rump and heart girth. On the other hand, farmer, age and the physiological stage had significant influenced ( $P<0.05$ ) on body length and heart girth. The results showed that the average height at withers, height at rump, body length, heart girth were  $70.3\pm7.9$  cm;  $72.6\pm8.1$  cm;  $63.5\pm4.7$  cm and  $79.2\pm5.4$  cm, respectively. Most of the goats (74.7%) were white, followed by black (13.3%) and brown (12.0%) dominant coat colors, respectively. In conclusion, the morphometry measurement of this breed reflects the average morphometry of the ettawa grade.

**Keywords:** Dairy goat, Ettawa grade, Morphometry

### **INTRODUCTION**

In Indonesia, goat farming has a significant contribution to farmer's income either as meat and or milk, leather and organic fertilizer production. Dairy goat farming is an alternative milk production in replacing to the availability of milk from dairy cattle. Dairy goat farming is relatively easy to manage, the breeds are well adapted to the local environment, well-utilized agriculture by-products for their feeds and relatively short generation interval. There are several dairy goat breeds available in the country such as Ettawa grade, Saanen, Anglo Nubian and their crossbreds. This is a great challenge for stakeholders to take advantage especially in providing milk.

North Sumatra Province has 866.255 head of goats and is considered as is one out of ten most densely populations of goats in the country (19.096.381 head) (DGLAHS 2020). A positive trend (0.86%) was derived from the national goat population from 2018-2021. Deli Serdang district was one of the areas in North Sumatra Province where farmers raised dairy goats as one of their sources of income.

A previous study by Waheed & Khan (2011) reported that teat measurement has a strong genetic correlation with milk production in 276 head of Beetal goats in Pakistan. Another study was also reported by Mucha et al. (2014) who investigated the genetic correlation among milk yield and conformation traits in several breeds of dairy goats (using 4229 first lactation data) of British Alpine, Saanen, and Toggenburg, belong to commercial farmers in the United Kingdom. Leg and feet conformation, udder traits, teat traits were used to correlate to milk yield. The study showed that. the highest correlations were found between udder depth and udder attachment (0.78), teat angle and teat placement (0.70), and back legs and back feet (0.64). However, the majority of correlations estimated between milk yield and the udder and teat traits were negative for the first lactation.

This study was purposed to investigate the measurement of dairy goats raised by farmers in sub-district of STM Hilir, Deli Serdang district. This measurement can be utilized for policymakers in the district to further develop dairy goats as one of the livestock suitable for development and to provide animal sources of protein in the market.

## **MATERIALS AND METHODS**

### **Location of the study and animal used**

A field study was conducted on 117 heads of dairy goats belonged to farmers located at STM Hilir Sub-district of Deli Serdang District of North Sumatra Province. The research method was a survey by purposive sampling based on the most population of dairy goats in the Deli Serdang Regency. The goats were housed intensively and received elephant grass around 3 kg per head per day and concentrate consisted of tofu by-product as much as 1 kg per head per day.

The parameters observed were qualitative traits such as dominant and patches coat color (white, black and brown) and the type of patches (big patches, small patches and spotted).

The quantitative traits include morphometry measurement such as body length (measured as the distance from the point at the top behind the scapular to the base of the tail), height at withers, (measured as the distance from the

ground to the withers), height at rump or hip height (measured as the distance from the ground to the rump) and heart girth (measured by taking the circumference of the heart using a tailor's tape calibrated in cm, taken as the circumference of the body immediately behind the shoulder blades in a vertical plane, perpendicular to the long axis of the body) as well as body weight (kg) (was taken using the digital hanging electric scale and measured to the nearest 0.01 kg). Each animal was identified by breed group, sex, estimated age class based on dentition and physiological stage (classified as open, dried, lactating does, early pregnancy and late pregnancy). The teat diameter (measured on the base of the teat) and teat length (measured on the length of the front teat). The measurement was conducted using a measuring tape and a measuring stick.

## Data analysis

Data were analyzed using a general linear model of SAS.10 (2002) statistical package, with the following model and assumption. The independent variables were body weight, body length, height at withers, height at rump and heart girth.

$$Y_{ijklmn} = \mu + A_i + B_j + C_k + D_l + E_m + \varepsilon_{ijklmn} \quad (1)$$

Where:

$Y_{ijklmn}$ : the observation of body weight from  $i$ th farmer,  $j$ th breed group,  $k$ th age,  $l$ th sex,  $m$ th physiology stage

$\mu$  : general mean

$A_i$  : the effect of  $i$ th farmers, where  $i = 1, \dots, 4$

$B_j$  : the effect of  $j$ th breed group, where  $j = 1, \dots, 4$

$C_k$  : the effect of  $k$ th age, where  $k = 1, \dots, 5$

$D_l$  : the effect of  $l$ th sex, where  $l = 1, 2$

$E_m$  : the effect of  $m$ th physiology stage, where  $m = 1, \dots, 5$

$\varepsilon_{ijklmn}$  : standard error from effects of farmers, breed group, sex, age and physiology stage

A similar model was applied to other dependent variables such as teat diameter and teat height, with model and assumption as follows:

$$Y_{ijklmn} = \mu + A_i + B_j + C_k + D_l + E_m + \varepsilon_{ijklmn} \quad (2)$$

Where:

Y ijklmnop : the observation of teat diameter from ith farmer, jth breed group, kth age and lth physiology stage

$\mu$  : general mean

A i : the effect of ith farmers, where i = 1,...4

B j : the effect of jth breed group, where j=1,...4

C k : the effect of kth age, where k = 1,.....5

D l : the effect of lth physiology stage, where l = 1.....5

$\varepsilon$  ijklmno : standard error from effects of farmers, breed group, age and physiology stage

Duncan's multiple range of test was performed if the independent variables were significant.

## RESULTS AND DISCUSSION

### Body weight performance

The results showed that age significantly influenced ( $P < 0.05$ ) body weight. The body sizes in livestock will experience growth from birth to body maturity (Sutiyono et al. 2006). The average body weight of Ettawah grade goats in this study was  $32.87 \pm 11.3$  kg, which was a little lower compared to the report by Kusminanto et al. (2020), where the body weight of Ettawah grade was 40 kg and five other breeds in the range of 21.03-27.87 kg. Batubara et al. (2016) stated that for Ettawa grade goats, the male body weight is 40 kg and the female is 35 kg, respectively in accordance with SNI 7325:2008. Body weight in livestock is an important trait, which is directly related to economic value, heavier body weight will yield higher goat prices (Kusminanto et al. 2020).

**Table 1.** The performance of body weight and morphometry traits

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Body weight					
Farmer	3	8243.2154100	2747.7384700	19.64	<0.0001*
Breed group	3	11279.3167300	3759.7722400	26.87	<0.0001*
Sex	1	1.7743600	1.7743600	0.01	0.9106
Age	5	1993.17725	398.6354500	2.85	0.0190*
Fisiologis	4	596.62084	149.1552100	1.07	0.3775
Height at withers					
Farmer	3	244.1716039	81.3905346	1.31	0.2749
Breed group	3	166.8608120	55.6202707	0.90	0.4460

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sex	1	850.9222913	850.9222913	13.71	0.0003*
Age	5	618.9075362	123.7815072	1.99	0.0859
Fisiologis	4	38.9563649	9.7390912	0.16	0.9594
Height at rump					
Farmer	3	196.0899807	65.3633269	0.99	0.4001
Breed group	3	271.1072535	90.3690845	1.37	0.2561
Sex	1	737.4896845	737.4896845	11.19	0.0012*
Age	5	502.9441262	100.5888252	1.53	0.1886
Fisiologis	4	112.3965330	28.0991332	0.43	0.7894
Body length					
Farmer	3	225.6446854	75.2148951	3.42	0.0201*
Breed group	3	78.2437861	26.0812620	1.19	0.3188
Sex	1	760.5397428	760.5397428	34.60	<0.0001*
Age	5	898.3480580	179.6696116	8.17	<0.0001*
Fisiologis	4	329.9963677	82.4990919	3.75	0.0069
Heart girth					
Farmer	3	252.9811780	84.3270590	2.86	0.0408*
Breed group	3	107.5108570	35.8369520	1.21	0.3085
Sex	1	544.3950120	544.3950120	18.45	<0.0001*
Age	5	1295.3053690	259.0610740	8.78	<0.0001*
Fisiologis	4	266.8730510	66.7182630	2.26	0.0678

\* significant influence ( $P < 0.05$ )

### Morphometry characteristic of dairy goats

The result of morphometry measurement of this study is presented in Table 1, whereas sex had a significant effect ( $P < 0.05$ ) on body length, height at withers, height at rump and heart girth. On the other hand, farmer, age and physiological stage had significant influence ( $P < 0.05$ ) on body length and heart girth. The results showed that the average height at withers, height at the rump, body length, heart girth were  $70.3 \pm 7.9$  cm;  $72.6 \pm 8.1$  cm;  $63.5 \pm 4.7$  cm and  $79.2 \pm 5.4$  cm, respectively.

The average body measurement characteristics are categorized as quantitative data that can be used to describe the type of goat. The body size characteristics of goats continuously increase by age until their body stops growing (Kusminanto et al. 2020). The body sizes of Ettawa grade goat have increased according to their age. Present results agreed with the findings of

Victori et al. (2016) that the body size characteristics of goats increase to 12-24 months and relatively fixed until 36-60 months. This happens along with the increase in body size, it will also be followed by other body sizes such as body length and heart girth. Trisnawanto et al. (2012) state that the value of body measurements increases with the increasing body weight of livestock. the body sizes in livestock will experience growth from birth to body maturity (Sutiyono et al. 2006). Body length, height at withers and height at rump are body measurements that affect the quantitative trait of livestock. The value of body size will increase with the age of the animal. Hamdani (2013) in Purwanti et al. (2019) state that growth in livestock is influenced by age, the rate of growth of livestock is very fast before entering sexual maturity and will slow down after body maturity and finally stops. This is also strongly stated by Sampurna (2013) in Putri et al. (2014) which states that the growth is influenced by internal factors are genetic, species, age, sex and external factors such as feeds and the environment. Farmers and breed group did not differ with respect to body sizes, this is because the existing management practices for all farmers in that area was similar, with respect to the type and amount of feeds given.

### **Coat color characteristics**

In this observation, most of the dairy goats (74.7%) had white, followed by black (13.3%) and brown (12.0%) coat color, respectively. From these data, it can be concluded that the dominant white color was of the characteristics from the Ettawa grade (PE). That color was in agreement as already been described according to the Decree of Ministry of Agriculture Number 695/Kpts/PD.410/2/2013 about the establishment of Ettawa grade (color combination of white, black and brown) as a local breed of goats This dairy goat perhaps the only that breed was dominantly found in Indonesia that was imported to Indonesia during the colonial era around 1800 – 1900. Nowadays, several dairy goat breeds are available such as Anglo Nubian and Saanen.

The most common patches found in this study was of small patches (65.1%), followed by spotted (19.1%) and large patches (15.9%), respectively. Such patches is a characteristic of PE goats, usually having black on the head and legs which are categorized as small patches. Meanwhile, the most common colors for patches were black (57.1%), followed by brown (28.6%) and white (14.3%), respectively. Goats with dominant white coat cover had black and or brown patches, whereas goats with black dominant color had white and or brown color patches. It seemed that the color variability of dairy goats in this area was white, black and brown, respectively.

**Table 2.** Distribution of coat color and the type of patches

Dominant coat color (%)		Type of patches (%)		Color of patches (%)	
White	74.7	Large patches	15.9	White	14.3
Black	13.3	Small patches	65.1	Black	57.1
Brown	12.0	Freckel	19.1	Brown	28.6

### Teat characteristics

As a type of dairy goat, the type and size of teat are very important in order to release milk from the udder. This is in relation to research conducted by Waheed & Khan (2011) who reported that teat length has a genetic and phenotypic correlation to milk lactation yield ( $0.42 \pm 0.08$  and  $0.38 \pm 0.07$ , respectively). The study also showed that length had a genetic and phenotypic correlation ( $0.29 \pm 0.09$  and  $0.26 \pm 0.07$ , respectively) to lactation length. Therefore it is very important to keep does with moderate teat length, since it has a strong genetic correlation to milk yield. However, the teat length did not show a strong genetic and phenotypic correlation to lactation length.

**Table 3.** The performance of teat diameter and teat height

Source	DF	Type I SS	Mean square	F Value	Pr > F
Teat diameter					
Farmer	3	26.0066094	8.6688698	0.63	0.5991
Breed group	3	22.3193078	7.4397693	0.54	0.6571
Age	5	229.1473496	45.8294699	3.32	0.0084*
Physiology stage	4	61.4721213	15.3680303	1.11	0.3555
Teat Height					
Farmer	3	22.5860039	7.5286680	0.83	0.4812
Breed group	3	26.6896638	8.8965546	0.98	0.4059
Age	5	194.4432496	38.8886499	4.28	0.0015*
Physiology stage	4	16.8279153	4.2069788	0.46	0.7626

\* significant different ( $P < 0.05$ )

Age of does had a significant ( $P < 0.05$ ) influenced on the diameter and height of teat, this was because that the older the does, the maturity of teats to grow in order to release milk from the udder. The general average of teat diameter and height were  $9.0 \pm 3.7$  cm and  $8.2 \pm 3.1$  cm, respectively. The number of senduro and sapera does were very limited and can be ignored. This

information represented the actual breed of dairy goats in the field and how farmers respond to different breeds available.

**Table 4.** Morphometry performance of dairy goats in Deli Serdang District of North Sumatra

Variable	Body weight (kg)(n)	Body length (cm)(n)	Heart girth (cm) (n)	Height at rump (cm)(n)	Height at withers (cm)(n)	Teat diameter (cm)(n)	Teat height (cm) (n)
Farmer							
1	21.0(38) <sup>b</sup>	65.2(38) <sup>a</sup>	80.2(38) <sup>a</sup>	74.1(38) <sup>a</sup>	72.0(38) <sup>a</sup>	8.2 (38) <sup>a</sup>	8.3(38) <sup>a</sup>
2	40.1(21) <sup>a</sup>	63.6(21) <sup>ab</sup>	80.7(21) <sup>a</sup>	73.5(21) <sup>a</sup>	71.1(21) <sup>a</sup>	9.1 (19) <sup>a</sup>	9.1(19) <sup>a</sup>
3	40.5(21) <sup>a</sup>	61.1(21) <sup>b</sup>	79.6(21) <sup>ab</sup>	71.3(21) <sup>a</sup>	69.4(21) <sup>a</sup>	7.6 (19) <sup>a</sup>	7.7(19) <sup>a</sup>
4	36.6(37) <sup>a</sup>	63.2(37) <sup>ab</sup>	77.1(37) <sup>b</sup>	71.4(37) <sup>a</sup>	68.6(37) <sup>a</sup>	8.0(35) <sup>a</sup>	8.0(35) <sup>a</sup>
Breed							
PE	32.6(90) <sup>a</sup>	63.3(90) <sup>a</sup>	78.9(90) <sup>a</sup>	72.8(90) <sup>a</sup>	70.2(90) <sup>a</sup>	8.4(86) <sup>a</sup>	8.4(86) <sup>a</sup>
PE cross	35.9(23) <sup>a</sup>	64.7(23) <sup>a</sup>	79.6(23) <sup>a</sup>	73.2(23) <sup>a</sup>	71.6(23) <sup>a</sup>	7.9(23) <sup>a</sup>	7.9(23) <sup>a</sup>
Sapera	10.1(3) <sup>b</sup>	59.8(3) <sup>a</sup>	83.3(3) <sup>a</sup>	65.3(3) <sup>a</sup>	63.7(3) <sup>a</sup>	5(1) <sup>a</sup>	5.0 (1) <sup>a</sup>
Senduro	51.6(1) <sup>a</sup>	67.0(1) <sup>a</sup>	87.0(1) <sup>a</sup>	70.0(1) <sup>a</sup>	70.0(1) <sup>a</sup>	10(1) <sup>a</sup>	10.0 (1) <sup>a</sup>
Age							
I0	27.8(2) <sup>b</sup>	54.5(2) <sup>d</sup>	72.0(2) <sup>d</sup>	62.0(2) <sup>b</sup>	62.0(2) <sup>c</sup>	4.5(2) <sup>c</sup>	4.5 (2 ) <sup>c</sup>
I1	33.1(13) <sup>ab</sup>	58.6(13) <sup>cd</sup>	73.6(13) <sup>cd</sup>	69.3(13) <sup>ab</sup>	66.2(13) <sup>bc</sup>	5.7(13) <sup>bc</sup>	5.6 (13) <sup>bc</sup>
I2	33.0(35) <sup>ab</sup>	61.9(35) <sup>bc</sup>	77.0(35) <sup>dc</sup>	72.3(35) <sup>a</sup>	69.8(35) <sup>abc</sup>	7.8(32) <sup>ab</sup>	7.8 (32) <sup>ab</sup>
I3	28.1(33) <sup>b</sup>	64.0(33) <sup>ab</sup>	79.0(33) <sup>bc</sup>	72.2(33) <sup>a</sup>	69.8(33) <sup>abc</sup>	8.6(31) <sup>ab</sup>	8.6 (31) <sup>ab</sup>
I4	31.9(15) <sup>ab</sup>	68.5(15) <sup>a</sup>	85.0(15) <sup>a</sup>	78.1(15) <sup>a</sup>	76.3(15) <sup>a</sup>	8.7(14) <sup>ab</sup>	8.8 (14) <sup>ab</sup>
> I4	41.9(19) <sup>a</sup>	66.2(19) <sup>ab</sup>	83.4(19) <sup>ab</sup>	73.1(19) <sup>a</sup>	71.0(19) <sup>ab</sup>	10.1(19) <sup>a</sup>	10.1 (6) <sup>a</sup>
Physiology stage							
1	46.6(5) <sup>a</sup>	70.7(5) <sup>a</sup>	82.6(5) <sup>b</sup>	70.0(5) <sup>b</sup>	70.6(5) <sup>b</sup>	8.2(5) <sup>a</sup>	8.2 (5) <sup>a</sup>
2	33.4(12) <sup>b</sup>	63.9(12) <sup>b</sup>	81.6(12) <sup>bc</sup>	71.7(12) <sup>b</sup>	69.3(12) <sup>b</sup>	7.3(12) <sup>a</sup>	7.3 (12) <sup>a</sup>
3	32.9(14) <sup>b</sup>	63.1(14) <sup>b</sup>	76.4(14) <sup>cd</sup>	71.1(14) <sup>b</sup>	68.5(14) <sup>b</sup>	7.7(14) <sup>a</sup>	7.7 (14) <sup>a</sup>
4	33.9(75) <sup>b</sup>	62.5(75) <sup>b</sup>	78.6(75) <sup>bcd</sup>	72.5(75) <sup>b</sup>	70.0(75) <sup>b</sup>	8.4(75) <sup>a</sup>	8.4 (75) <sup>a</sup>
5		61.6(5) <sup>b</sup>	74.8(5) <sup>d</sup>	74.3(5) <sup>ab</sup>	70.7(5) <sup>b</sup>	9.2(5) <sup>a</sup>	9.2 (5) <sup>a</sup>
Sex							
Male	35.6(6) <sup>a</sup>	72.5(6) <sup>a</sup>	88.5(6) <sup>a</sup>	81.3(6) <sup>a</sup>	79.8(6) <sup>a</sup>	-	-
Female	32.7(111) <sup>a</sup>	63.1(111) <sup>b</sup>	78.7(111) <sup>b</sup>	72.2(111) <sup>b</sup>	69.8(111) <sup>b</sup>	-	-

*a,b,c differences between means with different superscripts within the same sub category and class within the experiment are statistically different (P<0.05). Numbers within the parenthesis indicates the number of observations.*

## CONCLUSION

This study showed that age and sex had a significant influence ( $P < 0.05$ ) on body weight and morphometry measurement. The morphology measurement of goats in this study showed that average shoulder height, wither height, body length, and heart girth were  $70.3 \pm 7.9$  cm,  $72.6 \pm 8.1$  cm,  $63.5 \pm 4.7$  cm and  $79.2 \pm 5.4$  cm, respectively. In this observation, most of the dairy goats (74.7%) had white, followed by black (13.3%) and brown (12.0%) coat color, respectively. Age, body weight and morphometry measurement were important factors to select dairy goats as potential breed stock. All authors were contributed equally to this work.

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## Breeding Soundness Examination and Sexual Dimorphism of Young Bali Cattle Bull

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### ABSTRACT

The objectives of the research were to evaluate the Breeding Soundness Examination and Sexual Dimorphism of young Bali bulls. There were 33 healthy, under normal condition and 25 heads had reddish-brown and 8 had dark chestnut brown skin color. The data collected were body weight (kg), scrotal circumference (cm), libido (score 1-3), semen volume (ml), color of semen, motility (%), concentration of sperm ( $\times 10^7/\text{ml}$ ), live spermatozoa (%) and testosterone levels (ng/ml). The data were analyzed descriptively with a T-test for statistical analysis. The results Breeding soundness examination showed that bodyweight of  $214 \pm 25.2$  (160-252) kg, Scrotal circumference was  $20.96 \pm 1.19$  (19-24) cm, libido (score 1-3) of  $1.12 \pm 1.19$  (0-3), semen volume of  $2.15 \pm 1.02$  (1-4) ml, motility of  $51.25 \pm 9.54$  (40-65)%, concentration of sperm of  $668.51 \pm 216.39$  (340-952)  $\times 10^7/\text{ml}$ , live spermatozoa of  $52.51 \pm 8.86$  (40-60)% and testosterone level of  $6.64 \pm 0.28$  (4.93-7.51) ng/ml. Results of the evaluation of sexual dimorphism of reddish yellow and dark chestnut brown skin color consist body weight, scrotal circumference, and testosterone levels were found not significantly different ( $P > 0.05$ ) those values were  $224.71 \pm 25.85$  (n=25) and  $210.8 \pm 24.69$  kg (n=8), scrotal circumference were  $20.75 \pm 1.28$  (n=25) and  $21.04 \pm 1.18$  cm (n=8),  $6.41 \pm 0.78$  (n=25) and  $6.72 \pm 0.56$  ng/ml (n=8) respectively. It can be concluded that breeding soundness examination in young Bali bulls can be used to evaluate phenotype variations. There was no effect of sexual dimorphism on body weight, scrotal circumference, and testosterone levels Bali bull age of 1,5 to 2 years.

**Keywords:** Breeding examination, Sexual dimorphism, Young bull, Bali cattle

### INTRODUCTION

Bali cattle (*Bos sondaicus*, *Bos javanicus*, *Bos/Bibos banteng*) is an indigenous species (Muhamand et al. 2009) of Indonesia and has been raised throughout the islands. It was reported that the population of these cattle has decreased due to the high demand for beef for consumption (Antara 2013). The demand for beef has been increased since the last decade, to protect the decreasing population, import beef cattle was performed as many as 600,000 heads per

year. Other efforts were to increase Bali cattle production by improving management, breeding, and feeding.

Recently local farmers are more interested to involve in fattening bull than that of breeding. Farmers could get more money by feeding bull for fattening 8 months, than that of breeding need 9 months for pregnancy, and raise calves for at least 18 months. For fattening usually, the best young bull 18 to 12 months old would be demanded and has a good price returned. When the better young bull went for fattened and from time to time they are slaughtered, it would be difficult for the farmer to get better bull for breeding.

There was reported that phenotypically, the growth and production performance of Bali cattle varied widely (Oka et al. 2013), as well as semen volume, and sperm motility, while the sperm concentration tends to fluctuate over the bull's age (Nugraha et al. 2019). These conditions would be the background need for the establishment of evaluation of breeding soundness for the breeding program (Porbandar et al. 2011). The breeding soundness examination (BSE) should be able to evaluate biological characteristics for selection purposes (Sonjata et al. 2021). The success story of the breeding examination was reported in the south of Brazil. BSE can be used to increase calf production by 31% per year (Menegassi et al. 2011) and it was proved that BSE may improve economic aspects of the beef cow-calf system. A satisfactory method for early selection and identification of the minimum standard of a fertile bull is important to be established (Lone et al. 2017). Hence, improving cattle production can be done by reducing phenotype variations, therefore selection would be the program to choose.

As an ancestor of Banteng, Bali cattle bulls also have sexual dimorphisms (Purwantara et al. 2011), young bulls have reddish-brown skin color, they change their skin color to become dark chestnut brown or black following the maturation phase. This sexual dimorphism was considered as a change due to the growth and puberty process. The increase in sexual activity was followed by the increase in plasma testosterone concentrations, libido, and sperm motility (Syarifuddin et al. 2017).

It seems Bali cattle should be selected from an early young age to provide the best bull for genetic materials preservation. The potentials selection which can be performed is BSE, which can be used to select the best bull for mating. BSE was performed to choose the best bull for breeding by evaluating body weight, scrotal circumference, sperm quality, and libido. However, farmers starting fattening their best Bali bull between 18-24 months old. It was interested to evaluate the BSE of Bali cattle at 18-24 months old by evaluating body weight, scrotal circumference, spermatozoa quality, sexual dimorphism, libido, and blood testosterone levels. It was reported that semen and testosterone evaluation for Simmental and Limousine young bull at 18-23

months old produce good quality semen even can be used as frozen-thawed semen for insemination (Srianto et al. 2021).

The objectives of this study were to evaluate early BSE and sexual dimorphism of Bali cattle at the age of 18-24 months.

## **MATERIALS AND METHODS**

### **Cattle management and sexual dimorphism**

This study used 33 healthy and anatomically normal Bali bull with age of 18 to 24 months. Estimation of age was performed by examining teeth before I 1 tooth worn off was considered before 2 years of age (Kahn & Line 2010). These young bulls were kept in individual animal house for 16 weeks and fed with mixed of rice straws, *Leucaena*, casava mill, supplemented with minerals and were also given water for drink *ad libitum*.

### **Reproductive evaluation**

Scrotal circumference was measured by fixing the bull in the crush with hind leg was tighten nicely. Then the scrotum was hold by left hand and the right hand. Measurement was done by letting tape around at the large part circumference at the middle of scrotum.

Libido is an eagerness to mating the cows, it was evaluated by using estrogen injection cows as teaser. The teaser was tightened to crush let the bull walk close to the teaser. Libido was measured by giving a score as follow. Score 0: when young bull has no response while let it walk through closed to the teaser. Score 1: when the bull stand to the teaser, without further response.

Score 2: when the bull stand to the teaser with their penis erection for a while. Score 3 when bull stand, erection and by using artificial vagina semen was ejaculated.

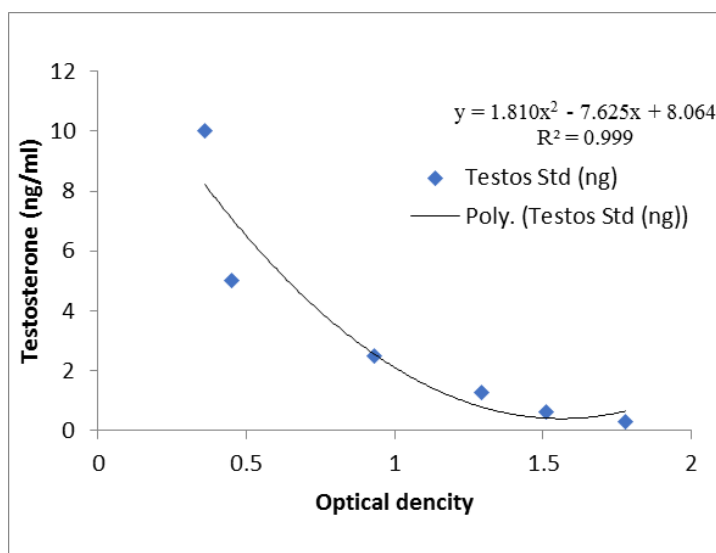
Semen collection was performed by using artificial vagina filled with 38°C water temperature with lubricated with Vaseline at the inside. When erection bull stand to the teaser, the bull penis was inserted into artificial vagina up to semen was collected.

Volume semen was measured, following collection by estimation from gitted tube. Mass spermatozoa motility was performed by put a drop of semen into object glass, then evaluated under microscope with 25× magnification. Motility of spermatozoa was scored by: score + or 1 when motility was low, score ++ or 2 when motility was moderate, and score +++ or 3 when motility was high (Yendraliza et al. 2019).

Viability and morphology were performed by placing 0,5 µl semen to the object glass, dropped and mixed with 20 µl nigrosine eosin, smeared and kept them dried and observed under microscope with magnification 400×. The viability of spermatozoa was calculated by counting 200 spermatozoa, then the spermatozoa which did not absorbed HE stain indicated live spermatozoa. Percentage of live spermatozoa was count by live spermatozoa divided by 200 sperm count which consist of live and dead spermatozoa (Susilawati 2011).

Morphology which indicates normality of spermatozoa was performed as evaluating viability (above) however abnormality spermatozoa was evaluated under 400× magnification of microscope to count abnormality of spermatozoa such as abnormality of head, mid piece and tail (Susilawati 2011).

Concentration. Spermatozoa concentration was evaluated by direct cells count method using standard hemocytometer counting blood cells (Susilawati 2011). This evaluation was performed by drawn semen into dilution pipette to the 0,05 mark, the pipet filled with hypotonic solution up to 101 marks. The hypotonic solution consists of 50 ml distillation water, 1 ml 2% eosin and 1 ml 3% sodium chloride. The counting chamber was then filled and let to settle for 5 minutes. Count spermatozoa in 5 large square in both chambers. Concentration of spermatozoa was calculating by multiplying the mean count by  $10^7$ .



**Figure 1.** Standard for calculation of testosterone levels

## **Testosterone assay**

Testosterone levels was evaluated by using blood samples and Elisa was performed in Laboratory. Blood samples were collected by using venoject through vena jugularis, then spined 25.000 g and blood plasma was used measured testosterone levels. Testosterone blood levels was measured using Bovine Testosterone Elisa kit (Wuhan Fine Biotech Co, Ltd) with sensitivity  $<0.118$  ng/ml. With procedure wash plate 2 times before adding standard, then add 50  $\mu$ l standard or samples into each well and immediately add 50  $\mu$ l Biotin labeled antibody. Followed by incubated for 45 minutes at 37°C then aspirate and washed 3 times. Add 100  $\mu$ l HRP-Streptavidin Conjugate (SABC) Working Solution into each well, incubated for 30 minutes at 37°C and aspired and wash 5 times. Add 90 $\mu$ l TMB substrate and incubate 15-20 minutes at 37°C Stop Solution. Finally read at 450 nm immediately and calculation of testosterone levels cab be performed. Precision of the assay with Intra-Assay Coefficient Variation less than 8% and Inter-Assay less than 10%, with solution and dilutions standard (Wuhan Fine Biotech Co, Ltd), can be seen in Figure 1.

## **Statistical analysis**

Data breeding soundness examination was analyzed descriptively using means and standard deviation and range minimum and maximum. Data male sexual dimorphism was analysis using t test while others by description as there was very small and unequal samples.

## **RESULTS AND DISCUSSION**

It was found that these Bali bulls had body weight of  $214 \pm 25.2$  kg with range between 160-252 kg. This range is relative wide. Similar body weight but with wider ranges of weight of young bull was found  $212 \pm 46.2$  kg have been reported by Nugraha et al. (2016) Bali bull's body weight have a positive correlation age (Nugraha et al 2019). This indicated that until 24 months old that some Bali bull have not reach their maturity (Rahma et al. 2019) ad tended to have late mature size. (Rahma et al. 2019). This indicated that there was a wide variations of body weight of young Bali bull.

The results of measurement of scrotal circumference in the present study were  $20.96 \pm 1.19$  cm, with range of 19-24 cm, this variation was also wide. Scrotal circumference of young Bali bull was reported  $32.50 \pm 2.65$  cm with wide variations (Susilawati et al 2020). Another study reported that scrotal circumference was  $28.02 \pm 2.23$  cm also with varied range 24.80-32.80 cm (Dasrul et al. 2019). It was reported that scrotal circumference correlated

positively with testosterone blood concentrations (Dasrul et al. 2019). Scrotal circumference can be used for the selection of bulls at young age (Lone et al 2017). Scrotal circumference measurement is a parameter for breeding is highly correlated with sperm output (Lone et al. 2017). There were wide variations of scrotal circumference of young Bali bull.

Libido evaluation in the present study showed that 15 bull (45.45%) was found without interest to the estrogen injected teaser, 5 bulls (15.15%) had stand to hormone treated teaser but without further response, 5 bulls (15.15%) have stand with a bit of erection, however without further response and 8 bulls (24.24%) had stand to the teaser with penis erection, and semen can be collected by artificial vagina. It was reported that libido have a positive correlation with the testosterone blood concentration (Hasbi et al. 2021). There wide variation of young Bali cattle bull in the present study.

Results of semen evaluation of 1.5 to 2 years Bali bull can be seen in Table 1. It showed that from 33 bulls, 8 (24.24%) had high libido and their spermatozoa can be collected using artificial vagina.

Semen volume measured in the present study was found  $2.15 \pm 1.02$  ml, with range 1-4 ml. Previous reseach found that higher volume of semen of young Bali bull was  $3.1 \pm 1.7$  ml (Ratnawati et al. 2020). Another report was found higher volume semen, those was  $4.70 \pm 0.61$  ml (Dasrul et al. 2019). Another researcher concluded that semen volume was correlated with spermatozoa motility (Nugraha et al. 2019).

Color of semen evaluation in the present study showed that from 8 young Bali bulls they had milky white semen. It was reported previously that color young bull semen was milky white (Ratnawati et al. 2020).

Percentage of motility of spermatozoa of 8 young Bali bull was  $51.25 \pm 9.54\%$  with range of 40-65%. This result was comparable to previous study, which found motility of  $56.7 \pm 15.6\%$  (Nugraha et al. 2016). The higher motility was reported up to  $73.33 \pm 2.88\%$  (Yendraliza et al. 2019) (%) and  $77.00 \pm 3.40\%$  with range 70.00-80.00% (Dasrul et al. 2019). The percentage of sperm motility was varied widely in Bali cattle.

Concentration of spermatozoa of 8 young Bali cattle in the present study was found  $668.51 \pm 216.39 \times 10^7/\text{ml}$ , with range of  $340-952 \times 10^7/\text{ml}$ . It was reported previously that spermatozoa concentration was lower than the results in the present study, those was  $404.8 \pm 286.6 \times 10^7/\text{ml}$  (Ratnawati et al. (2020). It was reported that numbers of sperm correlated positive to testosterone concentrations (Dasrul et al. 2019). It was reported that spermatozoa concentration fluctuated by the ages of bull (Nugraha et al. 2019).

Live spermatozoa of 8 young Bali cattle in the present study were found  $52.51 \pm 8.86\%$  with range of 40-60%. This finding was lower than previous study

which found that live spermatozoa was 71.82 to 83.66% (Nugraha et al. 2021) there were individual variation among the Bali bull (Indriastuti et al. 2020).

The average of testosterone levels of 33 young Bali bulls was  $6.64 \pm 0.28$  ng/ml with range of 4.93-7.51 ng/ml. This finding comparable to the results of Hasbi et al. (2021), who found that testosterone levels in Bali bull was  $6.94 \pm 2.43$  ng/ml. The serum testosterone in the present study was slightly lower than  $7.95 \pm 2.85$  ng/ml (Dasrul et al. 2019). The results of the present study were lower than report in Holstein bull, which was found between 6 to 12 ng/ml in blood (Kholghi et al. 2020).

Variables evaluated for breeding soundness examination in young Bali bulls were found varied between varied widely. From thirty-three (33) bulls of 1.5 to 2 years old, eight (8) bulls' semen were collected. As twenty-five (25) bulls had showed low libido semen cannot be collected. (Table 1). Breeding soundness examination can be used to evaluate and select young bull before I 1 lower teeth worn out. This would be able to fascilitate bull selection as early as possible.

**Table 1.** Breeding soundness examination of all Bali cattle 18-24 months old\*

Breeding soundness evaluation	n	Average $\pm$ SD	Min-max
Body weight (kg)	33	214 $\pm$ 25.2	160-252
Scrotal circumference (cm)	33	20.96 $\pm$ 1.19	19-24
Libido (score 1-3)	33	1.12 $\pm$ 1.19	0-3
Semen volume (ml)	8	2.15 $\pm$ 1.02	1-4
Color of semen	8	Milky white	Milky white
Motility (%)	8	51.25 $\pm$ 9.54	40-65
Concentration of sperm ( $\times 10^7$ /ml)	8	668.51 $\pm$ 216.39	340-952
Live spermatozoa (%)	8	52.51 $\pm$ 8.86	40-60
Testosterone levels (ng/ml)	33	6.64 $\pm$ 0.28	4.93-7.51

\* From 33 bulls, 8 bulls have high libido, erection and semen can be evaluated

The results evaluation of sexual dimorphism of Bali bull of 1,5 to 2 years old were reported in Table 2. From 33 bull consists of 25 heads (75.75%) had reddish yellow skin color and 8 heads (24.24%) had dark chestnut brown. Both groups were evaluated their value such as body weight, scrotal circumference, testosterone levels, using t test unequal number of samples. It was found that there was no significant different between reddish yellow and dark chestnut brown skin collar ( $P > 0.05$ ).

**Table 2.** Comparison physical, semen and testosterone of young bull with sexual dimorphism

Examination	Male sexual dimorphism of the skin collar			
	Reddish yellow	n	Dark chestnut brown	n
Before semen collection*				
Body weight (kg)	224.71±25.85	25	210.8±24.69	8
Scrotal circumference (cm)	20.75±1.28	25	21.04±1.18	8
Testosterone levels (ng/ml)	6.41±0.78	25	6.72±0.56	8
Following semen collection**				
Body weight (kg)	210.8±24.69	2	224.71±25.85	6
Scrotal circumference (cm)	19.75±0.35	2	20.58±0.73	6
Semen volume (ml)	2.85±0.49	2	2.00±1.09	6
Concentration (x 10 <sup>7</sup> )	611.5±331.63	2	685.83±203.44	6
Live spermatozoa (%)	55.00±7.07	2	51.66±9.83	6
Testosterone levels (ng/ml)	6.93±0.14	2	6.84±0.45	6

\* Non significant different ( $P>0.05$ ) \*\*With a small number of samples this data can not statistically analyzed.

Examination following semen collection from 33 young bulls 8 bull semen can be collected, those bull consist of two heads with reddish yellow and sixes head with dark chestnut brown. This comparison consists of body weight, scrotal circumference, semen volume, concentration, live spermatozoa, testosterone levels (ng/ml). This comparison cannot be evaluated statistically as sample reddish yellow skin collar only 2 heads of bull, hence they were compared descriptively however their values look the same. Hence the sexual dimorphism Bali cattle at age of 1.5 to 2 years old showed dimorphic diferent however it seems all variable evaluated similar between dimorphic was not different.

Young males have reddish brown collar of the skin, this collar turns to a dark brown following maturation. It was reported that maturation of Bali cattle was influenced by male hormone or testosterone. Hormone testosterone produced by cells interstitial or Leydig of testis are reddish-yellow, and males are reddish brown, turning to a dark brown with maturity following puberty. From Table 2. It can be seen that testosterone blood levels in young bull with reddish brown and dark chestnut brown skin collar were  $6.93 \pm 0.14$  ng/ml and  $6.84 \pm 0.45$  ng/ml respectively. As the samples was not enough for statistical analysis, it seems that the level of data presented in Table 2 similar or the same. May be at the age of 1.5 year to 2 years old male Bali cattle still developing for

maturation and there was no effect of sexual dimorphism body weight, scrotal circumference and testosterone levels.

It was reported that testosterone concentrations correlated positively with scrotal circumference, numbers of sperm, sperm of motility and sperm of abnormality. (Dasrul et al. 2019; Nugraha et al. 2019) however there was no correlation between testosterone concentrations with semen volume (Dasrul et al. 2019). It seems that in this study there no indication that testosterone initiated to induced changes colour of the skin of Bali cattle of 1.5 to 2 years old.

## **CONCLUSION**

It can be concluded that there were wide variations of productivity and reproductivity which can be identified by breeding soundness examination performed in Bali cattle as early as 18 to 12 months old. There were had no effect of reddish yellow and dark chestnut brown skin collor due to sexual dimorphism to body weight, scrotal circumference and testosterone levels of Bali bull age of 1.5 to 2 years.

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# Veterinary Science



## **Trematode and Nematode Gastrointestinal Infections in Livestock from Different Geographical Regions in Indonesia**

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### **ABSTRACT**

Helminthiasis remains a major factor for animal health and causes huge economic losses in the livestock industry of Indonesia, particularly infection of trematodes and nematodes. The study aimed to identify the level of trematode and nematode gastrointestinal infections at some farms from different geographical areas in Indonesia. A total of 376 fecal samples were examined using the floating method (Whitlock chamber) for nematodes and the sediment method (methylene blue 1%) for trematodes. The results of the examination were based on samples of livestock feces obtained from several different locations Malang (feces of cattle), Banten (feces of cattle and buffaloes), Waingapu-Sumba (feces of cattle, buffaloes, horses, pigs), and Denpasar-Bali (feces of pigs), showed that the percentage of helminth infections among examined areas were relatively similar (62.7-68.9%) with various range of trematode and nematode infections for 7.8-58.6% and 18.6-68.6%, respectively. Trematode can infect cattle, buffalo, and horses, and the highest infection is *Trematodes paramphistomum* spp. found in buffalo in Banten (45.00%) and *Trematoda fasciola* spp. detected in cattle in Malang (44.1%). In addition, Nematode can infect all types of livestock, the highest infection of nematodes was found in pigs in Denpasar (68.6%). The distribution of helminthiasis prevalence was considerably high at some farms in the examined areas in Indonesia. Helminthiasis in the gastrointestinal tract can cause digestive disorders and growth retardation. Accordingly, helminthiasis control needs to be concerned to improve livestock productivity.

**Keywords:** Fasciola, Paramphistomum, Strongyle, Floatation method, Sedimentation method

### **INTRODUCTION**

One of the government policies to cover the national demand for meat is the 2010 Beef Self-Sufficiency Program (Soedjana et al. 2007), but the results of the evaluation show that meat self-sufficiency has not been achieved. Areas that support the meat self-sufficiency program include Malang, Banten, Sumba, and Bali. The East Java Provincial Government does not accept imports from outside

and Malang Regency does not touch imported meat. The population of cattle in Malang Regency reaches 243 thousand head of cattle, and each year, buffalo cattle are also developed in Indonesia. Banten is one of the largest buffalo centers in Indonesia, contributing a population of 9.4%. The government of Sumba supports self-sufficiency in meat because Sumba is an area that has various numbers and types of livestock, namely cattle, buffalo, horses, and pigs. The fulfillment of pork in Bali is relatively safe due to self-sufficiency with a population of 890,000 heads (Ditjennak 2014). In developing countries, the growth and development of healthy livestock have not been maximally exploited due to obstacles such as malnutrition, mismanagement, and diseases (Hanafiah et al. 2019; Ibrahim et al. 2014; Adzitey 2013).

A parasitic infection (Helmintiasis) remains a major factor that disturbs animal health and causes huge economic losses in the livestock industry of Indonesia, particularly infection of both trematodes and nematodes. Recently, the identification of the worm parasite has mostly focused on the examination of eggs as a basis for genus determination, although commonly known that a lot of parasites' eggs are relatively similar. The prevalence of gastrointestinal helminths in cattle in Indonesia was about 47.7% for Nematode and 28.4%-33.0% for Trematode (Sawitri et al. 2020). Helminth can cause major losses in cattle, including weakness, loss of appetite, weight loss, slow growth, anemia, diarrhea, low productivity, and decreasing reproductive rates of livestock, all of which can lead to death. Parasite-related economic losses arise from a decrease in the cattle population as well as harm to specific animal components, such as the liver, that are parasite-infested and must be removed (Nurcahyo et al. 2021; Rodríguez-Vivas et al. 2017; Tantri et al. 2013). Helminthiasis is often neglected (Affroze et al. 2013, Khedri et al. 2015). Widjajanti (2004) stated that *Fasciola* sp. and *Paramphistomum* sp. is a species of trematode commonly found in Indonesia.

In Indonesia, studies about helminthiasis (Trematode and Nematode infections) have been widely reported (Nurcahyo et al. 2021; Sawitri et al. 2020; Ekawasti et al. 2019; Satyawardana et al. 2018). However, most of these studies focus on one region in cattle in Indonesia. There have not been many studies that discuss comparison the helminthiasis based on geographical region and host species. Because prevalences can vary depending on several parameters, such as the examined location, the hosts, and the methods used for detections, it is helpful to accumulate data across many areas to evaluate the parasitic distribution. Here, helminthiasis in cattle, buffalo, horses, and pigs from the different locations was investigated by the flotation method (Whitlock) for Nematoda and by the sedimentation method for Trematoda. The study aimed

to identify the prevalence of trematode (*Fasciola* spp. and *Paramphistomum* spp.) and gastrointestinal nematode (*Strongyle*) infections at some farms (cattle, buffaloes, horses, and pigs) from different geographical areas in Indonesia.

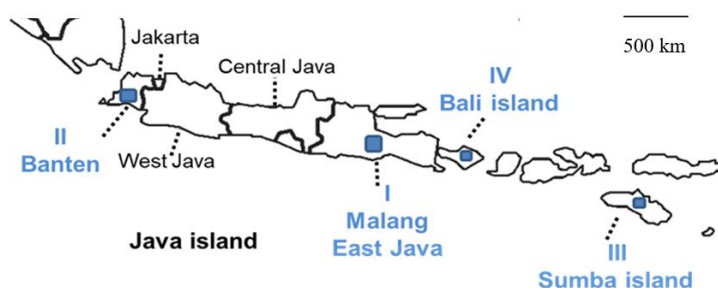
## MATERIALS AND METHODS

### Ethical approval

The study was approved by Research Ethics Committee, IACUC (Institutional Animal Care and Use Committee), Indonesia agency for agricultural research and development, agricultural of ministry (Nomor: Balitbangtan/BB Litvet/Rm\_NRm/06.01/2019)

### Location and samples collection

In the present study, a total of 376 fecal samples from several different locations Malang (feces of cattles), Banten (feces of cattles and buffaloes), Waingapu-Sumba (feces of cattles, buffaloes, horses, pigs), and Denpasar-Bali (feces of pigs), and each examined village had more than two farms. The location of these areas is shown in Fig. 1. The average annual temperature is 25°C. All samples were taken from the rectums of cattle, placed in individual plastic bags, and stored at 4°C until laboratory examination.



**Figure 1.** The locations of the examined region in Indonesia

### Fecal examination

Fecal samples were examined by flotation method using Whitlock universal chambers for detection of Nematode eggs (Gordon and Whitlock 1939) and by a sedimentation method for detection of Trematode eggs (Charlier et al. 2008). For the flotation method, 3 g of fecal samples were

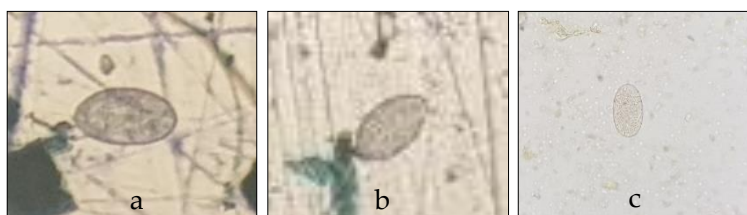
diluted in 17 ml of distilled water, and a 40 ml saturated sodium chloride solution was added. Next, 0.5 ml of the sample solution was placed onto a Whitlock universal chamber. After 5 min, we examined and counted the number of nematode eggs in the entire field under light microscopy at a magnification of  $\times 200$  or  $\times 400$ . The Whitlock method was highly sensitive for quantitative detection egg of Nematode. However, we could not identify the species of Nematode in the positive samples (Ekawasti et al. 2019).

For the sedimentation method, 3 g of fecal samples were mixed in 250 ml of water in a measuring cup and filtered through a tea sieve. Filtrates were allowed to stand for at least 10 min to allow the eggs to settle, and the supernatant then was discarded. This step was repeated twice. Finally, the collected sediments were stained with 5% methylene blue and observed by microscopy (Charlier et al. 2008).

## RESULTS AND DISCUSSION

### Identification of trematode and nematode

Based on the identification of length and width, color and shape of eggs, in sedimentation method found 2 types of trematode eggs (*Fasciola* spp. and *Paramphistomum* spp.), and in floatation method found 1 Nematode genus (Strongyle). The results of observations on the characteristics and egg shape of trematode species and nematode in feces samples in research can be seen in Figure 1.



**Figure 1.** Results of identification of trematode and Nematode eggs (100 $\times$  magnification) a) *Fasciola* spp. (b) *Paramphistomum* spp. (c) *Strongyle*

Identification of worm eggs based on morphology and color differences. *Paramphistomum* spp. eggs are brown blue while *Fasciola* spp. eggs are pink-grey yellowish (Purwaningsih et al. 2018). Size of *Paramphistomum* spp. eggs 113-175  $\mu$   $\times$  73-100  $\mu$  and is slightly translucent light yellow. *Fasciola* spp. egg has an operculum, are golden in color, and measure 150-190 $\mu$   $\times$  90-100 $\mu$ , while the nematode eggs of the strongyle type are smaller than trematode eggs, the

eggs are elliptical, thin enveloped, segmented, and thin shell, with a size of  $71\text{-}107\mu \times 41\text{-}54\mu$  (Firdayana 2016).

### Helminthiasis from different locations

Based on the identification of the genus of gastrointestinal parasites at 4 locations in Indonesia, it was found that the livestock from these locations suffered from helminthiasis. Fecal samples from 4 locations in Indonesia, from Malang (cattle), Banten (cattle and buffalo), and Sumba (cattle, buffaloes, horses) were examined for the presence of gastrointestinal parasites (Table 1).

Table 1. summarized the prevalence of helminthiasis in 4 locations in Indonesia. In a recent study, the incidence of helminthiasis was the highest in livestock caused by *Strongyle* was the highest prevalence (38.6%), *Fasciola* spp. (24.8%), *Paramphistomum* spp. (17%). Several studies reported that the prevalence of nematodiasis in livestock in Indonesia was more dominant than trematodiasis. Sawitri et al. (2020) reported that in cattle in the Tangerang area of Indonesia, nematode infection (*Strongyle* 47.7%) was higher than in trematodes (*Fasciola* spp. 33.0% and *Paramphistomum* spp. 28.4%). Prevalence of Trematode in Malang was found *Fasciola* spp. (44.1%) and *Paramphistomum* spp. (0%); in Banten was found *Fasciola* spp. (19.5%) and *Paramphistomum* spp. (39%); in Sumba was found *Fasciola* spp. (4.3%) and *Paramphistomum* spp. (3.5%); in Bali, no trematode examination was carried out on samples of pig feces. Helminthiasis in pigs was dominated by the Nematode genus (Tolistiawaty et al. 2016). Nematode infection was found in Malang (18.6%), Banten (6.8%), Sumba (56.8%), and Bali (68.6%). Thus, the study revealed that the percentage of helminthiasis among examined areas was relatively similar (62.7-68.6%) with various ranges of trematode and nematode infections for 7.8-58.6% and 18.6-68.6%, respectively. Our results for Nematode prevalence (38,6%) were found to be higher than those of previous reports in Indonesia: 23.6% in East Java by the Whitlock method (Ekawasti et al. 2019). Differences in the prevalence could be due to differences in location, management at the examined farms, or detection methods, we were nonetheless able to report the latest prevalence of Nematode. Environmental factors in different locations were known to influence the development of nematode larva on grass, including temperature, humidity, and rainfall (Das et al. 2016).

**Table 1.** Prevalence of Trematode and nematode in livestock in Indonesia based on different locations

Name of location	Sample	Total sampel of examined	Fecal examinations			Total infection
			Trematoda		Nematoda Strongyle	
			<i>Fasciola</i> spp.	<i>Paramphistomum</i> spp.		
Malang	Cattle	102	45 (44.1%)	0	19 (18.6%)	64 (62.7%)
Banten	Cattle	18	1 (5.5%)	1(5.5%)	0	
	Buffalo	100	22(22%)	45(45%)	8 (8%)	
		118	23 (19.5%)	46 (39%)	8 (6.8%)	77 (66.5%)
Sumba	Cattle	31	3(9.7%)	0	24 (77.4%)	
	Buffalo	22	2(9%)	4(18%)	1(4.5%)	
	Horse	61	0	0	32 (52.5%)	
	Pig	31	- *	-*	26 (83%)	
		146	5 (4.3%)	4 (3.5%)	83 (56.8%)	92 (64.6%)
Bali	Pig	51	-*	-*	35 (68.6%)	35 (68.6%)
Total		376	73/294 (24.8%)	50/294 (17%)	145/376 (38.6%)	

\*(unexamined)

The result of Trematoda, including *Fasciola* spp. (24.8%) and *Paramphistomum* spp. (17%) were found to be lower than those of previous reports in Indonesia: 35.59% *Paramphistomum* and 44.12% *Fasciola* in Yogyakarta by sedimentation method (Rinca et al. 2019). Helminthiasis is the influence of climate change in an area and there is a development of the host (Bennema et al. 2011). Helminthiasis can occur in areas with moist rainy areas of the tropics. *Paramphistomum* spp. and *Fasciola* spp., in particular, were found living in watery, swampy areas that are ideal for the intermediary host's development and reproduction, resulting in a high prevalence (Fromsa et al. 2011). Overall, this study performs that the percentage of helminthiasis was the highest in Bali (68.6%) and the lowest in Malang (62.7%). Probably Warm and wet climatic conditions in Bali match the development of eggs and the survival of worm larvae in nature when compared to the cold climate in Malang. The prevalence of trematodosis is higher in moist areas (warm and wet) with the availability of water, vegetation, and humidity needed by intermediate host snails and metacercariae to grow and develop (Karim et al. 2015). Climatic conditions play a very important role in the incidence and intensity of trematode infection. Climate change can affect the geographical distribution of parasitic diseases (Satyawardana et al. 2018).

The prevalence nematode of strongyle infection can be affected due to differences in temperature, humidity, and soil conditions that affect the development of infective larvae in a location. In addition, it can also be influenced by several factors, including livestock management, age of livestock, use of anthelmintics, quality of cages, sanitation, education, and economic status of farmers (Dina et al. 2021). In this study, the prevalence of strongyle was high in the Sumba area in cattle (77.4%) and pigs (83%). The area has a suitable area for strongyl nematode worms to breed. Generally, differences of geographical region origin can also affect the existence of different management in a region based on the climate and rainfall. Infection of helminthiasis can be influenced by some factors, such as the difference of geographical region origin of the sample, which is influenced by season, air temperature, rainfall, humidity, and soil conditions. It is can affect the development of eggs and infective larvae (Novobilsky et al. 2013; Widisuputri et al. 2020). Variation may be due to change in management practices of different herds and opportunities for grazing (Hanafiah et al. 2019). The livestock system in the Malang is already intensive, whereas livestock in the Bali region is mostly managed extensively, without pens and semi-intensively by grazing them during the day and then penning them during the night. This system provides a lot of opportunities for worm larvae in grasses to infect livestock. Tolistiawaty et al. (2016) stated that herd farming management highly influences parasite infection prevalence. Livestock farming management patterns in Indonesia can

be grouped into three categories: Extensive (pasturage), intensive (penned), and semi-intensive combination pattern (traditional). The use of the semi-intensive system by letting the cattle graze for feed (pasture system) and traditional system which are cattle not penned in the least will increase the danger of worm infection while cattle that managed intensively, the infection risk can be reduced since feed is given in the pen (Setiadi et al. 2012).

Furthermore, the difference in helminthiasis prevalence in various regions is influenced by several factors, for instance, infection agent, farming management, and host breed (Hanafiah et al. 2019). The incidence of gastrointestinal parasitic infections that occurs is probably caused by different regions. This is supported by research from Supriadi et al. (2014) which was stated that gastrointestinal parasitic infections in pigs can be caused by poor management. Poor cage sanitation is also a factor that increases the risk of parasitic infection. In addition, the most important factors associated with gastrointestinal parasitic infections are related to sanitation and said the variation in prevalence depends on the difference in agro-climatic condition and availability of a susceptible host (Singh et al. 2017; Roesel et al. 2017).

### **Helminthiasis from different host**

Examination of feces samples for Trematodes was carried out on feces samples of cattle, buffaloes, and horses. Prevalence of *Fasciola* spp. In cattle was higher (32.5%) than in Buffaloes (19.7%), *Paramphistomum* spp. Infection in Buffaloes was higher (40.2%) than in cattle (0.6%). Moreover, in the horse feces samples, no trematode infection was found. In addition, Nematode infection was found in all livestock feces samples 74.4% in pigs, 52.4% in horses, 28.5% in cattle, and 7.4% in buffaloes. The prevalence of Nematode infection is relatively dispersed from high to low (Table 2).

As shown in Table 2, overall, the percentage of helminthiasis was higher in pigs (74,4%) and followed by buffalo (67,3%), cattle (61,6%), and horses (52,4%). Other important factors influencing parasite infection are host species including body weight and breed. Significantly the highest prevalence in the pig as compared to another susceptible host. The other factors influencing the distribution of nematode worms between animals are sanitation and pen hygiene (Purwaningsih et al. 2018). The feces that accumulates in the pens attract the flies and are a suitable environment where nematode larvae can flourish. When the skin of the pig makes contact with the dirt, the larvae can infiltrate into the host's body. In addition, the infection rate in a host is very closely influenced by the management of the enclosure concerning sanitation. Among the specific host's pigs, cows, buffaloes, and horses have different living systems.

**Table 2.** Prevalence of trematode and nematode in livestock in Indonesia based on host species

Host	Total Sample of Examined	Fecal examinations			Total infection
		Trematoda		Nematoda	
		<i>Fasciola</i> spp.	<i>Paramphistomum</i> spp.	Strongyle	
Cattle	151	49 (32.5%)	1 (0.6%)	43 (28.5%)	93 (61.6%)
Buffalo	122	24 (19.7%)	49 (40.2%)	9 (7.4%)	82 (67.3%)
Horse	61	0	0	32 (52.4%)	32 (52.4%)
Pig	82	-*	-*	61 (74.4%)	61 (74.4%)
Total	376	73/294 (24.8%)	50/294 (17%)	145/376 (38.6%)	

\* (unexamined)

Based on this study, pigs, which were the animals that had the highest percentage of worm infections, were followed by buffalo, cattle, and horses. Nematode infection in pigs is higher in Sumba than in Bali. When viewed from the perspective of sanitation, generally, pigs are traditionally raised with low nutritional value and poor hygiene. This condition makes pigs are more vulnerable to various diseases and has the potential to spread the diseases. The possibility of a parasitic infection occurs due to a lack of public awareness about good sanitation, besides the habit of pigs by eat in soil contaminated with feces can be predisposed to infection. Management of buffalo in Indonesia is usually rarely penned, as well as some cows that are caged and some are not. Unlike the case with horses that are more often penned so that more attention is paid to, hygiene and nutrition are maintained. Livestock kept in pens have a lower rate of gastrointestinal nematode infection (Nurcahyo et al. 2021).

This result is due to the cage maintenance system, which is more kept clean than cattle that are not penned. The cleanliness and health of the animals in captivity depending on the breeder's treatment of their livestock. Cattle that are not housed are not paid attention to by the breeders, this causes the feed nutrition and hygiene of the livestock to be very poor so that it is easily infected by parasites including gastrointestinal nematodes. Gastrointestinal parasite infection rates are lower in livestock kept in pens than in pastures. Infected herd cattle will excrete feces containing nematode eggs and then hatch into infective larvae in the grazing area. These infectious larvae move among the grass in the grazing area which at any time can be swallowed by livestock grazing in contaminated grazing areas (Junaidi et al. 2014). Feed factors also influence gastrointestinal nematode parasitic infection. The feed

has an important role to form the immune system of livestock as a form of defense and also feed can be a medium of infection for gastrointestinal parasites. Livestock that has good health and nutrition will be able to develop resistance to worms or other types of parasites (Agus et al. 2017).

In this study, the prevalence of helminthiasis from 4 locations in Indonesia was 62.7-68.6% and from the different host was 52.4-74.4%, which is quite high compared to previous reports in several different locations in Indonesia (Ekawasti et al. 2019; Sawitri et al. 2020; Nurcahyo et al. 2021; Dina et al. 2021). Monitoring of disease control efforts is needed to reduce the prevalence of helminthiasis rate. Helminthiasis control includes improving maintenance management, use of anthelmintics, and control of intermediate hosts. So far, the cattle are kept in cages together with the owner's house and most of the livestock are grazed. It is hoped that the maintenance pattern will be changed by making one group cage so that livestock health monitoring can be more easily carried out. With the existence of group cages, it is hoped that livestock grazing patterns can be abandoned so that they will break the life cycle of trematode worms and nematodes in livestock areas.

## CONCLUSION

The results showed that the prevalence rate of helminthiasis in 4 locations in Indonesia (62.7-68.6%) and the type of host 52.4-74.4%, there were differences in prevalence rates based on location and host. The data from the present study indicated that the infection rate with Trematodes, including *Fasciola* spp. was highest in Malang (cattle), *Paramphistomum* spp. in Banten (buffalo) and nematodes (strongyle) in Bali and Sumba (Pig). These findings should be used to establish a management strategy to reduce the prevalence of helminthiasis in these locations. The prevalence of helminthiasis was found to be quite high on several farms in the Indonesian areas studied.

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## AUTHOR CONTRIBUTIONS

Fitrine Ekawasti as the main contributors, while others as member contributors

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## Utilization of Bali Traditional Medicines Formula as an Additional Feed for Bali Cattle

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### ABSTRACT

The Balinese people know medicine whose formulas have been recorded in various manuscripts. A study was conducted to determine the utilization of Bali traditional medicines formula as an additional feed for Bali Cattle in the Wira Kencana Tani Farmers Group, Bukti village, Kubutambahan sub-district, Buleleng Regency. Twenty Bali Cattle with an average body weight of  $140.28 \pm 67.44$  kg were used in this research for six months. This study was arranged in a completely randomized design with four treatments and five replications. The treatments were A: Bali Cattle fed Napier grass *ad libitum*, B: Napier grass + Prodef-1 as much 10 cc/head/day, C: Napier grass + Prodef-2 as much 10 cc/head/day and D: Napier grass + Prodef-3 as much 10 cc/head/day. The Parameters observed were average daily gain (ADG), feed intake, and feed conversion ratio. Business profits were calculate based on economic analysis, B/C ratio, and R/C ratio. This study suggests that the best average daily gain (ADG) in treatment D was 356.08 g/head/day. The results of the economic analysis also showed that Bali Cattle that received treatment D had the highest profit of Rp 1,120,000 with the B/C ratio of 0.02 and R/C ratio of 1.02. It can be concluded that the Bali cattle that received Prodef 3 had the highest ADG and the highest profit.

**Keywords:** Bali cattle, Traditional medicine, Napier grass, Feed additive

### INTRODUCTION

Ruminants can digest and utilize fibrous feeds as a source of energy and nutrition. They have a reticulo-rumen as an ecosystem where anaerobic microbes live, consisting of bacteria, fungi, and protozoa (Durrand & Ossa 2014; Punia et al. 2015). Bacteria and fungi play more of a role in helping the digestion of feed in crude fiber, whereas protozoa play a role in controlling the bacterial population. Setiyaningsih et al. (2021) states that the activity of microorganisms influences digestibility in the rumen. Optimum rumen conditions affect fermentation for microbial growth in the rumen.

In general, Bali cattle are given grass as a main of forage. To increase productivity, sometimes farmers also provide rice bran as additional feed. The provision of supplemental feed will increase the livestock production cost, so it is necessary to pay attention to make it a more efficient production cost (Sukanata et al. 2014). The grass is a type of forage that has high crude fiber content. Other than that, forage is also bulky, so the intake is limited by the capacity of the rumen. Therefore, it is necessary to manipulate the condition of the rumen by increasing the bacterial population while reducing (defaunating) the number of protozoa in the rumen.

Dinata et al. (2019a) reported that rumen manipulation with molasses solution containing liquid extract of hibiscus tiliaceus leaves could increase body weight gain in Bali cattle fed by elephant grass and pollard about 1.5 kg/head/day of 30.53% and increased the digestibility of BETN and crude fiber. It is necessary to add microbes in the rumen free from pathogenic bacteria with a manipulation process in the rumen. To reduce pathogenic microbes in the rumen fluid can be done by manipulated the population by using extracts of traditional herb plants.

Traditional herb plants, which can be used as medicinal plants, have been known and widely used by Balinese as local knowledge. Balinese people already note and recorded it in various types of "Lontar Usada Bali," which contains a collection of herbal medicinal compositions and how to use them (Sujarwo et al. 2015). One of the terms of a mixture of traditional medicine that which often mentioned is "Triketuka." Triketuka is the name of 3 types of medicinal plants consisting of kesuna (garlic = *Allium sativum* L), jangu (dringo = *Acorus calamus* L.) mesuwi (mesoyi = *Massoia aromatic* Becc.) (Adiputra & Trapika 2018). Many experimental and clinical studies mention that garlic has many beneficial health effects. The benefits of garlic include (1) antioxidant and antimicrobial, (2) reduced risk of cardiovascular disease, (3) reduced risk of cancer, (4) increased detoxification of foreign compounds, and (5) hepatoprotection (Colin- González et al. 2012). The rhizome of jangu contains antimicrobial substances that can suppress the growth of bacteria strains of *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Vibrio cholera* (Nayak et al. 2016). The rhizome of jangu has medicinal properties to treat cough, fever, asthma, bronchitis, digestive problems, nerve tonic, and expectorant (Liu et al. 2013). Masoyi peel contains massoia lactone, which can act as an antibacterial compound (Pratiwi et al. 2015).

Based on the description above, medicinal plant extracts in the rumen manipulation process are expected to reduce the protozoa population while increasing the population of bacteria that digest feed. With the increased feed digestibility in the rumen then the productivity of Bali cattle was expected to

increase. This study was to determine the effect of traditional medicinal plant extract formulas on the productivity of Bali cattle.

## MATERIALS AND METHODS

The study was conducted in the Wira Kencana farmer group in the Bukti village, Kubutambahan sub-district, Buleleng Regency. The cattle used in this study as many twenty bulls with an average initial body weight of  $140.28 \pm 67.44$  kg, which were reared for six months. Traditional medicinal plant extracts name Prodef were made in 3 specific formulas. The study used a randomized block design with four treatments and five replications. The treatments given were: Bali cattle were fed with Napier grass *ad libitum* (A), Bali cattle were fed Napier grass *ad libitum* + prodef-1 formula (B), Bali cattle were fed Napier grass *ad libitum* + prodef-2 formula (C), and Bali was fed Napier grass *ad libitum* + prodef-3 formula (D). Prodef formula was given as much as ten cc/head/day.

Prodef's formula was made from the fresh rumen contents of Bali cattle obtained from abattoirs. Fresh rumen was taken from slaughtered cattle and then filtered after the liquid portion from the rumen content was added with 5% medicinal plant extract. Rumen fluid formula then fermented for three days. The medicinal plants extract that was used in the Prodef formula was a mixture of the medicinal plant were: garlic (*Allium sativum* L), dringo rhizome (*Acorus calamus* L), and masoyi peel (*Massoia aromatica* Becc). Three medicinal plants extract formulas from a mixture of garlic extract: dringo rhizome: masoyi peel with a ratio of 2:1:1 (Prodef-1), ratio mixture 1:2:1 (prodef-2), and proportion of mixture 1:1:2. (prodef-3). After three days of fermentation, as much as 100 ml of this rumen fluid, pour and mix into 600 ml of Hibiscus leaf liquid extract and 300 ml of molasses to become a 1-liter mixed formula.

The variables measured in this research included: (1) Average daily gain (ADG); (2) Feed consumption; and (3) Feed Conversion Ratio (FCR). Bodyweight measured weighing was carried out once a month in the morning before the animals were given food and drink. To determine body weight gain by subtracting the final body weight from the initial body weight. ADG can be determined by dividing the difference in body weight by the length of the experiment.

The daily dry matter consumption (DM) of the ration can be calculated by subtracting the ration with the remaining ration for each individual. Feed conversion ratio (FCR) was calculated by dividing the amount of feed consumed per day by the additional bodyweight per head per day obtained during the study. The data result obtained from this study was analyzed by analyzing variance with an error rate of 1-5%. If the variance test shows a

significant difference, then the trial between the mean of the two treatments was carried out with Duncan's multiple distance test.

## RESULTS AND DISCUSSION

### Bali cattle productivity

The provision of Prodef gave an excellent response to the average daily gain (ADG) of Bali cattle during six months of rearing. Overall, cattle that received Prodef had a higher average ADG than cattle without additional Prodef. Cattle that obtained Prodef-3 formula (treatment D) had the highest body weight gain of 356.08 grams/head/day (Table 1). This mean ADG of cattle that were given treatment D was significantly higher ( $P<0.05$ ) compared to cattle that were treated with treatment A, B, and C. Cattle that were given treatment C had a PBB of 60.59% significantly higher ( $P<0.05$ ) compared to treatment A, but not significantly different ( $P>0.05$ ) compared to treatment B. Cattle that were given treatment B had a 22.35% higher PBB than treatment A, but statistically not significantly different ( $P>0.05$ ).

The higher ADG in cattle that obtained the Prodef formula showed that rumen microbes had an influential role in increasing the efficiency of feed digestibility. Giving a formula with a higher dose of masoyi peel extract plays a role in killing pathogenic bacteria in the rumen. Prodef also contains saponins which play a role in reducing the population of protozoa in the rumen because saponins bioactive substances can lyse protozoan cells (Susanti & Marhaeniyanto 2014). The reduced protozoa population will increase the bacteria population in the rumen (Goel et al. 2008). Reducing the number of protozoa will lead to the increased availability of N in the digestive tract (Herdian et al. 2011), the flow protein of microbial from the rumen, increases the efficiency of feeding, and reduces methanogenesis (Wang et al. 2011).

**Table 1.** Bali cattle productivity given Prodef formula

Variables	Treatment			
	A	B	C	D
Initial Body weight (kg)	129.10±49.84 <sup>a</sup>	149.20±80.48 <sup>a</sup>	150.90±90.42 <sup>a</sup>	131.90±68.13 <sup>a</sup>
Final body weight (kg)	149.34±25.90 <sup>a</sup>	173.96±44.23 <sup>a</sup>	183.40±35.10 <sup>a</sup>	196.00±71.80 <sup>a</sup>
Average daily gain (ADG) (g/head/day)	112.43±90.00 <sup>a</sup>	137.57±90.02 <sup>ab</sup>	180.56±37.07 <sup>b</sup>	356.08±78.88 <sup>c</sup>
Feed consumption (gDM/day)	4.18±0.98 <sup>a</sup>	4.85±0.78 <sup>a</sup>	5.01±0.65 <sup>a</sup>	4.92±0.73 <sup>a</sup>
FCR	37.15±4.35 <sup>a</sup>	35.24±6.32 <sup>a</sup>	27.77±9.54 <sup>a</sup>	13.81±4.56 <sup>a</sup>

The content of the rumen microbial consortium as probiotics in Prodef has been able to maximize the increased bacterial population in the rumen. Probiotic microbes can increase the microbial population, improve function and health, and absorb nutrients in the digestive tract (Mus et al. 2009). Probiotics were an effort to increase the digestibility of feed ingredients to absorb more nutrients (Astuti et al. 2015). The addition of probiotics causes the dry matter that was degraded in the rumen to be high. After that, the feed flow rate increases (Sugoro 2010); this was proved that additional Prodef treatment leads to higher feed consumption in cattle. The increased digestibility of feed causes more nutrients absorbed so that the ADG Bali cattle that gain Prodef becomes higher.

The increase in feed digestibility efficiency can be seen in Bali cattle which received additional Prodef 3 (treatment D) with the lowest Feed Conversion Ratio (FCR) value of 13.81. In general, cattle that received additional Prodef had a lower FCR value than treatment A. This was because cattle that received Prodef had higher ADG, even though their feed consumption was also increased. The FCR value was related to the efficiency of the use of feed by livestock. The lower the FCR indicates that the efficiency of feeds consumed and absorbed in the body was higher.

### **Revenue cost ratio**

The total cost incurred in fattening cattle business that obtain napier grass-based feed with ownership of 5 bulls were Rp. 61,475,000 (Table 2). There were an additional fee of Rp. 225,000 if giving Prodef. From the total costs, the cost of purchasing breeds was the largest with the amount of 81.04-81.33%. For the cost of forage in the form of elephant grass only Rp. 8,100,000 or about 13.33-13.18% from the total cost.

From the results of the farming analysis, it was shown that only cattle that received additional Prodef C formula (treatment D) were profitable. The higher value of profits obtained was due to the highest ADG, which affects the selling value of cattle to be higher. The highest B/C ratio and R/C values were also found in cattle treated with D treatment. The highest R/C value indicates that cattle treated with D treatment have the highest farming efficiency, so it was economically feasible to expand. In addition, to increase profits in the cabusinessness with forage-based Napier grass, it is necessary to add a Prodef formula.

**Table 2.** Analysis of Bali cattle fattening business given Prodef probiotics (per 5 heads/180 days)

Parameter	Treatment			
	A	B	C	D
Input				
Depreciation cages and equipment	750,000	750,000	750,000	750,000
Cattle breed 150 kg @ Rp. 10,000,000 × 5 head	50,000,000	50,000,000	50,000,000	50,000,000
Feeds				
- Elephant grass 25 kg/day@Rp 500	8,100,000	8,100,000	8,100,000	8,100,000
- Prodef Rp 40,000		225,000	225,000	225,000
Vitamin and Medicine	375,000	375,000	375,000	375,000
Labor (2 hour/person/day) @ Rp. 50,000	2,250,000	2,250,000	2,250,000	2,250,000
Total input	61,475,000	61,700,000	61,700,000	61,700,000
Output				
Average daily gain (ADG) (g/head/day)	112.43	137.57	180.56	356.08
Total ADG =B1 × 180 day × 5 head (kg)	101.20	123.80	162.50	320.50
Sale value				
- Additional selling points (Rp. 40,000/kg liveweight)	4,048,000	4,952,000	6,500,000	12,820,000
- Total selling value	54,048,000	54,952,000	56,500,000	62,820,000
Revenue	(7,427,000)	(6,748,000)	(5,200,000)	1,120,000
B/C Ratio	-0.12	0.11	0.08	0.02
R/C Ratio	0.88	0.89	0.92	1.02

*The depreciation cost of the cage is calculated by assuming the price per cage block is Rp. 1,500,000 with a technical life of 5 years. Forage 12% of body weight*

From the results of the farming analysis, it was shown that only cattle that received additional Prodef C formula (treatment D) were profitable. The higher value of profits obtained was due to the highest ADG, which affects the selling value of cattle to be higher. The highest B/C ratio and R/C values were also found in cattle treated with D treatment. The highest R/C value indicates that cattle treated with D treatment have the highest farming efficiency, so it was economically feasible to expand. In addition, to increase profits in the cabusinessiness with forage-based Napier grass, it is necessary to add a Prodef formula.

In general, farmers usually never calculate the price of forages, shed depreciation, and labor costs. The feed cost calculation was only done if the cattle were given additional feed, which was concentrate. If the cost of feed, shed depreciation, and labor was eliminated, then the entire treatment practice will benefit. The value of these benefits ranges from Rp. 3,673,000-Rp.12,220,000. The benefit was the cause for Bali farmers to be interesting in raising Bali cattle because they are still economically profitable.

## CONCLUSION

Bali cattle with provision Prodef-3 formula (treatment D) had the highest productivity and was the most economically profitable business.

## AUTHOR CONTRIBUTIONS

Anak Agung Ngurah Badung Sarmuda Dinata as the main contributor, Anastasia Sischa Jati Utami, Yusti Pujiawati dan Gresy Eva Tresia as the member contributor.

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## **Pathogenicity Study of Ducks Infected with Local Isolate of Highly Pathogenic Avian Influenza-H5N1-Clade 2.3.2**

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### **ABSTRACT**

The early outbreak of highly pathogenic avian influenza in poultry in Indonesia was caused by H5N1 clade 2.1.3, whereas the second outbreak in ducks was caused by 2.3.2 clade introduced from abroad. The study aims to determine the pathogenicity of local isolates of H5N1 clade 2.3.2 in ducks based on histopathological and immunohistochemical approaches, compared to clade 2.1.3 local isolates. Fifteen ducks were used and kept in the BSL-3 modular laboratory. Three ducks were served as negative control and six ducks were infected with 10<sup>6</sup> EID<sub>50</sub> viruses intranasally: two ducks were infected with 2.1.3 isolate (A/chicken/West Java/Krw54/2012), two were infected with 2.3.2 isolate (A/Environment/East Java/Lbm/2012) and two were infected with 2.3.2 isolate (A/duck/Sukoharjo/BBVW1428-9/2012). Another six were infected by direct contact with infected ducks. Visceral organs were collected for histopathology and immunohistochemistry. Intranasal and contact infection the clades caused mortality 50% in 3-7 dpi, produced nonsuppurative inflammations in the brain, heart, lungs, liver, intestines, pancreas, and kidneys with various degrees of severity and antigen distribution level. Lamongan and Wates isolates belong to clade 2.3.2, they can produce different degrees of severity and amount of antigen for the same route of infection. This study shows that the origin of the isolate plays a more important role in determining pathogenicity rather than the route of infection. The study showed that Wates isolate (clade 2.3.2) was the most pathogenic, followed by Lamongan (clade 2.3.2) and Karawang isolates (2.1.3), respectively. The order of pathogenicity indicates that clade 2.3.2 is more pathogenic than clade 2.3.1.

**Keywords:** HPAI-H5N1-clade 2.3.2, Pathogenicity, Duck

### **INTRODUCTION**

Waterfowl are known hosts for all Influenza A virus subtypes, posing a very serious health risk to other animal species and allowing genetic mixing to occur. Although several subtypes of Influenza A viruses found in natural hosts are non-pathogenic or non-virulent, their presence causes these natural hosts to become AI reservoirs (Hewajuli & Dharmayanti 2012). In Indonesia, at the end of 2012, a high mortality rate was reported in ducks in Central Java,

East Java, and DI Yogyakarta, and the cause of the outbreak was identified as AI virus subtype H5N1 clade 2.3.2. (Wibawa et al. 2012).

Based on the phylogenetic relationship, the clade 2.3.2 viruses had circulated in Myanmar and spread to Bangladesh and India. The most probable route of this transmission could be the movement of land-based poultry or local migratory bird (Nagarajan et al. 2012). Furthermore, sequence analyses revealed all genes clustered with clade 2.3.2.1 rather than previously clade 2.1.3, indicating the introduction of an exotic H5N1 clade into Indonesia and the new clade 2.3.2. had different glycosylation sites and several conserved amino acid changes, indicating that it might be differed antigenicity (Dharmayanti et al. 2014).

In addition, although several studies have investigated the pathogenesis of Asian H5N1 viruses in several bird species, available data was limited concerning the response of ducks to Indonesian H5N1 viruses (Wibawa et al. 2013). HPAI-H5N1 virus clade 2.3.2 produced severe disease as evidenced by clinical signs, presence of marked lesions, and abundant viral antigen in several tissues, especially the central nervous system and declared as highly pathogenic for ducks (Bröjer et al. 2015). This virus has not previously been reported circulating in Indonesia, because the predominant H5N1 virus in Indonesia is the H5N1 virus clade 2.1 which is divided into clade 2.1.1; 2.1.2; 2.1.3 (Dharmayanti et al. 2018).

The purpose of this study is to determine the pathogenicity of the new clade, 2.3.2, and compared to the previous clade, 2.1.3 in terms of histopathological aspects and immunohistochemical staining to detect the distribution of H5N1 antigen in visceral organs.

## MATERIALS AND METHODS

### Experimental animals

The ducks used in this study did not contain antibodies against AI-H5N1 and were also negative when tested with Polymerase Chain Reaction (PCR). The ducks have then adapted for three days in the insulator of the Biosafety Level-3 (BSL 3) modular laboratory, the Indonesian Research Center for Veterinary Science. The total of 15 ducks was divided into three groups, three as negative control ducks and six ducks were infected intranasally with local isolate virus and six ducks were infected by direct contact with infected ducks by placing them in the same isolator unit. Ducks were given food and drink *ad libitum*, enough rest at a comfortable temperature to prevent stress and at the end of the experiment (14 days) if any ducks survived they were necropsied according to the animal welfare standard for poultry.

## **Inoculum (local isolate of H5N1)**

As control animals, three ducks were injected intranasally with Phosphate Buffer Saline (PBS) solution pH 7.4, while the second group consisted of six ducks were infected with three kinds of AI local isolates originating from an outbreak in Indonesia. Six ducks were each infected with a dosage of  $10^6$  EID<sub>50</sub> intranasally, two ducks were infected with clade 2.1.3 isolate from Karawang (A/chicken/West Java/Krw54/2012), two were infected with clade 2.3.2 isolate from Lamongan (A/Environment/East Java/Lbm/2012) and two were infected with clade 2.3.2 isolate from Wates, Jogja (A/duck/Sukoharjo/BBVW1428-9/2012). In addition, another six ducks were infected by direct contact with infected ducks as described previously (Dharmayanti et al. 2013).

## **Visceral organ sampling collection**

Visceral organ samples were taken from ducks that had just died and ducks that survived up to 14 days after infection. The types of organs collected are brain, trachea, heart, lung, proventriculus, liver, intestine, pancreas, spleen, kidney, and bursa. The organs were cut approximately 0.5 cm thick and fixed in 10% buffered formalin solution (BNF) until the organs were completely fixed.

## **Histopathological examination**

Visceral organs that had been fixed in 10% BNF solution were processed as paraffin blocks, cut 3-4  $\mu$ m thick, and stained with hematoxylin and eosin (H&E) according to standard procedures. Histopathological examination was carried out descriptively with the aid of a microscope to determine the severity of the lesions and the final diagnosis of the disease.

## **Hyperimmune sera to H5N1**

This H5N1 avian influenza hyperimmune serum was produced in adult rabbits according to the previously used method (Damayanti et al. 2017). Positive control monoclonal antibody to AI (Mab AI, ID No. 8904-26-1500) and negative control monoclonal antibody against non-AI (Mab NDV, ID No. Q 24-1) were obtained from the AAHL-CSIRO laboratory, Geelong, Australia.

## **Immunohistochemical staining using avidin-biotin-peroxidase**

Immunohistochemistry was conducted as described previously (Damayanti et al. 2017), the slides were reacted with rabbit antisera anti-virus AI subtype H5N1 which had been standardized by checkerboard titration with

a concentration of 1:1000 in PBS solution. Subsequently, a secondary antibody labeled with biotin/biotinylated secondary antibody (DAKO, Denmark) was added and streptavidin peroxidase (DAKO, Denmark) was then applied. To visualize the antigen, diaminobenzidine (DAB) (Sigma Chem. Co., USA) was used.

## **RESULTS AND DISCUSSION**

### **Histopathological findings**

Intranasal infection with Karawang, Lamongan, and Wates isolates resulted in deaths at 6 dpi, 3 dpi, and 5 dpi, respectively whereas contact transmission with those isolates caused deaths at 6 dpi, 3 dpi, and 7 dpi, respectively. Intranasal and contact infection with three clades caused mortality 50% in 3-7 dpi, produced nonsuppurative inflammations in many organs with various degrees of severity and antigen distribution level. Previous pathogenicity studies of clade 2.3.2 and clade 2.1.3 also resulted in death in ducks 3-6 days post-infection (Dharmayanti et al. 2013). Table 1 shows that the type of organ affected and the degree of severity varies as mild, moderate to severe. In uninfected control ducks, no clinical symptoms or death were found, nor were specific lesions (NSL). Ducks infected intranasally with isolates of Karawang clade 2.1.3 (A/chicken/West Java/Krw54/2012) died at 6 dpi (days post-infection) characterized by severe encephalitis, moderate myocarditis, multifocal necrosis of the liver, and intestinal mucosal damage. Ducks that were infected intranasally but survived up to 14 dpi had no specific lesions (NSL). Meanwhile, ducks infected by contact with sick ducks died at 6 dpi with severe encephalitis and moderate myocarditis, while ducks that survived up to 14 dpi had NSL. The survived ducks with NSL could be explained from a previous report, stated that domestic ducks infected with Indonesian H5N1 showed a short infectious period and its termination coincides with the rise of antibodies (Wibawa et al. 2014).

Ducks infected intranasally with an isolate of Lamongan clade 2.3.2 (A/Environment/East Java/Lbm/2012) died at 3 dpi with severe encephalitis, moderate myocarditis, and diffuse fatty liver while ducks that survived up to 14 dpi had NSL. Meanwhile, ducks infected by contact died at 3 dpi had severe encephalitis, moderate myocarditis, pneumonia, and necrotic pancreas, and mild enteritis and nephritis, while ducks survived up to 14 dpi only had moderate encephalitis.

Ducks infected intranasally with the isolate of Wates clade 2.3.2 (A/duck/Sukoharjo/BBVW1428-9/2012) died at 5 dpi with severe encephalitis, moderate pulmonary hemorrhage, enteritis and nephritis multifocal necrosis

of the liver, severe multifocal necrosis of the pancreas, while those that survived up to 14 dpi had NSL. Ducks infected by contact died at 7 dpi with severe encephalitis and mild myocarditis. While those who survived up to 14 dpi had severe encephalitis and necrotizing pancreatitis, mild pneumonia, hepatitis, enteritis, and nephritis.

The main lesion observed in this study showed systemic hemorrhages in all visceral organs with a non-suppurative type of inflammation characterized by mononuclear inflammatory cells, associated with necrotizing liver and pancreas. In this study, the type of organ affected and the degree of severity varied. Multifocal necrosis of the liver, pancreas, and spleen as well as encephalitis and myocarditis were also reported previously by Pantin-Jackwood et al. (2013) and Pantin-Jackwood et al. (2017). Contact transmission with Lamongan isolate (clade 2.3.2) produced the most severe lesions, followed by intranasal infection with Wates isolates (clade 2.3.2) and intranasal infection with Karawang isolate (2.1.3), respectively. The rest of the infections produced similar mild to moderate lesions, namely: intranasal infection with Lamongan isolate (2.3.2), contact transmission with Wates isolate (2.3.2) and contact transmission with Karawang isolate (2.1.3). These findings suggest that clade 2.3.2 in ducks were more pathogenic than clade 2.1.3, as also reported by (Dharmayanti et al. 2014). In addition consistent and severely affected organs by these viruses was probably related to tissue tropism (Abou-Rawash et al. 2012). No specific lesions found in ducks survived up to 14 dpi suggested that antibody had been developed, as reported by Cagle et al. (2012) and Wibawa et al. (2013).

### **Immunohistochemical staining**

Table 2 shows the distribution and degree of antigen expressed as the number of antigenic cells per one microscopic field of view per organ (20× magnification), as described previously (Damayanti et al. 2017). Antigen was not detected in the negative control group. Intranasal infection with isolates of Karawang, Lamongan, and Wates caused death in ducks at 6 dpi, 3 dpi, and 5 dpi, respectively, and antigens were detected in various visceral organs. However, in the isolates that were infected intranasally in ducks that survived up to 14 dpi, the antigen could not be detected in all organs, probably the immune system had efficiently controlled virus replication and spread, thus prolonging the death of the duck (Cagle et al. 2012).

Karawang isolates clade 2.1.3 which was transmitted by contact caused death at 6 dpi and antigens were found enormously in the brain and moderately in the heart while in ducks that survived up to 14 dpi, antigen

could not be detected in all organs. Contact transmission by Lamongan isolate clade 2.3.2 caused death at 3 dpi with antigens detected enormously in the brain, moderately in the heart, lung, pancreas, and few antigens were detected in the intestines and kidney, whereas in ducks that survived up to 14 dpi, the antigen could only be detected in the brain. Contact transmission by Wates isolates clade 2.3.2 caused death at 7 dpi with massive antigens detected in the brain and few in the heart. However, in ducks that survived up to 14 dpi, antigen could be detected enormously in the brain and pancreas, but few in the lungs, liver, kidneys, and intestines.

In this study, the antigens were distributed either individually or in groups to form a cluster or chain-like with low, moderate to high antigen levels. Figures 1 and 2 showed massive antigen distribution in the brain, heart, kidney, and pancreas of duck that died 5 dpi after being infected intranasally with Wates isolate clade 2.3.2. The antigens occupied both intranuclear and intracytoplasmic sites, as previously reported (Damayanti et al. 2020). Both clade 2.3.2 and clade 2.1.3 had histopathological lesions correlated strongly with the amount of antigen distribution by immunohistochemistry, the more severe the histopathological lesion, the greater the amount of antigen detected, as previously reported in H5N1 infection (Damayanti et al. 2004). However, lesions caused by HPAI could vary in severity in different types of birds and this depends on the strain and concentration of the virus, route of infection, bird species, age of birds, and health status of birds (Pantin-Jackwood et al. 2013).

Contact transmission with Lamongan isolates (2.3.2) showed a massive antigen than intranasal infection and the antigen persisted in the brain at 14 dpi. Contact transmission with Lamongan isolate tends to be the most pathogenic, followed by Wates and Karawang isolate, respectively. The result was in accordance with the microscopical changes. However, intranasal infections with Wates isolate (2.3.2) produced more severe lesions and more antigens compared to contact transmission. This might be due to the shedding pattern of HPAI H5N1 viruses in ducks that were often detected at higher concentrations from oropharyngeal/tracheal swabs than from cloacal swabs (Wibawa et al. 2013). The route of inoculation of the HPAI-H5N1 virus was also an important variable affecting the presentation of the disease (Bröjer et al. 2015). However, although both Lamongan and Wates isolates belong to clade 2.3.2, they can produce different degrees of lesion severity and amount of antigen for the same route of infection. This study shows that the origin of the isolate plays a more important role in determining pathogenicity than the route of infection.

**Table 1.** Histopathological lesions in pathogenicity study of HPAI H5N1 in ducks

Code No	Treatment	Clade	Degree of severity lesion									
			Brn	Tra	Hrt	Lng	Prv	Lvr	Spl	Int	Pan	Kdn
NC.AE/Dk.1/14 dpi	Negative control	-	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL
NC.AE/Dk.2/14 dpi	Negative control	-	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL
NC.AE/Dk.3/14 dpi	Negative control	-	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL
Krw.AE/Dk1/6 dpi	Intranasal 10 <sup>6</sup> EID <sub>50</sub>	clade 2.1.3	+++	NSL	++	NSL	NSL	++	++	++	NSL	NSL
Krw.AE/Dk2/6 dpi	By contact	clade 2.1.3	+++	NSL	++	NSL	NSL	NSL	NSL	NSL	NSL	NSL
Krw.AE/Dk.3/14 dpi	Intranasal 10 <sup>6</sup> EID <sub>50</sub>	clade 2.1.3	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL
Krw.AE/Dk.4/14 dpi	By contact	clade 2.1.3	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL
Lm.AE/Dk.1/3 dpi	Intranasal 10 <sup>6</sup> EID <sub>50</sub>	clade 2.3.2	+++	NSL	++	NSL	NSL	++	NSL	NSL	NSL	NSL
Lm.AE/Dk.2/3 dpi	By contact	clade 2.3.2	+++	NSL	++	++	NSL	NSL	NSL	+	++	+
Lm.AE/Dk.3/14 dpi	Intranasal 10 <sup>6</sup> EID <sub>50</sub>	clade 2.3.2	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL
Lm.AE/Dk.4/14 dpi	By contact	clade 2.3.2	++	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL
Wates.AE/Dk.3/5 dpi	Intranasal 10 <sup>6</sup> EID <sub>50</sub>	clade 2.3.2	+++	NSL	+++	++	NSL	++	NSL	++	+++	++
Wates.AE/Dk.2/7dpi	By contact	clade 2.3.2	+++	NSL	+	NSL	NSL	NSL	NSL	NSL	NSL	NSL
Wates.AE/Dk.1/14dpi	Intranasal10 <sup>6</sup> EID <sub>50</sub>	clade 2.3.2	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL	NSL
Wates.AE/Dk.4/14dpi	By contact	clade 2.3.2	+++	NSL	NSL	+	NSL	+	NSL	+	+++	+

*dpi: day post-infection; Lng: Lung; NSL: No specific lesion; Prv: Proventriculus; Int: Intestine; Pan: Pancreas; Lvr: Liver; Brn: Brain; Spl: Spleen; Tra: Trachea; Kdn: Kidney*

*+ : mild lesion*

*++ : moderate lesion*

*+++ : severe lesion*

**Table 2.** Antigen detection of AI-H5N1 in the study of duck pathogenicity using the immunohistochemical method

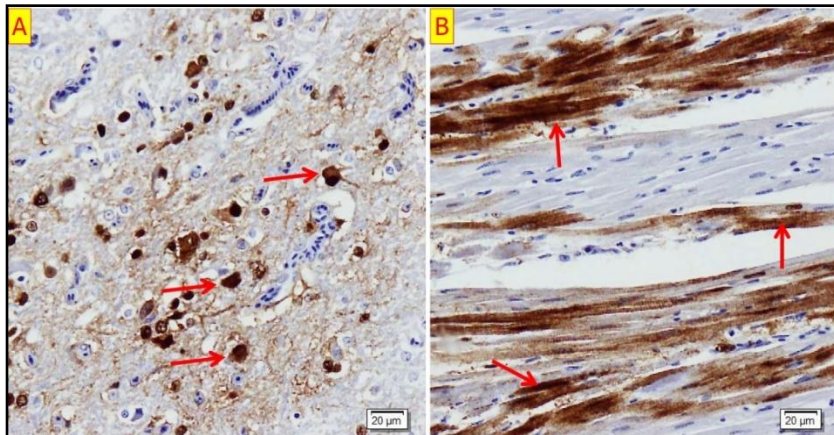
Code No	Treatment	Clade	IHC results		Antigen distribution
NC.AE/Dk.1/14 dpi	Negative control	-	Negative	Not detected	
NC.AE/Dk.2/14 dpi	Negative control	-	Negative	Not detected	
NC.AE/Dk.3/14 dpi	Negative control	-	Negative	Not detected	
Krw.AE/Dk .1/6 dpi	Intranasal 10 <sup>6</sup> EID <sub>50</sub>	clade 2.1.3	Positive	Brain +++, heart ++, liver ++	
Krw.AE/Dk .2/6 dpi	By contact	clade 2.1.3	Positive	Brain +++, heart ++	
Krw.AE/Dk.3/14 dpi	Intranasal 10 <sup>6</sup> EID <sub>50</sub>	clade 2.1.3	Negative	Not detected	
Krw.AE/Dk.4/14 dpi	By contact	clade 2.1.3	Negative	Not detected	
Lm.AE/Dk.1/3 dpi	Intranasal 10 <sup>6</sup> EID <sub>50</sub>	clade 2.3.2	Positive	Brain +++, heart ++	
Lm.AE/Dk.2/3 dpi	By contact	clade 2.3.2	Positive	Brain +++, heart ++, lung ++, Intestine +, pancreas ++, kidney +	
Lm.AE/Dk.3/14 dpi	Intranasal 10 <sup>6</sup> EID <sub>50</sub>	clade 2.3.2	Negative	Not detected	
Lm.AE/Dk.4/14 dpi	By contact	clade 2.3.2	Positive	Brain ++	
Wates.AE/Dk.3/5 dpi	Intranasal 10 <sup>6</sup> EID <sub>50</sub>	clade 2.3.2	Positive	Brain +++, lung ++, heart +++, intestine ++, pancreas +++, kidney ++	
Wates.AE/Dk.2/7 dpi	By contact	clade 2.3.2	Positive	Brain +++, heart +	
Wates.AE/Dk.1/14 dpi	Intranasal 10 <sup>6</sup> EID <sub>50</sub>	clade 2.3.2	Negative	Not detected	
Wates.AE/Dk.4/14 dpi	By contact	clade 2.3.2	Positive	Brain +++, lung+, liver +, intestine +, pancreas +++, kidney +	

*dpi: days post infection*

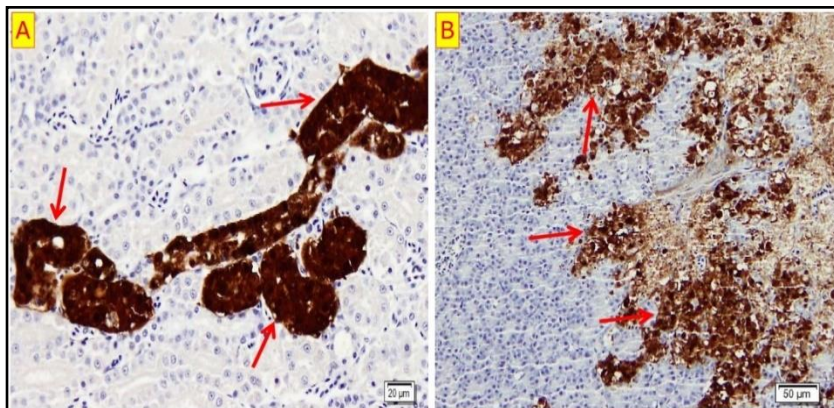
+ : few (0-5) cells containing antigens/microscopic field ( ×20)

++ : moderate (5-10) cells containing antigens/microscopic field ( ×20)

+++ : enormous (10-20) cells containing antigens/microscopic field ( ×20)



**Figure 1.** Massive antigen distribution in duck infected intranasally with Wates isolate clade 2.3.2, 5 dpi in the brain (A) and heart (B). Immunohistochemistry, DAB substrate



**Figure 2.** Massive antigen distribution in ducks infected intranasally with Wates isolate clade 2.3.2, 5 dpi in the kidney (A) and pancreas (B). Immunohistochemistry, DAB substrate

## CONCLUSION

This study shows that intranasal infection and contact transmission with clade 2.1.3 from Karawang (A/chicken/West Java /Krw54/ 2012), clade 2.3.2 from Lamongan (A/Environment/East Java/Lbm/2012), and clade 2.3.2 from Wates (A/duck/ Sukoharjo/ BBVW1428-9/2012) were classified as pathogenic and caused deaths, as indicates by various degree of lesion severity and the amount of antigen detection in various tissues. This study shows that the origin of the isolate plays a more important role in determining pathogenicity

than the route of infection. Isolate Wates was the most pathogenic, followed by Lamongan and Karawang isolate, respectively.

## AUTHOR CONTRIBUTIONS

Damayanti R, Wiyono A, and Dharmayanti NLPI were equally contributed to this work.

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## **Enrofloxacin and Ciprofloxacin Residues in Broiler Livers in East Java, Indonesia**

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### **ABSTRACT**

Antibiotics are widely used as veterinary medicine for therapeutic purposes as well as for prophylaxis and growth promotion. The present study quantitatively analyzed the occurrence of enrofloxacin and ciprofloxacin residues in a total of 55 broiler liver samples from small-holder farms in the Districts of Malang and Blitar (East Java Province). Detection of enrofloxacin and ciprofloxacin in the chicken liver was conducted using high-performance liquid chromatography (HPLC) equipped with a UV detector. Analysis results show forty samples did not contain both ciprofloxacin and enrofloxacin residues, 7 samples were detected for ciprofloxacin and enrofloxacin residue at the total concentration of 286.47-859.11 ng/g, 15 samples were detected for enrofloxacin residue in the range of 88.04-801.82 ng/g, 7 samples were detected for ciprofloxacin residue in the range of 100.47-430.03 ng/g. Total enrofloxacin (sum of enrofloxacin and ciprofloxacin) from 55 samples with a concentration of 88.04-859.11 ng/g was found in 15 samples and 12 among those samples exceeded 200 ng/g. The occurrence of enrofloxacin and ciprofloxacin residues in some liver samples in this research indicated that enrofloxacin was still used by broiler farmers especially in the District of Malang. Therefore, proper actions should be taken to avoid the emergence of antibiotic resistance in consumers as well as in the environment.

**Keywords:** Enrofloxacin, Ciprofloxacin, Residue, Chicken liver, HPLC

### **INTRODUCTION**

Antibiotics have become an integral part of livestock production and play an important role in the maintenance of animal welfare, mainly for therapeutic purposes as well as for prophylaxis and increasing the productive capacity of animals. Fluoroquinolones such as enrofloxacin are powerful options among the antimicrobial agents employed in the treatment of various bacterial-caused poultry diseases (Trouchon & Lefebvre 2016). They are widely used in the treatment of poultry for mycoplasma infections, colibacillosis, and pasteurellosis (Šandor et al. 2012) at the recommended dosage of 10 mg/kg body weight for 3 to 5 days (EMA 1998).

Poultry meat including liver is widely consumed throughout the world. However, the liver is considered a highly risky poultry product due to its role in the detoxification of toxic substances, including antibiotics (Bani-Asadi et al. 2021). Residue formed due to overuse or neglecting of withdrawal time. Administered of enrofloxacin will be metabolized by the liver and converted into the main metabolite form ciprofloxacin and several metabolites such as oxociprofloxacin, enrofloxacin amide, dioxociprofloxacin, oxoenrofloxacin and dihydroxyenrofloxacin (EMA 1998).

The presence of residues beyond the allowable limits in food for human consumption may pose some toxicological effects such as allergies and may contribute to the selection of resistant bacteria in humans gut microflora (Kim et al. 2012) and needs attention due to its reactions to humans such as allergy and increasing resistance to treatment for infections caused by *Campylobacter* (Gouvêa et al. 2015). Furthermore, ciprofloxacin as metabolite is a heat-stable compound by any cooking methods except microwaving at 800 W with one spoonful of sunflower oil for 15-20 minutes for muscles and 3-5 minutes for liver and kidney (Faten et al. 2016).

To ensure human food safety, different countries and regions set various maximum residue limits (MRLs) on fluoroquinolone residues and some countries have more rigid regulations than others. Therefore, to ensure human food safety, the European Union (EU), under Commission Regulation (EU) no. 37/2010 established that the sum of enrofloxacin and ciprofloxacin MRLs must be 100 mg/kg in muscle and 200 ng/g in the liver. Indonesia does not have the MRL of enrofloxacin residue in the liver. Furthermore, the use of antibiotics has been prohibited since January 2018 as regulated by the Ministry of Agriculture of the Republic of Indonesia through 14/Permentan/PK.350/2017.

The presence of quinolone residues in broiler liver had been monitored in different countries such as in Pakistan (Aslam et al. 2016), Turkey (Arslan-Acaröz & Sözbİlir 2020), Iran (Attari et al. 2014; Moghadam et al. 2016) and Egypt (Faten et al. 2016), but none from Indonesia for the past ten years. Therefore, this study aimed to investigate the presence of enrofloxacin and ciprofloxacin residue in broiler liver samples collected from farms in the District of Malang and Blitar, in East Java Province before the prohibition on the use of antibiotics in Indonesia. The method used was a validated HPLC with UV detection.

## **MATERIALS AND METHODS**

### **Samples**

This study was reviewed and approved by The Animal Care and Ethics Review Committee of the Institution Review Board Indonesian Research

Center for Veterinary Science, Bogor (Balitbangtan/Bblitvet/A/06/2017). Fifty-five broiler livers (aged 30 days) were collected from 28 local farmers in East Java province, namely the District of Malang (42 samples from 21 farmers) and the District of Blitar (13 samples from 7 farmers) in April 2017. Malang District was chosen as the location with the second-highest population and Blitar District as the less population of broiler chickens in East Java Province (BPS, 2016). The samples were randomly selected and each sample was placed in polyethylene plastic, transported to the laboratory under cold conditions in a foam box containing chiller packs. All samples were frozen at -20°C until analyzes.

### **Chemicals and reagents**

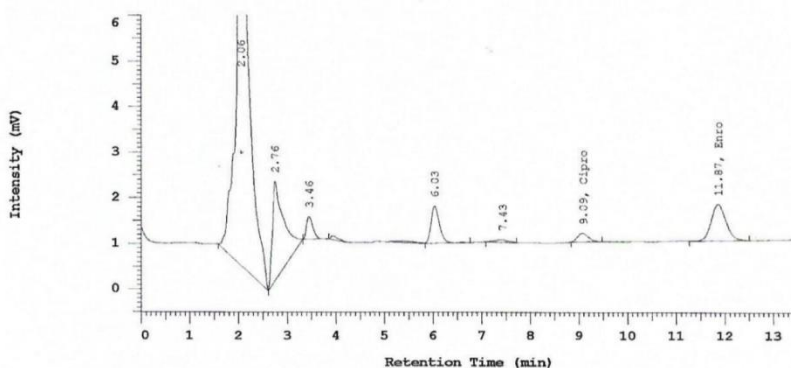
All reagents and chemicals were analytical grade and HPLC grade quality were supplied by Merck (Darmstadt, Germany). HPLC-grade water was produced by a Milli Q Direct 8/16 System (Millipore SAS, 67120 Molsheim, France). All analytical standards were of high purity grade (>90%) and were purchased from Vetranal (Sigma-Aldrich, Darmstadt, Germany). The quality of reagents and standard solutions were of analytical grade.

### **Sample preparation and analysis of enrofloxacin and ciprofloxacin residues in chicken broiler liver**

The frozen broiler liver samples were thawed at room temperature (25°C) and then cut into fine pieces for homogenization. The homogenized samples were then prepared according to a method developed by Ovando et al. (2004) with minor modifications. Briefly, 0.2 gr of the fresh homogenized chicken liver sample was extracted with the addition of 2 mL of phosphate buffer and 8 mL of dichloromethane, then vortexed for 1 min, and centrifuged at 4,000 rpm for 20 min and the upper layer was discarded and the organic layer was taken and placed in a clean tube. The tissue was extracted twice with the addition of 6 mL of dichloromethane. The combined organic layers were then evaporated at 30°C under a nitrogen stream. The residue was dissolved with 200 µL mobile phase and then separated on an HPLC Hitachi L-7000 (Hitachi, Inc. Japan) with a C18 Sunfire column (5 µm; 4.6 × 250 mm) (Waters, Ireland) using a mobile phase of a mixture of 0.2 M trichloroacetic acid-methanol-acetonitrile (74:4:22) at a flow rate of 1.2 mL/min and detected with UV detector at 277 nm. Identification of ciprofloxacin and enrofloxacin were obtained by finding the retention time, area, and spectra of peaks of unknown substances with the standards.

## Method performance

Partial validation method in terms of linearity, accuracy, and limit of quantification (LOQ) was conducted according to the criteria specified in EU Commission Decision 2002/675/EC (EC, 2002). Ciprofloxacin and enrofloxacin were separated at 9.09 min and 11.87 min, respectively as shown in Figure 1. Linearity of the method was evaluated at five points matrix matched calibration curves were obtained through spiking different blank samples with enrofloxacin and ciprofloxacin in the concentration of 10 to 200 ng/g. The calibration curves provided high correlation coefficient ( $r^2 > 0.99$ ). The accuracy (recovery) was conducted by spiking blank chicken liver at three levels of 50, 100, and 200 ng/g. The accuracy (recovery) was 86.39% and 99.74% for ciprofloxacin and enrofloxacin, respectively. Limit of detection (LOD) and limit of quantitation (LOQ) were conducted by spiking 10 different blank samples with 100 µg/kg level for each analyte. The LOD of the method for each sample were calculated using the equation follows:  $LOD = 3 \times SD/s$ , and  $LOQ = 10 \times SD/s$ , where  $s$  and  $SD$  were the slope and standard deviation of the y-intercept of three individual calibration curves. The LODs were 3.62 ppb and 4.17 ppb for ciprofloxacin and enrofloxacin, respectively. Whereas the LOQs were 5.64 and 5.87 ng/g for ciprofloxacin and enrofloxacin, respectively.



**Figure 1.** Chromatogram separation of ciprofloxacin (0.09 min) and enrofloxacin (11.07 min) in a positive sample

## RESULTS AND DISCUSSION

### The use of antibiotics by farmers

Chicken livers are widely consumed throughout the world. However, overuse or incorrect application of antimicrobials in animal production has led to the development of antimicrobial resistance and antibiotic residue

persistence in animal tissues including the liver which can harm human health. In early 2018, the use of antibiotics as an antibiotics growth promoter (AGP) has been banned in Indonesia by the Ministry of Agriculture of the Republic of Indonesia as regulated in 14/Permentan/PK.350/2017 regarding the classification of veterinary drugs.

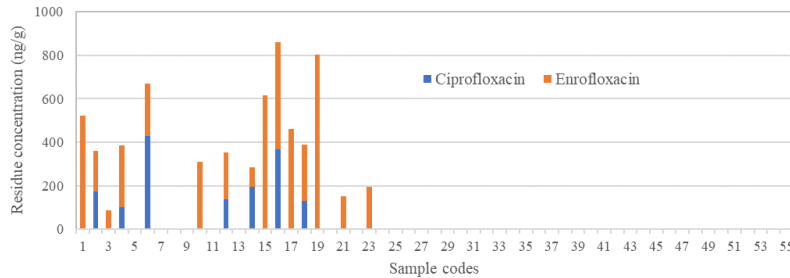
Even though enrofloxacin and/or ciprofloxacin are not used as a growth promoter, some farmers used these antibiotics to maintain animal health, as they told when the interview was conducted during sample collection. It was revealed that the most widely used antibiotics were amoxicillin (71.43%) and enrofloxacin (53.57%) as shown in Table 1.

**Table 1.** Types of antibiotics used by 28 broiler farms in Districts of Malang and Blitar

Antibiotics	n (%) of antibiotic user
Amoxicillin	20 (71.43%)
Enrofloxacin	15 (53.57%)
Sulfadiazine	12 (44.64%)
Tylosin + colistin	6 (21.43%)
Oxytetracycline + neomycine sulfate	4 (14.28%)
Erythromycin or Ciprofloxacin + sulfadiazine	2 (7.14%)
Ampicillin or Gentamycin or Streptomycin or Tetracycline	1 (3.57%)

### Ciprofloxacin and enrofloxacin in liver samples

Antibiotics of fluoroquinolones (including enrofloxacin) contain both carboxylic acidic and basic amino groups and are therefore amphoteric, having pK values of 5.5-9.3, so that at physiological pH they exist as zwitterions, at pHs of most body fluids mainly un-ionized. The drugs are most lipophilic and close to blood pH. Enrofloxacin is metabolized to ciprofloxacin by a de-ethylation reaction in the liver to a microbiologically active metabolite (Lees & Toutain 2012). Residues are measured as the sum of enrofloxacin and ciprofloxacin.



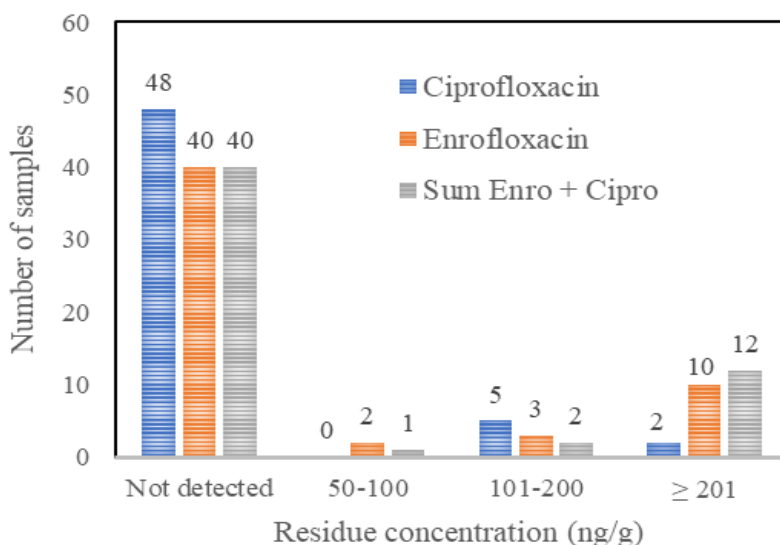
**Figure 2.** Ciprofloxacin and enrofloxacin residues in broiler liver samples analysed from District of Malang (sample codes: 1-42) and District of Blitar (sample codes: 43-55)

Enrofloxacin and its metabolite, ciprofloxacin analyzed using an HPLC were present in some of 55 samples collected from farmers in the District of Malang (42 samples) and Blitar (13 samples) in 2017 (before the prohibition on the use of AGP in Indonesia) as shown in Figure 2 and 3. The results show that all positive samples were detected from the District of Malang, but none from the District of Blitar. Forty samples did not contain both ciprofloxacin and enrofloxacin residues, 7 samples were detected for ciprofloxacin and enrofloxacin residue at the total concentration of 286.47-859.11 ng/g, 15 samples were detected for enrofloxacin residue in the range of 88.04-801.82 ng/g, 7 samples were detected for ciprofloxacin residue in the range of 100.47-430.03 ng/g, and the highest concentration was found in sample no 16 (859.11 ng/g). Total enrofloxacin (sum of enrofloxacin and ciprofloxacin) from 55 samples from Districts of Malang and Blitar was found in 15 samples with a concentration of 88.04-859.11 ng/g and 12 among those samples exceeded 200 ng/g.

The study indicates that the use of antibiotics in an area with a high population of broiler farms (District of Malang) is more intensive than in an area with less population of broiler farms (District of Blitar). This study also reveals that the metabolite of ciprofloxacin occurred less than its parent (enrofloxacin) which is due to intensively use of enrofloxacin (Table 1) and also meant in accordance with the statement that enrofloxacin does not metabolize in a huge part to ciprofloxacin (Trouchon & Lefebvre 2016).

Figure 3 shows that all positive samples contained enrofloxacin residue. Ciprofloxacin and enrofloxacin residues were not found in 72.7% of samples, 1.8% of samples contained a sum of ciprofloxacin and enrofloxacin residue less than 100 ng/g, 3.6% of the samples contained a sum of ciprofloxacin and enrofloxacin residue between 101 to 200 ng/g and 12 (21.8%) of the samples contained some of the ciprofloxacin and enrofloxacin residues exceeded 200 ng/g. The overall mean concentration

of the residues in broiler's liver samples was 27.82 ng/g and 89.35 ng/g for ciprofloxacin and enrofloxacin, respectively, or 118 ng/g for a sum of enrofloxacin and ciprofloxacin. The results could not be concluded due to the absence of the MRL in Indonesia, but if referring to the MRL's of European Union countries, it was within the permissible limit, except for those which exceeded 200 ng/g and poses an alarming situation. The occurrence of enrofloxacin and ciprofloxacin residues in some samples in this research indicated that enrofloxacin was still used by some farmers especially in the District of Malang.



**Figure 3.** The levels of enrofloxacin and ciprofloxacin in broiler chicken liver samples (n=55)

The presence of drug residues in some samples might be due to the overuse or misuse of some farmers and lack knowledge on the proper withdrawal times of drugs as they admitted during the questionnaire was made and lacked authorities to perform regular monitoring antibiotics residues in animal food products, mainly because there was no regulated prohibition on the use of antibiotics at that time. Farmers did not know the content of commercially purchased feed due to unclear feed labeling and do not list nutritional information or ingredients including antibiotics added (Coyne et al. 2019). Therefore, withdrawal times and MRLs of drugs should be determined to prevent the formation of residues in the animal tissues.

The withdrawal time of ciprofloxacin was 23 days (Khan et al. 2015), therefore the maximum administration of this drug should be on day 17th if

the chicken is slaughtered at the age of 40 days. Verma et al. (2020) were also suggested the use of enrofloxacin appears to be overuse both for prophylactic and therapeutic purposes, not observing the withdrawal period, the nonexistence of restrictive legislation, or their inadequate enforcement. They observed 63/113 samples positive for enrofloxacin in the range of 28-284 ng/g and ciprofloxacin in 57/113 in a concentration range of 28-284 ng/g. Widiastuti (2008) found that enrofloxacin persisted in the liver of broiler chicken up to 168 hours after administration of 50 mg enrofloxacin/kg BW/day for 9 days. Whereas Wijayanti et al. (2015) showed that the same application dose of ciprofloxacin in broiler caused the high level of drug left in muscle and liver until seven days post-injection.

Our results are higher than Faten et al. (2016) who used HPLC and found that 86.7% of 15 chicken liver samples marketed in Egypt tested were positive for ciprofloxacin at concentrations level of 96.33 to 300.27 ng/g. Arslan-Acaröz & Sözbilir (2020) who analyzed 100 chicken livers marketed Afyonkarahisar, Turkey by LC-MS/MS method found that the enrofloxacin contamination was found in 39% of samples between 1.42 and 30.23 ng/g, and the ciprofloxacin contamination in 31% of samples between 1.25-6.92 ng/g, but none of those samples, exceed the MRL. However, our study was lower than the study conducted by Sultan (2014) revealed that the range concentration of enrofloxacin in the 30 liver samples examined was 10-10,690 ng/g with the mean concentration of 4,290 ng/g, and 17 (56.66%) among them exceeded the MRL of 200 ng/g.

The findings of our study are in line with other studies for that quinolone-analysis conducted by ELISA on 45 broiler liver from local shops of Jhang city in Pakistan and found 40 (88.8%) contained enrofloxacin residues and that 62% among those samples were above the MRL of 200 ng/g (Younus et al. 2017) or (Nizamlioglu & Aydın 2012) who evaluated a total of 50 chicken liver samples which analyzed by ELISA method and reported that 34% samples contained quinolones. Attari et al. (2014) also reported that in 85% of 20 chicken liver samples collected from the Northwestern part of Iran, the enrofloxacin residue analyzed by ELISA level ranged from 5.30 to 90.70 ng/g, with a mean concentration of 39.54 ng/g. Whereas Sarker et al. (2018) performed a monitoring study in Bangladesh by thin-layer chromatography (TLC) detection and found contamination levels in chicken liver samples of 57% and 83% for enrofloxacin and ciprofloxacin, respectively. Suprisingly, Tavaloki et al. (2015) showed that the percentage of contamination in winter (100% and mean concentration of 2.57 µg/kg) was higher than in summer (100% and mean concentration of 2.57 ng/g). In contrast, Meena et al. (2020) and did not find any ciprofloxacin residue in 60 samples analyzed.

In contrast, Meena et al. (2020) from India and Rahman et al. (2021) from Bangladesh did not found any ciprofloxacin and enrofloxacin residues in all samples analyzed. The results found may vary from lab to lab due to variations in the collection time of meat samples and withdrawal period followed by poultry farms.

## CONCLUSION

The study investigating the occurrence of antibiotics residues of enrofloxacin and ciprofloxacin in broiler liver samples from Districts of Malang and Blitar in 2017 showed that those antibiotics were found in some liver samples which belong to the District of Malang. The results are very useful for consumers and authorities responsible for law enforcement in applying strict regulations and to perform regular monitoring and establishing national MRL. It is also suggested the need to take proper actions and strategies of controlling the concentration levels to avoid the emergence of antibiotic resistance in consumers as well as in the environment.

## ACKNOWLEDGEMENT

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## AUTHOR CONTRIBUTIONS

Both authors were the main contributor.

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## Detection of Nitrite in Cleaned Edible Bird Nest from Sumatra Island

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### ABSTRACT

Edible bird nest (EBN) is a bird's nest commonly made from the saliva of a swiftlet from the species *Aerodramus fuciphagus*. As the largest exporting country, Indonesia must comply with the nitrite content standard set by China, which is 30 ppm. The maximum limit of nitrite content in EBN exported to China currently refers to the regulation of nitrite content in processed baby food because nitrite can cause poisoning and harm human health. This project aims to study and analyze the nitrite content in EBN from Sumatra as a reference and levels of nitrite policy in Indonesia. The number of samples was calculated proportionally from the data on the swiftlet houses using OpenEpi software. A total of 18 samples cleaned EBN from swiftlet houses were obtained from various regions of Sumatra Island. Samples were washed with water one time. Samples were tested with the spectrophotometric method at The Center for Diagnostic of Agricultural Quarantine. The results showed that from the total 18 samples cleaned EBN; five samples were higher than the nitrite content standard (30 ppm). The average nitrite in cleaned EBN is 30.1913 ppm. Swiftlet farmers should be managing swiftlet houses and apply good farming practices.

**Keywords:** Edible bird nest, Nitrite, Sumatra

### INTRODUCTION

Edible bird nest is made from the compressed saliva of swiftlet species, *Aerodramus fuciphagus*. Edible bird nest (EBN) needs about 35 days to complete one nest during the breeding season (Teh & Ma 2018).

Indonesia is currently the largest exporter and production country of edible bird nests globally (Hamzah Z et al. 2013; Looi & Omar 2016). The number of exports of EBN from Indonesia to China in 2015 was 14 tons. Subsequently, this number increased to 22 tons in 2016 and 52 tons in 2017. The conversion value of EBN exports to China totalled US\$87.4 million or equivalent to Rp1.18 trillion. (Gumilar 2018).

The high demand for EBN in the international market is due to the belief that the properties contained in EBN, especially that of EBN, have medicinal properties. (Amriani et al. 2019), as food or health drink for gastronomy (But et al. 2013), and its ability to boost the immune system (Chantakun & Benjakul 2020). Sialic acid in EBN has also known as an anti-influenza (Haghani et al. 2016) and anti-diarrhoea (Chua et al. 2014).

Edible bird nest from Indonesia has a high protein amount of 59.8-65.8%, and sialic acid as much as 10%. The potential quality of EBN from Indonesia is quite popular with foreign countries, so that the international market demand for EBN from Indonesia increases from year to year (Hamzah Z et al 2013). Variations in the composition and protein content of EBN are highly dependent on geographical, food sources and differences in the genus or gallus of swallows (Zukefli et al. 2017).

The swallow's nest industry is faced with various food safety for consumers demands, related to the quality of EBN and the fulfilment of nitrite level (Chan et al. 2013; Looi & Omar 2016). The maximum limit for nitrite levels in EBN exported to China is 30 ppm. The maximum limit for nitrite levels in EBN exported to China is 30 ppm. The maximum limit refers to the rules for nitrite levels in processed baby food. Therefore, it is crucial to study the nitrite levels in EBN because nitrite can cause poisoning and harm human health. Nitrite can be toxic and dangerous because it can cause methemoglobinemia conditions resulting in impaired oxygen flow, difficulties in breathing (Saputro et al. 2016) and an increased risk of colon cancer (Thorburn 2015).

## **MATERIALS AND METHODS**

### **Research location**

This study was conducted to determine the nitrite level in EBN by sampling swiftlet houses in various areas in Sumatra. There are six, eight and four samples from A, B, and C areas, respectively. Sampling was carried out from August 2020 to March 2021. A spectrophotometric method is used to test the nitrite content of EBN and carried out at the Center for Diagnostic of Agricultural Quarantine. Samples were washed at the EBN processing company in Tangerang Jakarta.

### **Sampling method**

This research is a cross-sectional study. A total of 18 samples of cleaned EBN were from swiftlet houses in various regions in Sumatra. Sampling is

taken randomly in groups (random cluster sampling) from A, B and C areas. The criteria for the EBN sample used were white EBN, measuring at least 6 grams per piece of the nest, ready to harvest, having a light to medium level of cleanliness and a weight of about 30-40 grams. The criteria for the swallow houses selected as the sampling location are the swiftlet houses located on the islands of Sumatra, which produce EBN regularly every month. Uncleaned EBN samples were washed with water at the EBN processing company for the washing process as declared in the washing process.

## **Data processing**

### ***Sample data processing***

Data on nitrite level in EBN was processed using Microsoft Excel and presented descriptively.

## **Nitrite measurement stage**

### ***Sample selection***

The criteria for EBN samples are white EBN, measuring at least 6 grams per piece of the nest, ready to harvest, has a light to moderate level of cleanliness and a weight of 30-40 grams. The uncleaned EBN was washed with water one time. The criteria for the swallow house selected as the sampling location is that the swallow house was located in various areas on Sumatra Island, which regularly produces EBN every month.

### ***Washing process***

Uncleaned EBN is pre-cleaned and scraped (approx.  $5 \pm 2$  seconds). Initial washing was for  $10 \pm 2$  seconds on each sample. The washing uses reverse osmosis (RO) water to remove dirt and soften (softening). The sample was dried in tissue paper and aerated for  $150 \pm 5$  minutes. The process of feather removal was conducted using tweezers made of stainless steel and brushed using a soft brush. The sample was rinsed using running water until the water touched the entire surface of the EBN with a time of  $15 \pm 2$  seconds. Samples were dried again for 24 hours (one night) and moulded. Then the sample was dried until completely dry (about 14 hours). Spectrophotometry methods tested samples of cleaned EBN weighing 0.5 grams.

## Nitrite examination

The materials used for this study were: solutions of 1 ppm nitrite; nitrite working standard; sulfanilamide; saturated sodium chloride (NaCl); N-(1-naphthyl) ethylene dihydrochloride (NED, Merck KGaA, Germany); saturated NaCl, and ion free water.

The tools used are: a set of UV-visible spectrophotometer, analytical balance, blender, ultrasonic digester, timer, test tube, measuring flask, beaker, funnel, Erlenmeyer, micropipette (10, 100, 1000 µl), icebox, refrigerator, freezer (-40 C), sterile tweezers, label paper, microtip/pipette tip (10, 100, 1000 µl), computer, stationery, gloves, hygienic sample plastic, and sterile disposable spatula.

### Stage of nitrite examination using spectrophotometry method

The first step is to determine the standard curve obtained from the dilution of the standard nitrite solution to 7 concentration levels, such as 0.0 µg.l-1, 0.2 µg.l-1, 0.3 µg.l-1, 0.4 µg.l-1, 0.5 µg.l-1, 0.6 µg.l-1, 0.7 µg.l-1, then the diluted standard solution was added with 0.6 ml of saturated sodium chloride (NaCl) and ionized water to reach 10 ml. After that, adding one ml of sulfanilamide. After 5 minutes, we added 1 ml of naphthyl ethylene diamine (NED).

The standard solution was allowed to settle for 15 minutes then put into a cuvette, and the absorbance spectrophotometer (UV-1800) was measured at a wavelength of 540 nm (Yusuf et al. 2020). The second step is 0.5 g of the blend and homogenized sample, then 40 ml of ion-free water is added until 50 ml. The next step is to add 3 ml of saturated sodium chloride (NaCl) solution. The mixed solution was heated in an ultrasonic digester at a temperature of 400C for 30 minutes. The hybrid solution was filtered using Whatman filter paper no. 41 (GE Healthcare, Germany). Transfer the supernatant into a 10 ml volumetric flask up to the mark of 10 ml, add 2.5 ml of sulfanilamide and after 5 minutes, add 2.5 ml of NED and homogenize. After 15 minutes, start measuring the absorption using a spectrophotometer at a wavelength of 540 nm (Yusuf et al. 2020). Furthermore, the concentration of nitrite level is calculated with the following formula:

$$\text{Nitrite level } (\mu\text{g}\cdot\text{g}^{-1}) = \frac{C \times V_{\text{solvent}}}{W}$$

Annotation:

C = the amount of nitrite in the sample is obtained from the calibrated curve (µgl<sup>-1</sup>)

V = sample solvent volume (ml)

W = sample weight (g)

## RESULTS AND DISCUSSION

The results showed five samples containing nitrite higher than the standard nitrite level of 30 ppm. All samples are from Sumatra, as shown in Figure 1. The average of nitrite in the cleaned EBN is 30.1913 ppm. Data on the nitrite in cleaned EBN are presented as shown in Table 1.



**Figure 1.** Map of Sumatra island

**Table 1.** Data on nitrite level in cleaned edible bird nest from Sumatra Island

Origin	Code of sample	Average of nitrite level in each area (ppm)	Total average of nitrite Level (ppm)	Median (ppm)	Standard deviation
Area A	00-08	51.7743			
Area B	09-12	11.9623	30.1913	15.4212	33.9257
Area C	13-18	14.3686			

Research on the nitrite content in EBN has been carried out today. The research results on white EBN in the Hong Kong Market (all samples imported from Indonesia, Malaysia, Thailand and Vietnam) showed variations in nitrite content between 0 ppm to 6430 ppm. The median value of nitrite content in white EBN from the four countries is 100 ppm (Chan et al. 2013). The test results for the nitrite level were higher than 30 ppm because the EBN sample was a cleaned EBN washed one time, and there was lower than previous research. The EBN that was washed one time did not significantly reduce the nitrite level. The average nitrite content of EBN one time cleaned from South Kalimantan was  $65.24 \pm 3.38$  ppm and decreased during the washing process. The average nitrite level of EBN cleaned in one time and two times cleaning was not significantly distinct. The most significant decrease in nitrite levels was obtained in EBN washed three times. The nitrite level of EBN, cleaned

three times, decreased to an average of  $30.87 \pm 2.11$  ppm (Susilo et al. 2016). Different washing frequencies give other nitrite reductions. In addition to cleaning the nest from feathers and dirt, washing can indirectly reduce the nitrite level of the EBN. The nitrite level in EBN is influenced by the length of time the EBN is exposed to water. The longer the EBN is exposed to water, the lower the nitrite level (Chan et al. 2013; Hamzah Z et al 2013). (Chan et al. 2013; Hamzah Z et al 2013). It is essential to know that nitrite on EBN is quickly reduced. EBN nitrite could be removed (98%) by soaking it in water (Chan et al. 2013). But it will reduce market interest for EBN.

Commonly, swiftlet constructs their nest in caves location, near the coastal region or tropical rainforest. The nest is built onto the smooth surface of the concave walls and located at least 2.5 m above the ground. Currently, urbanization has reduced nesting sites for swiftlets to seek out unoccupied buildings for new nesting sites. Dark, damp and cooling conditions in the building supported appropriate environmental conditions. Over the years, many activities have grown in swiftlet farming. Swiftlet houses imitate a cave-like environment with the purpose to provide nesting sites and attract swiftlet. Swiftlet farmers do not control the birds' movement, breeding or diets. Swiftlets can move freely to hunt for insects. The newly constructed swiftlet houses are primarily in rural or agricultural land (Chua & Zukefli 2016).

Nitrite contamination on EBN occurs from its habitat (Utomo et al. 2018). The environmental cleanliness of the swiftlet house is an important thing and has a strong correlation with the amount of nitrite in the EBN. Various studies have shown that the EBN can be contaminated with nitrite from the environment (Hamzah et al. 2013). Environmental conditions also influence nitrite levels in EBN. The environment affects mainly the floor of the swallow houses when there is a decay of organic material (Amriani et al. 2019). Generally, EBN exists swiftlet droplets that contain ammonia. The ammonia will be oxidized by oxygen to nitrite and then oxidized again to nitrate. The formation of nitrite in the swallow's nest results from a natural process of changing nitrogen in the swiftlet house environment (Chan et al. 2013). Nitrite in the EBN comes from the oxidation of swiftlet droplets or faeces in the air. The manure that remained in the EBN contains ammonia. Ammonia will be oxidized by oxygen to nitrite, then oxidized to nitrate (Amriani et al. 2019). Nitrite is formed naturally by the oxidation of sodium nitrate ( $\text{NaNO}_3$ ) by nitrogen in the air (Leonanda & Zolanda 2018; Yenil & Yemiş 2018). Nitrogen in the atmosphere in large quantities cannot be directly utilized by living things. Nitrogen must be converted into ammonia to nitrite and then nitrite to nitrate (Kiding et al. 2015). The high nitrite levels may also be due to anaerobic fermentation by specific bacteria in the presence of ammonia in EBN (Paydar

et al. 2013). Variations in nitrite are due to the variability of maintenance practices in the swiftlet house and harvest time in the EBN (Yusuf et al. 2020).

Nitrite has been used in processed foods since a long time ago, and its use is strictly limited to prevent poisoning in humans (Paydar et al. 2013). The positive effects of nitrite include colour development and a source of antioxidants to protect flavours from rancidity (Saputro et al. 2016). Nitrite was also used as an antimicrobial food additive, especially useful for killing *Clostridium botulinum* which produces the deadly botulism toxin. The properties of nitrite can support colour stability, enhance aroma, and inhibit the formation of oxidation products (Thorburn 2015). Nitrite is added to food as an additive and has been widely used in meat and meat products, smoked fish, cheese, fruit juices and mineral water. (Thorburn 2015; Saputro et al. 2016; Yenil & Yemiş 2018).

The use of very high nitrite can cause food poisoning. Large amounts of nitrite in humans can cause gastrointestinal disturbances, bloody diarrhoea and even death. Chronic poisoning from nitrites can cause generalized depression and headaches (Abdurriyai & Syamsinar 2019). Nitrite on food has been associated with methemoglobinemia in infants (Thorburn 2015). Methemoglobinemia is a condition where nitrite will bind to haemoglobin (Hb) in the blood and inhibit oxygen transport in the bloodstream (Rosita 2014; Amanati 2016). This condition will result in a lack of oxygen in the blood (hypoxia) and is characterized by blue skin (cyanosis), shortness of breath, vomiting and death (Samsuar et al. 2020). Patients with methemoglobinemia will have breathing difficulties due to an impaired oxygen respiration system. Nitrites react with strong acids in the stomach (Saputro et al. 2016). Nitrite can increase cancer risk factors due to the formation of N-nitroso compounds which are carcinogenic (Paydar et al. 2013). In addition, it will formated of the carcinogen N-nitrosamine as a high carcinogenic compound. N-Nitrosamines can cause colorectal cancer (Saputro et al. 2016). The maximum daily intake dose of nitrite is limited to 0-0.07 mg/kg body weight (Amriani et al. 2019). According to the Food and Agriculture Organization, the acceptable daily intake of nitrite in EBN is 30 µg/g (Chua & Zukefli 2016). The maximum limit for nitrite level in EBN exported to China has been set at 30 ppm (Keputusan Kepala Barantan 2013).

## CONCLUSION

The average nitrite in cleaned EBN from Sumatra is 30.1913 ppm. The cleaned EBN one time washing with running water did not significantly reduce the nitrite level. Cleanliness of the swiftlet house environment is essential and strongly correlates with the amount of nitrite in the EBN; various studies have shown that the EBN can be contaminated with nitrite from the

environment. Swiftlet farmers should be emphasized on swiftlet house management and apply good farming practices.

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### AUTHORS CONTRIBUTION

Widiyani P, Sudarwanto MB, Latif H and Lukman DW were contributed equally to this work.

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## Characteristics of Carcass and Total Microbials of Broiler Chicken Meat as the Impact of Zonation in Closed House Cages

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### ABSTRACT

This study aims to determine whether zonation closed house cages affect the carcass characteristics and shelf life of broiler chickens. Closed house enclosure of the Faculty of Animal Husbandry, Udayana University with an area of 120 × 12 m which was divided into 4 zonations, and the area of each zonation was 30 × 12 m. The research design used was a Completely Randomized Design with 4 cage zoning treatments, namely zonation 1, zonation 2, zonation 3, and zonation 4, with 30 replications. The research material was 120 heads of broiler. The variables observed were carcass characteristics and meat storage life. The results showed that cage zonation had a significant effect ( $P < 0.05$ ) on final weight, slaughter weight, carcass weight, carcass percentage, breast weight, and whole thigh weight, but had no significant effect ( $P > 0.05$ ) on wing cut, back and total microbial count of meat. In zone 3, the average final weight of 4-5% was significantly ( $P < 0.05$ ) higher than the final weight in other zones. Storage at room temperature (25°C) for 3-9 hours, broilers in zone 3 obtained the highest microbial growth in meat ( $P < 0.05$ ). The conclusion of this study was that the zoning of the cage in the closed house causes differences in the parameters of the broiler carcass characteristics. Broilers in cages 3 zoned had the best carcass characteristics, the highest total microbial count of meat, and the lowest meat storage capacity.

**Keywords:** Zonation, Broilers, Carcass characteristics, Total microbe of meat

### INTRODUCTION

The application of a cage with a closed house system is one way to increase broiler production. Closed house cages are closed cages equipped with feed, drinking, lighting, heating/brooder systems, exhaust fans, cooling pads, sensors, electrical panels, and curtains. In the Closed house type cage, there are 4 maintenance areas or zoning, where in zone 1 it is close to the air entrance (inlet) which has a lower temperature compared to the other 3 zones. Zone 4 is the maintenance area closest to the air outlet (exhaust fan). This causes differences in temperature, humidity, and ammonia levels in the closed

house, which in turn can affect broiler productivity (Renata et al. 2018). Broilers that are farther away from the air entrance (inlet) are found to have a decreasing red blood profile (Brilianto et al. 2019).

Closed house cages can reduce mortality, morbidity and accelerate growth so that it can affect the income level of farmers. The basic principle of closed house cages is to prevent environmental influences, such as temperature and excessive sunlight intensity entering the cage. The closed house cage system can also minimize the spread of disease from outside into the cage and can record data on changes in temperature inside the cage in real-time (Adnyana et al. 2020; Putra et al. 2019). It is very important to pay attention to the density of broilers in the cage, because a high number of broilers in the cage will have an impact on their activities and production.

The density of broilers in the cage has an effect on pH, water holding capacity and has no effect on tenderness and cooking loss of meat (Fausiah et al. 2019). Factors before broiler slaughter (ante mortem) including maintenance and feed management can affect the quality of results after slaughter, such as carcass and broiler meat quality (Soeparno 2011). Carcass and meat quality that is not good and followed by poor handling, will have an impact on the growth of destructive microbes in broiler meat (Hajrawati et al. 2016). Based on the description above, a study was carried out with the title "Carcass characteristics and total microbial count of broiler chicken meat as the impact of zoning in closed house cages".

## MATERIALS AND METHODS

### Research material

The research material consisted of (a) One day old CP Ross Platinum strain broiler (DOC) weighing  $44.7 \text{ g} \pm 1.13$ ; (b) Complete commercial ration in the form of crumble (BR0/S0) for 1-7 days old chicken (pre-starter); (c) BR-1/S11 commercial ration for 8-21 days old chicken (starter); (d) BR-2/S12 commercial ration for 22 days old chicken (finisher); and (e) Drinking water sourced from bore well water in the cage area. The nutritional content of the ration is shown in Table 1.

**Table 1.** The nutritional content of the ration used\*)

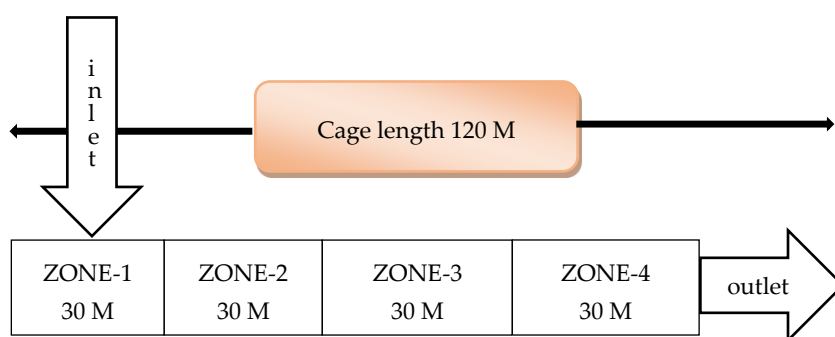
Nutrition		Nutrient content (%)		
		BRO	BR1	BR2
Water content	Max	14.00	14.00	14.00
Crude protein	Min	22.00	20.00	19.00
Crude Fat	Min	5.00	5.00	5.00

Nutrition		Nutrient content (%)		
		BRO	BR1	BR2
Crude fiber	Max	4.00	5.00	6.00
Ash	Max	8.00	8.00	8.00
Ca	-	0.80-1.10	0.80-1.10	0.80-1.10
P	-	0.50	0.50	0.45
Lysine	Min	1.30	1.30	1.05
Metionine	Min	0.50	0.50	0.40
Metionine+Cystine	Min	0.90	0.90	0.75
Tryptophan	Min	0.20	0.20	0.18
Threonine	Min	0.80	0.80	0.85

Source: \*) Produced by PT Carroen Pokphand Indonesia Tbk.

## Cages and equipment

The cage used was 1 unit of closed house cage complete with automatic facilities and infrastructure. The cage area was 120 × 12 m divided into 4 zones/maintenance areas, with the length of each zone was 30 m (Figure 1). The provision of feed and drinking water, as well as vitamins, were carried out automatically/mechanically. The temperature in the cage was regulated by a system/thermoregulator that can regulate the fan/exhouse fan automatically according to the temperature required by the DOC/broiler chickens in the cage.



**Figure 1.** Floor plan of the closed house at the Faculty of Animal Husbandry, University of Udayana

## Research design

This study used a completely randomized design (CRD) with 4 treatment areas of maintenance/zoning (Figure 1). Each treatment consisted of 30 broilers as replicates, and all samples were marked according to zoning.

Research variables: final weight, cut weight, carcass percentage, carcass cut weight (breast, wings, back, and whole thighs), and microbial count during storage. The results of the study were analyzed using analysis of variance with the SPSS-1.7 system software.

## RESULTS AND DISCUSSION

### Carcass characteristics

One way anova analysis (Table 2), showed that there were significant differences ( $P < 0.05$ ) in final weight (FW), slaughter weight (SW), carcass weight (CW), % carcass, breast, and thigh pieces intact (TI) as a result of the maintenance/zoning area in the closed house enclosure. Zoning was not significant effect ( $P > 0.05$ ) on the back and wing pieces. The broiler closest to the air inlet had the lowest FW, SW, CW, and breast piece ( $P < 0.05$ ). This was caused by the humidity in the Z1 above 80%. High humidity causes the litter to get wetter, which in turn can speed up the fermentation process. This is in accordance with the opinion of Renata et al. (2018) which states that wet litter will accelerate the production of  $\text{NH}_3$ . Hidayat et al. (2020) and Brilianto et al. (2019) stated that the increase in microclimatic ammonia in the cage can reduce the appearance before slaughter (*ante mortem*) of broilers and also have an impact on all parameters after slaughter (*post mortem*). High levels of ammonia cause oxidative stress and irritation of the respiratory tract, then there will be disruption of nutrient absorption by the intestinal mucosa of broilers. This condition will have an impact on increasing daily body weight.

**Table 2.** Carcass characteristics of broiler chickens as impact of retention area/zoning in closed house cages

Zonation (Z)	Parameters							
	FW	SW	CW	C	Breast	IT	Wing	Back
Z-1	2000 <sup>b</sup>	1990 <sup>b</sup>	1500 <sup>b</sup>	75 <sup>b</sup>	583 <sup>b</sup>	447 <sup>b</sup>	150 <sup>a</sup>	320 <sup>a</sup>
Z-2	2090 <sup>a</sup>	2050 <sup>a</sup>	1550 <sup>a</sup>	76 <sup>a</sup>	595 <sup>b</sup>	478 <sup>a</sup>	152 <sup>a</sup>	325 <sup>a</sup>
Z-3	2100 <sup>a</sup>	2080 <sup>a</sup>	1600 <sup>a</sup>	77 <sup>a</sup>	628 <sup>a</sup>	488 <sup>a</sup>	150 <sup>a</sup>	334 <sup>a</sup>
Z-4	2040 <sup>b</sup>	2070 <sup>b</sup>	1523 <sup>b</sup>	74 <sup>b</sup>	588 <sup>b</sup>	452 <sup>b</sup>	155 <sup>a</sup>	328 <sup>a</sup>
SEM	0.44	0.41	0.44	0.01	0.45	0.43	0.45	0.35

FW: final weight (g); SW: slaughter weight (g); CW: carcass weight (g); C: carcass percentage (%); IT: intact thigh (g). Wing (g); Back (g). The same superscript in the same column shows a non-significant difference ( $P > 0.05$ )

The carcass characteristic data obtained were better than that informed by (Pakage et al. 2020) with the achievement of final weight (FW): 1.99 kg and

performance index (PI): 336. According to (Setiawan & Adisti 2018; Putra et al. 2019) the final weight of the broiler was (1567-1614) g. The final weight data in this study is lower than the results of Hidayat et al. (2020) research, namely: 2226,67 g in the zone near the inlet. This may be due to the closed house of the Faculty of Animal Husbandry in the lowlands with hot weather. Prasetyo et al. (2021) stated that to get the performance and quality of broiler meat, the location of closed house farms must be carried out on the higher ground. The zoning of the cage had no significant effect on the back and wing pieces of broilers ( $P < 0.05$ ). This was because on the wings and back there is no accumulation of reserve energy in the body (Soeparno 2009).

### Total microbial count

Healthy broiler meat before slaughter is basically sterile or contains only very few levels of microorganisms, but after slaughter, these tissues begin to be contaminated by microbes from the surrounding environment (Komariah et al. 2004). The effect of cage zoning (Z) on total microbial/total plate count (TPC) in meat and stored at room temperature 25°C for 9 hours are shown in Table 3. Zoning had no significant ( $P < 0.05$ ) effect on the TPC at 0 hours of storage. With the increase in storage time to 3, 6, and 9 hours the zoning effect became real ( $P < 0.05$ ) on the TPC during 6 hours of storage and TPC was still on the safe threshold for consumption. The Standard Nasional Indonesia (2009), stipulates that the biological quality requirement for the TPC of broiler chicken is a maximum of  $1 \times 10^6$  CFU/g. This was similar to that reported by (Hajrawati et al. 2016) who get TPC on broiler chicken in the market:  $2.9 \times 10^5$  to  $8.19 \times 10^6$  CFU/g. Antari et al. (2017) also reported that the total microbial count in meat in the Denpasar city market was  $2.22 \times 10^4$  CFU/g.

**Table 3.** The number of broiler meat microbes (TPC/Log CFU/g ) in different zoning, for 9 hours of storage at room temperature (25°C)

Zonation (Z)	Storage time (hours)/TPC/log CFU/g			
	0	3	6	9
Z1	$8.3 \times 10^2^a$	$2.7 \times 10^3^b$	$5.3 \times 10^5^b$	$1.4 \times 10^6^b$
Z2	$1.2 \times 10^3^a$	$4.0 \times 10^3,4^b$	$2.6 \times 10^4^b$	$1.1 \times 10^6^b$
Z3	$1.8 \times 10^3,5^a$	$2.9 \times 10^5^a$	$4.5 \times 10^6^a$	$9.0 \times 10^7^a$
Z4	$1.4 \times 10^3^a$	$4.1 \times 10^4^b$	$7.2 \times 10^5,4^b$	$1.7 \times 10^6^b$
SEM	0.03	0.04	0.02	0.05

*The same superscript in the same column shows a non-significant difference ( $P > 0.05$ )*

Broiler chicken meat at Z3 with 9 hours of real storage had the highest TPC ( $P < 0.05$ ) and exceeded the recommended SNI-2009. This may be because broiler chickens at Z3 have the highest temperature (30-31)°C and the lowest air humidity (60%). This condition causes broiler chickens to experience heat stress. Prolonged stress before slaughter will have an impact on meat quality (Soeparno 2009). It was further explained that low Water Holding Capacity (WHC) of meat and high cooking loss would increase microbial contamination (TPC), and meat would spoil quickly.

The total bacteria in the meat will be safe for consumption if it is cooked at a high temperature, because the bacteria cannot tolerate heat (Hajrawati et al. 2016).

## **CONCLUSION**

Zoning in a closed house at the Faculty of Animal Husbandry, Udayana University causes differences in the parameters of the carcass characteristics of broiler chickens. Broiler chickens in cage zoning 3 (Z3) have the best carcass characteristics, but the highest total microbial count (TPC) and the lowest shelf life of meat.

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## **AUTHOR CONTRIBUTIONS**

Tirta Ariana IN: Conceived the study, contributed to the discussion and correction the final manuscript.

Sumerta Miwada IN: Contributed to the method and discussion.

Bulkaini: collected the data and the method and discussion.

Gde Suranjaya: contributed analyses the data.

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## **Determination of Trenbolone Acetate Hormone Residue on Imported Beef Meat and Imported Beef Liver at Slaughter House and Cold Storage**

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### **ABSTRACT**

The concentration of trenbolone acetate (TBA) hormone residues in samples were tested using enzyme linked immunosorbent assay (ELISA). Beef meat and beef liver samples used in the study were collected from slaughterhouses and cold storages in Jakarta, West Java and Banten. A total of 247 samples consisting of approximately 50-150 g fat-free portions of beef or beef liver were collected and transported to the laboratory in the shortest time possible. From the test results obtained some samples of both beef and beef liver containing residues of trenbolone acetate above the maximum residue limit (MRL) that has been set by CODEX (10 ppb for meat and 2 ppb for liver). In Jakarta area found one beef sample (3.4%) and two beef liver (9.5%) containing TBA above MRL. In Banten area, two samples (8.5%) of beef liver from slaughterhouse were found containing TBA residues above the MRL. Meanwhile, in West Java, only one sample (7.1%) of beef from slaughterhouse was found with the residual content of TBA above the MRL. From the results above can be seen almost all the results that showed TBA concentration above MRL obtained from slaughterhouse. To obtain quantitative test results confirm, a number of samples which give concentrations above the highest standard in the ELISA standard curve will then be tested using High Pressure Liquid Chromatography (HPLC). The results of this study indicate that the residual concentration of trenbolone acetate still need further attention for the discovery of several samples containing residues of hormones trenbolone acetate with a number that exceeds the maximum residue limits.

**Keywords:** Trenbolone acetate, TBA, ELISA, Growth promotor hormone

### **INTRODUCTION**

Chemical residues in food are an important food safety issue both in terms of consumer concern and food trade. Among all the food related problems, food safety is a major issue to tackle out at present. Food safety is basically an approach to control the hazards to the consumers through several safety measures (Irfan 2016).

Increased welfare and public awareness of the importance of consuming animal protein sources, also contributes to increasing the level of beef consumption in Indonesia. In 2019, the national demand for beef is estimated to be around 686,271 tons, assuming a consumption of 2.56 kg/capita/year. The availability of beef based on domestic production is 404,590 tons, which is produced from 2.25 million slaughtered cows. Based on these data, an additional 281,681 tons are still needed which are met through imports of beef (92 thousand tons), buffalo meat (100 thousand tons), and feeder cattle (500 thousand heads, equivalent to 99,980 tons of meat) (Ternak 2014). The shortage of beef stock is due to the imbalance between domestic demand and supply of beef. The livestock population that produces meat for consumption may not be able to meet all the needs of beef in Indonesia. Low productivity and animal health are one of the reasons for the low population of livestock producing meat. Import policies of both feeder cattle and frozen beef from several countries that still allow the use of several growth-stimulating hormones need attention. The importation of frozen beef without supervision will have a detrimental impact on public health. This loss occurs when the amount exceeds the threshold so that the animal product becomes unsafe for consumption. In several countries that are the destination for importing including Australia, New Zealand, USA and Canada, the use of growth stimulating hormones is still allowed (Danial et al. 2016).

Growth stimulating hormone residues can be found in animal products if the use of the drug is not in accordance with the instructions given, for example the stopping period of a drug that is not obeyed before slaughtering the animal. The use of growth-promoting hormones in Indonesia including Trenbolone Acetate, Melengestrol Acetate and Zeranol in livestock has been prohibited since 1983. Government Policy regarding Hormone Residues: based on the Decree of the Minister of Agriculture Number 806 of 1994; The Circular of the Director of Animal Health Number 328 / XII-C dated 4 October 1983 and the results of the meeting of the Indonesian Veterinary Drug Commission on 12 August 1998 confirmed that growth stimulating hormones are not permitted for use in production animals for consumption. This is also reinforced by the existence of Government Regulation No. 22 of 1983 which essentially protects public health from hazards that can interfere with health due to consuming animal-derived food ingredients containing drug residues. Another thing that supports the prohibition is the inadequate level of education and knowledge and awareness of breeders who use growth-promoting hormones to comply with the withdrawal time provisions before the livestock is slaughtered.

This study aims to determine the presence of TBA residue in imported beef and beef liver. The expected benefit is that it can be used as consideration

in drafting regulatory policies for imported cattle from importing countries that still allowed use the growth promoting hormones.

## **MATERIALS AND METHODS**

### **Materials**

Samples were imported beef and liver (n = 247) in a number of slaughterhouses and cold storage in the Jabodetabek, Banten and Sumatra areas. The total sample taken was divided into 4 sample groups, namely beef from RPH-R (n = 90), beef from cold storage (n = 62), beef liver from RPH-R (n = 90) and beef liver from cold storage (n = 5).

The materials used include methanol pa, methanol 40%, methanol 80%, tert-butylmetileter pa, phosphate buffer saline (PBS), buffered Na-acetate, distilled water, and Ridascreen Trenbolon ELISA kit (negative and positive control of trenbolone acetate hormone. and its residues (17 $\beta$ -trenbolon), washing solution, conjugate, substrate solution, stopping solution).

### **Methods**

The concentration of trenbolone acetate hormone residues in the samples was tested using the Enzyme Linked Immunosorbent Assay (ELISA) method. Reagents and samples are conditioned at room temperature before being used/tested. The test stages are adjusted according to the procedure included with the ELISA kit. Trenbolone acetate residual hormone concentrations were calculated using Ridawin software based on a standard curve that had been prepared.

### **Sample preparation**

Homogenize 10 g of ground sample with 10 ml of 67 mM PBS buffer and shake for 5 min. Mix 2 g of the homogenized sample with 5 ml of tert-Butyl methyl ether in a centrifugal screw cap vial and shake vigorously for 30 – 60 min and centrifuge 10 min 3000g 10 – 15°C. Transfer the supernatant into another glass vial with screw cap. Repeat the extraction procedure with another 5 ml of tert-Butyl methyl ether. Evaporate the combined ether layers and then dissolve in 1 ml of methanol (80%). Dilute the methanolic solution with 2 ml of 20 mM PBS. Rinse the column with 3 ml MeOH (100%) and equilibrate the column with 2 ml 20 mM PBS. Ensure that all liquid is removed from the column by pressing air or N<sub>2</sub> through the column. Elute slowly with 1 ml MeOH (80%), flow rate: 15 drops per min. Dilute eluate 1:2 (1 + 1) with distilled wate. Use 50  $\mu$ l per well in the test.

## **Assay protocol**

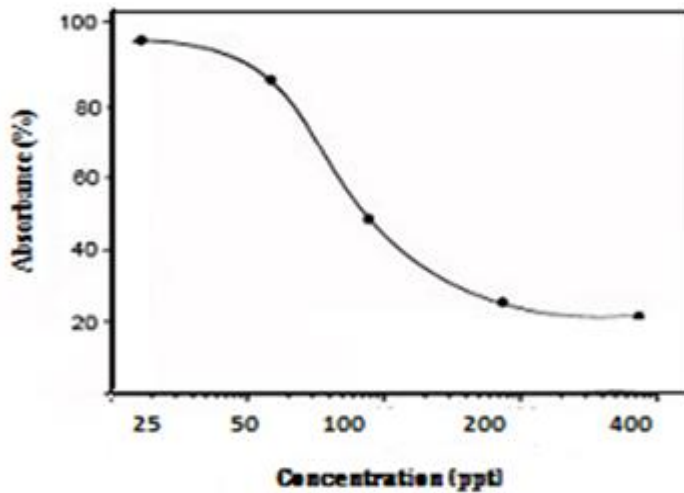
All standards and samples should be simultaneously tested in duplicate. Pipette 100 µl of zero standard in duplicate. Pipette 50 µl of zero standard in duplicate. Pipette 50 µl of standard solution in duplicate. Pipette 50 µl of each sample solution in duplicate to the remaining wells of the microtiter plate. Pipette 25 µl of conjugate (Trenbolone-HRP) to all wells, except wells H1 and H2. Pipette 25 µl antibody solution to all wells, except wells H1 and H2. Seal the microtiter plate and shake the plate for a few seconds. Incubate for 1 hour in the dark at 20 - 25°C. Discard the solution from the microtiter plate and wash 3 times with rinsing buffer. Pipette 100 µl of substrate solution into each well. Incubate 30 minutes in the dark at 20 - 25°C. Pipette 100 µl of stop solution to each well. Read the absorbance values immediately at 450 nm.

## **Interpretation of results**

The O.D. values of the six standards and the samples (mean values of the duplicates) are divided by the mean O.D. value of the zero standard and multiplied by 100. The zero standard is thus made equal to 100% (maximal absorbance) and the other O.D. values are quoted in percentages of the maximal absorbance.

## **RESULTS AND DISCUSSION**

The calculation of the gained results was made by RIDAWIN software. For construction of the calibration curve the mean of the absorbance values obtained for the standards was divided by the absorbance value of the first standard (zero standards) and multiplied by 100. The absorption is inversely proportional to the concentration of trenbolone. The amount of trenbolone in the samples is expressed as trenbolone equivalents. The Trenbolone equivalents in the sample (ng/ml) corresponding to the % maximal absorbance of each sample can be read from the calibration curve. As can be seen in Fig.I, the trenbolone calibration curve was found to be virtually linear in the 25 to 400 ppt.



**Figure 1.** Calibration curve of trenbolone standards (25 - 100 ppt)

All of the cattle taken as samples in the slaughterhouses are imported cattle that were previously fattened in the feedlot. From the test results, several samples of both from beef and beef liver contained TBA residues above the maximum residue limit (MRL) as shown in Table 1.

From the test results, it was found that several samples of both beef and beef liver contained TBA residue above the Maximum Residue Limit (BMR). Maximum Residue Limit (MRL) was determined by CODEX, namely 2 ppb for meat and 10 ppb for liver (FAO/WHO 2018). In Jakarta area found one beef sample (3,4%) and two beef liver (9,5%) containing TBA above MRL. In Banten area, two samples (8.5%) of beef liver from slaughterhouse were found containing TBA residues above the MRL. Meanwhile, in West Java, only one sample (7.1%) of beef from slaughterhouse was found with the residual content of TBA above the MRL. From the results above can be seen almost all the results that showed TBA concentration above MRL obtained from slaughterhouse. With this result, we must also be aware of imported feeder cows that will be slaughtered at the slaughterhouse. Feedlotters should be more vigilant about the withdrawal time of drug. To obtain confirmatory quantitative test results, a number of samples that provide concentrations above the highest standard on the ELISA standard curve will then be tested using High Performance Liquid Chromatography (HPLC). The content of drug residues that exceeds the maximum residue limit set can cause animal products to be unsafe for consumption because they can cause allergic reactions, poisoning and carcinogenic (A)(Okocha RC, Olatoye IO 2018).

**Table 1.** Trenbolone acetate concentration on beef and beef liver

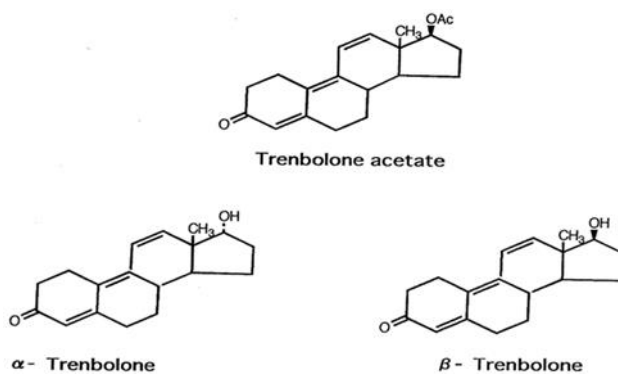
District	Sample Location	Sample Type	Number of Sample (n)	Trenbolone Acetate Concentration (ppb)		Above MRL (%)
				Mean±SD	Min-Max	
Jakarta	Slaughterhouse	Beef	29	0.63±0.23	0.12-2.01	3.4
		Beef Liver	21	3.34±2.97	0.18-15.14	9.5
	Cold Storage	Beef	23	0.11±0.09	0.07-0.31	0
		Beef Liver	9	0.36±0.21	0.16-0.62	0
Banten	Slaughterhouse	Beef	25	0.43±0.96	0.19-1.32	0
		Beef Liver	27	3.28±3.21	0.21-13.24	8.5
	Cold Storage	Beef	29	0.32±0.19	0.10-0.72	0
		Beef Liver	11	4.05±3.62	0.26-11.01	0
West Java	Slaughterhouse	Beef	21	0.62±0.12	0.09-1.12	0
		Beef Liver	14	3.25±2.81	0.27-11.24	7.1
	Cold Storage	Beef	24	0.11±0.09	0.06-0.19	0
		Beef Liver	14	0.28±0.12	0.19-0.23	0
Total			247			

*The Maximum Residual Limit (MRL) value for Trenbolone Acetate is 2 ppb for meat and 10 ppb for liver (CODEX). The detection limit is 0.2 ppb, if the concentration is ≤ 0.2 ppb, the sample is categorized not detected.*

Hormones that are commonly used as growth promoters include zeranol, progesterone, estradiol benzoate, 3 estradiol, testosterone propionate, and trenbolone acetate. Trenbolone acetate (TBA) is a synthetic androgenic anabolic steroid hormone (Sang-Hee jeong and Myung-Woon Lim 2010). TBA was implanted in the ear of cattle for ± 60 days before slaughtering (Schneider et al. 2007). Beef fattening in Australia generally implants about 90% growth-promoting hormone. Implantation the right one results in a 5-15% increase in acceleration growth, improve feed conversion, muscle building, and reduce fat (Fritsche S, Solomon MB, Paroczay EW 2000).

Trenbolone acetate hormone (TBA) is a growth-promoting hormone that is implanted into cows to increase body weight and increase feed conversion efficiency. The impact of the excess TBA hormone in the body can cause delayed puberty due to inhibition of estrogen production, deviations and changes in behavior, sexual deviations, impaired liver and kidney function, loss of sexual appetite and the risk of developing degenerative diseases such as Parkinson's and Alzheimer's, spermatogenesis, oligospermia, testicular atrophy (A Passantino 2012) and carcinogenic (Yongo et al. 2016). Trenbolone

acetate is classified as a hard drug that is not permitted for registration and distribution, therefore the MRL for trenbolone acetate in animal foods is not established. The maximal residue limits according to the Codex Alimentarius Commission for TBA in muscles are 2 ppb and 10 ppb in the liver (FAO/WHO 2018). The European Economic Community (EEC) banned the use of anabolic compounds as growth accelerators in food animals (Nazli et al. 2005) while the United States Food and Drug Administration (USFDA) permitted the limited use of some hormones with natural origins (such as estradiol and testosterone) and some synthetic hormones such as trenbolone in animal husbandry (Passantino 2012).



**Figure 2.** Chemical structure of trenbolone (Donner 2015)

In recent years there is growing suspicions and evidence about a connection between the consumption of high amountsof red meat, especially processed meat products, with some important and common diseases such as diabetes, coronary heart disease, heart failure, stroke and cancer (Yongo et al. 2016). With these worries, the European Union Food Safety Agency (EFSA) has reported that all six types of hormones can cause endocrine, developmental, immunological, neurobiological, immunotoxic, genotoxic, and carcinogenic effects for the susceptible risk groups, and has forbidden the import of meat obtained from countries which do not regulate their meat productions accordingly (Donner 2015; Yongo et al. 2016).

ELISA is a method of testing hormone residue sensitive, accurate, relatively inexpensive, and easy to handle for routine testing (Mahgoub et al. 2006) . This method is an initial test (screening) to determine the residual content TBA for imported cattle ready for slaughter. ELISA can be used as a screening tool for hormone residue screening because of its high sensitivity and precision (Yongo et al. 2016). In addition, the advantages of ELISA include having a simple test method, more affordable, portable equipment and a

larger number of samples that can be tested so that the examination time is more efficient (Jiang & Zhang 2011). However, the use of ELISA is less specific and accurate because of the cross reaction with other related chemical substances (Oveisi et al. 2007). Therefore, further testing is needed on the positive results obtained. Confirmatory testing can be done by chromatographic and mass spectrometric tests. This test is useful for knowing the existence of a false positive value on the test being carried out.

Almost all samples of beef and beef liver from cold storage gave trenbolone acetate residue below the predetermined limit. This indicates that the country of origin of livestock has paid attention to aspects of veterinary public health by importing both beef and beef liver from cows that have passed the drug stopping period. Our test results are of important as they give information about the use of trenbolone acetate in national animal husbandry and in the food industry (Donovan 2015).

## CONCLUSION

The results of this study indicate that the residual concentration of the TBA hormone in both meat and liver samples, especially those from slaughterhouse, still needs more attention because there are still several samples containing TBA hormone residues in an amount that exceeds the predetermined maximum residue limit. Import policies in the form of live livestock and frozen meat from countries that still use growth-promoting hormones will increase the risk of products containing growth-stimulating hormone residues, which, if the amount exceeds the threshold, will make animal products unsafe for consumption.

Determination of trenbolone acetate hormone residues using the ELISA method is only screening, it is necessary to continue quantitative confirmation testing with the HPLC/LC-MS method to ensure the true residue concentration.

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# **Agricultural Social Economics and Policy**



## **Livestock Products' Price Behavior during Covid-19 Pandemic Era in Java, Indonesia**

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### **ABSTRACT**

The pandemic era of Covid-19 may affect decreasing willingness to pay people in Indonesia, especially for livestock products that consider high-income elastic such as beef, broiler meat, and eggs. A study was conducted to analyze price behaviour on livestock products before and during the Covid-19 pandemic to anticipate future price fluctuations. A total of 480 weekly consumer prices of beef, broiler meat, and eggs had been used in the study from July 2019 until December 2020. The data has been gathered from National Online Market Information System-Livestock by the Food Security Agency of the Ministry of Agriculture divided into two periods, *i.e.* before March 2020 as a period of non-pandemic and after March 2020 that considered as during pandemic era. Data from six provinces in Java were analyzed in the study. The data used were the prices of three main livestock products, namely beef, broiler meat, and eggs, and weekly data from six provinces in Java (DKI Jakarta, West Java, Central Java, DI Yogyakarta, East Java, and Banten). The results indicated that all provinces in Java were significantly affected by consumer price changes of beef, broiler meat, and eggs ( $P < 0.001$ ). The beef price, region, and time variables and their interactions were significantly affected by price changes ( $P < 0.001$ ). However, the price of eggs has been affected by the time variable ( $P < 0.05$ ), while that of their interaction was not affected significantly ( $P > 0.3$ ). The results indicated that consumers' beef price has the most affected due to the Covid-19 pandemic era in Java, while price eggs and broiler meat were not too affected by price changes.

**Keywords:** Price behavior, Livestock products, Covid-19 pandemic, Java

### **INTRODUCTION**

Nowadays, the global world, including Indonesia, is still facing the Covid-19 pandemic with complex consequences for almost all aspects of people's lives. Indonesia declared the Covid-19 pandemic a national disaster through Presidential Decree No. 12 of 2020 concerning the Determination of Non-Natural Disasters for the Spread of Corona Virus Disease 2019 (Covid-19). This is followed by Government Regulation No. 21 of 2020 concerning Large-Scale Social Restrictions in the context of accelerating control and

prevention of Corona Virus Disease (Covid-19) by March 31, 2020. Due to this regulation, people experience massive changes in attitudes and behaviour, especially in daily mobility in all aspects of life.

Households, in general, are affected by this pandemic due to beef, broiler meat, and eggs being considered regular products. Households have been and are still expected to experience an impact on their consumption ability due to the limitations of the community to do activities outside the home, thus affecting their purchasing power ability.

The first Covid-19 case was detected in Indonesia on March 2, 2020, in West Java and has spread to 34 provinces on April 9, 2020. As of February 21, 2021, positive confirmed cases in Indonesia have reached 1.28 million, of which 63.43% contributed from the cases of six provinces on the island of Java. Meanwhile, 93.53% of cases on the island of Java came from the provinces of DKI Jakarta, West Java, Central Java, and East Java, respectively. Confirmed cases continue, and it is difficult to predict when the outbreak will occur even though a vaccine has been found and has begun to be implemented. Indonesia has planned a vaccination target of 181.55 million with a temporary realization for the 1st and 2nd vaccinations of 1.23 million and 736.71 thousand, respectively (Satuan Tugas Penanganan Covid-19 2021). (Satuan Tugas Penanganan Covid-19 2021).

Per capita consumption of beef, broiler meat, and eggs in 2019 was 2.56 kg; 5.69 kg; and 6.74 kg, respectively (Pusdatin 2020a; 2020b; 2020c). Meanwhile, BPS (2020) stated that 56.24% of Indonesian people were 271 million, mostly in Java. The high contribution of animal protein livestock-based in Java is the risk of people's declining purchasing power due to the Covid-19 pandemic, which is feared to cause massive market distortion. On the other hand, conditions are also faced with food logistics, which causes production costs to be more expensive and leads to inflation due to rising food prices. As of December 2020, broiler eggs were considered a commodity that contributed to 0.10% of total national inflation of 2.25%. In terms of consumption, eggs and broiler meat contributed 0.12% and 0.10%, respectively to changes in the national household consumption index of 2.54% (BPS 2020).

Therefore, monitoring food prices are very relevant to avoid uncontrolled price fluctuations due to the Covid-19 pandemic. Specifically, through the Food Price Stabilization Team under the authority of the Ministry of Trade and the Ministry of Agriculture, the government carries out these duties and functions. Food price stabilization at the consumer level is carried out by the Ministry of Trade, while price stabilization at the producer level is carried out by the Ministry of Agriculture (Priyanti & Inounu 2018a).

Livestock-based animal protein is very important to meet people's balanced nutrition, and it is irreplaceable with other foods. Therefore, price behaviour in Java during the Covid-19 pandemic is important to know the pattern of change and anticipate more extreme fluctuations in different regions of Indonesia, leading to uncertainty and social turmoil. This will be important as a reference in considering strategic policies to evaluate policies during the Covid-19 pandemic crisis and the implications of further welcoming new adjustments to ensure the adequacy and affordability of livestock-based animal protein for the community.

## MATERIALS AND METHODS

The study has been carried out using secondary data from the National Online Market Information System-Livestock by the Food Security Agency of the Ministry of Agriculture. It consists of two periods, i.e. before March 2020 as non-pandemic and after March 2020. The data used were the prices of three main livestock products, namely beef, broiler meat, and eggs. Weekly data from six provinces in Java (DKI Jakarta, West Java, Central Java, DI Yogyakarta, East Java, and Banten) were gathered. Therefore, 480 observations were obtained and analyzed using the General Linear Model (GLM) procedure by Statistical Analysis System (SAS 9.0).

The behaviour of prices of livestock products considered essential staple food commodities has strong relevance to being studied, referring to the occurrence of the Covid-19 pandemic outbreak starting in early March 2020. In general, beef, broiler meat and eggs during the pandemic experienced various fluctuations in each region on the island of Java. Therefore, to determine the effect of region and time during the pandemic on its price behavior, the following model was developed in the analysis.

$$P_{kom} = a_0 + a_1 \text{Region} + a_2 \text{Time} + \text{Region} * \text{Time} \dots\dots\dots (1)$$

- $P_{kom}$  = Prices of livestock products (beef, broiler meat, and eggs) (Rp/kg)
- Region = Provinces in Java Island (DKI Jakarta, West Java, Central Java, D.I Yogyakarta, East Java, and Banten)
- Time = Dummy variable of the weekly period, before the Covid-19 pandemic (July 2019-March 2020) and during the Covid-19 pandemic (April-December 2020)
- Region\*Time = Interaction variables between regions and periods

Equation (1) regressed sequentially for each retail price of beef, broiler meat, and eggs, respectively.

## RESULTS AND DISCUSSION

### Price of animal food products

Nationally, the price of animal food products during the study period experienced an upward trend. Until June 2020, the price increase was still followed by an increase in the farmers' exchange rate (*NTP = Nilai Tukar Petani*-Farmer's Exchange Rate) of 1.69 in the livestock sub-sector. This was due to an increase in the price index received (*It*) by 1.77 percent, higher than the increase in the price index paid (*Ib*) by 0.07 percent. However, the increase of national *NTP* was not enough to be contributed from the region of Java, where East Java is the only province in Java that experienced positive *NTP* growth. Broiler meat and beef were commodities with the largest contribution to the increase of *It*. Meanwhile, based on the value of the agricultural business exchange rate (*NTUP = Nilai Tukar Usaha Pertanian*-Agricultural Farming Exchange Rate), the livestock subsector experienced an increase of 1.62 percent in June 2020. The livestock sub-sector was the only one that experienced positive changes among other agricultural sub-sectors. The increase was considered high above the *NTUP*'s national average of 0.08 percent (BPS 2020).

The price increases that were not followed by increases in *NTP* and *NTUP* in most areas of Java were an early indication that prices formed are not sufficient to impact farmers' welfare. High prices only occur at the consumer level, while farm gate prices are relatively low. This condition occurs in connection with the high demand for livestock products due to restricted community activities in Java, which directly affects the distribution flow of food logistics, which may affect a higher cost margin between prices at the farmer and consumer level.

Until the end of 2020, *NTP*'s livestock subsector was recorded at 98.08, or 5.17% lower than the average national level, or decreased by 0.75% from the previous period (YoY). Livestock is the only subsector with *NTP* below 100. That was caused by the rate of change in the price index received (*It*), which was 0.77% smaller than the price index paid (*Ib*). However, there was an increase in *It* as of December 2020 due to rising prices of various commodities in all components of the livestock sub-sector. The products with the largest *It* contribution were broiler meat and eggs. *NTUP* followed the increase in prices of various commodities as of December 2020, which increased 0.56% from the previous month (BPS 2020).

The decline in *NTP* and *NTUP* of the national livestock sub-sector until the end of 2020 was relatively high, contributed mainly by beef. As conveyed by Darwis et al. (2020), the decline in farmers' welfare occurred due to the high price of heifers, which are mostly imported due to the decreasing exchange

rate of the rupiah. Furthermore, it stated that a significant decrease in purchasing power occurred, increased operational costs due to the increase in the price of feed raw materials, and the obstruction of trade and logistics systems due to the lockdown policy in some areas of Australia.

Livestock products have income elasticity that continues to change dynamically. These changes generally tend to fluctuate more rapidly than those in industrial products. Ilham & Haryanto (2020) the decline in farmers' welfare occurred due to the high price of heifers, which are mostly imported due to the decreasing exchange rate of the rupiah. Furthermore, it stated that a significant decrease in purchasing power occurred, increased operational costs due to the increase in the price of feed raw materials, and the obstruction of trade and logistics systems due to the lockdown policy in some areas of Australia.

Livestock products have income elasticity that continues to change dynamically. These changes generally tend to fluctuate more rapidly than those in industrial products. Ilham & Haryanto (2020) have shown that livestock products generally have a higher level of income elasticity than those of crops products. This implies that livestock products are more sensitive to changes in income. Therefore, the pandemic period, which causes a decrease in people's income and purchasing power, should generally lead to a decrease in demand.

The dynamics of the price behaviour of livestock products in Java is illustrated by the Coefficient of Variance (CV), which shows relatively low price fluctuations during the pandemic; however, they vary between regions (Table 1). This may indicate the good performance of livestock products during the pandemic. Controlled prices of livestock products in the aggregate are a positive contribution to the food inflation rate. Prices of food products have an important side, as mentioned by Lakollo (2015). In addition to affecting the income and standard of living of farmers and rural residents, changes in the price of food products may also affect trade in other goods.

**Table 1.** Coefficient of Variance on average livestock products' in Java

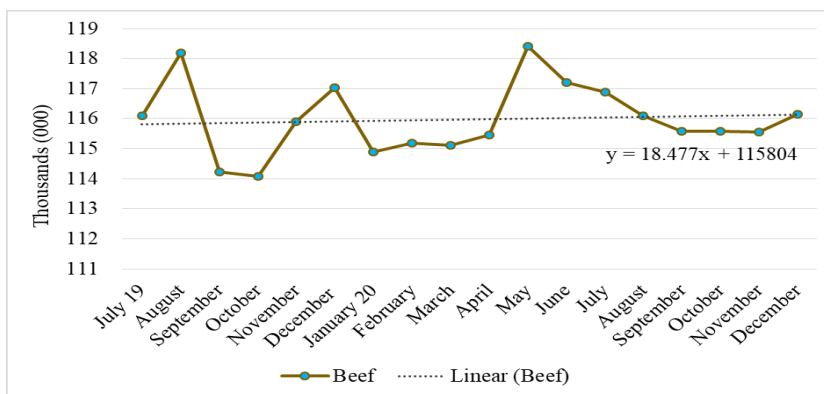
Province	Average coefficient of variance (%)		
	Beef	Broiler meat	Eggs
DKI Jakarta	2.19	7.04	6.25
West Java	1.53	8.19	7.19
Central Java	1.66	8.23	7.71
DI Yogyakarta	1.96	10.88	7.79
East Java	1.29	9.55	7.76
Banten	2.39	7.96	7.77
Mean of Java	1.84	8.64	7.41

*Source: Food Security Agency, 2021 (analyzed)*

The price of broiler meat and eggs had shown higher fluctuation than beef, where DKI Jakarta and Banten were the highest. The price of eggs is more stable across regions compared to that of broiler meat. DI Yogyakarta experienced high fluctuation in both prices of eggs and broiler meat. This may be caused due to social assistance received by the community, so that spending more on the needs of side dishes also increased, which was dominated by broiler meat and eggs (KRJogja 2020).

### Price of beef

Beef prices before the pandemic were 0.74% lower, with price fluctuations occurring even higher in the period before the Covid-19 pandemic. Although it caused an increase in the price of the imported heifer, on the one hand, restrictions on trade activities between countries during the pandemic have benefited domestic production so that domestic prices tend to be more controlled. The highest price occurred in May 2020 due to increased demand for the needs of Eid al-Fitr (Figure 1). Furthermore, prices continued to sag due to the tendency of public consumption to switch to broiler meat and eggs, either purchased independently or with basic food assistance from the government or the private sector. Not only from the decline in household consumption, but the price decline was also the result of the accumulation of the cessation of the hotel, restaurant, and tourism sectors, as reported by a decrease in GDP, providing accommodation and food and drink up to 11.46% in the third quarter of 2020 (Kementerian PPN/Bappenas 2020). However, the price disparity between regions for Java coverage is generally still relatively low, which can be interpreted from an average CV of 1.84%. Price fluctuations were the most dominant contribution from the 2019 period and ahead of Eid al-Fitr.



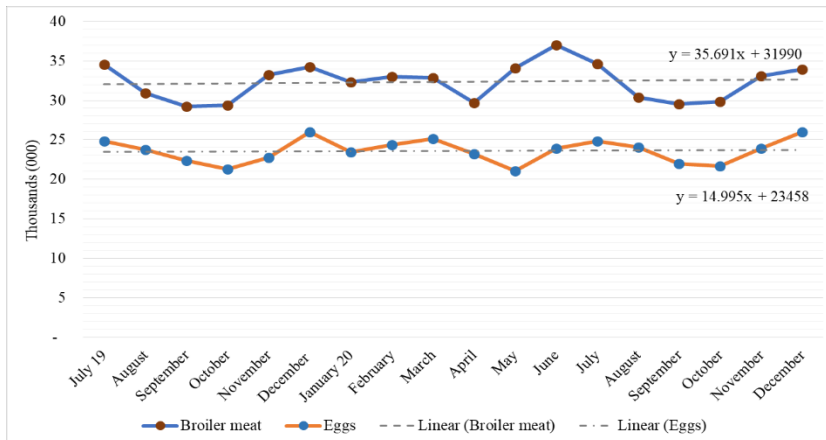
**Figure 1.** Beef price in Java (National Online Market Information System-Livestock by Food Security Agency 2021)

Wahyuni et al. (2016) reported that beef is a commodity with high income or expenditure elasticity and is very responsive to changes in income or expenditure. Beef also has a market segment, most of which are upper-middle class and urban economic groups. During a pandemic where households experience significant income pressure, it is natural that DKI Jakarta and Banten, as a reflection of urban society, experience the most noticeable price fluctuations compared to other provinces on Java Island, although they are still relatively low, indicated by the CV of 2.19% and 2.39%, respectively. Good price control on beef minimizes the pervasiveness of price increases on broiler meat and eggs, considering that both are substitutes for beef. Both are common animal protein sources for the lower-middle class because the prices are relatively more affordable. During the Covid-19 pandemic, the contribution of broiler meat and eggs became important in maintaining adequate protein intake for people's immunity.

### **Price of broiler meat and eggs**

Broiler meat is the commodity that experiences the most considerable price fluctuations in almost all provinces on the island of Java. The Covid-19 pandemic has disrupted the supply chain for DOC, feed, veterinary medicines, operational processes, distribution, and product marketing (Budastra 2020). Supply chain disruptions coupled with a decline in people's purchasing power leads to an imbalance of supply and demand, so price formation becomes unreasonable. One of the efforts made to respond to this is preparing a scheme for the absorption of live birds from farmers to be included in the distribution of Non-Cash Food Assistance (BPNT) and food packages by the Ministry of Trade through the assignment of SOEs (Kementerian Perdagangan 2020). These efforts were quite successful in bringing prices back in May. However, they subsequently experienced a significant increase in June as demand soared ahead of Idul Fitri and lasted until July.

The relatively high fluctuation of broiler meat prices during the January-July 2020 period (Figure 2) has disappeared since the end of 2019 due to oversupply production. However, until the pandemic, production did not decrease, so the absorption efforts that had been carried out previously again led to a decline at the farmer level due to a decrease in demand. To maintain people's purchasing power, the government has also implemented a fiscal stimulus of Rp. 405.1 trillion, of which Rp. 10 trillion has been allocated for social safety nets (Hirawan & Verselita 2020).



**Figure 2.** The Prices of Chicken Meat and Egg in Java (National Online Market Information System-Livestock by Food Security Agency 2021)

The behaviour of the price of eggs shows a similar pattern to that of broiler meat. However, the price of chicken eggs experienced the peak of the decline in prices in May, a month after the height of the reduction in the price of broiler meat. After that, the price of eggs continued to creep up, with a peak in July (Figure 2). The increase in the price of eggs was triggered by the rejection of laying hens at the farmer level in June 2020 because the price of live birds was high at that time, which resulted in a reduction in the production of eggs. The increased demand for chicken eggs for the distribution of social assistance for handling Covid-19 also contributed to the price increase. At the beginning of the pandemic, eggs were also the target of public panic buying, which in aggregate led to a significant increase in demand. The Ministry of Trade carried out the price stabilization step through coordination with the association of chicken egg breeders to carry out independent market operations for the public so that prices gradually returned to sloping effective since August 2020 (Tempo 2020). However, the price went up again towards the end of the year in line with high demand, followed by the impact of draining rejects in the previous period. The price increase is increasingly unavoidable, along with the increased price of imported feed during that period (Pusat Pengkajian dan Perdagangan Domestik 2020).

### **The influence of regions and time on the prices of animal food products during the Covid-19 pandemic period in Java**

The government has made various efforts to maintain market balance in production and consumption as the key to establishing reasonable prices to reduce fluctuations in animal food prices during the pandemic. Four main

factors determine the price of animal food products in the market, namely supply, demand, distribution system, and marketing chain (Priyanti & Inounu 2018). The primary strategy is to ensure that food stocks are available for up to 3-4 months and that the food logistics system runs smoothly. Given Indonesia's geography, the logistics system plays a vital role in ensuring the availability and affordability of food. Besides providing that the logistics infrastructure is well-available, incentives in the logistics sector are needed to encourage its implementation in the field.

In general, broiler meat is the most volatile livestock product and varies between regions. This indicates a significant difference between regions, as mentioned by (Wahyuni et al. 2016) that geographical area affects the proportion of expenditure on animal food, which means differences in consumption preferences. Some of the things that cause these differences in preferences are natural resources, culture, tastes, and income. Generally, urban households have a higher preference for beef compared to its substitutes. The same is true for consumption patterns over time. Changes from time to time are also determined by changes in income, public awareness of the importance of food and nutrition, and lifestyle changes (Ariani et al. 2018).

The effect of the region and time was further confirmed that both regions, time, and interaction had a significant effect on beef, broiler meat, and egg prices. These fluctuations also indicated the disparity of beef prices in Java with a 95% confidence level. Therefore, it cannot be simply concluded that the price of beef is more influenced by differences between regions but still depends on factors between times. This is different from what happened to broiler meat and eggs. In general, only regional variables had a significant effect on price disparities for each of these products in Java. In addition, the price disparity for eggs is also affected by the time variable, even at the 90% confidence level.

Observations on the effect of region and time on the pattern of price changes become information that carries other implications, namely the importance of knowing regional and time-specific food consumption patterns. Complete information on the performance of consumption patterns will greatly assist the policy-making process in maintaining food price stability under various scenarios. Through the Ministry of Agriculture, government continues to strive for food security in general by changing the budget posture to strengthen production, which is also expected directly to maintain and improve the welfare of farmers/breeders. In terms of financing, the People's Business Credit (KUR) is allocated for agriculture and livestock in 2020, up to Rp 50 trillion out of a total of Rp 190 trillion. This has received a positive response from farmers/breeders, indicated by the absorption as of mid-May 2020, which has reached Rp 17 trillion (Andri 2020).

**Table 2.** General linear model test of the influence of region, time and their interaction on consumer prices for animal food products in Java

Commodity	Sig. ( $\alpha = 5\%$ )				
	R-Square	Corrected model	Region	Time	Region*time
Beef	0.8605	0.0001	0.0001*	0.0007*	0.0001*
Broiler meat	0.3574	0.0001	0.0001*	0.3777	0.9519
Eggs	0.3345	0.0001	0.0001*	0.0515**	0.9725

Description: \*) significant at = 5%; \*\*) significant at = 10%)

Source: National Online Market Information System-Livestock by Food Security Agency, 2021 (analyzed)

The regional influence on the price disparity of animal food products can also be reviewed by comparing the subsets between regions as presented in Table 3. Regional variables influence the significant disparity in animal food product prices, as evidenced by the estimation results, mostly  $\leq 5\%$ . In beef, the price disparity was only not significantly different between West Java (2) and Banten (6), with an estimated result of 0.2069 ( $P > 0.05$ ). In broiler meat, there was no significant difference in price disparities between West Java-DI Yogyakarta, Central Java-DI Yogyakarta, Central Java-Banten, and DI Yogyakarta-Banten with each estimated value of 0.0551; 0.7738; 0.8410; and 0.9308 ( $P > 0.05$ ). West Java, Central Java, and Banten are producing regions that control 76.14% of broiler production in Java (Ditjen PKH 2020). The insignificant difference between these producing areas and DI Yogyakarta indicates that the broiler meat market between these regions is operationally better integrated so that changes in the producer area can be quickly recognized by the market and transformed into a balanced consumer price. DI Yogyakarta also influences this integration to play a dual role as both producer and consumer (Priyanti & Inounu 2018). The regional influence on the price disparity of eggs shows no significant difference between prices in DKI Jakarta-West Java, West Java-Banten, Central Java-DI Yogyakarta, and Central Java-East Java with each estimated value of 0.0727; 0.7610; 0.5923; and 0.1242 ( $P > 0.05$ ). Each of these subsets shows a similar pattern, namely the proximity of two areas that directly border. During the pandemic, many eggs were distributed for social assistance, which is thought to have influenced the clustering of distribution patterns in adjacent areas, which led to low price disparities.

**Table 3.** Estimation of the influence of regional factors on the disparity of animal food prices in Java

Beef	Region	1	2	3	4	5	6
	1		0.0001	0.0001	0.0001	0.0001	0.0001
	2	0.0001		0.0001	0.0001	0.0001	0.2069 <sup>ns</sup>
	3	0.0001	0.0001		0.0001	0.0001	0.0001
	4	0.0001	0.0001	0.0001		0.0001	0.0001
	5	0.0001	0.0001	0.0001	0.0001		0.0001
	6	0.0001	0.2069 <sup>ns</sup>	0.0001	0.0001	0.0001	
Broiler meat	Region	1	2	3	4	5	6
	1		0.0002	0.0001	0.0001	0.0001	0.0001
	2	0.0002		0.0275	0.0551 <sup>ns</sup>	0.0001	0.0450
	3	0.0001	0.0275		0.7738 <sup>ns</sup>	0.0046	0.8410 <sup>ns</sup>
	4	0.0001	0.0551 <sup>ns</sup>	0.7738 <sup>ns</sup>		0.0018	0.9308 <sup>ns</sup>
	5	0.0001	0.0001	0.0046	0.0018		0.0024
	6	0.0001	0.0450	0.8410 <sup>ns</sup>	0.9308 <sup>ns</sup>	0.0024	
Eggs	Region	1	2	3	4	5	6
	1		0.0727 <sup>ns</sup>	0.0001	0.0001	0.0001	0.0360
	2	0.0727 <sup>ns</sup>		0.0025	0.0004	0.0001	0.7610 <sup>ns</sup>
	3	0.0001	0.0025		0.5923 <sup>ns</sup>	0.0384	0.0065
	4	0.0001	0.0004	0.5923 <sup>ns</sup>		0.1242 <sup>ns</sup>	0.0012
	5	0.0001	0.0001	0.0384	0.1242 <sup>ns</sup>		0.0001
	6	0.0360	0.7610 <sup>ns</sup>	0.0065	0.0012	0.0001	

Description: ns (non-significant); = 5%; 1 (DKI Jakarta); 2 (West Java); 3 (Central Java); 4 (DI Yogyakarta); 5 (East Java); 6 (Banten)

Source: National Online Market Information System-Livestock by Food Security Agency, 2021 (analyzed)

The characteristics of the region's influence on prices can be interpreted from the grouping of Table 4, where East Java showed the lowest average price. East Java represents a beef producer, so that before and during the Covid-19 pandemic, supply in the retail market did not experience significant obstacles due to logistical limitations. The highest average price of beef has consistently occurred in DI Yogyakarta both before and after the Covid-19 pandemic. Culturally, the people of Yogyakarta prefer local beef for daily consumption. Meanwhile, imported meat or frozen meat is used more for consumption in hotels and fast-food restaurants. Continuing with the pandemic with imports closed, beef prices remain high even though consumption for hotels and fast-food restaurants tends to decrease.

**Table 4.** LS means the price of animal food products by the interaction effect of region and time in Java

Variable		Least Square Mean (Rp)		
Region	Time	Beef	Broiler meat	Eggs
DKI Jakarta	1	118,908	34,346	24,716
	2	121,351	34,727	24,482
West Java	1	115,080	32,784	24,128
	2	115,913	32,972	24,072
Central Java	1	111,149	31,614	23,421
	2	111,946	32,171	23,095
DI Yogyakarta	1	124,361	31,816	23,367
	2	123,977	32,226	22,852
East Java	1	107,934	30,565	22,909
	2	109,096	30,680	22,455
Banten	1	116,364	32,126	24,161
	2	115,477	31,838	23,870

**Description:** Time 1 (before the Covid-19 pandemic); 2 (after the Covid-19 pandemic)

**Source:** National Online Market Information System-Livestock by Food Security Agency, 2021 (analyzed)

Unlike the case with beef, the highest prices for broiler meat and eggs before and during the pandemic have consistently occurred in DKI Jakarta. Relatively close prices also occur in the area around DKI Jakarta, covering the areas of West Java and Banten. The similarity in prices is thought to occur due to the similarity of consumption patterns up to the pandemic with various supporting policies, even though West Java and Banten are producing regions. However, in terms of fluctuations in the price of broiler meat and eggs in the DI Yogyakarta region, it is the highest, as shown in Table 1. The most significant price fluctuations occurred in broiler meat with a year of year (YoY) comparison as of July 2020, also indicated by the CV value of 15%. Another peak of price fluctuations occurred in May 2020, when demand for broiler meat peaked in connection with Eid al-Fitr. Generally, the price of broiler meat at the highest price peaks, namely May and July 2020, shows two important things: high price fluctuations in the eastern part of Java Island. Still, the higher average price in the same period occurred in the western part. The price of broiler meat seems to be polarized into two regional groups.

## CONCLUSION

It is concluded that all provinces in Java were significantly affected by consumer price changes of beef, broiler meat, and eggs ( $P < 0.001$ ). For the beef price, region and time variables and their interactions were significantly affected by price changes ( $P < 0.001$ ). However, the price of eggs has been affected by the time ( $P < 0.05$ ), while that of their interaction was not significantly affected ( $P > 0.3$ ). The results indicated that consumers' beef price has the most affected due to the Covid-19 pandemic era in Java, while price eggs and broiler meat were not too affected by price changes. A deeper understanding of the aspects that affect consumption performance between these regions becomes important as a consideration in determining price control policies.

## AUTHOR CONTRIBUTIONS

All authors were contributed equally to this work

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## **Income Analysis of Broiler Retailers in Traditional Manado City Markets**

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### **ABSTRACT**

This research aims to analyse income and affects factors of broiler retailers in Bersehati Traditional Manado City Market. The collected data were from primary and secondary sources. Primary data were obtained directly from interviews using respondents' questionnaires and secondary data from relevant agencies and other research results. The purposive sampling method was used to appoint the location. The respondents of this research based from the consideration that the Bersehati Traditional Market and Pinasungkulan Market are the most considerable number of retailers. Both of them are non-permanent and permanent traders. The number of respondents is determined based on criteria of permanent traders of 30 people. Based on the results of this research, the income of the retailers' traders in Bersehati Traditional Market and Pinasungkulan Market was IDR 2,639,945,070/year. The factors that significantly affect the income of broiler sales in the Bersehati Traditional Market and the Manado City Pinasungkulan Market ( $P < 0.05$ ) are the volume of broilers sold and the selling price broilers and the marketing costs.

**Keywords:** Income, Retailer broilers, Traditional market

### **INTRODUCTION**

The development of livestock-based agribusiness is directed at increasing the community's income and standard of living, expanding employment opportunities, and filling market segments, domestic and foreign markets. One of the activities in livestock agribusiness is marketing. One of the parties involved in marketing activities is retailers. Retailers in the traditional markets of Manado City are part of a marketing agency that has an important role because they deal directly with consumers. Retailers in the traditional markets of Manado City have been running a business, especially in broiler marketing, for a long time. They have been able to meet the demand for broilers by consumers. The marketing activities of broilers in the traditional markets of Manado City, run by retailers, are supported by the smooth operation of the previous marketing agencies by providing ready-to-sell broiler stocks. In traditional markets, the buying and selling process is generally carried out by bargaining

to find a price agreed between the two parties, namely between the seller and the buyer; if there is a price agreement, then a sale and purchase transaction is made. Traditional markets in Manado City with the highest number of broiler retailers are Bersehati Traditional Market, Wenang, Pinasungkulan and Wanea District. The retailers in the Market consist of permanent and non-permanent retailers. The retailer still means retail traders who intensively carry out activities at the Bersehati Traditional Market and the Pinasungkulan Market every day. Based on background that the formulation of the problem in this study is how much income broiler retailers were selling, broiler volume sold, broiler selling price, marketing costs, level of education, and length of business effect.

## **MATERIALS AND METHODS**

### **Location and time of research**

This research at Bersehati Traditional Market and Pinasungkulan Market, Manado City, on December 22, 2020 to December 30, 2020.

### **Types and sources of data**

The data were collected from primary and secondary sources. Primary data were obtained directly from interviews using questionnaires of respondents, and secondary data were gathered from relevant agencies and other research results.

### **Sample method**

The method used was purposive sampling, based on certain criteria requirements. The purposive sampling method was used to determine the location and respondents. The location was determined based on the consideration that the market had more than ten broilers selling traders. The broiler retailers were based on the consideration that the traders who were the respondents were permanent retailers' activities in the market every day. There were 38 broiler retailers active in Pasar Bersehati and Pinasungkulan, consisting of permanent and non-permanent traders. The number of samples used as respondents was 30 permanent retailers.

### **Data analysis model**

The first objective of this study is to analyze the amount of broiler sales revenue in the Bersehati Traditional Market and the Pinasungkulan Market, analyzed using the following equation (Porwanto et al. 2019):

$$I = TR - TC,$$

Description:

I = Income

TR = Total Revenue

TC = Total Cost

The second objective of this study is to analyze the factors that affect the income of broiler sales in the Bersehati Traditional Market and the Pinasungkulan Market using multiple linear regression analysis with the following natural logarithmic model:

$$\ln Y = b_0 + b_1 \ln X_1 + b_2 \ln X_2 + b_3 \ln X_3 + b_4 \ln X_4 + b_5 \ln X_5 + e$$

Description:

Y = Income

b<sub>0</sub> = Constant

b<sub>i</sub> = coefficient

X<sub>1</sub> = Volume of broilers sold

(Kg / year)

X<sub>2</sub> = selling price of broiler (IDR / kg)

X<sub>3</sub> = Marketing costs (IDR/ year)

X<sub>4</sub> = the respondent's education level

(year)

X<sub>5</sub> = Length of business (Year)

e = error

Statistical testing of the regression equation was carried out using the f test, t test and R<sup>2</sup>.

## **RESULTS AND DISCUSSION**

### **Research location overview**

Manado City is located between 124°40'-124°50' East Longitude and 1°30'-1°40' North Latitude. The city of Manado has an area of 157.3 km<sup>2</sup>. The city of Manado has various types of markets as shopping places for food and clothing needs for the community, both supermarkets and traditional markets. Traditional markets in Manado City are scattered in several locations including in Wenang District, namely Bersehati Market and also in Wanea District, namely Pinasungkulan Market. Bersehati Traditional Market is located in Calaca Village, Wenang District, Manado City. As for the boundaries of Wenang Subdistrict, namely the north by the Jengki River and the Singkil District, the East by the Tikala District, the south by the Sario District and Wanea District, the west by the Manado Bay. Pinasungkulan Market is one of the markets in Manado City and is located in Wanea District.

## **Characteristics of respondents**

The characteristics of respondents are one element that can affect the ability of traders to carry out marketing activities. The characteristics of the respondents collected included age, the amount of time allocated for selling, and gender.

### **Retailer trader age**

The results of research on 30 respondents in Bersehati Traditional Market and Pinasungkulan Market showed that the age of the retailers selling broilers varied. There are 2 retailers aged between 21-30 years or 6.67% of the total respondents, 15 people 31-40 years old or 50% of the total respondents, 8 people aged 41-50 years or 26.66% From the number of respondents, 3 people aged 51-60 years or 10% of the total respondents, and 2 people over 60 years old or 6.67% of the total respondents. Retailers in the Bersehati Traditional Market and Pinasungkulan Market are people with an average age who are still productive to work and develop their business into a larger and more advanced scale.

### **Amount of time allocated for broiler sales**

Research data shows that broiler retailers allocate varying amounts of time to complete sales at Bersehati Traditional Market and Pinasungkulan Market every day. The allocation of time provided by retailers in carrying out broiler marketing activities every day is 6 hours as many as 9 people or 30% of the number of respondents, 7 hours as many as 14 people or 46.67% of the number of respondents, 8 hours as many as 13.33% of the number of respondents, and the time allocation is around 9 hours as many as 3 people or 10% of the total respondents.

### **Gender**

Research data based on gender in Bersehati Traditional Market and Pasar Pinasungkulan towards 30 respondents stated that 13 people were male or 43.33% of the total number of respondents, while 17 people were female, 56.67% of the total respondents. Based on the research data, the highest number of broiler retailers is in Bersehati Traditional Market and Pinasungkulan Market according to gender, namely women, this shows that women have enthusiasm and are actively involved in earning income to meet family needs.

## Broiler retailer merchant income analysis

### Marketing costs

Marketing cost can be observed in Table 1. Research data showed, the amount of marketing costs in running a broiler sales business in the Bersehati traditional market and the Manado City Pinasungkulan market is IDR 12,675,600,000/year. The cost of purchasing a broiler is the largest amount of the costs incurred, which is IDR 12,176,500,000/year or 96.06% of the total cost, while depreciation of equipment is the smallest cost of marketing costs, which is IDR 7,500,000/year or 0.06% of the total costs incurred.

**Table 1.** Broiler sales business marketing costs

Description	Amount (IDR/year)	Percentage (%)
Broiler purchase costs	12,176,500,000	96.06
Retribution	281,820,000	2.22
Electricity	19,320,000	0.15
Plastic packaging	73,200,000	0.58
Transportation	117,260,000	0.93
Shrinkage of tools	7,500,000	0.06
Total marketing costs	12,675,600,000	100.00

*Source: Primary data processed (2021)*

### Revenue analysis

Revenue from broiler sales by retailers at Bersehati Traditional Market and Pinasungkulan Market in Manado City can be seen in Table 2.

**Table 2.** Broiler retailer acceptance

Description	Amount (IDR/year)	Average (IDR/month)	Percentage (%)
Meat	14,314,958,570	1,192,913,214	93.47
Head	244,140,000	20,345,000	1.59
Chicken feet	216,123,500	18,010,291,67	1.41
Innards	540,323,000	45,026,916,67	3.53
Total receipts	15,315,545,070	1,276,295,423	100.00

*Source: Primary data processed (2021)*

The revenue received by retailers at Bersehati Traditional Market and Pinasungkulan Market from the sales of meat is IDR 14,314,958,570/year or 93.47% of the total revenue. Revenue originating from the sale of non-carasses, namely

sales of heads amounting to IDR 244,140,900,000/year or 1.59% of the total proceeds, the sale of claws is IDR 216,123,500/year or 1.41% of the total revenue, and the sale of offal is IDR 381,555,000/year or 3.53%. The total revenue obtained by retailers is IDR 15,315,545,070/year with an average monthly income of IDR 1,276,295,423/month.

Based on the research data, the revenue was obtained from the sales of broilers, both from the sale of carcass and non-carcass. The number of broilers that are more and more sold or the size of the business scale which is getting bigger will increase the amount of revenue received by retailers. This is in accordance with the results of research by Momongan, et al. (2020), which states that the amount of revenue is influenced by the scale of the business being run.

### *Income analysis*

Total amount of income earned by broiler retailers in the Bersehati Traditional Market and the Pinasungkulan Market in Manado City can be seen in Table 3.

**Table 3.** Total revenue of retailers

Description	Amount (IDR/year)	Average income of each trader (IDR/month)
Total revenue	15,315,545,070	42,543,181
Total marketing costs	12,675,600,000	35,210,000
Income	2,639,945,070	7,333,181

*Source: Primary data processed (2021)*

The data of the research results show that the income of broiler retailers is IDR 2,639,945,070/year, with an average income of each retailer of IDR 7,333,181/month. Based on the research data, that income is strongly influenced by the amount of revenue and marketing costs incurred by retailers.

### **Factors affecting retailers' income**

The results of the regression analysis of the factors that affect broiler sales business income, where the independent variables (independent) are broiler volume sold ( $x_1$ ), broiler selling price ( $x_2$ ), marketing costs ( $x_3$ ), education level ( $x_4$ ), and length of business ( $x_5$ ), can be seen in Table 4.

The results of the regression analysis in Table 4, it is known that the R-Square value is 0.98, meaning that in this study the ability of the independent variables is the volume of broilers sold ( $x_1$ ), selling price of broilers ( $x_2$ ), marketing costs ( $x_3$ ), education level ( $x_4$ ), and the length of effort ( $x_5$ ) in explaining the dependent variable (income) is 98%, the remaining 2% is

**Table 4.** Results of regression analysis of factors affecting income of broiler retailers

Variables	TH	Coefficient
Broiler volume sold ( $x_1$ )	+	5.611***
Broiler selling price ( $x_2$ )	+	5.384***
Marketing costs ( $x_3$ )	-	4.617***
Education level ( $x_4$ )	+	0.002
Length of business ( $x_5$ )	+	0.003
R-Square		0.980
F-count		16.578

\*\*\*) significant at  $\alpha = 5\%$

explained by other variables not discussed in this study. The F test is used to determine the effect of the independent variable on the dependent variable. Based on the results of the regression analysis in Table 4, the independent variables (broiler volume sold, broiler selling price, marketing costs, length of education, and length of business) have an F-count value of 16,578,831, while the F-table is 2.60 so it can be concluded that the value of the F-count in this study is greater than the F-table at the level of  $\alpha = 0.05$ . The probability value of F-count is 0.00 and this shows that together all independent variables, namely the volume of broilers sold, the selling price of broilers, marketing costs, education level, and length of business have a significant effect on the income of broiler sales in the Bersehati Traditional Market. and Manado City's Pinasungkulan Market.

### ***Broiler volume sold***

The variable volume of broilers sold had a very significant effect on income ( $P < 0.05$ ) and the regression coefficient was 5.611. The regression coefficient of the volume of broilers sold is positive in accordance with the hopeful sign, meaning that every 1% increase in broiler volume sold means that broiler retailers get an increase in income of 5.611% per year. Based on the results of the research, broiler retailers in the Bersehati Traditional Market and the Pinasungkulan Market in Manado City were able to sell 576,360 kg of broilers/year with an average number of sales per retailer of 19,212 kg/year.

### ***Broiler selling price***

The broiler selling price variable has a very significant effect on income ( $P < 0.05$ ), and the regression coefficient is 5.384. The regression coefficient of the broiler selling price variable is positive in accordance with the hopeful sign,

meaning that every 1% increase in the selling price of the broiler, the trader will receive an increase in income of 5.384% per year. The average selling price of broilers per year is IDR 24,846/year. The selling price of broilers to consumers before the Covid-19 pandemic ranged from IDR 29,000 to IDR 30,000 per kilo (Mandak et al. 2016). The selling price of broilers adjusts to market prices and the situation in the midst of the Covid-19 pandemic, such as on religious holidays the selling price of broilers has increased according to market needs so that at the end of the year the broiler price reached IDR 16,000 due to the Covid-19 pandemic situation in Indonesia.

### ***Marketing costs***

The marketing cost variable has a significant effect on income ( $P < 0.05$ ), and the regression coefficient is 4.617. The regression coefficient is negative and according to the expectation sign, it means that every 1% increase in marketing costs will reduce retailers' income by 4.617% per year. Marketing costs in selling broilers consist of costs for purchasing broilers, user fees, electricity, transportation, depreciation of equipment, and packaging.

### ***Level of education***

The variable level of education has no significant effect on income ( $P > 0.05$ ). The results of this analysis indicate that the level of education possessed by retailers has no effect on income.

### ***Length of business***

The length of business variable has no significant effect on income ( $P > 0.05$ ). This means, the increase in the length of the business run by retailers does not affect the total income.

## **CONCLUSION**

The income earned by broiler retailers in Bersehati Traditional Market and Pinasungkulan Market in Manado City was IDR 2,639,945,070/year with the average income of each trader is IDR 7,333,181/month. Factors that significantly affect broiler sales business income in Bersehati Traditional Market and Pinasungkulan Market in Manado City ( $P < 0.05$ ) are the volume of broilers sold, the selling price of broilers, and marketing costs.

## **AUTHOR CONTRIBUTIONS**

Jolanda K.J. Kalangi, Ingriet D.R. Lumenta and Dian K. Harefa as authors; Jolanda K.J. Kalangi, Ingriet D. R. Lumenta and Dian K. Harefa as data collector and information; Jolanda K.J. Kalangi and Ingriet D.R. Lumenta and Dian K Harefa as data analyzer and editor manuscript and discussant.

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## **Economic Liability for Superior Duck Innovation of Alabimaster-1 Agrinak in South Kalimantan**

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### **ABSTRACT**

The development of selected local duck originally from South Kalimantan, Indonesia Alabio, has a high opportunity and prospect in line with increasing market demand for duck products. A new variety of superior duck, *i.e.* Alabimaster-1 Agrinak has been created and produced by the Indonesian Agricultural Research and Development Agency *c.q.* Indonesian Research Institute for Animal Production. Alabimaster-1 Agrinak has been released by Agriculture Ministerial Decree No.360/Kpts/PK.040/6/2015 through a series of the genetic selection process of local Alabio ducks from Hulu Sungai Utara District in South Kalimantan that remain high variability in its performance. Therefore, research to increase the performance through enhancing the potential genetic of local Alabio ducks is strategically carried out to achieve better value-added for farmers. The objective of the study was to analyze the liability of Alabimaster-1 Agrinak that farmers in South Kalimantan have raised. Survey methods with an in-depth interview, observation, and field verification were chosen in this study from August – October 2017. The research location was determined by purposive sampling at the farm with an economies size of scale for 500 females and 150 males Alabimaster-1 Agrinak respectively in the district of Hulu Sungai Utara and Hulu Sungai Tengah, South Kalimantan. The results showed that by 14% discounting factor, Net Present Value achieved IDR 21.808 million with B/C ratio of 1.21 and 17.96% of return on investment. This can be concluded that the innovation of Alabimaster-1 Agrinak enables enhancing the added value of raising ducks for a better living of farmers. The availability of good breeding stock of Alabimaster-1 Agrinak needs to be supported by the local government by empowering a group of farmers that are supervised intensively for technical aspects.

**Keywords:** Profit analysis, Cash flow analysis, Alabimaster duck

## INTRODUCTION

Duck farming is considered a prospective market that has a high potential and opportunity for development along with increasing people's awareness to meet livestock-based animal protein consumption. Commercial duck farming is becoming more popular because of the increased demand for duck meat, eggs, and products (Adzitey & Adzitey 2011). The national population of ducks has increased slightly from 2014 to 2018 by 3.16 %/year (Ditjen PKH 2019) and one of the duck population centres in Indonesia is South Kalimantan. Nowadays, the culinary food base of duck meat is very popular and its demand tends to increase from time to time (Irma 2014). This situation may enhance to increase in livestock-based animal protein consumption of Indonesian people. Policy and action support from multi-stakeholders need to be in line with the enthusiasm of duck farmers', one of those is the urgency to have a duck breeding policy and technical guidance to supervise duck farming that is standardized in feeding, management as well as veterinary management.

Local duck is one of the local germplasm that raised quite a long time in Indonesia that need to be improved in the years to come. One of the problems faced by farmers in raising duck is a shortage of good quality breeds. Farmers traditionally raising ducks for eggs production and culling ducks would become a source of duck meat, besides male DOD that raised for 6 months.

For that reason, Indonesian Research Institute for Animal Production (IRIAP), based in Ciawi-Bogor, as one of the technical units of Indonesian Agency for Agricultural Research and Development (IAARD) has invented a superior breed of ducks that has a higher production and productivity. Alabimaster-1 Agrinak has been released by Agriculture Ministerial Decree No.360/Kpts/PK.040/6/2015 through a series of the genetic selection process of local Alabio ducks from Hulu Sungai Utara District in South Kalimantan that remain high variability in its performance.

In reality, the distribution of those inventions for the superior breed of ducks has not massively met the demand yet. Alabimaster-1 Agrinak has been distributed to group of farmers in the District of Hulu Sungai Utara, South Kalimantan, and enhancing to support breeding development in the field for the availability of good quality breeds. Susanti & Kumalawati (2019) has stated that the selection process may increase egg production for Alabio duck as Alabimaster-1 Agrinak. The average egg production of 24 weeks aged of Alabio duck was  $107.81 \pm 14.15$  egg/bird equal to  $64.17 \pm 8.42\%$ .

The economic liability of this superior duck of Alabimaster-1 Agrinak has been assessed in this paper to evaluate how big is the value-added achieved by introducing this innovation. This economic analysis is very important to implementation massively through dissemination program to farmers, so that

will be an indicator for responses from the farmers as well as other end users for further superior ducks development in the future.

## **MATERIALS AND METHODS**

Survey methods with in-depth interview techniques, observation, and field verification were chosen in this study was from August – October 2017. The research location was determined by purposive sampling at the farm with an economies size of scale for 500 females and 150 males Alabimaster-1 Agrinak in the district of Hulu Sungai Utara, South Kalimantan. Financial analysis criteria that have been used for this study include profit analysis, Net Present Value (NPV), Benefit-Cost (B/C), Internal Rate of Return (IRR), and Return on Investment (ROI). The cash flow analysis may give information as consideration for investment in further development of duck farming. The parameters used in this research consist of technical parameters and economic parameters. Technical parameters consist of business scale, culled duck period, egg production, feed consumption, use of production inputs, feed conversion ratio, mortality, litter/manure production, and electricity usage. Meanwhile, the economic parameters included of product prices, production input prices, investment, revenue, fixed costs, and operational costs.

## **RESULTS AND DISCUSSION**

There are four separate duck farming practices in South Kalimantan, i.e. (a) breeding; (b) hatching eggs; (c) rearing pullet; and (d) egg production for consumption. This is in accordance with Wibowo (2016) that duck farming based on its objectives includes egg production, hatching eggs, rearing ducks, fattening male ducks, processing salted eggs, and trading eggs. The study more focuses on breeding aspects for evaluating and analyzing how big is the value-added achieved by introducing the innovation of superior breeding duck. Financial analysis was observed at the farm with an economies size of scale for 500 females and 150 males Alabimaster-1 Agrinak which is obtained from BPTU Pelaihari. The technical parameters observed and collected consisted of the average hatching egg production, mortality, feed consumption, use of medicines/vitamins, vaccines, and disinfectants. One of the technical parameters showed that farmers purchase pullets that are ready to lay eggs/pullet (aged about 4-6 months), then reared to produce hatching eggs for a year. Ducks will be culled after the age of about 16-18 months.

Financial analysis shows that cash flow information is running from the farm and can be used as a consideration for investment and farm development. Financial analysis criteria that have been used for this study include profit

**Table 1.** Profit analysis of superior duck innovation Alabimaster-1 Agrinak breeding farm

Items	Volume	Unit	Price/Unit	Total
Revenue				
Hatching egg sales	90,000	eggs	2,200	198,000,000
Consumption egg sales	19,500	eggs	1,600	31,200,000
Manure sales	114	sack	10,000	1,140,000
Culled female duck sales	495	head	45,000	22,275,000
Culled male duck sales	149	head	40,000	5,960,000
Cost				
Fixed cost				
Breeding cage depreciation	1	year	2,500,000	2,500,000
Warehouse depreciation	1	year	1,000,000	1,000,000
Sago/snail chopping machine depreciation	1	year	1,000,000	1,000,000
Purchase of female Alabimaster-1 Agrinak ducks	500	head	85,000	42,500,000
Purchase of male Alabimaster-1 Agrinak ducks	150	head	75,000	11,250,000
Variable Cost				
Feed	39,146.25	kg	4,200	164,414,250
Medicine/vitamin	1	year	464,200	464,200
Vaccine	1	year	650,000	650,000
Disinfectant	1	year	429,900	429,900
Electricity cost	12	month	75,000	900,000
Labor	12	month	2,000,000	24,000,000
Profit analysis				
Total revenue				258,575,000
Total fixed cost				58,250,000
Total variable cost				190,858,350
Profit				9,466,650
R/C Ratio				1.04

Net Present Value (NPV), Benefit-Cost (B/C), Internal Rate of Return (IRR), and Return on Investment (ROI). Profit analysis results showed that the

breeding farm obtained profit by IDR 9,466,650 in a year. Profit analysis of superior duck innovation Alabimaster-1 Agrinak breeding farm in detail at the field can be seen in Table 1.

Based on Table 1, the share of feed costs in duck breeding farms was 66 , of the total costs. The feed cost that must be spent was IDR 164,414,250 of the total cost of IDR 249,108,350 within one year. The results of this analysis were in accordance with Molnar & Szollosi (2017) that the largest proportion of duck production costs in Hungary were the feed cost and the purchase of duck breeds. This large proportion of feed costs is better than the common one because farmers use mixed feed which has a lower price than the price of commercial concentrate feed. The average feeding mix given to laying ducks was 150 grams/day/head at a price of IDR 4,200 per kg. On the other hand, it is important to use snails as an additional source of protein for laying ducks. The addition of 22.5% fermented snail flour to feed ration was reported to be able to increase the income over feed cost of IDR 7,316/kg egg sales (Biyatmoko 2014). Another study also used gold snails 5% and noni fruit 0.06% in the feed ration produced the best Rambon duck performance with the value of income over feed cost IDR 11,675.74 per head (Tanwiriah et al. 2019). The second biggest cost was the cost of purchasing pullet ducks and male ducks which have a contribution of 21.58 , of the total cost. Farmers purchase pullet ducks at an average price of IDR 85,000/head and male ducks at an average price of IDR 75,000/head. Other cost sources were labor costs (9.63 ,), depreciation (1.81 ,), medicine/vitamins (0.62 ,), and electricity costs (0.36 ,).

Revenue sources from duck breeding farms come from hatching eggs sales, consumption egg sales, manure sales, culled female duck sales and culled male duck sales. The largest revenue was sourced from the sale of hatching eggs which reached 76.57, of the total revenue. Based on observations, the average egg production in the field was around 60%, so that egg production in a year was 109,500 eggs. Based on the total egg production, as many as 90,000 eggs (82.19 ,) can be sold as hatching eggs and the remaining 19,500 eggs (17.81 ,) were sold as consumption eggs. Therefore, the second-largest revenue source comes from the sale of consumption eggs with a contribution of 12.07 , of the total revenue. Erlina's research (2013) in Hulu Sungai Utara Regency showed that egg production and income of Alabio duck farmers were influenced by the production facilities subsystem, production subsystem, marketing subsystem, and supporting institutions.

Sales of culled female ducks contributed 8.61 , of the total revenue, while sales of culled male ducks contributed 2.30 ,. Farmers purchase pullet ducks at the age of 6 months and then produce eggs for 12 months. Sales of manure, although its contribution is quite small (0.44 , of total revenue), but in a year can produce 114 sacks for IDR 10,000 per sack. The results of profit analysis

showed that the profit received by the farmer was IDR 9,466,650 in a year and the ratio between revenue and the total cost was 1.04. This means that duck breeding is still profitable with an R/C ratio greater than one. In addition to being economically profitable, factors that support the development of duck farming in Hulu Sungai Utara Regency include the availability of breed, distance to markets, location of duck breeding, availability of labor, distance from settlements, and mastery of technological developments (Yunandar et al 2021). The duck breeding farm was also analyzed in Blitar Regency, East Java with a scale of 500 ducks with an R/C value of 1.35 (Wibowo et al. 2002; Prasetyo 2006).

Based on the results of the profit analysis and technical parameters, a cash flow analysis simulation of the Alabimaster-1 Agrinak breeding practices was compiled for 10 years with the main components consisting of revenues, expenses, loan principal installments, and income flows. The calculation of the cash flow analysis simulation in this study used a discount factor (df) of 14 %. The analysis did not include a credit mechanism since the farmer was relatively managed his operational duck breeding farm by themselves. The simulation results of the cash flow analysis of Alabimaster-1 Agrinak duck breeding farm can be seen in Table 2.

Financial analysis with a discounted factor of 14% in Table 2 showed an average NPV value of IDR 21.808 million and a B/C ratio of 1.21. This means that the Alabimaster-1 Agrinak breeding farm was profitable with a positive NPV value and a B/C ratio  $\geq$  of 1. NPV is obtained by calculating all operating values minus the current operating costs and investment costs with the discounted factor of 14%. A positive NPV means that investment for duck breeding practices is considered profitable, and vice versa for negative NPV during a certain period. B/C ratio is one method of investment feasibility that emphasizes the magnitude of the benefits to the total costs incurred from an investment. The B/C value of duck breeding practices if  $\geq 1$  means that the business is feasible. Research results of Sari et al (2020) also showed that the financial analysis of laying duck farming business with intensive practices in Gadingrejo District, Pringsewu Regency, Lampung showed a positive NPV and B/C value of more than 1.

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[illegible]

Items	Years									
	1	2	3	4	5	6	7	8	9	10
Total-II B	190,858	190,858	190,858	190,858	190,858	190,858	190,858	190,858	190,858	190,858
Total costs (II A + IIB))	249,108	249,108	249,108	249,108	249,108	249,308	249,308	249,308	249,508	249,508
Cashflow (I-IIA-IIB)	9,447	18,893	28,340	37,786	47,233	56,479	65,726	74,973	84,019	93,066
•Total benefit	258,555	268,002	277,448	286,895	296,341	305,788	315,034	324,281	333,528	342,574
•Total costs	249,108	249,108	249,108	249,108	249,108	249,308	249,308	249,308	249,508	249,508
•Net benefit	9,447	18,893	28,340	37,786	47,233	56,479	65,726	74,973	84,019	93,066
•Discount factor (0,14)	0.88	0.77	0.67	0.59	0.52	0.46	0.40	0.35	0.31	0.27
•Net present value (NPV)	8,286	14,538	19,128	22,373	24,531	25,731	26,267	26,282	25,837	25,104
•Discounted benefit	226,803	206,219	187,270	169,865	153,910	139,313	125,900	113,680	102,562	92,407
•Discounted cost	218,516	191,681	168,141	147,492	129,379	113,582	99,633	87,397	76,726	67,303
•Benefit-cost (B/C) ratio	1.04	1.08	1.11	1.15	1.19	1.23	1.26	1.30	1.34	1.37
•Return on investment (ROI)	17.96									
•Internal rate of return (IRR)	30.82									

the factor of 14%. A positive NPV means that investment for duck breeding practices is considered profitable, and vice versa for negative NPV during a certain period. B/C ratio is one method of investment feasibility that emphasizes the magnitude of the benefits to the total costs incurred from an investment. The B/C value of duck breeding practices if  $\geq 1$  means that the business is feasible. Research results of Sari et al (2020) also showed that the financial analysis of laying duck farming business with intensive practices in Gadingrejo District, Pringsewu Regency, Lampung showed a positive NPV and B/C value of more than 1. Baruwa et al. (2018) who conducted research in Oyo and Lagos States, Nigeria also reported that all types of duck farming were profitable, but duck hatchery farming was more profitable than duck rearing farming and pullet enlargement farming.

The results also showed that the IRR and ROI values obtained from the Alabimaster-1 Agrinak duck breeding farm were 30.82% and 17.96%, respectively. The value of both indicates that the duck breeding farm was financially profitable because it was able to provide greater profits than the current commercial banking interest rate. The results of Elly et al. (2019) also showed that the integration of the duck and rice business results in an IRR value greater than the current interest rate, as well as a positive net B/C and NPV value.

## **CONCLUSION**

Innovation of Superior duck Alabimaster-1 Agrinak based on the results of profit analysis and cash flow analysis within 10 years is considered economically liable as breeding practices of duck farmers. Alabimaster-1 Agrinak ducks need to be developed intensively and massively in South Kalimantan, to meet the demand for people as well as actors in hatchery farming and rearing ducks farming to obtain hatching eggs.

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## **AUTHOR CONTRIBUTIONS**

Priyono, Priyanti A, Susanti T, and Rohaeni ES were contributed equally to this work.

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## Development of Smallholders Beef Cattle Farming: Support Resources

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### ABSTRACT

The development of animal husbandry can not be separated from the support of various resources. The high and low access of farmers to resources is determined by the farmer's human resources. The research objective was to assess the development of smallholder beef cattle farming. The research was conducted in Grujugan Kidul Village, Grujugan SubDistrict, Bondowoso District. Grujugan Kidul Village is one of the villages fostered by the Universitas Jember in the Community Assistance Village Service Program (PPDB) in accordance with the Decree of the Chancellor of the Universitas Jember Number 12716 / UN25 / KL / 2018 concerning Universitas Jember Assisted Villages. Research variables include human resources (X), financial resources (Z1), technological resources (Z2), physical resources (Z3), and livestock farming business development (Y). Respondents of this study were independent farmers, non-partnership system, beef cattle farmers 174 people. Data obtained by using the method of Focus Group Discussion (FGD) and survey with a likert scale. Data were analyzed by the Partial Least Square (PLS) method with SmartPLS 2.0. The results showed that the farmers' human resources had a positive and significant effect on access to financial, technological, and physical resources, each of which was .383; .474; and .324. The conclusion of this research is that 64.2% of the development of smallholder beef cattle farming is influenced by farmers' resources. The findings of this study are that human resources do not directly affect the development of smallholder beef cattle farming, but through various aspects of resources that support these development efforts.

**Keywords:** Beef cattle farming development, Smallholder beef cattle

### INTRODUCTION

Meat self-sufficiency is national issue that require attention from various parties. Efforts to develop beef cattle business are still being carried out by the government and private sectors, both through breeding and fattening (Rusdiana & Praharani 2018). Livestock business development is closely related to sustainable livestock business efforts. The definition of sustainability

according to the Big Indonesian Dictionary (KBBI) is continuous, while development is a gradual and regular development effort that leads to the desired target. Sustainable livestock development is closely related to livestock business development when viewed from the theory of resource management (David 2009; Hunger & Wheelen 2003; Pearce & Robinson 2013). Indicators of livestock business development are increasing farmer income, increasing livestock population being kept, increasing labor or working time allocation, and increasing production units (Amam & Harsita 2019; Amam et al. 2019). Livestock business development cannot be separated from resource support (Amam et al. 2019).

Beef self-sufficiency in Indonesia seems not just a dream. The population growth rate of beef cattle was recorded at 3.02 (Animal Husbandry & Health Statistics 2020) which is still higher than the population growth rate of 1.25 (Central Statistics Agency 2020). Such conditions have an impact on the efforts that the Indonesian people continue to make to fulfill beef self-sufficiency. Various efforts and strategies for developing livestock businesses include determining livestock areas and regional planning approaches (Mayulu et al. 2010; Susanti et al. 2014; Soetriono et al. 2019; Zahrosa et al. 2020), internal factors and approaches. external (Otoluwa et al. 2016; Hajirin et al. 2020; Rusman et al. 2020), resource accessibility approach (Amam et al. 2019; Amam & Soetriono 2020).

The accessibility of farmers to resources affects the development of livestock businesses (Amam et al. 2019). The greater the access of farmers to resources, the greater the potential of farmers in developing livestock business (Amam et al. 2019). Livestock business resources include financial resources, technological resources, and physical resources (Amam et al. 2019), in addition, resources also consist of economic resources, environmental resources, and social resources (Amam et al., 2019). Farmer access to resources is influenced by each farmers human resources (Amam et al. 2021; Amam et al. 2021), the institutional performance of farmer groups (Amam et al. 2020; Soetriono & Amam 2020), and also vulnerability aspects of livestock business as part of business risk (Amam et al. 2020; Amam & Solikin 2020).

This study aims to examine the development of smallholder beef cattle farming with a resource approach, namely resources that support the development of smallholder beef cattle farming. The resources referred to in this study are financial resources, technological resources, and physical resources (Amam et al. 2019). The novelty of this research is to apply the development of livestock business with a resource approach to the commodity of smallholder beef cattle, which has previously been applied to broiler and dairy cattle farms. The benefits and contributions of this article are as a means of developing knowledge considering that scientific development can be

carried out with research findings, and also as a public policy database considering that public policy requires an academic text as its foundation.

## MATERIALS AND METHODS

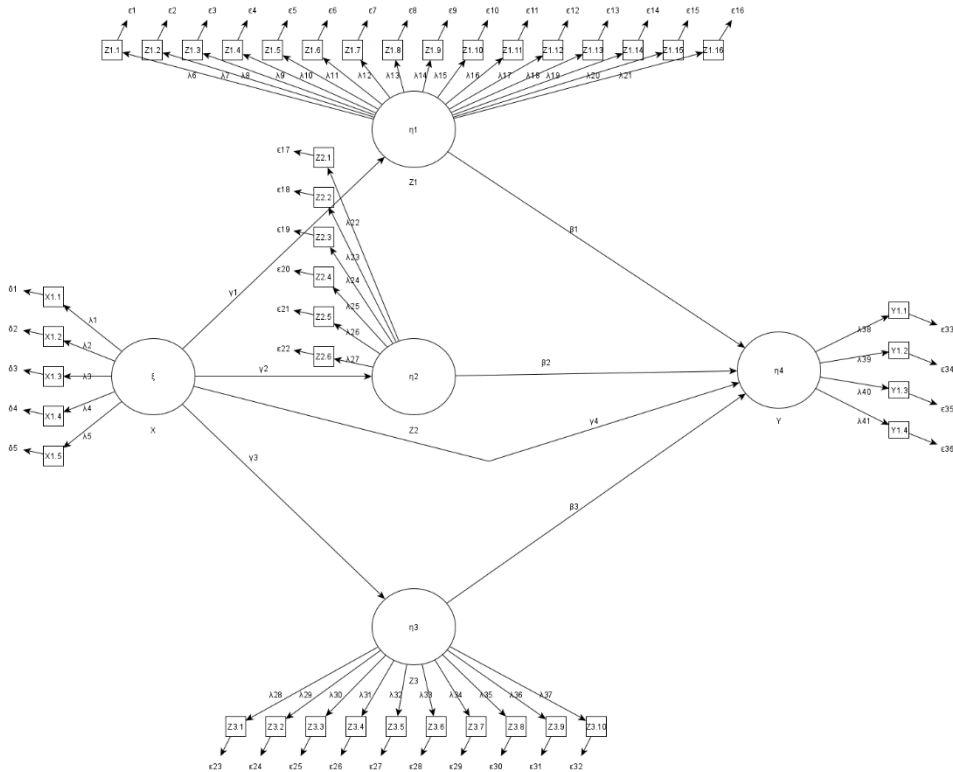
The research was conducted from August to December 2019 in Grujugan Kidul Village, Grujugan SubDistrict, Bondowoso District. Grujugan Kidul Village is one of the assisted villages of the Universitas Jember in the Assisted Village Service Program (PPDB) in accordance with the Decree of the Chancellor of the Universitas Jember Number 12716/UN25/KL/2018 regarding the Villages Assisted by the Universitas Jember. Respondents of this study were smallholder beef cattle farmers with an independent maintenance system, not partnership system (*gaduhan* in Indonesia indigenous language), as many as 174 people. Research variables include human resources (X), financial resources ( $Z_1$ ), technological resources ( $Z_2$ ), physical resources ( $Z_3$ ), and livestock business development (Y). Research variables and indicators are described in Table 1, while the relationship between each variable is shown in Figure 1. The data were obtained using the Focus Group Discussion (FGD) and *servei* methods. The survey was conducted by means of interviews and filling out Likert scale questionnaires. Data analysis using Partial Least Square (PLS) method with SmartPLS 2.0.

The variables in this study consisted of 6 (six) main variables (Table 1), namely: Competency Standards for Graduates (SKL) of the Undergraduate Animal Husbandry Study Program (X) and 5 (five) dimensions of sustainable livestock development, namely the ecological dimension ( $Y_1$ ), the economical dimension ( $Y_2$ ), social and cultural dimensions ( $Y_3$ ), institutional dimensions ( $Y_4$ ), and technological dimensions ( $Y_5$ ). The research method uses a digital survey with the google form feature. The survey was conducted using a likert scale of +1 to +5. The data were then analyzed partially using simple linear regression using IBM SPSS Statistics 26 software.

**Table 1.** Research variables and indicators

Variable	Indicator	Notation
Farmer HR (X)	knowledge and skills	X <sub>1.1</sub>
	farmer's health	X <sub>1.2</sub>
	farmer motivation	X <sub>1.3</sub>
	the ability of farmers in language	X <sub>1.4</sub>
	farming experience	X <sub>1.5</sub>
Financial resources ( $Z_1$ )	main income	Z <sub>1.1</sub>
	income from beef cattle business	Z <sub>1.2</sub>

Variable	Indicator	Notation
	income from non-farm business	Z <sub>1.3</sub>
	income from other livestock businesses	Z <sub>1.4</sub>
	total income for family living needs	Z <sub>1.5</sub>
	savings amount	Z <sub>1.6</sub>
	debt amount	Z <sub>1.7</sub>
	amount of debt repayment	Z <sub>1.8</sub>
	male calf ownership	Z <sub>1.9</sub>
	female calf ownership	Z <sub>1.10</sub>
	male possession	Z <sub>1.11</sub>
	female possession	Z <sub>1.12</sub>
	adult bull ownership	Z <sub>1.13</sub>
	adult cow ownership betina	Z <sub>1.14</sub>
	ownership of pregnant cows	Z <sub>1.15</sub>
	number of cattle ownership	Z <sub>1.16</sub>
Technological resources (Z <sub>2</sub> )	seed selection	Z <sub>2.1</sub>
	feed technology	Z <sub>2.2</sub>
	livestock health	Z <sub>2.3</sub>
	home system	Z <sub>2.4</sub>
	fattening management	Z <sub>2.5</sub>
	marketing Management	Z <sub>2.6</sub>
Physical resources (Z <sub>3</sub> )	residential house	Z <sub>3.1</sub>
	cattle pen	Z <sub>3.2</sub>
	means of transportation	Z <sub>3.3</sub>
	means of communication	Z <sub>3.4</sub>
	information facility	Z <sub>3.5</sub>
	household electricity	Z <sub>3.6</sub>
	land ownership	Z <sub>3.7</sub>
	land use	Z <sub>3.8</sub>
	water availability	Z <sub>3.9</sub>
	feed availability	Z <sub>3.10</sub>
Livestock business development (Y)	additional income	Y <sub>1.1</sub>
	increase in livestock population	Y <sub>1.2</sub>
	addition of manpower or additional allocation of working time	Y <sub>1.3</sub>
	addition of cages (production unit)	Y <sub>1.4</sub>



**Figure 1.** Variable relationship model

Based on the pattern of variable relationships in Figure 1, the mathematical approach to measuring each indicator using the PLS method is as follows:

$$X_i = \lambda_{xi} \xi_i + \delta_i ; Y_i = \lambda_{yi} \eta_i + \epsilon_i$$

Data analysis using the PLS method using the SmartPLS 2.0 application system. Wiyono (2011) suggests that one of the advantages of the PLS method is that it can strengthen weak theories and/or can design (find) new theories. The PLS method consists of 2 (two) working steps, namely testing the outer model and testing the inner model. The outer model test consists of at least 4 (four) criteria, namely the indicator test with the outer loading value, the Average Variance Extracted (AVE), Cronbach's Alpha (CA) value, and the Composite Reliability (CR) value. The test of the inner model at least includes the coefficient of determination (R<sup>2</sup>), the value of t-statistics, and the value of the parameter coefficient koefisien.

## **RESULTS AND DISCUSSION**

### **Profile of Grujugan Kidul Village as a assisted village of the University of Jember**

Grujugan Kidul Village, Grujugan SubDistrict, Bondowoso District, East Java Province has an area of 2.86 km<sup>2</sup> which is geographically located at an altitude of 313 masl with an average rainfall of 217 mm. The profile of Purnama Village consists of a moor area of 134 ha, building and residential area of 28.4 ha, pond (pool) covering an area of 0.8 ha, yard area of 102.4 ha, and others covering an area of 20 ha in the form of unproductive lands. or not used. Grujugan Kidul Village consists of 6 hamlets, 4 Rukun Warga (RW), 26 Rukun Tetangga (RT) with a total population of 5,327 people consisting of 2,536 men and 2,791 women, so the population density is 1,876 people/km<sup>2</sup>.

The potential for the development of beef cattle in Grujugan Kidul Village is supported by the availability of yard land (102.4 ha), upland area (134 ha), and unutilized land (20 ha). This potential of land can be used as a forage area for animal feed, as a source of animal feed, especially during the dry season when the availability of forage becomes a major problem for beef cattle business (Harsita & Amam 2019). The carrying capacity of potential development of beef cattle is also obtained from agricultural waste in the form of straw, bran, and bran from a rice harvest area of 363 hectares with and from other agricultural wastes in the form of corn cobs and rejected corn from a planting area of 18 hectares with a production of 76 tons or with an average 4.22 ton/ha.

The large population of beef cattle in Grujugan Kidul Village is 1,457 heads and the high motivation of farmers to the sustainability of the beef cattle business is the reason for the formation of the Universitas Jember Community Assistance Village Service Program (PPDB) for the development of beef cattle business in accordance with the Decree of the Chancellor of the Universitas Jember Number 12716/UN25 /KL/2018 regarding the Assisted Villages of the Universitas Jember. The PPDB program is based on Law Number 6 of 2013 concerning Livestock Empowerment. Livestock empowerment is focused on the three pillars of the livestock business consisting of breeding, feeding, and management (Amam & Harsita 2019).

The objectives and targets of the PPDB Program include: (1) Governance based on the establishment and strengthening of livestock institutions; (2) Basic social services through the implementation of the Junior High School Education Program (School of Animal Husbandry Community); (3) Local economic development based on superior commodities, namely beef cattle; (4) Improving the quality of the living environment using the concept of zero

waste, by utilizing agricultural waste for animal feed and using livestock manure for agricultural fertilizer; and (5) Empowering village women through mentoring programs oriented to diversification of processed beef products.

### Test outer model

The outer model test in the PLS method has at least 4 (four) criteria, namely indicator testing (outer loading value), Average Variance Extracted (AVE), Cronbach's Alpha (CA) value, and Composite Reliability (CR) value. The results of the indicator test (Table 1) by determining the value of the outer loading are shown in Table 2 and the results of the test for the values of AVE, CA, and CR are shown in Table 2.

**Table 2.** Indicator test results

Notation	X	Z <sub>1</sub>	Z <sub>2</sub>	Z <sub>3</sub>	Y	Information
X <sub>1.1</sub>	0.903					valid
X <sub>1.2</sub>	0.516					valid
X <sub>1.3</sub>	0.865					valid
X <sub>1.4</sub>	0.523					valid
X <sub>1.5</sub>	0.887					valid
Z <sub>1.1</sub>		0.612				valid
Z <sub>1.2</sub>		0.914				valid
Z <sub>1.5</sub>		0.804				valid
Z <sub>1.6</sub>		0.725				valid
Z <sub>1.9</sub>		0.862				valid
Z <sub>1.11</sub>		0.934				valid
Z <sub>1.13</sub>		0.849				valid
Z <sub>1.14</sub>		0.882				valid
Z <sub>1.15</sub>		0.908				valid
Z <sub>1.16</sub>		0.951				valid
Z <sub>2.1</sub>			0.878			valid
Z <sub>2.2</sub>			0.735			valid
Z <sub>2.3</sub>			0.528			valid
Z <sub>2.4</sub>			0.506			valid
Z <sub>2.5</sub>			0.729			valid
Z <sub>2.6</sub>			0.939			valid
Z <sub>3.2</sub>				0.556		valid
Z <sub>3.3</sub>				0.671		valid
Z <sub>3.4</sub>				0.739		valid
Z <sub>3.5</sub>				0.757		valid
Z <sub>3.7</sub>				0.511		valid

Notation	X	Z <sub>1</sub>	Z <sub>2</sub>	Z <sub>3</sub>	Y	Information
Z <sub>3,8</sub>				0.511		valid
Z <sub>3,9</sub>				0.825		valid
Z <sub>3,10</sub>				0.883		valid
Y <sub>1,1</sub>					0.895	valid
Y <sub>1,2</sub>					0.724	valid
Y <sub>1,3</sub>					0.621	valid
Y <sub>1,4</sub>					0.515	valid

*Description: outer loading value after removing invalid indicator (valid >500)*

**Table 3.** Outer model test results

Variable	Notation	AVE	CA	CR
Farmer human resources	X	0.834	0.911	0.819
Financial resources	Z <sub>1</sub>	0.848	0.867	0.735
Technology resources	Z <sub>2</sub>	0.721	0.885	0.752
Physical resources	Z <sub>3</sub>	0.707	0.754	0.747
Livestock business development	Y	0.852	0.896	0.856

### Test inner model

The test of the inner model in the PLS method has at least 3 (three) criteria, namely the coefficient of determination (R<sup>2</sup>), the value of t-statistics, and the value of the parameter coefficient. The test results of the inner model in Figure 1 are shown in Table 4.

**Table 4.** Inner model test results

Test	Value	Description
Coefficient of determination (R <sup>2</sup> )		
a. Financial resources	0.429	
b. Technology resources	0.312	
c. Physical resources	0.226	
d. Livestock business development	0.642	
t-statistic		
X → Z <sub>1</sub>	3.412	significant
X → Z <sub>2</sub>	2.915	significant
X → Z <sub>3</sub>	1.668	significant
X → Y	1.647	not significant
Z <sub>1</sub> → Y	4.221	significant
Z <sub>2</sub> → Y	2.876	significant
Z <sub>3</sub> → Y	1.819	significant
Coefficient of parameter		

Test	Value	Description
$X \rightarrow Z_1$	0.383	positive effect
$X \rightarrow Z_2$	0.474	positive effect
$X \rightarrow Z_3$	0.324	positive effect
$X \rightarrow Y$	0.087	positive effect
$Z_1 \rightarrow Y$	0.315	positive effect
$Z_2 \rightarrow Y$	0.273	positive effect
$Z_3 \rightarrow Y$	0.149	positive effect

*t* table: 1.652 (*n* = 174)

### The effect of livestock human resources on resource access

The financial, technological, and physical resources of beef cattle business in Grujugan Kidul Village are influenced by the human resources of farmers by 42.9, 31.2, and 22.6%, respectively. Financial resources are positively and significantly influenced by the human resources of farmers (significance level 5%) (Table 4), meaning that the higher the human resources of farmers, the greater access to financial resources. Farmers who have high human resources, for example, have experience raising cattle and have knowledge and skills, generally have access to high financial resources, such as high incomes, ownership of more than 1 (one) commodity, so that they are able to meet the needs of family life, and even have family savings. The increase in farmer income indicates that livestock farming is no longer a motive for family savings (Harsita & Amam 2021), but has turned to the livestock business (Amam & Harsita 2021).

Technological resources are positively and significantly influenced by the human resources of farmers (significance level 5%), meaning that the higher the human resources of farmers, the greater the access to technological resources. Farmers who have high human resources, for example, have experience raising cattle and have knowledge and skills generally have access to high technological resources, such as being able to choose seeds, being able to process animal feed, understanding about housing, understanding how to fatten cattle, and understanding cattle marketing management (Amam et al. 2019; Amam et al. 2019). Cattle marketing management which is professionally managed by livestock institutions (livestock groups) with a contract system (forward contract) as a form of price and value protection (hedging) can reduce the risk of farmer losses (Amam & Soetrisno 2019), because marketing is closely related to consumer attitudes. (Amam et al. 2016; Harsita & Amam 2019), as well as consumer satisfaction and loyalty (Amam & Harsita 2017), thus having an impact on increasing farmers' income.

Physical resources are influenced by the human resources of farmers positively but not significantly (significance level 5%), meaning that the quality of human resources of farmers means greater access to physical resources. Farmers who have high human resources, for example, have experience raising cattle and have knowledge and skills, generally have access to high physical resources, such as being able to use land to plant forage for livestock, being able to provide clean water sources for drinking livestock by making wells, and being able to provide feed. livestock to meet the nutritional needs of livestock. Farmers who have high human resources are able to process forage with fermentation technology to increase nutritional value by utilizing biotechnology (Amam et al. 2019), besides that it is useful for maintaining the availability of forage during the dry season (Harsita & Amam 2019).

### **Smallholders beef cattle farming business development**

Human resources of farmers and access to resources simultaneously affect the development of smallholder beef cattle farming business by 64.2% (Table 4). Livestock business development as part of sustainable livestock development efforts (Suyitman et al. 2009; Setyawan & Amam 2021), so that development efforts always pay attention to ecological dimensions, economic dimensions, social and cultural dimensions, institutional dimensions, and technological dimensions (Suyitman et al. 2009; Zhao et al. 2020; Linden et al. 2020; Martin et al. 2020; Tarawali et al. 2011). Sustainable livestock development is expected to reduce the negative environmental impacts caused by livestock business (Hammond et al. 2017).

The development of smallholder beef cattle farming is directly influenced by the human resources of farmers positively but not significantly ( $t$  statistic  $< t$  table). This condition shows that the high human resources of farmers do not guarantee that farmers are able to develop smallholder beef cattle business without the support of various resources. The unstable selling price of live cattle is one of the main problems for farmers in Grugugan Kidul Village (Harsita & Amam 2019). Amam & Harsita (2019); Xu et al. (2019) stated that marketing is one aspect of the vulnerability of livestock business. The decline in the selling price of livestock can also be caused by a poor livestock transportation system (Stojkov et al. 2020).

The development of smallholder beef cattle farming is influenced by the strength of financial resources positively and significantly ( $t$  statistic  $> t$  table). This condition shows that the large access of farmers to financial resources enables the efforts of farmers to develop their businesses. The amount of income of farmers is one of the reasons farmers are able to develop their businesses. One of the supporting factors is to strengthen livestock institutions

at the livestock group level (Amam & Soetriono 2019). The roles of livestock institutions include reducing the risk of farmer losses and as a forum for empowering farmers so that they are able to increase breeder human resources (Amam & Harsita 2019).

The development of smallholder beef cattle farming is positively and significantly influenced by the strength of technological resources ( $t$  statistic  $> t$  table). Such conditions indicate that the large access of farmers to technological resources enables the efforts of farmers to develop their businesses. Selection of good seeds is one of the efforts of farmers in conducting business development. Seedlings are one of the three main pillars of livestock business. Amam & Harsita (2019) stated that the three pillars of the livestock business consist of breeding, feeding, and management. Seed selection can also be influenced by consumer demand (Menger & Hamm 2021), in addition to selecting seeds for future breeding strategies (Sunds et al. 2021).

The development of smallholder beef cattle farming is influenced by the strength of physical resources positively and significantly ( $t$  statistic  $> t$  table). This condition shows that the large access of farmers to physical resources enables the efforts of farmers to develop their businesses. Utilization of land to plant forage forage is one of the efforts of farmers in conducting business development. Integrated agriculture with integrated systems can increase agricultural productivity (Nayak et al. 2018; Esteves et al. 2018; Jeswani et al. 2018; Hai et al. 2020; ). Utilization of land for animal feed is also useful for meeting the nutritional needs of livestock, so that it can increase the body weight of livestock (Amam & Haryono 2021)

## CONCLUSION

Farmer human resources have a positive and significant impact on access to financial, technological, and physical resources, respectively 0.383; 0.474; and 0.324. The development of smallholder beef cattle farming is simultaneously influenced by the human resources of farmers and access to resources by 64.2%. The findings of this study are that human resources do not directly affect the development of smallholder beef cattle farming, but through various aspects of resources that support these development efforts.

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## **Sustainability Analysis of Beef Cattle and Development Strategy Based on Collective Cages in Lombok Island**

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### **ABSTRACT**

Beef cattle raising in Lombok Island mostly uses collective cages where the level of its implementation has not been optimal yet as expected. This study aims to determine a strategy for raising beef cattle based on collective cages on smallholder farms in Lombok Island based on their sustainability status. The research has been conducted with a survey method to collect primary data from December 2018-March 2019. The method used to determine the sustainability status is Multidimensional Scaling with the Rapid Appraisal Beef Cattle Smallholder approach. There are six dimensions studied that include hygienic aspects of collective cages, management of cages, animal health, feed management, drinking water management, socio-economic and cultural related aspects. The development strategy formulation was carried out by analyzing the sensitive attributes of the six dimensions, which were the top priority based on the root mean square value. The results showed that there were 28 sensitive attributes with a prospective assessment by experts 10 critical factors for the success of beef cattle development were determined. In conclusion, there are four strategies for developing beef cattle based on collective cages on smallholder farms in Lombok Island; those were improved collective cage health management; increasing productivity, efficiency, and income of beef cattle farmers based on the existing collective cages; increasing access to information, technology, capital and cattle marketing and increasing the role of government, business, and other stakeholders.

**Keywords:** Beef cattle, Collective cages, Development strategy, Smallholders, Sustainable status

### **INTRODUCTION**

West Nusa Tenggara (NTB) is one of the producing regions and has a surplus of beef production, of which almost 99% of the 1,242,749 heads of the total cattle population were smallholder cattle farming (Dinas Peternakan dan Kesehatan Hewan Provinsi Nusa Tenggara Barat 2019) and dominated by Bali

cattle. Therefore, the position of beef cattle farming becomes very strategic to the contribution to meet national beef demand (Mashur 2017a).

Beef cattle smallholder farmers in NTB faced 28 obstacles to meet the ASEAN Economic Community (AEC) in the last year of 2015 (Mashur 2017). Beef cattle raising have been carried out by farmers considered in a traditional system with low productivity. Since 1990, the productivity of Bali cattle in NTB has continued to decline from year to year. In addition to decreasing cattle productivity, there is also a decrease in land resources as a feed component for cattle farming (Dinas Peternakan dan Kesehatan Hewan Provinsi Nusa Tenggara Barat 2019). Raising beef cattle on smallholder farms in NTB is carried out intensively in Lombok Island and extensively instead in Sumbawa Island.

Raising beef cattle in Lombok Island mostly uses collective or communal cages, where cattle are penned continuously throughout the day without being released from the pen; however, some farmers grazed the cattle to the fields and gardens during the day and brought them back to the pen at night. Developing smallholder beef cattle farms based on collective cages is a model of intensive beef cattle maintenance. This may include providing health services and avoiding theft. This model has been built by implementing integrated cattle farmer group management in terms of technical, social, economic, and cultural aspects in cattle practices, such as management of maintenance, feed, breeding, animal health services, marketing, manure waste treatment, and cattle safety systems (Mashur 2020).

Cattle housing is one of the 28 obstacles faced by farmers in NTB. Apart from protecting cattle from various health problems, the cage also functions to prevent cattle theft. The use of collective cages for beef cattle is expected to provide a solution for cattle safety and comfort to produce optimally. Mashur et al. (2020) has shown that the application of beef cattle feed health management on community farms based on collective cages in Lombok Island is in a moderate to good value with an average score of 3.80 based on a Likert scale. Of the 21 aspects of feed health management that have been implemented by beef cattle farmers on collective farm-based community farms, there is not a single aspect of feed health management that has been implemented with a good to very good score (valued between 4- 5). A total of 52.43% of beef cattle farmers on collective cage-based community farms on the island of Lombok have implemented feed health management with a value of moderate to good, and 38.38% of farmers have implemented feed health management with a good to very good value. In connection with this, research has been conducted to determine the strategy for developing smallholder beef cattle farms based on collective cages in Lombok Island.

## **MATERIALS AND METHODS**

Primary data need to be gathered in the form of attributes related to six dimensions to analyze the sustainability and development strategy of beef cattle based on collective cages in Lombok Island. These were technical and collective cage health requirements, collective pen management methods, availability and health requirements of feed, availability and health requirements of drinking water, disease incidence, and social, economic, and cultural aspects. Interviews of a total of 185 respondents using a questionnaire had been carried out in five regencies/cities in Lombok Island from December 2018-March 2019. Eight expert respondents and stakeholders were selected, who were deliberately chosen and have competence, experience, credibility, neutrality, and being willing to provide answers. The technique of determining the respondents was done by purposive random sampling, namely farmers who have at least five years of cattle experience and members of collective cage management.

The determination of the strategy for developing smallholder beef cattle farms based on collective cages is carried out based on sustainability status using the Multi-Dimensional Scaling (MDS) method called the RAP-BCS (Rapid Appraisal Beef Cattle Smallholder) approach, with a bit modification by a previous study (Kavanagh 2011). Several previous researchers have applied this approach, *i.e.* in Beef Cattle Farming Areas for Agropolitan Area Development in Bondowoso Regency (Ramadhan et al. 2014) and Seaweed-Based Coastal Area Development in West Sumbawa Regency (Nuryadin 2015). The scores for each attribute were analyzed multi-dimensionally to determine one or several points that reflect the position of sustainable development of beef cattle farming based on collective cages. Through the MDS method, the position of the sustainability point can be visualized through the horizontal and vertical axes, with the rotation process, the position of the point can be visualized on the horizontal axis with the sustainability index value given a score of 0% (bad) and 100% (good). If the system under study has a sustainability index value of 50%, the system is said to be sustainable and unsustainable if the index value is <50% (Susilo 2013). The sustainability analysis results are stated in the Sustainability Index of Beef Cattle Farming Development based on collective cages (ikb-BCS). The sustainability index value of each dimension can be visualized in the form of a kite diagram. The formulation of a strategy for developing beef cattle farming based on sustainable collective cages on the island of Lombok is based on the sensitive attributes of the leverage analysis using Rapid Appraisal Beef Cattle smallholder (RAPBCS) on each sustainability dimension. Based on the

most dominant sensitive attribute, various efforts or improvement strategies can be carried out without underestimating the meaning of other attributes.

The data were analyzed through seven stages: (1) Determining the attributes that include the six dimensions of sustainability. The number of attributes to be analyzed is 96 attributes, consisting of 20 dimensions of technical requirements and cage health attributes; 17 attributes of collective cage management dimensions; 20 attribute dimensions of feed health requirements; 9 attribute dimensions of drinking water health requirements; 20 attributes of disease incidence dimensions, and 10 attributes of social, economic and cultural dimensions; (2) Assessment of each attribute on an ordinal scale based on the sustainability criteria of each dimension; (3) Preparation of the index and status of development sustainability both in multi-dimensional and in each dimension; (4) The stage of ordination; (5) A sensitivity analysis (leverage analysis) to determine the variables sensitive to sustainability; (6) Carlo's system analysis takes into account aspects of uncertainty, and (7) Formulates a strategy for developing beef cattle farming based on collective cages (Mashur 2021).

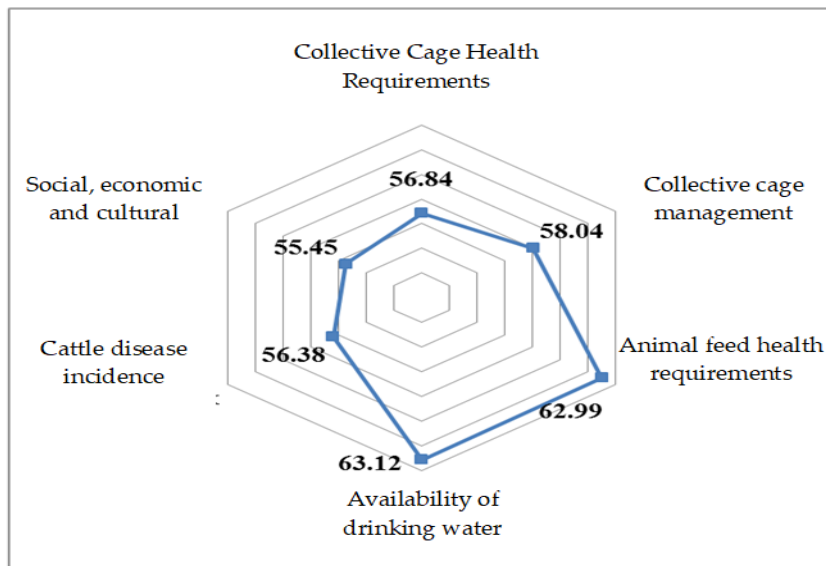
## **RESULTS AND DISCUSSION**

### **Multidimensional sustainability status**

Determining the value of the sustainability index in a multi-dimensional manner is a picture of the sustainability of the development of beef cattle in smallholder farms based on collective cages on Lombok Island. Multi-dimensional value is obtained by multiplying the index value between dimensions by weights based on expert opinion. Budiharsono (2017) stated that the multi-dimensional value between the six dimensions could not be done with an average but have to be done with a pairwise comparison test obtained from expert assessments in the field of smallholder farmer (technical requirements and cage health, collective cage management, animal feed, drinking water, disease incidence, along with socio-economic and cultural) so that the weights of each dimension can be obtained (Table 1). The results have shown that the combined value of the six dimensions is a total of 58.98. This value indicates into the category of quite sustain, refer to sustainability standards (Susilo 2013). The combined sustainability index value of the six dimensions is illustrated in the kite diagram, as shown in Figure 1.

**Table 1.** The value of weight, the value of sustainability index and the value of multidimensional weighting index of the six dimensions of the development of smallholder beef cattle farms based on collective cages in Lombok Island

Dimension	Weight value (%)	Sustainability index value	Weighted result index value
Collective cage health requirements	15.45	56.84	8.78
Collective cage management	14.16	58.04	8.22
Animal feed health requirements	23.61	62.99	14.87
Availability of drinking water	12.88	63.12	8.13
Cattle disease incidence	19.31	56.38	10.89
Social, economic and cultural	14.59	55.45	8.09
Total	100.00	58.80	58.98



**Figure 1.** Kite diagram of the six dimensions of sustainability index value for development of smallholder beef cattle farms based on collective cages in Lombok Island

### Stress value and coefficient of determination

The stress value and the coefficient of determination ( $R^2$ ) are used to estimate the accuracy of the results on sustainability index value, whether it is necessary to add attributes to reflect the level of accuracy of the six dimensions to be appropriately justified. The stress value is defined as a measure to estimate whether the accuracy of the results obtained is close to the original data (goodness

of fit); if the stress value is getting closer to zero, it indicates that the resulting data is considered accurate. The stress values and the R2 of each dimension of sustainability status and strategy for developing community farms based on collective cages in Lombok Island are shown in Table 2.

**Table 2.** Stress values and coefficient of determination of the six dimensions for developing smallholder beef cattle farms based on collective cages in Lombok Island

Dimension	Stress value	Percentage of stress value	Coefficient of determination (R2)	Percentage of Coefficient determination
Collective cage health requirements	0.1386808	13.87	0.9525459	95.25
Collective cage management	0.1404049	14.04	0.9516730	95.17
Animal feed health requirements	0.1372888	13.73	0.9533278	95.33
Availability of drinking water	0.1499501	14.99	0.9474304	94.74
Cattle disease incidence	0.1380562	13.81	0.9530509	95.31
Social, economic and cultural	0.1481608	14.82	0.9481055	94.81

The stress value of the six dimensions of sustainability in the development of smallholder beef cattle farms based on collective cages in Lombok Island ranges from 0.13-0.15 (13-15%) with R2 ranges from 0.94-0.95 (94-95%). This means that all the attributes studied from the six dimensions of sustainability of smallholder beef cattle farms based on collective cages are accurate enough to provide good analytical results and can be scientifically justified. This is in accordance with the results of research findings by Fauzi & Anna (2015) in a good Rapfish model that indicated by a stress value of <0.25 (25%) and the coefficient of determination (R2) is close to greater than 80% or close to 100% (Kavanagh 2011).

The results of the Monte Carlo analysis had shown that points in the scatter plot are in a clustered position, this means that the results of the ordinance points used in determining sustainability status for the development of beef cattle farms based on collective cages in Lombok Island are quite stable, so that errors or disturbances can be overcome. Kavanagh & Pitcher (2014) stated that errors or disturbances in the ordinance results are indicated by points spread out or separated from other sets of points in the scatter plot. These may be caused by: (1) The influence of attribute scoring errors caused

by lack of information, misunderstood attributes or how to make attribute scores; (2) The effect of variations in scoring due to different opinions or assessments by different researchers; (3) The stability of the MDS analysis process that is repeated (unstable anchor position); (4) Data entry errors or missing data; and (5) The high value of "stress" analysis results. In detail, the results of the Monte Carlo ordinance of the six dimensions of sustainability for the development of smallholder beef cattle farms based on collective cages in Lombok Island are presented in the form of a scatter plot (Table 3).

**Table 3.** Attributes sensitive to each dimension of sustainable development of smallholder beef cattle farms based on collective cages in Lombok Island

Dimension		Attribute sensitive (Leverage factor)	RMS*
Collective cage health requirements	1	Cattle waste does not interfere	0.66
	2	Cage floor material	0.59
	3	Main cage size	0.59
	4	Cage for calves	0.57
How to manage collective cage	1	The floor of the cage is cleaned and not slippery. The floor of the cage must not	0.94
	2	have holes	0.92
	3	Spacious cage aisle	0.86
Availability of animal feed	1	Affordable feed price/cost	0.90
	2	Meet nutritional needs	0.90
Availability of drinking water for livestock	1	There are no snails in drinking water sources	0.31
	2	Drinking water is changed every day	0.31
Disease occurrence	1	Diarrhea disease incident	0.73
	2	Scabies disease	0.58
	3	Stomach bloating disease	0.58
	4	Itching disease	0.57
	5	Demodex disease	0.56
	6	Pink Eye Disease	0.55
	7	Anthrax disease	0.55
	8	Reproductive disorders	0.51
	9	Epizootic Septicemia Disease	0.51
Social, economic and cultural	1	Animal feed costs	2.77
	2	Labor cost	1.33
	3	Cattle treatment costs	1.25
	4	Cattle become skinny	1.24
	5	Number of sick cattle	1.20
	6	Livestock insurance	1.14
	7	Farmer's income	1.10
	8	Number of cattle died	0.96

\*RMS = root means square

## **Development strategy**

The development strategy of smallholder beef cattle farms based on collective cages in Lombok Island has been analyzed using sensitive attributes of the six dimensions that need to be a top priority. These sensitive attributes are the main factors in supporting the sustainable development of smallholder beef cattle farms. For this reason, various efforts are needed from multi-stakeholders involved, such as cattle farmers, government, and community, to improve attributes. These sensitive attributes and maintain or re-enhance well-identified attributes to achieve sustainability (Table 3).

Furthermore, experts conducted a prospective assessment from the 28 sensitive attributes, and 10 critical success factors were determined. These were: controlling anthrax and SE disease, avoiding reproductive disorders, increasing farmer/breeder income, feed cost efficiency, paying attention to the density of cow cages, improving management manure waste, cleaning the floor of the cage every day to avoid slippery, fulfills the need for animal feed both in quantity and quality and provides cattle insurance guarantees to farmers on community farms based on collective cages in Lombok Island (Mashur 2017b). Based on the priority order of dominant/sensitive attributes, the results of leverage analysis that affect sustainability and critical success factors, a development strategy has been prepared for community-based beef cattle farming collectively in Lombok Island.

Strategy 1 with improvement of collective cage health management through (a) Improve cattle health service system (mainly periodic Anthrax and SE vaccinations) and avoid reproductive disorders; (b) Improve management of cattle manure to have a negative impact on environmental hygiene and health; (c) Clean the cage every day to avoid slippery; and (d) Pay attention to the size of the cage, especially the main cage, to overcome exceed capacity.

Strategy 2 with increasing productivity, efficiency, and income of existing collective cage-based beef cattle farmers through (a) Supply of liable price, sustainable feed based on agricultural and industrial by-products in sufficient quantity and quality; (b) Optimizing the use of family labor to increase the economy of scale on cattle farming; (c) The use of technology by the mating calendar to apply the 3S system (one year, one cow, one calf); (d) Processing cattle manure into solid and liquid organic fertilizers; and (e) Facilitating the provision of machine tools for processing feed and cattle waste.

Strategy 3 with increasing access to information, technology, capital, and cattle marketing through (a) Build an information network system that is easily accessible to farmers, including through social media; (b) The introduction of feed processing and storage technology that is easy to use by

farmers; (c) Facilitate access to capital assistance with procedures and low interest; and (d) Facilitate access to cattle marketing in favor of farmers.

Strategy 4 with increasing the role of government, business, and other stakeholders through (a) Capacity building for farmer group through training, technical guidance, counselling and farmer internships; and (b) Improve the role and function of agricultural extension workers, veterinarians and related service/institutional officers in guidance and assisting farmers and livestock health services.

## **CONCLUSION**

The sustainability status of beef cattle development on community farms based on collective cages in Lombok Island is considered moderately, with a sustainability index value of 58.98. Four strategies have been proposed to proceed on cattle development strategy community farms based on collective cages in Lombok Island, namely: (1) improved collective cage health management; (2) increasing productivity, efficiency, and income of beef cattle farmers based on the existing collective cages; (3) increasing access to information, technology, capital and cattle marketing, and (4) increasing the role of government, business, and other stakeholders.

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## **AUTHOR CONTRIBUTIONS**

Mashur and Kholik, a writing original draft preparation and analisis data, M.Munawaroh funding aquisiton, M. Sriasih, D. Oktaviana and SN Sya'diyah editing manuscript.

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## **Effect of Water-Saving Technology Innovation (Reservoir and Trench Dam) on Beef Cattle Farming**

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### **ABSTRACT**

Water-Saving Technology using reservoirs and dam trench in the dry land area carried out in the District of Grobogan and Maros. A structural survey has been done on 17 and 24 respondent farmers to obtain income information before technology implementation. Carrying capacity analysis was calculated after the implementation of technology based on the dry matter requirement of crossbred cattle in Grobogan and Bali cattle in Maros. A sampling of crops by-products in Grobogan taken from paddy rice production, while that in Maros was taken from corn and peanut during dry season. Data were analyzed descriptively which results show that the main income of the respondents in Grobogan was cattle farming (48.59%) while in Maros was crops farming (51.87%). The implementation of water-saving technology in Grobogan was able to increase the farmer income by Rp. 12.80 million/farmer, while in Maros by Rp. 12.83 million/farmer. The impact of this innovation in Grobogan was the increase of rice straw with the carrying capacity for 73.13 crossbred cattle (10 ha), while that in Maros of corn stover and peanut straw had to carry a capacity of 168.97 head of Bali cattle (6.5 ha). Rice by-products were used as cattle feed in the dry season. While in Maros utilization of maize and peanut by-products for cattle feeding was in the rainy season when the cattle could not be grazed in a paddy field during rice planting. In conclusion the implementation of Water-Saving Technology was able to increase farmer income, therefore it is potentially developed in dryland agricultural activity in Indonesia.

**Keywords:** Water-saving technology, Dry land, Beef cattle farming

### **INTRODUCTION**

Water-Saving Technology using Reservoir and Dam Trench is one of the government projects that have a target to build 30.000 reservoirs as a water source for irrigation in the dryland and rainfed land. Until the end of 2017, the government organizations such as the Agricultural Ministry, Ministry Disadvantaged Areas and Transmigration, and Ministry of Housing and Public Works have built 2700, 90, and 50 units of reservoirs respectively. This

project should be continued to reach the reservoir build target. Due to these conditions, a model of reservoir management in the 4 million hectares (ha) of land was needed (BBSDLP 2014). This program was used to increase crop index so that may increase national food production to reach sustainable food self-sufficiency to improve the food security and world food barns in 2045. Dryland is one of the sub-optimum that has a mediate productivity rate caused by soils in the sub-optimal land category commonly is a fertile soil with pH around neutral to alkaline and also contains high base (Wahyunto & Shofiyati 2014). Water supply in the dry season is one of the important factors for agricultural improvement in the dryland. The priority of agricultural improvement in dryland is using the water source effectively that can be done by using properly cost-effective technology, collaborated with in situ management for the organic matter, so the land productivity and crop index can be increased by 2-3 times higher and increasing farmers income (Mulyani et al. 2014).

On the other hand, the potential of dry land is quite potential for livestock development, especially beef cattle with grazing management such as in NTT Province (Priyanto 2016; Priyanto & Diwyanto 2014). Similarly, in NTB beef cattle had raised under dryland area conditions plays an important role to supply the Java Island region where prices are worth trading out of the region (Priyanto et al. 2016). The development of food crops and plantations has prospects in supporting cattle farming through crops and livestock integration (Priyanto 2017). Feed supply related to nutrients requirements for livestock reported as the main factor in beef cattle farming in Indonesia (Tangendjaja 2017).

Improving the model of crops in the dry season may support feed supply to beef cattle based on agricultural by-products. Beef cattle farming practiced by farmers is commonly focusing on calf production (cow-calf operation/CCO) and feeder cattle that can be done with extensively (grazing), intensively (impounded), integrated with crops and plantation (crop-livestock system/CLS) to decrease production feed cost (Diwyanto and Handiwirawan 2004). Research from Nappu and Taufik (2016), showed that cacao-cattle integration can improve cacao productivity and cattle farming by using cacao by-products as feed resources, cow dung to compost cocoa plants, thus being able to increase income 45.9% with an operating profit of Rp. 13.03 million/ha/2 heads/year. This activity is a reflection of Low External Input Sustainable Agriculture (LEISA) and zeroes waste energy which produces food, feed, fertilizer, and fuel (4 F) (Kementarian Pertanian 2014). This article discusses the effect of an application model of reservoir/dam trench utilization and management in the dryland area using properly integrated innovative

technology in crops to improve integrated crops-beef cattle which in turn may increase farmers' income.

## **MATERIALS AND METHODS**

### **Developing model of reservoirs/dam trench utilization and management**

Innovation technology of water saving in dryland has been done in crop development areas, that is (a) Dam trench utilization model in Tambirejo village, Toroh District, Grobogan Regency, Central Java Province conducted in 10 ha area; (b) Dam Trench utilization Model in Bado Ujung village, Tompobulu Sub District, Maros Regency, South Sulawesi Province conducted in 6,5 ha area. This study was done in 2018 at dry season (2nd dry season) to improve crop management. Reservoirs were built in every main water source in the village. There is 2 model of water utilization and management used in this study they are: (a) Model of Reservoir/Dam Trench utilization and management in dryland of the crops area using innovative integrated technology; and (b) Innovative model of a farming system to utilize and manage the water in dryland of crops area by implemented an integrated technology which technically, socially, and economically. To find out the condition of the innovations that will be applied to do agro-ecosystem analysis activities (Gibbs 1985) and Participatory Rural Appraisal (PRA) to agree with cooperator farmers (Conway 1986).

A model of water utilization and management from small reservoirs and other water storage structures will be used by applying innovative integrated technologies in the dry land. These may include: (a) Water harvest technology; (b) Distribution and irrigation technology; (c) Technology on land utilization and fertilization; (d) Cultivation technology and food crops according to local conditions; (e) Forming and strengthening Farmer Group; and (f) Technical, social, and economic analysis. Two models will be developed depends on the infrastructure of the harvesting water reservoirs and dam trench. In the area where the model will be conducted, innovations of research results from the Indonesian Agency of Agricultural Research and Development (IAARD) will be implemented according to local conditions with its principle to use water effectively and efficiently.

### **Analysis economic of farming business system**

Both of these locations have the potential to develop beef cattle farming by utilizing the potential of forage and agricultural by-products (rice straw, corn stover, and peanut straw). Before analyzing the effect of developing

Reservoirs and Dam Trench and introducing technology, a structured survey and Focus Group Discussion (FGD) had been carried out to 17 and 24 cooperators farmers in Grobongan and Maros, respectively as ex-ante analysis.

The data will be analyzed using Net Cash Benefit (NCB) to estimate cash cost using the below formula Amir and Knipscheer 1989):

$$NCB = TR - TC,$$

$$TR = Q_p \times P$$

$$TC = Q_c \times P, \text{ where:}$$

$$TR = \text{Total Revenue (Rp)}$$

$$TC = \text{Total Cost (Rp)}$$

$$Q_p = \text{Quantity of production (cattle/head, crops/kg)}$$

$$Q_c = \text{Quantity of inputs}$$

$$P = \text{Price (Rp)}$$

Typology of Farming System method has been used to estimate percentage contribution of revenue from crops and beef cattle as well (Soehadji 1992).

### **Carrying capacity analysis on innovation technology of reservoirs and dam trench developing**

This analysis was used to evaluate the potential feed produce from implementing the technology on dry matter base feed requirements. The cattle used in Grobogan was cross beef cattle, while that in Maros was Bali cattle. Crops by-products harvested of paddy rice in Grobogan and corn stover and peanut straw in Maros would be a proxy of agricultural by-products during 3 months of the dry season. The formula used for analysis of the carrying capacity was:

$$CC = P/R$$

$$P = WLD$$

$$R = (3/100) \times B \times 90 \text{ days,}$$

where:

CC = Carrying capacity during dry season  
(Animal Unit)

P = Dry Matter (DM) of by-products crops  
(kg/3 months of dry season)

R = DM requirement (kg/animal unit/3  
months of dry season)

W = Crop by-products production (kg/ha)

L = Harvesting area (ha)

D = DM of crops by-products  
(%)/100

B = Body weight of beef cattle (kg)

## RESULTS AND DISCUSSION

### **Characteristic and potentially developing of dryland for farming activity of water-saving innovation**

Dryland available in Indonesia is around 144.47 million ha, of which 99.65 million ha (68.98%) is potentially used for agricultural production, and around 44.82 million ha is not potentially used due to being located in the forest area. Regarding potency land analysis, around 29.39 million ha (29.50%) of potential land used for dryland crops, around 1.12 million ha (1.13 %) potential used for highland vegetable plants, around 66.72 million ha (66.95%) potential used for annual plants included fruits and around 2.42 million ha (2.43%) potential used for grazing area (BBSDL 2014) (Table 1). Higher potential dryland available in Kalimantan, for about 30.48 million ha, then follow by Sumatra around 28.56 million ha, Papua 13.35 million ha, Sulawesi 9.12 million ha, Jawa 8.79 million ha, Maluku 5.08 million ha and both Bali and Nusa Tenggara around 4.29 million ha. Technology innovation is needed to optimize the use of dryland for farming activity, *i.e.* water-saving innovation. This will increase the intensity of the plantation. This condition will lead to an increase of feed available for cattle from crops by-products that in turn will farmer's income.

This is related to Wahyunto and Shofiyati (2014) which explained that dryland is one of the high potency resources for agricultural improvement, for crops, horticulture, plantations, and animal husbandry. Physical soils characteristic slightly affected by the water available for about 21.5% and about 78.5% affected by other factors. The result from the study showed that water available in Gunung Kidul's dryland in sugar cane plantations 34.16-42.81%, forest teak production 30.34-35.68%. dry field 29.48-32.62%, and the lowest is mixed plantations are 23.39-28.28% respectively (Khalimi & Kusuma 2018). This condition causes the care needed during water-saving innovation and technology implementation to guarantee that water management in dryland can provide enough water for a year. Demand for land use in the agricultural sector is increased, one of the potential agro-ecosystem to be improved is dryland in the dry season, caused by the lower real productivity (Yulianto 2014). Land and water shortages are the main limiting factors in agricultural production (Yin et al. 2020).

Dam trench as irrigation source in dryland was implemented in some area (West Java, Central Java, Daerah Istimewa Yogyakarta, East Java, South East Sulawesi, and South Sulawesi (Heryani et al. 2002; Karama 2003; Sutrisno et al. 2003; CIRAD 2004). The study showed that dryland productivity potentially increasing if (a) Water available fluctuation problem can be minimalized; (b) Watershed capacity can be optimized both naturally or

artificially; and (c) Optimization of water use efficiency and commodities use (Heryani et al. 2003). A reformation of dryland management must be done to reach the food self-sufficient to decrease imported products (Idjudin & Marwanto 2008). The use of dry land provides foods when effective technology and strategy can be rightly implemented. There are few drylands technology management that can be implemented, such as conservation, increasing the chemical, physical and biological fertility, organic matter management, and supplement irrigation. Strategies used to manage the potential dryland are (a). Identification and delineation of land which uses for crops; (b) appropriate Agricultural technology selection; (c) Intensively disseminated of the technology; and (d) The increasing number of research for dryland uses in agricultural (Abdurachman et al. 2008).

**Table 1.** Potency of dryland for crops activity, highland vegetable plants, annual plants, and grazing area

Islands	Potency of lands (ha)				Total
	Food crops (FC)	Vegetable plants (VP)	Annual plants (AP)	Grazing area (GA)	
Sumatra	10.812.354	40.203	17.703.303	-	28.555.860
Java	1.909.124	1.008.677	5.868.687	-	8.786.487
Bali & Nusa Tenggara	1.139.258	44.449	2.515.790	586.335	4.285.831
Kalimantan	7.333.249	-	22.940.823	206.452	30.480.524
Sulawesi	1.905.998	26.974	6.190.556	996.285	9.119.813
Maluku	824.533	5.194	3.689.136	560.256	5.079.119
Papua	5.468.840	-	7.808.768	67.434	13.345.042
<b>Indonesia</b>	<b>29.393.356</b>	<b>1.125.497</b>	<b>66.717.062</b>	<b>2.416.761</b>	<b>99.652.676</b>
Percentage	29.50	1.13	66.95	2.43	100.00

## Crops-livestock opportunity and impact of developing a reservoirs/dam trench on dry land

### *Profile of beef cattle farming*

Survey results in Grobogan's reservoirs developing area showed that beef cattle were the main livelihoods of farmers that reached 76.48%, and the rest 23,52% was cropping farmers (Table 2). The primary livelihood was identified from higher-income contributions during a year, which determined that beef cattle activity has a higher economic-contribution for farmers in Grobogan than crops activities. The different condition was identified in Maros, which

crops activity is a significant income for the farmer compared to beef cattle farming (58.34% vs 41.66%). This is inseparable from the broader land area for the development of food crops in Maros compared to Grobogan. Beef cattle found in Grobogan are dominated by cross-bred, such as Simmental, Limousin and Ongole breeds. As many as 88.23% of farmers have Simmental cross-bred, 17.64% have Limousin cross-bred, and 17.64% have Ongole cross-bred. Simmental cross-bred, and Limousin cross-bred were big framed cattle famous in the market with higher prices. Bali cattle are primarily found in Maros, which are very adaptive to the feed available locally.

**Table 2.** Business profile beef cattle of the development of dam trench in Grobogan and Maros

Parameter	Grobogan (n=17)		Maros (n=24)	
	Percentage	Average	Percentage	Average
Livelihood				
Farmer (crops activity)	23.52	-	58.34	-
Farmer (cattle farming)	76.48	-	41.66	-
Breed of cattle				
Simmental-crossbred	88.23	-	-	-
Limousin-crossbred	17.64	-	-	-
Ongole-crossbred	17.64	-	-	-
Bali	-	-	100.00	-
Physiological cattle status				
Cows	88.23	1.33	44.54	2.75
Bulls	29.41	1.40	14.66	0.42
Calves	35.29	1.5	40.55	2.50
Sub total	100.00	2.47	100.00	6.17
Cattle farming				
Intensive (in a pen)	100.00	-	-	-
Ekstensive (grazing)	-	-	100.00	-

Average cattle owned by farmers in Grobogan was lower than farmers in Maros (2.47 vs 6.17 heads/farmer). This is due to more availability of grazing land in Maros. Both sites have shown that farmers did not practice for fattening operation, feeder cattle instead. Cattle manage by intensively farming in Grobogan, while that in Maros were extensively managed by grazing. Extensive grazing management can not be applied due to limited land and prioritized for planting crops as staple food. In the other hand, major cattle farming in Maros more into grazing extensively by raising in the forest mostly throughout the year, except that during post harvest paddy rice where

cattle be grazed in the field. Priyanto et al. 2016 had shown that cattle farming in NTT would have a potential economies of scale to be implemented due to availability grazing land area, beside opportunity for selling cattle outside of region with higher price.

Feed is the main component to produce beef, that should be in an efficient way to minimize cost with liable output. Cattle farmers in both sites have used rice straw and corn stover as based feed (Table 3). Almost 82% of farmers also use high-quality grass and 76% of farmers use native grass as a mixed-feed in Grobogan. While that in Maros, around 16,66% of farmers have used native and high-quality grass. Concentrate such as rice bran also added to feeding cattle and had to pay for around Rp 4000/kg in Grobogan. Cattle farmers in Maros had to buy rice bran in a big scale and feeding the cattle as much as 1 kg/head/day.

**Table 3.** The use of animal feed by cattle farmers

Parameter	Grobogan (n=17)		Maros (n=24)	
	Percentage	Remark	Percentage	Remark
Feed stuff				
Rice straw	100	Rank 1	100	Rank 1
Corn stover	100	Rank 2	100	Rank 2
High quality grass	82.35	Rank 3	16.66	Rank 3
Native grass	76.47	Rank 4	16.66	Rang 3
Feed supplement				
Rice bran	100	buy: 1 kg/head/ for Rp.4,000/kg	100	1 kg/head (self-produced)
Feed shortage				
Yes	64,70	Scale >3 heads	100	Grazing in the forest
No	35,28	Scale 1-2 heads	-	-
Feed Buying	52,94	Rp 800,000/truck (dry season)	From Forest	Paddy rice (rainy season)

Most of the farmers in Grobogan (64.70%) said that forage supply was limited in the dry season from July to September, especially difficult for farmers with more than three heads of beef cattle dry season. Almost all farmers buy feed shortage from other districts with price Rp 800,000 per truck or equal to 4 tons. As much as 52.94% of respondents, on average, will buy 2-3 trucks depends on their demand. Therefore, innovation of reservoirs building is essential to increase crop plantation index, which may produce

higher crops by-products that can be used for cattle as feed sources. On the other hand, all farmers in Maros said that cattle feed becomes limited during the rainy season because the field has been planted by paddy rice, so that minimal area for cattle grazing and relocated to the forest. Therefore, building a dam trench in the dry season is very important to increase crop production, leading to increased crop by-products for cattle feedstock during the rainy season.

### *Economic analysis of beef cattle farming*

Table 4 shows that the average revenue from cattle farming in Grobogan was Rp 22.06 million/farmer/year. The selling price of beef cattle reached Rp 13.78 million/head and was categorized in a high price due to cross-bred of Simmental or Limousin, even though considered young cattle. This would be different compared to that in Maros, which average farmers' revenue from cattle farming was Rp 10.41 million/farmer/year. The selling price of beef cattle was lower than in Grobogan, that is Rp 8.33 million/head due to Bali cattle breed.

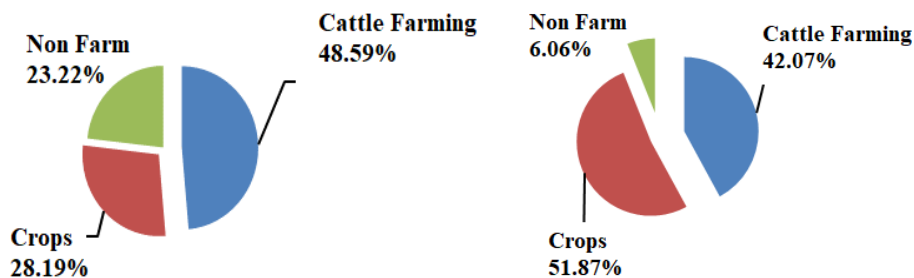
Revenue from crops plantation activity in Grobogan with yearly crops pattern on paddy-paddy-corn reached Rp 12.87 million/farmer/year. These crops had planted on rented land with an average area of 0.37 ha/farmer. While that of farmers in Maros, its revenue was Rp 12.83 million/farmer/year with yearly cropping pattern of paddy - paddy - corn/peanut. Another income source that is non-farm activities in Grobogan reached Rp 10.54 million/farmer/year (trading activities, stalls and others). In contrast, that of farmers in Maros achieved their income of Rp 0.13 million/farmer/year. This is due to the geographic condition of both districts, where Grobogan is relatively close to the centre city compared to that of Maros so that non-farm activity will be a greater opportunity for other jobs. The total revenue of cattle farming in Grobogan was higher than in Maros (Rp 45.40 million/farmer/year vs 24.77 million/farmer/year), which implied beef cattle farming is an important contribution to household farmers' income even though the land area was smaller.

**Table 4.** Farm typology analysis in Grobogan and Maros

Variable	Grobogan (n=17)			Maros (n=24)		
	Percentage	Average	Total	Percentage	Average	Total
Beef cattle sold /year	100	-	-	66,66		
Cattle sold (head)	-	1.6	27	-	1.25	15
Price (Rp 000/head)		13,780			8,330	
Sub total of cattle revenue (Rp 000/year)	-	22,060	353	-	10,410	125
Paddy field						
Land ownership	100	-	-	100	-	-
Land area (ha)	-	0.37	6.3	-	0.69	8.3
Crops revenue /year						
Paddy rice first harvest (Rp 000)	100	5,230	890	100	5,380	64,650
Paddy rice second harvest (Rp 000)	100	5,170	88 0	100	6,220	74,660
Corn	100	2,470	42,000	66,66	1,230	14,700
Sub total revenue of crops revenue	-	12,870	219,000	-	12,830	154,010
Non-farm (Rp 000/year)						
Merchant, Etc.	41,17	10,540	179,200	0,25	0,130	1,50
Total revenue (Rp 000/year)	-	45,400	-	-	24,770	-

### ***Typology of farming activity analysis***

Analysis on “Typology of Farming Activity” showed that beef cattle farming was a major farming activity of farmers in Grobogan that reached yearly of Rp 26,06 Million (48.59% of total farmer income). Respectively, crop farming and non-farm activity would follow that of cattle farming with 28.19% and 3.22% of total farmer income (Figure 1). A different result was shown by that analysis in Maros, which crop farming was the major activity to yield farmers’ income of Rp 12.85 Million (51.97%). This would be followed by cattle farming and non-farm activity, with 42.07 and 6.06%, respectively. These results showed that farmers in Grobogan need to be supported by improving cattle feeding management to support livestock production.



**Figure 1.** Topology of farming activity analysis in Grobogan (Left) and Maros (Right)

A categorized typology of farming activity showed that beef cattle farming in Grobogan can be categorized as “Branch business”, while that and in Maros considered as “Complementary business” (Soehadji 1992). Later, there were 4 classification of livestock business, *i.e.* (a) Complementary business (subsistence) for fulfill family needs that provide around 30% of total income; (b) Branch business (mixed farming), providing 30-70% of total income; (c) Main business (single commodity), providing 70-100% of total income; and (d) industrial business (specialized farming), providing 100% of total income. The crops-livestock integration is a recommended pattern of farming activity. Reservoirs and dam trench development are recommended to be implemented in both locations to support the economy of farmers in dryland, as a source of income from food crops and beef cattle farming.

### ***Innovation Technology of Water-Saving to Support Feed Resources of Beef Cattle Farming***

This analysis also considered the impact of ditch reservoir/dam development on the additional waste generated and the overlay target

achieved during the research activities. Farmer's cooperators as part of farmer organizations (farmer groups) involved intensively. The result showed that the use of reservoirs in Grobongan for three months during the dry season could be feeding beef cattle as much as for 73.13 heads of crossed-bred cattle (Carrying capacity = 73.13 charges) with the paddy field area of 10 ha (Table 5). Bodyweight (BW) for crossed-bred cattle for Ongole-crossed (PO), Simmental-crossed-Ongole (Simp), and Limousin-crossed-Ongole (Limpo) were 428.67 kg, 458.68 kg, and 471.32 kg, respectively as consideration of feed requirements (Setiyono et al. 2017). Feed requirement for these crossed-bred cattle was 450 kg/head (animal unit/AU) with additional rice bran of 1 kg/head/day as a supplement. The dry matter (DM) requirement for cattle is estimated at 3% of BW.

Meanwhile, Maros, which produce corn stover and peanut straw, has reached its carrying capacity of as much as 169.97 AU of Bali cattle in 3 months during the dry season. BW of Bali cattle is 210.1 kg for males and 207.89 kg for females (Hikmawaty et al. 2014). That feed produces in Maros can be used as feedstock in the rainy season. The carrying capacity value is calculated based on Bali cattle, where half of the needs of prominent types of cattle are because they have a lower BW (Hikmawaty et al. 2014).

The resulting capacity is only limited to the achievement of the area of land that the intensity of cropping patterns can increase. This is primarily related to crop by-products planted and utilized by beef cattle livestock in each location (calculated for three months of feed use). The wider the target of the Reservoirs/Dam Trench is built, it will produce a carrying capacity of more feed that can be utilized more for beef cattle. Agricultural by-products can be used as forage substitutes for which availability is limited to beef cattle. Study in Parepare city with land use for the crop is 7.505 ha can provide feed for 688.43 AU (Rauf & Rasbawati 2015), while that in Sumedang with potentially land use can provide feed for 26,174.21 AU of small ruminants and 59,565.58 AU of large ruminants from potential protein and TDN of natural grass and crops by-products as much as 20,366.39 ton/year and 147,553.67ton/year, respectively (Tanuwiria et al. 2006). This showed that dryland used for beef cattle farming by providing feed from crop by-products could be improved by increasing the target area for implementing this program.

**Table 5.** Carrying capacity predicted for beef cattle in Grobogan and Maros

	Commodity	Area (ha)	Prod (kg/ha)	DM Prod (kg/3 months of dry season)	DM Requirement (kg/AU/3 months of dry season)	Carrying capacity (AU/3 months of dry season)
	Paddy waste					
Grobogan (reservoirs)	Paddy straw	10	14783.14	36319.71	1215	29.89
Crossed-bred cattle	Rice bran	10	439.20	3892.63	90	43.24
	Total Grobogan					73.13 AU
	Corn waste					
Maros (dam trench)	Corn straw (stem)	6.5	7200	10960.56	567	19.33
Bali cattle	Corn cob/corn hump	6.5	5300	16163.94	567	28.51
	Peanut waste					
	Peanut straw	6.5	19000	69246.45	567	122.13
	Total Maros					169.97 AU

*Data of area (ha) is get from observation during the study. Paddy waste production (kg/ha) and DM of paddy straw are getting from unit equalization in Azis et al. 2014; Rice bran production predicting need DM of rice bran that finds in Akbarillah et al. (2007); Production of paddy waste, corn waste, and peanut waste (kg/ha) got from sampling in both location of study ; DM of corn waste got from Ardiana et al. (2015); DM of peanut waste got from Syamsu (2007); DM of production (kg/3 months of the dry season) from every crop waste calculating depends on crops waste production and DM.*

In Indonesia, the dryland potential of food crops reaches 29 million ha (BBSDLP 2014). Assume that if 10% can be applied "Water-saving technology", it will support the carrying capacity of 21,239.600 heads of beef cattle cross-bred (2.9 million ha/10 ha  $\times$  73.14 chairs) in the development of rice plants during the dry season. The result of crops (maize and peanut) can support feed availability as 54,488.789 of Bali cattle (2.9 million ha/6.5 ha  $\times$  122.13 heads). The condition of the real Indonesia still has a lower carrying capacity than the potential (Sitindaon 2013; Arifin and Riszqina 2016; Mirah et al. 2015). It was reported by Kleden et al. (2015) that livestock holding capacity is 0.42 AU/ha/year for coffee plantation areas, while 0.38 AU/ha/year for pasture (grazing area). In intercropping, planting grasses and legumes is carried out in semi-arid locations to meet livestock feed needs (Menezes et al. 2019). Corn and wheat intercropping is also carried out to reduce carbon emissions and water use efficiency in agricultural activities (Hu et al. 2016). Corn and legume intercropping was also carried out by Masvaya et al. (2017). Intercropping cereals with legumes increases the production and stability of the production of these crops (Raseduzzaman & Jensen 2017).

The resulting capacity is only limited to the achievement of the area of land that the intensity of cropping patterns can increase. This is primarily related to crop by-products planted and utilized by beef cattle livestock in each location (calculated for three months of feed use). The wider the target of the reservoirs/dam trench is built, it will produce a carrying capacity of more feed that can be utilized more for beef cattle. Agricultural by-products can be used as forage substitutes for which availability is limited to beef cattle. Study in Parepare city with land use for the crop is 7,505 ha can provide feed for 688.43 AU (Rauf & Rasbawati 2015), while that in Sumedang with potentially land use can provide feed for 26,174.21 AU of small ruminants and 59,565.58 AU of large ruminants from potential protein and TDN of natural grass and crops by-products as much as 20,366.39 ton/year and 147,553.67 ton/year, respectively (Tanuwiria et al. 2006). This showed that dryland used for beef cattle farming by providing feed from crop by-products could be improved by increasing the target area for implementing this program.

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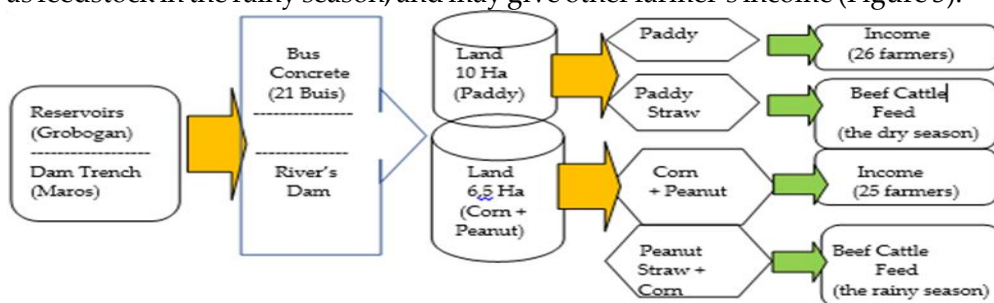
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### *Effect of time using innovation of feed in the dry season*

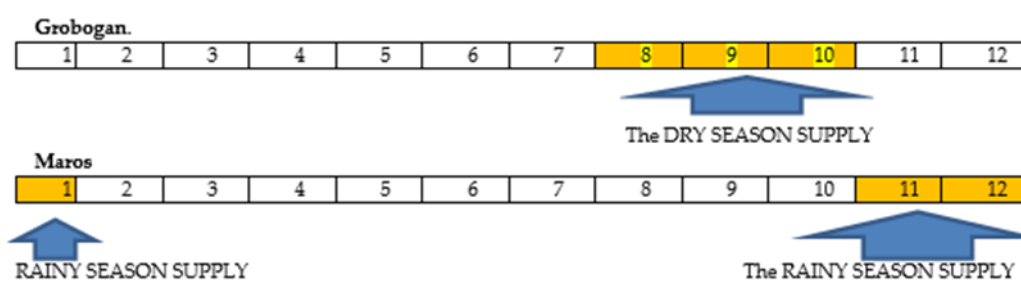
Grobogan has reservoirs that distribute water to 21 wells from buis concrete to provide water for crops activity around 10 ha nearby the water containers in the dry season. This condition supports the paddy plantation in the dry season (August - November) while the water-saving program is created. Increasing the activity of the crops due to rising water available for plantation can improve the paddy production by 7.2 tons/ha in 10 ha area owned by 26 farmers. The calculation showed that the farmer's income improved by Rp 12,728,461/farmer. Besides, rice straw was produced so that it can be used as beef cattle feed. On the other hand, feed for beef cattle are limited in the dry season and force the farmer to buy feed for a different location, so the existence of crops by-products as feed was needed. Relocating the water program can give the added value of rice straw in the dry season and impact feed demand for beef cattle. While that in Maros, Dam trench developing has provided water for 6.5 ha area planted by corn and peanut (intercropping), which produce 5.3 ton and 0.7 ton of main product, respectively and sold in Rp 7,000/kg and Rp 15,000/kg. This condition may add value to farmer's income by Rp. 7,800,000/farmer, in addition to the utilization of crops by-products used as animal feed. Crops-livestock integration is the appropriate program to be implemented as an effect of reservoirs/dam trench developing programs in both locations (Figure 2).

The utilization of feed looks different at the two observation sites. The use of paddy rice in Grobogan as an available feed may impact decreasing feed cost in the dry season caused by reducing looking feed for other locations (August-November), especially for the farmer that has more than three heads of beef cattle. Using reservoirs for irrigation can increase the crop plantation intensity correlated with raising the rice straw available for feed. Meanwhile, the rainy season in Maros usually uses the land for crop activity (paddy), so the farmer hardly provides grazing areas for their cattle. Hence, feedstock was a critical point to provide feed during the rainy season. Dam trench can be used

to provide water for corn and peanut were in turn crops by-products available as feedstock in the rainy season, and may give other farmer's income (Figure 3).



**Figure 2.** The integration model is applied from the impact of developing irrigation management to the contribution of income and animal feed during the dry season



**Figure 3.** Utilization of waste by farmers in both locations (Grobogan and Maros regency)

## CONCLUSION

The development of innovation in "Water Saving Technology" (Reservoir and Dam Trench) in the dryland areas can be concluded that in Grobogan, the role of beef cattle (48.59%) is the main source of income. Instead, in Maros, the main source is food crops (51.87%) due to the broader ownership of land agricultural. This innovation increased the farmer income of food crop in Grobogan by Rp. 12.80 million/farmer (rice), while in Maros, it reached Rp. 12.83 million/farmer (corn and peanut). The impact of this innovation in Grobogan can supply rice straw and rice bran with its carrying capacity of 73.13 beef cattle (10 ha). While that in Maros support from corn straw and peanut (planted intercropping) potential as Bali cattle feed as much as 168.97 heads (area 6.5 ha), when there is a shortage of feed through the model of crop-livestock integration. The allocation utilization in Grobogan is during the dry season when many farmers purchase feed. Otherwise, the case in Maros

prepared for the supply of feed in the rainy season because cattle can not graze (planted paddy rice). Implementation of program innovations made very precise as on dry land development policy in the future, given the potential for developing prospective land food crops that are still widely available in Indonesia.

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# **Livestock Nutrition and Feed Technology**



## Methane Mitigation the *In Vitro* Rumen Fermentation using Combination of Bioindustrial Products of Cashew Nutshell

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### ABSTRACT

One of the strategies to mitigate methane emissions is by using feed additives derived from plant extracts that contain secondary compounds. The study aimed to mitigate methane and improve other rumen fermentation products by adding combination of two bioindustrial products biofat (BF) and biosmoke (BS) of cashew nut shells in the *in vitro* study. This experiment used a randomized block design with 6 treatments and 4 replications. The treatments were different combinations of biofat and biosmoke as follows: Control= substrate without addition of biofat or biosmoke; BFBS1 = 0%BF: 100%BS; BFBS2 = 25%BF:75%BS; BFBS3 = 50%BF:50%BS; BFBS4 = 75%BF:25%BS; BFBS5 = 100%BF: 0%BS. The variables that observed were: total gas and methane productions, ruminal NH<sub>3</sub> and partial VFA concentrations, DM, OM and NDF degradabilities, The results showed that the CH<sub>4</sub> production reduced significantly ( $P<0.01$ ) by the addition of combinations of biofat and biosmoke into the substrates.. Methane production was lower compared to control by 11.50% (BFBS1), 36.85% (BFBS2), 38.50% (BFBS3), 41.84% (BFBS4) and 26.07% (BFBS5) compared to control. Significantly higher propionate and total VFA concentration and similar NH<sub>3</sub> concentration were obtained in all combinations except BFBS5 compared to control. DMD and OMD in the presence of combination of biofat and biosmoke at different ratios were similar as the control ( $P>0.05$ ). In conclusion, the effort to mitigate methane and enhancing other rumen fermentation products was obtained by addition of the best combination of biofat and biosmoke with the ratio of 75%BF:25%BS as feed additive in the *in vitro* rumen fermentation.

**Keywords:** Methane, Biofat, Biosmoke, Cashew nutshell, *In vitro* rumen

### INTRODUCTION

The development of beef cattle farming continues to receive fully support from the government in an effort to be self-sufficient in animal protein. In the era of the COVID-19 pandemic, the recommendation to consume protein, especially animal protein, is increasing in the community. It is expected that

the increased consumption of animal protein can increase the community's immunity to fight the COVID-19 virus.

At present, significant efforts from the Indonesian government have been conducted to increase the local livestock population. However, this increase results in higher methane emissions from ruminant. Estimated global CH<sub>4</sub> emissions in the last decade was around 574 million tonnes with a contribution and global population of 15% ruminants (Van Amstel 2012). The contribution of CH<sub>4</sub> emissions of ruminants in Indonesia to global CH<sub>4</sub> emissions was 0.14% (0.79 million tons of CH<sub>4</sub>/year) (Thalib et al. 20012). The formation of methane in the rumen was a result of the binding of carbon dioxide with hydrogen through the activity of methanogenic bacteria. The methane was then emitted through eructation (about 83%), respiration (about 16%) and anus (about 1%) (Vlaming 2008).

Therefore, strategies to reduce methane emission from ruminant should be conducted without affecting ruminant productivity. One of these strategies is through feed additives which derived from plant extracts that contain secondary metabolic compounds such as saponins (Wina 2012; Yuliana et al. 2014), tannins (Jayanegara et al. 2009; Bhatta et al. 2013), an extract from cashew nut shell (CNSL) (Watanabe et al. 2010) and essential oil (Patra & Yu 2012, Bodas et al. 2012). The main product of cashew plants (*Anacardium occidentale* Linn) is their nuts, and one of the wastes is the nut shells which are 45-50% of the total nut. Utilization of this nut shell to be more beneficial can be done by hexane extraction, followed by pyrolysis to become biofat (CNSL), biochar and biosmoke (Saenab et al. 2016). Each of these three products have shown to decrease *in vitro* CH<sub>4</sub> production (Saenab et al. 2018). The synergistic effect of combination between biofat and biochar have been published previously (Saenab et al 2020)

The experiment aimed to observe the addition of biofat (0.25 µl/ml) with biosmoke (2.5 µl/ml) with different ratio as feed additive in the *in vitro* rumen fermentation and to reduce methane production and to improve rumen fermentation products.

## MATERIALS AND METHODS

### Experimental procedures

The experiment was conducted from January to April 2016, at the Feed Laboratory of the Research Institute of Animal Production (Balitnak) in Bogor. The experiment has been approved by the Animal Welfare Commission of the Indonesian Agency or Agricultural Research and Development (Balitbangtan/Balitnak/Rm/05/2016).

The cashew nut shells were obtained from farmers in Pati Regency, Central Java Province. Bioindustrial products of cashew nut shell (biofat and biosmoke) were used as feed additive in this experiment. The method of preparing biofat (BF) and biosmoke (BS) was described in Saenab et al. (2016).

A complete feed for cattle consisted of grass, *Gliricidia sepium* leaves, corn, coconut cake, molasses, bran, urea, salt (NaCl), limestone (CaCO<sub>3</sub>), and premix used as a substrate in the *in vitro* rumen fermentation. The CP and TDN of this complete feed were 15.63% and 69.7%, respectively (Saenab et al. 2018).

Buffer medium was prepared by mixing bicarbonate buffer solution, macro-mineral solution, micro-mineral solution, resazurin, distilled water, reducing solution and rumen fluid as described in Makkar (2003).

### ***In vitro* rumen fermentation**

Treatments were different combinations of biofat (BF, 0.25 µl/ml) and biosmoke (BS, 2.5 µl/ml), that were as follows: 1) Control (Substrate without any addition of Biofat or Biosmoke), 2) Substrate + BFBS1 = 0%BF : 100%BS; 3) Substrate + BFBS2 = 25%BF : 75%BS; 4) Substrate + BFBS3 = 50%BF : 50%BS; 5) Substrate + BFBS4 = 75%BF : 25%BS; 6) Substrate + BFBS5 = 100%BF : 0%BS.

Different combinations biofat and biosmoke were each added to the substrate. A total of 750 mg of substrate was weighed into the bottle. Rumen buffer solution (75 ml) was added and the rubber stopper was quickly applied on the bottle. The bottle was placed in the water bath and incubated at 39°C for 48 hours. Rumen fluid was collected just before morning feeding from rumen of a fistulated Friesian Holstein cow fed with commercial concentrate and elephant grass. The total gas and methane production were recorded at 3, 6, 9, 12, 24, 30, 36, 48 hours of incubation. At the end of incubation, the supernatant was separated by filtration to obtain residue and supernatant. The residue was dried in the oven 105°C for 24 hours and weighed. Ash content of feed and residue was determined according to AOAC method (2005) and NDF analysis was conducted following Van Soest method without addition of amylase. The *in vitro* dry matter (DM) and organic matter (OM) of digested fractions were calculated from the dry matter and organic matter of initial sample minus those of residue. The DM or OM of digested fractions divided by the DM or OM of initial sample was calculated as *in vitro* dry matter or organic matter degradabilities. Other residue samples of *in vitro* incubation were digested using Neutral detergent solution to obtain residual NDF fraction. The NDF of digested fractions divided by the NDF of initial sample was calculated as *in vitro* NDF degradability. pH, NH<sub>3</sub> and VFA were measured after 4 hours of incubation. Ammonia content in the supernatant was determined using Conway microdiffusion technique. The supernatant for

volatile fatty acid (VFA) analysis was kept in low pH by adding sulphuric acid. Volatile fatty acid (VFA) products from fermentation were analysed by GC using gas chromatography (Bruker Scion 436 GC) with capillary column BR-Wax fame containing WCOT fused silica with the length of column 30 m × 0.32 mm ID. The carrier gas was Nitrogen 25 ml/min and the burning gas was Hidrogen 30 mL/min. Injector temperature was 250°C, while the column temperature gradient was 70-150°C in 11 minutes. The detector used was FID with temperature of 275°C.

### **Statistical analysis**

This study used a Randomized Block Design (RBD) with 6 treatments and 4 replications. The experimental data from different combinations of biofat and biosmoke and control (substrate without any addition of biofat or biosmoke) were analyzed separately by PROC GLM using SPSS Program Package 16. Further analysis using Duncan test was done for obtaining significant differences among treatments.

## **RESULTS AND DISCUSSION**

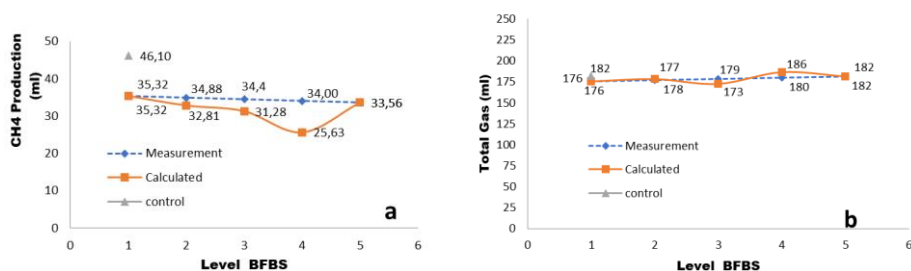
The effects of combination of biofat with biosmoke with various levels on CH<sub>4</sub> production, total gas, ammonia, pH, are shown in Figures 1a, 1b, 2a and 2b. There are 2 curves, namely curves (dashed lines) indicating values predicted or calculated as written in the research method. The thick line curve shows the measurement results of in vitro fermentation. The triangle point shows the measured value of the variable measured from the control treatment.

Figure 1a shows that methane production measured decreased to the level of administration of a combination of biofat and biosmoke (BFBS4 = 75 : 25%) compared to the control and vice versa at the level of combination administration (BFBS1 = 100 : 0%) methane production increased. The results of the analysis showed that the addition of a combination treatment of biofat and biosmoke caused a very significant decrease ( $P < 0.01$ ) on the production of methane gas in the rumen.

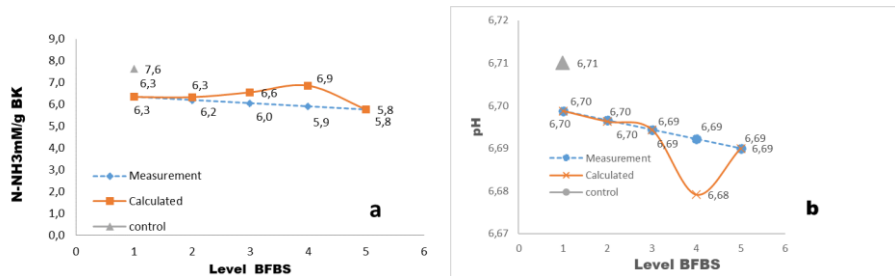
From the analysis, the results showed a decrease in methane production by 23.37% (BFBS1 level = 0%BF : 100%BS), 28.83% (BFBS2 level = 25%BF : 75%BS), 32.14% (BFBS3 level = 50%BF : 50%BS), 44.40% (BFBS4 level = 75%BF : 25%BS) and 27.21 (BFBS 5 level = 100%BF : 0%BS) (Figure 1a). The highest decrease in methane production (44.40%) was obtained at BFBS4 level (= 75%BF : 25%BS)

The synergistic effect that can be seen from the higher decrease in the production of methane measured compared to the predicted methane or the value obtained from the calculation is produced by a combination of biofat with biosmoke. Synergy in suppressing methane may be caused by biofat containing bioactive compounds, namely anacardic acid and its derivatives which can reduce methane production (Saenab et al. 2017). The effect of anacardic acid is very strong (dominant) in reducing methane gas production. This is because anacardic acid is a surfactant that can disrupt bacterial cell membranes in the rumen, thereby reducing the microbial population in the rumen and inhibiting methanogenic activity which causes decreased methane gas production (Watanabe et al. 2010). Anacardic acid is a phenolic compound that has antimicrobial activity that can inhibit the growth of methanogens and several other bacteria, thereby reducing methane (Kamra et al. 2006).

Synergy in suppressing methane has also been reported by Jayanegara *et al.* (2012) who use the term 'associative effect'. By combining several plants containing tannin compounds (*Carica papaya*, *Clidemia hirta*, *Swietenia mahagoni* and *Eugenia aquea*), it can provide an associative effect on suppressing methane in in vitro rumen fermentation (Jayanegara et al. 2012). High phenolic compounds in a plant will interact with chemicals from other plants such as proteins and carbohydrates through hydrogen bonds, so that the degradation of carbohydrates in the rumen is disrupted and causes a decrease in H<sub>2</sub> production which is the substrate for methanogenesis. This mechanism shows that methane production is declining. According to Saenab et al. (2016) that biofat and biosmoke contain different phenolic compounds. Biofat contains phenolic compounds that bind to long chain fatty acids (C15) while biosmoke contains simple and acidic phenolic compounds. Besides that, biofat also contains bioactive compounds, namely anacardic acid, cardol and cardanol which can reduce methane (Saenab et al. 2017). Both of the cashew shell's bioindustrial products can reduce methane.



**Figure 1.** a. Methane of production; and b. Total gas of production from *In vitro* Fermentation with combination of Biofat: Biosmoke with different levels.



**Figure 2.** a. Ammonia Production; and b. The pH value of in vitro Fermentation with Combination of Biofat: Biosmoke with different levels

Figure 1b shows the results of the analysis of the effect of the combination treatment of biofat levels and biosmoke which increased insignificantly ( $P > 0.05$ ) on total gas production in vitro in the rumen compared to controls. This shows that the effect of the combination treatment of biofat with biosmoke did not interfere with the fermentation process which led to similar total gas production.

Not as the effect of the combination treatment of biofat and biochar, that the addition of a combination of biofat and biosmoke did not provide a synergistic effect on ammonia levels in feed fermentation in vitro (Figure 2a). The effect of the combination treatment between plants containing phenolic was reported between plant cocksfoot (*Dactylis glomerata* cv. Cristoss) and Sainfoin (*Onobrychis viciifolia* cv. Zeus); the combination of Hypericum perforatum and pine oil (pine mugo), and a combination of ginger, peppermint and garlic. These combination also showed no synergistic effect on ammonia levels *in vitro* feed fermentation in the rumen (Niderkorn *et al.* 2012; Soliva *et al.* 2008; Wanapat *et al.* 2013).

In line with the results of ammonia, the combination of biofat with biosmoke showed no synergistic effect on rumen pH in feed fermentation in vitro (Figure 2b). Rumen pH decreased significantly ( $P < 0.05$ ) at the BFBS4 combination level = 75: 25% compared to the control but the pH value from the results of this study is in the normal range, namely the average of 6.67-6.73. The results of this study are in line with the results obtained by Saenab *et al.* (2016) that the administration of biosmoke reduced rumen pH and it is known that biosmoke is acidic or has a low pH so that its effect might cause decreased rumen pH. This is in line with the results of the study of Niderkorn *et al.* (2012) and Yang (2017) that the combination of plants containing phenolic which is between Cocksfoot plants (*Dactylis glomerata* cv. Cristoss) and Sainfoin (*Onobrychis viciifolia* cv. Zeus) showed no synergistic effect on rumen pH.

The proportion values of Acetate, Propionate, Valerate, BCVFA, Total VFA (mM) and Acetate/Propionate, from the treatment of a combination of biofat and cashew shell biochar at different levels can be seen in Table 1.

**Table 1.** The effect of biofat/biosmoke combination at different ratios on molar proportion of Acetate, Propionate, Butyrate, Valerate, BCFVA and Total VFA (mM) concentration, and Acetate/Propionate of feed incubated 48 hours in the in vitro rumen fermentation

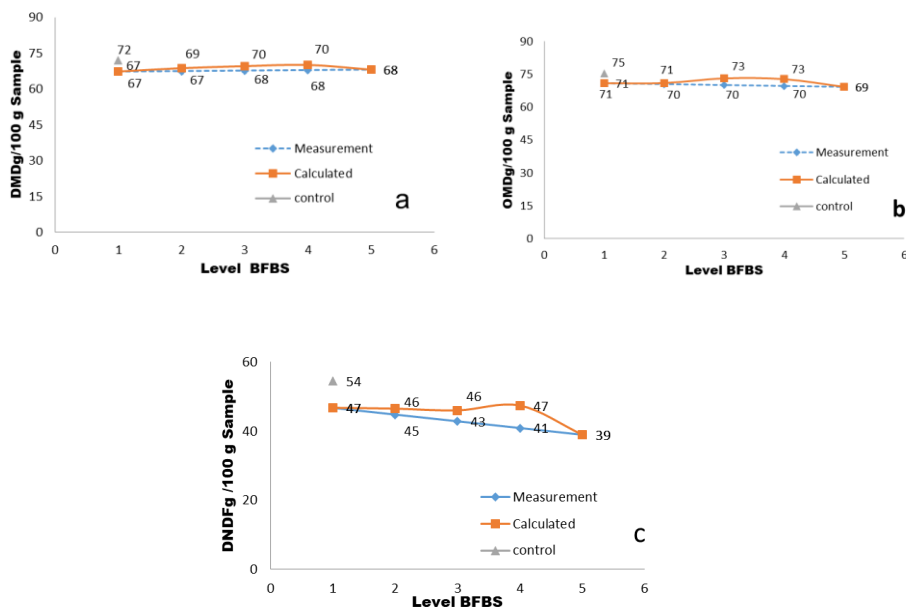
Treatment	Level	Acetate	Propionate	Butyrate	Valerate	BCVFA	A/P	Total VFA
		mol/100mol						(mM)
Control	0 %	62.060 <sup>c</sup>	20.140 <sup>a</sup>	10.330	2.180	5.270 <sup>bc</sup>	3.110 <sup>b</sup>	72.420
Biofat (BF)/ Biosmoke (BS)	0% BF : 100 % BS	59.840 <sup>bc</sup>	22.570 <sup>ab</sup>	10.150	1.790	4.640 <sup>ab</sup>	2.660 <sup>a</sup>	69.130
	25% BF : 75% BS	58.040 <sup>bc</sup>	24.570 <sup>b</sup>	11.300	2.070	4.010 <sup>a</sup>	2.360 <sup>a</sup>	70.940
	50%BF : 50% BS	57.410 <sup>ab</sup>	24.350 <sup>b</sup>	11.890	2.040	4.290 <sup>ab</sup>	2.380 <sup>a</sup>	74.820
	75%BF : 25%BS	55.000 <sup>a</sup>	24.690 <sup>b</sup>	12.230	2.240	5.830 <sup>c</sup>	2.250 <sup>a</sup>	79.400
	100% BF : 0% BS	59.390 <sup>bc</sup>	24.990 <sup>b</sup>	11.900	1.830	4.420 <sup>ab</sup>	2.390 <sup>a</sup>	78.300
P-value		0.022	0.024	0.109	0.716	0.009	0.009	0.622
SE		1.277	1.007	0.598	0.240	0.324	0.151	4.847

BCVFA = branched chain short chain fatty acids, A / P = Acetate / Propionate

Different letters in the same column show significant ( $P < 0.05$ ) or very significant ( $P < 0.01$ ) difference. Statistical analysis of each product was tested separately against the control

Table 1 shows the combination treatment of biofat and biosmoke (BFBS4 = 75 : 25%) increased propionate and BCVFA but reduced acetate production significantly ( $P < 0.05$ ). Increasing molar proportion of propionate may be due to the ability of biosmoke containing simple phenolic compounds to and biofat which also contained some phenolic compounds in increasing the population of propionate-producing bacteria. This result is in line with the results of the study of Wanapat et al. (2013) who combined several plants containing simple phenolic compounds (ginger, peppermint and garlic) and added them in the in vitro rumen fermentation resulted in an increased propionate, reduced acetate and butyrate.

The increase in BCVFA at BFBS4 = 75 : 25% showed that the effect of the combination treatment of biofat and biosmoke could not inhibit protein degradation or did not inhibit the protease enzyme or population of protein breaking bacteria into BCVFA, but when the level was increased to 100% biochar the treatment effect the combination of biofat and biosmoke against BCVFA decreased.



**Figure 3.** DMD (a), OMD (b) and NDFD (c) profiles of feed in the in vitro rumen fermentation with different combination level of biofat and biosmoke

The results of the analysis showed that the degradation value of the measurement results decreased but did not have a significant effect ( $P > 0.05$ ) on the level of administration of the combination of biofat and biosmoke compared to the control (Figure 3a, 3b dan 3c). This might be due to the

influence of giving anacardic acid biofat. The structure of lipids in anacardic acid which had unsaturated bond could reduce the degradation of fiber in the rumen because unsaturated fatty acids were toxic to fiber-breaking bacteria (Maia et al. 2007). According to Watanabe et al. (2010) that the addition of CNSL (biofat) inhibited the growth of *Ruminococcus flavefaciens* and *R.albus* which have the ability to digest fiber. This also resulted in degradation of DM, OM and fiber degradation (NDF) decreased with increasing levels of biofat administration, although it has been combined with biosmoke But the effect was not significant may be due to the low level of biofat or biosmoke added into the in vitro rumen fermentation

In contrast with the results of the above research, the combination of plants containing plant compounds such as plants (ginger, peppermint and garlic) reduced DM, OM and NDF (fiber) digestibility in goats (Wanapat et al. 2013) and Seongjin (2017). Also reported by Niderkorn et al. (2012) that the combination of plants containing phenolic compounds such as plant cocksfoot (*Dactylis glomerata* cv. Cristoss) and Sainfoin (*Onobrychis viciifolia* cv. Zeus) decreased degradation of NDF (fiber).

## CONCLUSION

It was concluded that the highest methane mitigation while improving other rumen fermentation products and not affecting digestibility was obtained at the combination of biofat and biosmoke at the ratio of 75% Biofat :25% Biosmoke. This combination level of biofat and biosmoke should be further tested as feed additive with different types of feed on animal responses are warranted.

## ACKNOWLEDGEMENT

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## **Effects of the Natural Feed Additive on Internal Organs and Abdominal Fat of Broiler Ducks**

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### **ABSTRACT**

The use of antibiotics in the poultry feed industry has been abandoned because it could become residues in livestock origin products. Phytobiotics are bioactive compounds of plants that can be used as alternatives antibiotics in animal feed. The study was conducted to assess the effects of different natural feed additives and levels on internal organs weight and abdominal fat of broiler ducks. A total of 180 two-week-old broiler ducks were randomly allotted to 9 treatments with 4 replicates, each with 5 birds per replicate. The treatments were T0 (basal diet as a control), T1 (basal diet + 1% of noni), T2 (basal diet + 250 ppm of clove oil), T3 (basal diet + 2% of noni), T4 (basal diet + 500 ppm of clove oil), T5 (basal diet + 1% of noni + 250 ppm of clove oil), T6 (basal diet 1% of noni + 500 ppm of clove oil), T7purba (basal diet 2% of noni + 250 ppm of clove oil), and T8 (basal diet 2% of noni + 500 ppm of clove oil). Data were collected obtain abdominal fat and internal organs weight. The data were analyzed of variance based on a Completely Randomized Factorial Design and continued with Duncan's multiple range test for differences. The result study showed that the abdominal fat of broiler ducks has no significantly reduction in different natural feed additive and levels. The natural feed additives have no negative effects on internal organs weight of broiler ducks.

**Keywords:** Phyto biotic, Noni fruit, Clove oil, Internal organ

### **INTRODUCTION**

Livestock production performance is not only achieved by providing the right amount and quality of nutrients, but also increases production by providing feed additives in the form of prebiotics, probiotics, phytogenics and antibiotics. Commonly used feed additives are antibiotics or Antibiotic Growth Promoters (AGP) that can help suppress the growth of pathogenic bacteria so as to increase livestock production. Antimicrobials, especially antibiotics in livestock are used as therapy, prevention of disease, and are also used as a growth promoter (Ventola 2016). Efforts to find alternative AGP continue to be carried out in line with the prohibition by government regulation Permentan No. 14/2017. Improve productivity is effectively obtained with the use of synthetic feed additives but can lead to pathogen contamination and develop resistant bacterial populations. The phytogenic

feed additives are alternative feed additives that aim to improve feed quality, animal health and animal products through their specific nutritional properties. They can be classified into several groups: sensory additives, technological additives, zootechnical additives and nutritional additives (Madhupriya et al. 2018).

Extraction or distillation of clove leaves (*Syzygium aromaticum* L) will produce essential oils of 2-4%, with a content of eugenol 70-90%. Clove essential oil has biological activities, such as antibacterial, antifungal and antioxidant properties, and is used traditionally as a flavor enhancer agent and antimicrobial ingredient in food (Lee & Shibamoto 2001; Velluti et al. 2003). The use of eugenol clove leaf essential oil in feed is expected to improve the performance of broiler chickens to produce high quality meat products. This is possible because eugenol essential oil clove leaves have antimicrobial and antioxidant properties and serve as a growth booster. In addition, eugenol compounds from clove leaf essential oil can increase digestive enzymes and improve the performance/growth of livestock, where the mixture of essential oil components stimulate the secretion of digestive enzymes in chickens (Zeng et al. 2015).

*Morinda citrifolia* or noni fruit contains nutritional values such as essential amino acids, minerals, vitamins- enzymes, carbohydrates, alkaloids, and other nutrients that directly or indirectly help the metabolism of nutrients and for the growth of cells and tissues (Blanco et al. 2006; Abou et al. 2015). Broilers given fresh Noni fruit juice (1.5 ml/head/day) showed better production appearance on weight gain, feed conversion, and feed efficiency (Sunder et al. 2011). The administration of 5% noni fruit extract in feed shows growth and egg production appearance in quails (Sunder et al. 2013). Antimicrobe activity in noni fruit as a feed additive is expected to improve the condition of microflora in the digestive tract of poultry, especially in the small intestine. Good microflora condition in the small intestine can optimize the absorption of food substances so that livestock are able to increase the digestibility of feed and produce optimal production appearance (Almeida et al. 2019).

Research on the performance of broilers given eugenol clove leaf essential oil and fruit flour curdling in feed aims to evaluate the dose of eugenol clove leaf essential oil as a feed additive that provides the best performance. The results of this study are expected to provide a solution to the use of synthetic antibiotics whose function is replaced by eugenol clove leaf essential oil and noni fruit powder to produce safer meat products. Based on the description above, there needs to be research on the application of use noni fruit powder and clove oil on the productivity of broilers ducks which include abdominal fat and weight of internal organs.

## MATERIALS AND METHODS

### Birds and dietary treatments

The research was conducted at Mitra Karya Farm Blitar. This research used 180 broiler ducks (unsexing) aged 2 weeks with a coefficient of diversity of body weight of less than 10%. The type of duck used was a broiler hybrid duck which is a cross between a Peking male duck and a female Khaki Chambell duck from local hatchery. The ducks were allocated into 36 plots, each contained 5 broilers ducks with a maintenance duration of 4 weeks. Each unit was equipped with a feed and drinking area. The feed provided was prepared in accordance with the nutritional needs of broilers ducks according to NRC standards (1994) in Table 1. Research materials noni fruit powder and clove oil were obtained from Materia Medica, Batu City.

**Table 1.** The composition and nutritional content of basal diet

Ingredients	Amount
Polished rice (% as fed)	35.00
Polished maize (% as fed)	27.51
Soybean meal (% as fed)	17.89
Local fish meal (% as fed)	17.00
Palm oil (% as fed)	2.00
Promix (% as fed)	0.50
Salt (% as fed)	0.10
Analyzed nutritional % of DM*:	
Gross energy (cal/g)	3,928.00
Crude protein (%)	16.19
Crude fat (%)	6.01
Fiber (%)	3.60
Calcium (%)	1.75
Phosphorus (%)	0.64

\*Analyzed by Laboratory of Animal Nutrition and Feed Science, Blitar District's Livestock and Fisheries Service

### Experimental bird management

The research method used is a biological test designed using a Complete Randomized Design (CRD) consisting of 9 treatments and 4 replays namely T0: basal feed (control); T1: 1% noni fruit powder; T2: clove oil 250ppm; T3: 2% noni fruit powder ; T4: clove oil 500ppm; T5: 1% noni fruit powder + clove oil 250ppm; T6: noni fruit powder 1% + clove oil 500ppm; and T7: 2% noni

fruit powder and 250ppm clove oil; T8: 2% noni fruit powder + 500ppm cloves. Biological testing procedure begins with preparing noni fruit powder, clove oil and preparation of feed materials used as basal feed and maintenance of broiler hybrid ducks. Broiler hybrid ducklings are kept in groups in cages measuring 70 × 80 × 40cm each 5 heads are grouped according to the treatment given. The floor plan of the broiler hybrid duck maintenance cage is arranged randomly. Noni fruit powder and clove oil are given as feed additives on basal feed in accordance with the treatment. *Ad libitum* feeding is given in the morning (at 07.00) and afternoon (15.00) with feed according to treatment. The slaughter of broiler ducks is carried out at the age of 42 days by fasting first. The slaughter process consists of bleeding, scalding, picking, and eviscerating. Eviscerating process is done by carefully separating the organs of the liver, spleen, gizzard, heart, and abdominal fat. The parameters tested include the appearance of production and carcass quality such as abdominal fat percentage and internal organ weight.

### Statistical analysis

The data obtained is analysed using ANOVA variety analysis, if there are differences followed by Duncan's Multiple Distance Test.

## RESULTS AND DISCUSSION

The effect of either noni fruit powder or clove oil on internal organ, giblets weight and abdominal fat of broiler ducks were summarized in Table 2. Statistical analysis of giblets weight showed there was no significant ( $P>0.05$ ) difference between experimental groups. This may be due to affected by the similarly nutrient content in the basal diet.

**Table 2.** Effect of feeding noni fruit powder and clove oil on internal organ of broiler ducks

Item	Treatments									P
	T0	T1	T2	T3	T4	T5	T6	T7	T8	
Liver (g)	42.54	41.91	39.86	44.17	41.29	44.87	39.80	43.06	44.89	0.989
Gizzard (g)	48.66	52.34	48.45	54.06	54.31	47.50	48.75	50.68	53.75	0.805
Heart (g)	10.16	10.65	9.60	9.61	9.84	10.12	9.99	9.39	10.30	0.591
Spleen (g)	2.82	2.02	1.50	1.39	1.28	1.68	1.47	1.67	2.17	0.099
Giblet (%) *	5.96	6.36	5.92	6.38	5.96	6.32	5.99	6.39	6.50	0.591
Abdominal fat (%) *	1.18	1.23	0.90	0.80	0.93	1.03	1.01	1.09	1.04	0.725

\*% of final weight

## Internal organs

The effect of noni fruit powder and clove oil on liver, gizzard, heart, spleen, and giblets weight of broiler duck showed there was no significant difference ( $P>0.05$ ). These results were in line with Kurniawan et al. (2020) reported that the dietary *Morinda citrifolia* and *Arthrospira platensis* powder were no significant different effects among treatments on liver, gizzard, heart, spleen, giblet, and abdominal fat of broiler ducks. Sjojfan et al. (2019) reported that the different form of probiotic had no effect on internal organs weight due to the same nutrient content in the ration of dietary and age of slaughter. The addition different levels amount of different probiotic forms ranged between 0-0.4% did not significantly affect the weight of internal organs. The factors affect internal organs weight are size, colour, and consistency of liver such as breed, genetics, age, sex, individual status, and feed intake. Furthermore, either powdered or encapsulated probiotic was anti-nutrition free reported that accumulation of toxins or anti-nutrition in the heart muscles will change the heart size (Ardiansah et al. 2020). Mukhtar (2011) reported that the effect of clove oil and antibiotic on the broiler performance showed no significant ( $P<0.05$ ) difference for chick performance, but the improved in feed intake in 400mg/kg clove oil could be due to these positive effects of clove oil on the digestive system. Feeding poultry more than the requirement cause extra-digestion and it would affect the size of gizzard due to the thickening of the muscle type of feed particle size (mash, crumble, or pellet) affected giblets weights, smaller particle size decrease gizzard activity (Tuli et al. 2014).

## Abdominal fat

The effect of noni fruit powder and clove oil on abdominal fat of broiler duck showed there was no significant difference ( $P>0.05$ ). Abdomen fat is a layer of fat found around the gizzard and the lining between the abdominal and intestinal muscles. The average abdominal fat weight of Tegal ducks was almost the same which ranged from 1.00 to 1.59 grams (Sahara et al. 2019). Subekti et al. (2012) report that abdominal fat is influenced by body weight. Except for that, the nutrients consumed by ducks will be used for the first time for life, after that for production. This is thought to cause a limitation of the fat depot and suppress excess body fat. As a result, each individual research duck in each treatment had balanced abdominal fat. This is understandable because energy consumption is prioritized for basic life and production. The weight of abdominal fat tends to increase with weight gain (Dewanti et al. 2013). Widjastuti (2019) report that the addition of noni juice into drinking by adding 1–3 ml of noni juice per litter of drinking water has not significantly affect

( $P>0.05$ ) on the abdominal fat of Sentul chicken but has significant effect ( $P<0.05$ ) when using 4 ml/l and 5 ml/l in drinking water. The decrease in abdominal fat because the bioactive substance as xeronine would improve the metabolism of carbohydrate and fat in the carcass.

## CONCLUSION

The finding of the present study suggests that the abdominal fat and of broiler ducks has no decreased significantly in different noni fruit level up to 2% and clove oil level up to 500 ppm. The natural feed additives have no negative effects on internal organs weight of broiler ducks.

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## AUTHOR CONTRIBUTIONS

D. Kurniawan, A. Widigdyo and A.S.W. Utama were contributed equally to this work.

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## **Substitution of Indigofera Leaf Meal in Feed on the Production and Quality of Senkub Chicken Eggs and its Business Feasibility**

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### **ABSTRACT**

Eggs are a food material with high nutritional value, which is needed by the human body. The desired quantity and quality of eggs, is inseparable from the quantity and nutritional quality of the feed given. In intensive chicken farming, feed is the largest cost, which can reach 70% of the production cost. *Indigofera zollingeriana* plant is a legume plant that has a high protein content, which has been widely used for a mixture of protein source rations. The research objective was to see the effect of Indigofera leaf flour substitution in rations on the production and quality of native chicken eggs and the level of farmer acceptance. The research was conducted in plasma farmers using 200 Senkub chickens aged 18 weeks consisting of 4 feed treatments. The variables observed were production performances and physical and chemical egg qualities. To determine the level of acceptance and feasibility of technology components, a farm analysis was carried out. The results showed that substitution of rations with 10% Indigofera leaf flour had no effect on production performances ( $P>0.05$ ). However, adding Bio b to drinking water (P2) reduced the ration conversion ( $P<0.05$ ). The physical quality of eggs, was not significantly affected ( $P>0.05$ ) egg fat content reduced by 24.27% and egg yolk protein content increased by 13.38%. Substitution of rations with Indigofera leaf flour reduced the in feed cost by 17% in the laying period so that it was feasible to be disseminated. In conclusion, Indigofera leaf flour substitution had no effect on production performance, increased egg protein content and decreased feed cost.

**Keywords:** Indigofera flour, Chicken, Performances

### **INTRODUCTION**

The Agricultural Research and Development Agency has been developing superior chickens strain called KUB Chicken. KUB chickens were developed as layer hens. while for broilers, a superior kampong chickens were also produced (SenSi-1 Agrinak). The cross between these two strains was given called Senkub chicken. This SenSi-1 Agrinak chicken was the result of 6 generations based on male line selection research within superior growth and higher body

weight. SenSi-1 Agrinak chickens were divided into 2 groups based on feather colour; SenSi-1 Agrinak has grey feather and SenSi-1 Agrinak has white feather. The results of the selection of live weight at 10 weeks of age in males, both SenSi-1 Agrinak chickens, were coloured feathers (white and grey) with a slightly higher average live weight. SenSi-1 Agrinak white feather; for rooster 1,051 g/head; for hen 751.00 g/head and SenSi-1 Agrinak grey feather with rooster weight 1,015 g/head; hen 739.00 g/head (Hasnelly et al. 2017).

In intensive chicken farming, feed is the biggest cost that can reach 70% of production costs. Therefore, the price of feed raw materials will determine the cost of production. To meet the needs of, Most farmers obtain this raw material from poultry shop or commercial feed mill. Local raw materials are mostly by-products of agro-industry and are generally of low quality and have low protein content and digestibility.

*Indigofera zollingeriana* is a type of legume that has a high protein content of 25-31%, a minimum TDN of 70% with a dry matter digestibility of 75-78%. This plant can also be used as feed source of various ruminants and poultry, these plant very good for increasing the production and quality of meat, eggs and milk, as well as producing animal food products with lower cholesterol levels and has high vitamins content (Herdiawan & Krisnan 2014). With regard to feeding to poultry, the results of research (Meidi et al. 2018) to ducks given up *Indigofera zollingerina* flour up to 16% in the ration of Peking ducks aged 2-7 weeks has the potential to increase slaughter weight by 988.75±218, 41 g 1,242.50±188.25 g with the same protein content of 18%. As for egg production, the research results (Akbarillah et al. 2011) on the administration of indigofera flour as a substitute for yellow corn as a protein source in quail showed not significantly different ( $P>0.05$ ). As for the yolk colour (Akbarillah et al. 2008) on quail eggs fed rations with 10% indigofera flour supplementation gave a different effect ( $P<0.05$ ) on egg yolk colour compared to without indigofera flour supplementation. The purpose of this study was to examine the effect of indigofera plant flour on the ration on egg production and egg quality of cross-bred chickens in particular and of native chickens in general and to see the level of acceptance received by farmers compared to only providing by commercial feed.

## MATERIALS AND METHODS

This study was conduct with Senkub chickens (SenSi-1 Agrinak crosses × KUB chickens) of 18 week old. Two hundreds chickens were reared for 16 weeks. Chickens are placed in 4 units of cages made of bamboo, each of which was equipped with a feed and drinking container. The complete composition of the feed and its contents is are presented in Table 1. The treatments consisted

**Table 1.** Nutrient content from each treatment

Description	P0	P1	P2	P3
Dry matter	86.35	86.65	86.65	86.71
Ash	5.14	4.51	4.51	4.63
Crude protein	13.94	12.71	12.71	13.74
Crude fat	7.32	7.82	7.82	7.60
Crude fiber	5.93	5.96	5.96	6.00
GE (cal/g)	3,005.00	3,028.75	3,028.75	3,009.88
Ca	0.26	0.19	0.19	0.25
P	0.83	0.75	0.75	0.75

*Proximate Analysis data collaborated with UNUD (Feed Lab Analytic)*

*P0= (25% concentrate + 40% corn + 35% rice bran; P1= P0 with 10% with indigofera flour; P2= P0 with 10% with indigofera flour + Bio B 2 cc; P3= (Concentrate 21.5% + Corn 36.5% + rice bran 31.5%) + Indigofera flour 10%*

of 4 types of rations and each treatment was repeated 5 times where each replication consisted of 10 chickens. The research was conducted using a completely randomized design (CRD) (Gomez & Gomez 1995) with treatments consisting of P0 = feed according to typically farmers' ration ( 25% concentrate + 40% corn + 35% bran); P1 = P0, with the concentrate substituted by 10% Indigofera flour; P2 = P1 + Bio B 2 cc/1 liter of water; P3 = Concentrate (21.5% + Corn 36.5% + Bran 31.5% + Indigofera flour 10%). Variables observed were : production performances (feed consumption, egg production and feed conversion); physical egg quality (egg weight, shell weight, egg yolk index, egg white index, egg white weight, egg yolk weight); chemical egg quality (egg yolk protein content, egg yolk fat content, Haugh Unit (HU) and egg yolk color score). The data were analyzed for variance and continued with Duncan's test. To determine the feasibility level of the technology component of indigofera plant flour on the production and quality of chicken eggs, qualitative and quantitative descriptive methods were carried out. The analytical tools used include the balance of revenues and costs or the R/C ratio (Suratiah 2006).

## RESULTS AND DISCUSSION

### Production performance

Substitution of ration material (concentrate) (P1) with 10% Indigofera flour or substitution of all feed ingredients (P3) with 10% Indigofera flour on ration consumption decreased in quantity but statistically had not significantly different ( $P>0.05$ ), as well as in egg production and weight increased in quantity, but statistically not significantly different ( $P>0.05$ ) with without indigofera flour

(Table 2). This was related to the proximate analysis data, the nutritional content of the mixed rations for the flour treatments was almost the same (Table 1). The results of this study are the same as those obtained by Akbarillah et al. (2011) for egg production, the addition indigofera flour as a substitute for yellow corn as a protein source in quail showed no different results ( $P>0.05$ ). Likewise, research by Pagala & Surajat (2018) with Arab chickens given with *Indigofera zollingeriana* leaf flour to feed also did not have a significantly different effect ( $P>0.05$ ) on the level of feed consumption was the nutrient content of the feed, especially the metabolic energy content.

Feed conversion was closely related to feed consumption and egg production (Puspita 2008). The lower the feed conversion value obtained, the more efficient the livestock production, in addition to the amount of feed consumed by the weight of the eggs produced, Anggorodi (1994) was explained that the level of feed conversion was largely determined by the balance between metabolic energy and nutrients, especially protein and amino acid. Moreover, the addition of 10% *Indigofera* flour to the ration gave a greater weight when compared to control. The weight of Senkub chicken eggs from the results of this study was higher than the results obtained from the research of Kustiningsing & Retnawati (2020) on KUB chickens who found that there was no difference egg weight in the group of chickens with 10% fresh *Indigofera* supplementation compared to the control group (0%). This may be due to *Indigofera* which was given in fresh form. According to Palupi et al. (2014), egg weight was influenced by feed nutrients such as protein content, certain amino acids such as methionine and lysine, energy, total fat, and essential fatty acids such as linoleic acid. The need for one of these nutrients was not met through ration intake, then it will reduce egg weight. *Indigofera* sp. leaves. which contains amino acids, especially the amino acids methionine and lysine, was able to maintain a normal egg weight.

**Table 2.** Productivities dan chicken feed conversion ration for forth month observation

Variable	Treatment			
	P0	P1	P2	P3
Ration consumption (g/head/day)	102.28 <sup>b</sup>	100.50 <sup>ab</sup>	98.16 <sup>a</sup>	100.32 <sup>ab</sup>
Daily eggs production (%)	41.19 <sup>b</sup>	51.93 <sup>ab</sup>	52.76 <sup>a</sup>	52.30 <sup>a</sup>
Average egg weight (g/each)	40.14 <sup>b</sup>	43.47 <sup>a</sup>	43.15 <sup>a</sup>	42.38 <sup>ab</sup>
Feed conversion ration (g/head/day)	6.19 <sup>b</sup>	4.45 <sup>a</sup>	4.3 <sup>a</sup>	4.53 <sup>a</sup>

P0 = (25% concentrate + 40% corn + 35% rice bran; P1 = P0 with 10% with indigofera flour; P2= P0 with 10% with indigofera flour + Bio B 2 cc; P3 = (Concentrate 21.5% + Corn 36.5% + rice bran 31.5%) + *Indigofera* flour 10%; Different superscript letters on the same line indicate that there is a significant difference with a 95% confidence level ( $P<0.05$ ).

## Physical egg quality

The substitution of 10% Indigofera flour on the physical quality of Senkub chicken eggs can be seen in Table 3. The results of the analysis showed that the egg weight both quantitatively and statistically has significant effect ( $P<0.05$ ) when compared without substitution of Indigofera flour (P0). However, when viewed from the physical quality, both weight and percentage of shells, weight and percentage of albumin, weight and percentage of egg yolks as well as egg albumin index and egg yolk index within Indigofera flour substitute as much as 10% had not significantly different ( $P>0.05$ ) when compared with without Indigofera flour. This result was in line with the results obtained by Mulyadi (2013) with the use of functional feed on the quality of Arabic chicken eggs does not significantly affect the physical quality of eggs.

**Table 3.** The effect of feeding different levels of Indigofera leaf meal of physical quality of Senkub chicken egg

Parameter	Treatment			
	P0	P1	P2	P3
Egg weight (g)	40.14±1.46 <sup>a</sup>	43.48±4.32 <sup>b</sup>	43.15±2.46 <sup>b</sup>	42.38±2.74 <sup>ab</sup>
Egg shell physique				
Egg shell thickness (mm)	0.35±0.06 <sup>a</sup>	0.32±0.02 <sup>a</sup>	0.33±0.04 <sup>a</sup>	0.33±0.02 <sup>a</sup>
Egg shell weight (g)	5.32±0.45 <sup>a</sup>	5.50±0.53 <sup>a</sup>	5.67±0.54 <sup>a</sup>	5.48±0.30 <sup>a</sup>
Egg shell percentage (%)	13.23±0.88 <sup>a</sup>	12.69±0.94 <sup>a</sup>	13.13±0.85 <sup>a</sup>	12.69±0.84 <sup>a</sup>
Egg white physique				
Weight (g)	18.03±2.49 <sup>a</sup>	20.85±3.57 <sup>b</sup>	22.08±1.87 <sup>b</sup>	21.30±2.43 <sup>b</sup>
Egg white percentage (%)	45.09±6.94 <sup>a</sup>	47.79±5.25 <sup>b</sup>	51.15±3.12 <sup>ab</sup>	50.18±3.48 <sup>ab</sup>
Index albumin	0.07±0.02 <sup>a</sup>	0.06±0.01 <sup>a</sup>	0.06±0.01 <sup>a</sup>	0.05±0.01 <sup>a</sup>
Egg yolk physique				
Weight (g)	16.05±2.59 <sup>b</sup>	16.24±1.37 <sup>a</sup>	15.52±1.56 <sup>a</sup>	15.71±1.40 <sup>a</sup>
Egg yolk percentage (%)	13.23±0.80 <sup>a</sup>	12.69±0.94 <sup>a</sup>	13.13±0.85 <sup>a</sup>	12.96±0.84 <sup>a</sup>
Egg yolk index	0.38±0.01 <sup>a</sup>	0.41±0.09 <sup>a</sup>	0.37±0.06 <sup>a</sup>	0.39±0.03 <sup>a</sup>

## Chemical quality of eggs

The results showed that the addition of 10% Indigofera flour into chicken rations increased number in egg yolk protein content, but not significantly different ( $P>0.05$ ) with others treatment Table 4.

The average fat content of Senkub chicken eggs treated with feed contained Indigofera leaf meal was presented in Table 3. The provision of 10% Indigofera leaf meal in the feed had no effect ( $P>0.05$ ) on the fat content of Senkub chicken eggs. The results of this study are in accordance with the results of research by Surajat & Ibrahim (2021) in Arabic chickens which were given additional 20% Indigofera flour in the feed, which had no effect on the fat content of the eggs produced. The low-fat content of Senkub chicken eggs may be influenced by the low-fat content of Indigofera leaf flour. Abdullah (2014) reported that the fat content of *Indigofera zollingeriana* leaf flour reached 3.62%. Furthermore, it was said that the addition of *Indigofera zollingeriana* leaf flour to Arabic chicken feed in large quantities, would reduce the fat content in Arabic chicken eggs.

On the egg yolk colour scale, the Roche Yolk Colour Fan Score was used to measure the level of yellowness of the yolk. The results of the study showed that 10% Indigofera flour substitution increased the colour of the egg yolk (more orange) when compared to the control ( $P<0.05$ ), it is in line with the results of Akbarillah et al. (2001) on quail where the administration of 10% Indigofera flour as a substitute for yellow corn in the ration mixture gave a higher yolk colour compared to the control using yellow corn. This result was confirmed by Akbarillah et al. (2010) who said that the increase in yolk colour in Indigofera flour was due to the presence of beta carotene and xanthophylls contained in Indigofera leaves so that it could improve the yolk pigmentation rate.

**Table 4.** The average of Senkub chicken eggs quality

Parameter	Treatment			
	P0	P1	P2	P3
Yolk egg fat (%)	30.90±0.10 <sup>a</sup>	30.63±1.85 <sup>a</sup>	34.67±0.85 <sup>b</sup>	32.72±1.68 <sup>ab</sup>
Yolk egg protein (%)	13.94±3.08 <sup>a</sup>	12.24±3.28 <sup>a</sup>	14.51±1.52 <sup>a</sup>	14.20±1.41 <sup>a</sup>
Yolk egg colour	10.60±0.74 <sup>b</sup>	10.80±0.63 <sup>ab</sup>	11.00±0.00 <sup>ab</sup>	11.00±0.82 <sup>a</sup>
HU	82.05±8.29 <sup>a</sup>	76.41±7.69 <sup>a</sup>	75.70±7.60 <sup>a</sup>	73.04±8.77 <sup>a</sup>

P0 = (25% concentrate + 40% corn + 35% rice bran; P1 = P0 with 10% with indigofera flour; P2 = P0 with 10% with indigofera flour + Bio B 2 cc; P3 = (Concentrate 21.5% + Corn 36.5% + rice bran 31.5%) + Indigofera flour 10%. Different superscript letters on the same line indicate that there is a significant difference with a 95% confidence level ( $P<0.05$ )

Giving 10% Indigofera flour had no effect on the haugh unit (HU) number of Senkub chicken eggs. The results showed that the HU number in the four treatments was more than 72, indicate that the quality of the eggs produced from the four treatments was very good (quality I HU value >72). Determination of egg quality based on the HU value was based on the standards of the United State Department of Agriculture, namely the HU value less than 31 was classified as C quality, the HU value less than 31-60 was classified as B quality,

the HU value was less than 60-72 classified as A quality and a HU score of more than 72 was classified as AA quality.

### Economic analysis

The results of the cost analysis Table 5 showed that the substitution of the ration mixture with a mixture of feed ingredients derived from 10% *Indigofera* flour reduced the total cost of raising Native Chicken. The more commercially substituted rations the lower the total production cost. The decrease in total costs was due to a decreased in variable costs, which was the cost of rations. This was because the price of rations that used *Indigofera* flour mixed substitution was cheaper per kilogram than the price of commercial rations for laying hens (saving up to Rp 1200). The lower the price of the ration was not necessarily the lower the cost of the ration. It depends on how much ration

**Table 5.** Economic analysis

Description	Treatment			
	P0	P1	P2	P3
<b>Cost</b>				
Number of chickens (head)	30	30	30	30
Pullet price (Rp)	25,000	25,000	25,000	25,000
Total	750,000	750,000	750,000	750,000
Feed for 4 months (kg)	589.65	543.90	514.26	560.48
Price (Rp)	7,000	6,800	6,900	5,800
Total (Rp)	4,127,550	3,698,520	3,548,394	3,250,784
Probiotic (Rp)	-	-	60,000	
Total (kg)	4,877,550	4,448,520	4,358,394	4,000,784
<b>Income</b>				
Chicken sale	30	30	30	30
Chicken weight	1.85	1.48	1.63	1.70
Amount (kg)	55.47	44.28	48.90	51.00
Price (Rp)	35000	35000	35000	35000
Total (Rp)	1,941,450	1,549,800	1,711,500	1,785,000
Production	1,236	1,558	1,583	1,569
Price (Rp)	2,000	2,000	2,000	2,000
Amount	2,471,400	3,115,800	3,165,600	3,138,000
Total income	4,412,850	4,665,600	4,877,100	4,923,000
Revenue	(464,700)	217,080	518,706	922,216
R/C ratio	0.40	0.35	0.39	0.45
B/C ratio	(0.10)	0.05	0.12	0.23

was consumed. The consumption of chicken rations studied from all treatments showed that the provision of 10% *Indigofera* flour was less when compared to the group of chickens consumed commercial feed, as well as in terms of the ration price per kilogram, the addition of 10% *Indigofera* flour to the ration mixture was cheaper than commercial feed (reduced input by 17%). In terms of profit, the addition of 10% *Indigofera* flour (P1, P2 and P3) to the ration mix actually provided an advantage compared to the farmer method (P0), so it was feasible to continue and can disseminated to the farmers.

## CONCLUSION

Substitution of 10% *Indigofera* flour in the ration mixture did not affect the physical qualities and production of eggs. Results on egg quality chemically substitution of ration ingredients with 10% *Indigofera* flour had no effect on egg yolk fat content but increased egg yolk protein content by 13.38%. In farming analysis, substitution of ration material with 10% *Indigofera* flour reduces input by 17% in the layer period so that it is feasible to be disseminated.

## AUTHOR CONTRIBUTIONS

All authors were contributed equally to this work

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## **The Effect of Local Feed Ingredient on the Performance of Superior Native Chicken and Income Over Feed Cost**

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### **ABSTRACT**

The nutrient requirements of native chickens are different from those of broiler chickens, but many breeders use commercial broiler or layer feed for native chickens. This study aims to examine the use of local feed ingredient on the performance of superior native chickens and IOFC. The study used a factorial design  $3 \times 2$  with 5 replicated cages, contained 10 unsexed chickens at 4 weeks old; the first factor was type of chicken, namely the Sensi chicken and the KUB chicken, the second factor was feed treatment. The feed treatments used were (D0) 100% commercial broiler diet as a control, (D1) 50% commercial broiler feed + 15% commercial layer concentrate + 25% rice bran + 10% rejected rice, (D2) 30% commercial layer concentrate + 50% rice bran + 20% rejected rice. Observations were made for 6 weeks, on body weight (BW), feed consumption, BW gain, feed conversion ratio (FCR) and income over feed cost (IOFC). The results showed that type of chicken had a significant effect on BW, FCR, BW gain and IOFC, while feed treatment had a significant effect on feed consumption, BW gain and FCR. There was no interaction between chicken type and diet on all observations. In this study, it can be concluded that Sensi chickens had higher final BW and BW gain, lower FCR and IOFC than KUB chickens had. P1 feed (50% commercial feed broiler + 15% commercial layer concentrate, 25% rice bran and 10% rejected rice) can replace P0 feed (commercial broiler diet) without affecting final BW, BW gain and IOFC.

**Keywords:** Superior native chicken, KUB chicken, Sensi chicken, Local feed

### **INTRODUCTION**

KUB chickens and Sensi chickens are superior native chickens that have been released by the Agricultural Research and Development Agency in 2017. The advantage of KUB chickens is that they have high egg production, while the advantage of Sensi chickens is their fast growth. The KUB chicken was selected for egg production subjected to be female line in supporting the production of native day old chick (DOC) for national meat production (Iskandar & Sartika 2014; Hidayat et al. 2011; Hidayat et al. 2014). To create

KUB as female line, the IRIAP (Indonesian Research Institute for Animal Production) has also produced SenSi-chicken as the candidate of male lines in the IRIAP's research project to create grand parent or parent stocks of improved meat type of native chicken (Iskandar et al. 2012; Hidayat et al. 2014)

SenSi-1 Agrinak chicken is a local broiler breed that has been released by the Ministry of Agriculture through the Decree of the Minister of Agriculture of the Republic of Indonesia No. 39/Kpts/PK.020/1/2017. SenSi-1 Agrinak chicken is a family of Sentul chickens selected for several generations based on body weight criteria of 10 weeks of age (Iskandar & Sartika 2015). The standard body weight of female and male SenSi-1 Agrinak chickens at the age of 10 weeks is 745 and 1,066 grams, respectively (Ditjen PKH 2017).

Iskandar et al. (2012) stated that the native chicken ration can use a single ration with 19% protein content with a final weight of 1134 g/head at the age of 12 weeks or with a more efficient double ration with 19% protein content in the starter ration (1-6 weeks), and 15% on the finisher ration (6-12 weeks) with a final body weight of 1159 g/head.

Rice bran is a local feed ingredient that is widely available. The rice milling process produces 70% of rice (endosperm) as the main product, as well as several by-products such as husks (20% ) and rice bran (8-10% ) (Chen et al. 2012). In the rice milling process, the husks are separated and brown rice is obtained. The next stage is the grinding process of rice which aims to remove bran and bran from the endosperm of rice. Rice bran is the outermost part of the rice grain that is wasted during the rice milling process (Thahir 2010). The nutritional content of rice bran according to Rao (2000) is 16.5% protein, 21.3% fat, 11.4% crude fiber and 49.4% total carbohydrates. Meanwhile, according to Hartati et al. (2015) the nutritional content of rice bran is protein  $13.37 \pm 0.02\%$ , fat  $16.8 \pm 0.05\%$ , crude fiber  $13.56 \pm 0.10\%$  and ash  $9.47 \pm 0.06\%$ .

Another feed ingredient that has high nutritional value and relatively cheap price is rejected rice. Rice is an energy source, with a carbohydrate content of 88%, and crude protein 10.9%, crude fat 0.6 and ash 0.56% (Loebis et al. 2017) while according to Hernawan & Meylani (2016), crude protein content 8.17% and 0.4% crude fiber. This study aims to evaluate the use of rice bran and rejected rice as local feed on the performance of KUB and Sensi chicken as superior native chickens. It is expected that the use of local feed with the right composition could reduce feed costs, hence, increase the farmers' income.

## **MATERIALS AND METHODS**

The study used a Completely randomized factorial design (2×3) The first factor was the type of chicken, namely Sensi chicken and KUB chicken, the second factor were the feed treatment. Each main factor used 150 chickens aged

4 weeks (unsex), which were divided into 3 feed treatments, each treatment was repeated in 5 cages, 10 chickens each. Composition of feed treatments is presented in Table 1. Nutrient content and the price of feed ingredients and commercial broiler and laying hen diets are presented in Table 2, while the nutritional content and the price of experimental feed are presented in Table 3.

**Table 1.** The composition of experimental feed ingredients

Diets	Ingredients			
	Commercial broiler diet (%)	concentrate laying hens (%)	Rice bran (%)	Reject rice (%)
D0	100	0	0	0
D1	50	15	25	10
D2	0	30	50	20

*D0 = 100% commercial broiler diet as a control, D1 = 50% commercial broiler feed + 15% commercial layer concentrate + 25% rice bran + 10% rejected rice, D2 = 30% commercial layer concentrate + 50% rice bran + 20% rejected rice*

**Table 2.** Nutrient content and price of feed ingredients

Feed and feedstuffs	DM (%)	Nutrient content based on dry matter (%)					Price (IDR/kg DM)
		Crude protein	Crude fat	Crude fiber	Ash	NFE	
Commercial broiler diet	90.32	22.92	5.87	2.75	6.61	61.96	8.813
Commercial laying hen concentrate	93.22	32.55	3.33	3.15	32.23	28.75	8.668
Rice bran	87.11	14.38	8.03	6.61	7.82	63.16	4.248
Rejected rice	88.14	8.38	0.37	0.42	2.20	88.63	4.538

*DM: Dry matter; NFE: Non nitrogen free extract*

**Table 3.** Nutrient content and price of experimental feed

Diets	DM (%)	Nutrient content based on dry matter (%)					Price (IDR/kg DM)
		Crude protein	Crude fat	Crude fiber	ash	NNFE	
D0	90.32	22.92	5.87	2.65	6.61	61.95	8,813
D1	89.73	20.77	5.48	3.49	10.31	59.94	7,263
D2	89.15	18.62	5.09	4.33	14.02	57.94	5,692

*DM: Dry matter; NNFE: Non nitrogen free extract; D0: 100% Commercial broiler feed  
D1: 50% Commercial broiler + 15% concentrate laying hens + 25 rice bran+ 10% reject rice; D2: 30% concentrate laying hens + 50% rice bran+ 20% reject rice*

The research was conducted in January – March 2021 for 6 weeks. The observed variables included feed consumption, body weight gain, feed conversion ratio and Income over feed cost (IOFC). Live weight and feed consumption were measured every week from each group of replication. Feed conversion ratio was calculated by feed consumed divided by the total live weight gain. Data were subjected to ANOVA (analysis of variance) using SAS 9.13 statistical software. The mean values of the treatments were the tested using Duncan's multiple range test the 5% degree of confident.

## RESULTS AND DISCUSSION

Live weight, feed consumption, body weight gain, feed conversion ratio and IOFC of KUB and SenSi chickens for each treatment are presented in Table 4.

**Table 4.** Weight of chickens aged 4 weeks and 10 weeks, Feed intake, body weight gain, FCR and IOFC

Factors	Initial weight (4 weeks) (g/head)	Final weight (10 weeks old) (g/head)	Feed Intake (g/head)	Body weight gain (g/head)	FCR	IOFC (IDR/head)
Type of chicken						
SenSi	263.20 <sup>a</sup>	940.65 <sup>a</sup>	2501.52 <sup>a</sup>	677.45 <sup>a</sup>	3.83 <sup>b</sup>	5521.0 <sup>a</sup>
KUB	243.20 <sup>b</sup>	797.19 <sup>b</sup>	2346.95 <sup>b</sup>	567.55 <sup>b</sup>	4.31 <sup>a</sup>	3002.7 <sup>b</sup>
Diets						
D0	253.20 <sup>x</sup>	968.80 <sup>x</sup>	2258.77 <sup>y</sup>	715.60 <sup>x</sup>	3.16 <sup>z</sup>	5140.1 <sup>x</sup>
D1	254.90 <sup>x</sup>	939.24 <sup>x</sup>	2629.36 <sup>x</sup>	684.34 <sup>x</sup>	3.89 <sup>y</sup>	4854.7 <sup>x</sup>
D2	251.50 <sup>x</sup>	698.72 <sup>y</sup>	2384.58 <sup>y</sup>	467.56 <sup>y</sup>	5.16 <sup>x</sup>	2790.8 <sup>y</sup>
Interaction	NS	NS	NS	NS	NS	NS

Values in the same column and factor, with difference superscript are significantly different ( $P < 0.05$ ); D0 = 100% commercial broiler feed; D1 = 50% commercial broiler feed + 15% concentrate laying hens + 25% rice brand + 10% reject rice; D2 = 30% concentrate laying hens + 50% rice brand + 20% reject rice; NS = not statistically significant ( $P > 0.05$ )

### Live body weight

SenSi and KUB chickens used in this study came from the same hatching period and were reared on the same feed until 4 weeks of age. The initial weight of the study (4 weeks old) SenSi chicken (263.20 grams) was

significantly higher than KUB chicken (243.20 g). However, the initial weight based on feed treatment was not significantly different (Table 4).

The weight of SenSi chickens at the end of the study (age 10 weeks) was 940.67 g significantly higher than KUB chickens (797.07 g). This is in accordance with the type of SenSi chicken, which is meat type of chicken, therefore has a higher body weight gain than KUB chickens which are laying types. The live weight of SenSi chickens aged 10 weeks in this study was higher than the research results of Hasnelly et al. (2017), that the live weight of male SenSi chickens aged 10 weeks was 908.76 g and females 750.53 g.

### **Feed intake**

The type of chicken has a significant effect on feed consumption. The average feed consumption of SenSi chickens was 2,501.60 g/head significantly greater ( $P < 0.05$ ) than KUB chickens, which was 2,346.93 g/head. The consumption of SenSi chicken feed in this study was the same as that of Hasnelly et al. (2017), that the cumulative feed consumption of Sensi chickens at the age of 10 weeks is approximately 2,500 g/head. Feed treatment also had a significant effect on feed consumption and there was an interaction between the type of chicken and the feed treatment. Feed consumption of D1 was 2,629.36 g significantly higher ( $P < 0.05$ ) than D0 and D2 with feed consumption of 2,258.77 and 2,384.58 g. The low consumption of D0 feed was due to the high nutrient content, namely protein content and higher NFE than D1 and D2. While the low consumption of D2 is thought to be due to low palatability.

### **Body weight gain**

The type of chicken and the feed treatment had a significant effect ( $P < 0.05$ ) on body weight gain, but there was no interaction between the type of chicken and the feed treatment. The increase in body weight of SenSi chickens was 677.47 g, significantly higher than KUB chickens, which was 567.53 g ( $P < 0.05$ ). This is because SenSi chicken is a broiler type chicken while KUB chicken is a layer type, broiler type chicken has a higher body weight gain than laying type chicken. Body weight gain D0 and D1 were not significantly different but significantly higher than D2, there was no interaction between the type of chicken and the feed treatment. The low D2 body weight gain was due to the low nutritional content, especially crude protein and NNFE compared to D1 and D0 (Table 3).

## **Feed conversion**

The type of chicken has a significant effect on feed conversion. The average FCR value of SenSi chickens was 3.85 significantly lower than the FCR of KUB chickens, which was 4.32 ( $P < 0.05$ ). The feed conversion ratio in this study was higher than previous studies, namely the FCR of KUB chickens ranging from 3.14 to 3.74 (Hidayat et al. 2017). The feed treatment also significantly affected the FCR ( $P < 0.05$ ) and there was an interaction between the type of chicken and the feed treatment. Feed conversion ratio D0 is 3.20, the lowest compared to D1 (3.89) and D2 (5.16). This is because the nutrient content of D0, especially crude protein and NFE is the highest compared to D1 and D2. According to Allama et al. (2012) a low feed conversion value indicates that the efficiency of feed use is good, because the more efficient the chicken consumes feed to produce meat. The feed conversion value of D0 treatment is better than that reported by (Suryana 2017) that the average FCR value in 10-month-old KUB hens is 3.50.

## **Income over feed cost**

The income over feed cost (IOFC) of SenSi chicken (5,521.1 IDR/head) was significantly higher than that of KUB chicken (3,002.7 IDR/head) ( $P < 0.05$ ). This is in line with the FCR value of SenSi chicken which is significantly better than KUB chicken. The feed treatment significantly affected the IOFC ( $P < 0.05$ ) and there was an interaction between the type of chicken and the feed treatment. Income over feed cost Diet D0 was 5,140.1 IDR/head higher than D2 (2,790.8 IDR/head) but not significantly different from D1 treatment (4,854.7 IDR/head). Income over feed cost is the result of selling chickens minus the cost of feed. Diet D1 with a lower price than D0 has the same IOFC value even though the FCR of D1 is higher than D0. Farmers can choose a profitable feed formula according to the price of feed Ingredients that apply at the time the chicken rearing activities are carried out.

## **CONCLUSION**

SenSi chickens had higher final body weight, higher body weight gain, lower feed conversion and higher income over feed cost (IOFC) than KUB chickens. P1 feed (50% commercial broiler + 15% concentrate laying hens, 25% bran and 10% reject rice) can replace P0 feed (Commercial broiler diet) without affecting final body weight, body weight gain and IOFC.

## AUTHOR CONTRIBUTIONS

Erna Winarti, Ari Widyastuti, Widodo Suwito, Gunawan, Soeharsono were contributed equally to this work

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## **Effect of Edamame Flour Filler Substitution on the Nutrition Facts of Culled Duck Meatballs**

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### **ABSTRACT**

This study objective was to determine the effect of substitution of edamame flour filler on the nutrition facts of culled duck meatballs. Materials used in this study consisted of culled duck meat, tapioca, edamame flour, egg white, garlic, onion, salt, pepper, monosodium glutamate, sodium tripolyphosphate, and ice. Edamame flour filler substitution treatments were 0, 5, 10, 15, and 20% of the full filler. The nutrition facts of meatballs were calculated based on the nutritional adequacy rate of meatballs which refers to the average energy sufficiency for the Indonesian population per person per day, which is 2,150 kcal, 60 g total protein, 67 g total fat, and 30 g fiber with a 50 g serving (5 meatballs). Samples were tested with 3 replications. The data from the calculation of the nutrition facts of meatballs were analyzed using analysis of variance and then tested by using Duncan's New Multiple Range Test. The results showed that the substitution of edamame flour filler had a highly significant effect ( $P < 0.01$ ) on the nutrition facts of meatballs. Culled duck meatballs substituted with up to 20% edamame flour had a higher content of total fat, total protein, and fiber than control meatballs.

**Keywords:** Culled duck meat, Edamame flour, Filler, Meatball, Nutritional fact

### **INTRODUCTION**

Duck is a kind of waterfowl whose meat is much less appealing to the general public as it is off-odor (Anggraini et al. 2017), has hard texture and tough so that the quality of the meat is low (Chang & Dagaas 2004). Volatile components are derived from the oxidation of unsaturated fat that cause duck meat to have a fishy odor (Hustiany et al. 2001; Hustiany 2001; Purba et al. 2010). Duck meat has higher fat and protein content and lower calories than other chicken meat (Utami et al. 2011). The color of culled duck meat is darker than chicken meat (Ali et al. 2007). The chemical composition of duck meat is 73.29-80.69% moisture, 19.99-24.34% protein, 1.05-1.18% ash (Qiao et al. 2017), 1.55-2.30% intramuscular fat (He et al. 2018), and 29.36-31.12% fat (Rukmiasih & Tjakradidjaja 2007; Rukmiasih et al. 2009).

The quality of culled duck meat can be improved through processing it into meatballs. The technique of processing culled duck meat into meatballs

can reduce the off odor of duck meat (Mega et al. 2009; Anggraini et al. 2017). Duck meat processed into meatballs is desired whilst compared to different processed products (Putra et al. 2011; Kusmayadi & Sundari 2019). Meatballs are one of processed meat products made through mashing meat and then mixing it with flour and spices, then forming balls and boiling them until cooked in warm water (Chakim et al. 2013).

Indonesia's growing population and high society activities resulted in a fast growth inside the consumption pattern of ready-to-cook and ready-to-eat meat (Prayitno et al. 2009) certainly one of which is meatballs. Meatballs have high acceptability and nutrition facts (Prayitno et al. 2016; Prayitno et al. 2019). Chemical composition of meatballs consists of no less than 9% protein, a maximum of 2% fat, maximum moisture content of 70%, and a maximum of 3% ash (SNI 2014). Meatballs as a comminution product are usually tapioca flour as a filler. Tapioca flour in meatball processing can be substituted with edamame flour as a filler. Substituting fillers in meat products is an innovation to utilize local resources (Syam et al. 2019). The chemical composition of edamame flour consists of 3.22% moisture, 40.02% protein, 18.43% fat, 34.65% carbohydrate, and 3.78% ash (Prayitno & Rahman 2020).

Meatballs produced with primary component of culled duck meat with edamame flour filler substitution can meet the dietary needs of human. The chemical composition of culled duck meatballs is 45.05-46.93% moisture, 10.61-16.13% protein, 17.16-19.86% fat, 0.11-2.24% fiber, 0.84-1.19% ash (Prayitno & Rahman 2020). The nutrition facts of culled duck meatballs substituted with edamame flour filler calculated based totally on the nutritional adequacy rate have by no means been evaluated earlier than. This study aims to evaluate the nutrition facts of culled duck meatballs substituted with edamame flour filler.

## **MATERIALS AND METHODS**

### **Materials**

Substances used in this study were culled duck meat, edamame, pepper, monosodium glutamate, sodium tripolyphosphate, garlic, onion, egg whites, tapioca flour, and ice.

### **Methods**

This study consists of several activities. The activities of these studies consist of edamame flour processing, meatballs processing, calculating nutrition facts, and data analysis.

### ***Edamame flour processing***

Edamame pods were peeled and the seeds were taken out and crushed. Edamame seeds were roasted at 60°C for 24 hours. The dried edamame seeds were ground till smooth after which sieved using a 60 mesh sieve. This sieved edamame flour was used as a filler for processing of culled duck meatballs.

### ***Meatball processing***

The formulation of meatball is 60% meat, 15% tapioca flour (filler), 10% egg white, 2.5% garlic, 1% onion, 1.5% salt, 1% pepper, 1% monosodium glutamate, 1% sodium tripolyphosphate, and 7% ice. Edamame flour filler substitution treatments were 0, 5, 10, 15, and 20% of the full filler. Duck meat cleaned and separated between bones, meat, and fats. Duck meat divided into small portions and then ground first, then the smooth meat placed into a grinder machine with salt, pepper, monosodium glutamate, sodium tripolyphosphate, garlic, onion, egg whites, tapioca flour, edamame flour, and ice cubes, then milled again so that each one ingredient are mixed homogeneously. The dough shaped manually into balls. Boil the meatballs until they float. The cooked meatballs then drained and the nutritional facts become calculated.

### ***Calculating nutrition facts***

The nutrition facts of meatballs are calculated based on the range of nutritional adequacy of meatballs which refers to the average energy adequacy for Indonesian population according to an individual per day, that's 2,150 kcal, total protein 60 g, total fat 67 g, and dietary fiber 30 g with a serving size of 50 g with the number of meatballs as lots as 5 (BPOM 2011; BPOM 2016; BPOM 2019).

### ***Data analysis***

The information from calculated nutritional facts of the meatballs was analyzed by using analysis of variance in a completely randomized design and if there was a significant difference, than tested further with Duncan's Multiple Range Test (Riadi 2014).

## **RESULTS AND DISCUSSION**

The recommended dietary allowance (RDA) is an average daily nutritional adequacy for anyone consistent with age group, gender, body size, body activity, and unique physiological conditions to achieve optimal health status (BPOM 2016). The nutrition facts (NF) written on processed food labels

should include the proportion of the recommended dietary allowance (RDA) that is calculated using the nutrition facts label (NFL). NFL calculated primarily based on the average energy adequacy for the Indonesian population, which is 2,150 kcal per person per day. The nutrient content in processed meals should not be more than 100% NFL per day (BPOM 2016). The nutrition facts and recommended dietary allowance of culled duck meatballs are presented in Table 1 and Table 2.

**Table 1.** The nutrition facts of culled duck meatballs substituted with edamame flour filler with a serving size of 50 g

Component	P0	P1	P2	P3	P4
Calories from fat (kcal)	77.22 <sup>a</sup>	80.15 <sup>b</sup>	83.30 <sup>c</sup>	86.31 <sup>d</sup>	89.37 <sup>e</sup>
Total fat (g) <sup>ns</sup>	8.58 <sup>a</sup>	8.91 <sup>b</sup>	9.26 <sup>c</sup>	9.59 <sup>d</sup>	9.93 <sup>e</sup>
Total protein (g)	5.31 <sup>a</sup>	5.84 <sup>b</sup>	6.56 <sup>c</sup>	7.30 <sup>d</sup>	8.07 <sup>e</sup>
Fiber (g)	0.06 <sup>a</sup>	0.29 <sup>b</sup>	0.56 <sup>c</sup>	0.78 <sup>d</sup>	1.12 <sup>e</sup>

*P0 = 0%, P1 = 5%, P2 = 10%, P3 = 15%, and P4 = 20% substitution edamame flour from full filler*

**Table 2.** The percentage of RDA\* of duck meatballs substituted with edamame flour filler with a serving size of 50 g

Component	P0	P1	P2	P3	P4
Total fat (%)	12.81 <sup>a</sup>	13.29 <sup>b</sup>	13.81 <sup>c</sup>	14.31 <sup>d</sup>	14.82 <sup>e</sup>
Total protein (%)	8.84 <sup>a</sup>	9.73 <sup>b</sup>	10.93 <sup>c</sup>	12.17 <sup>d</sup>	13.44 <sup>e</sup>
Fiber (%)	0.18 <sup>a</sup>	0.95 <sup>b</sup>	1.85 <sup>c</sup>	2.58 <sup>d</sup>	3.73 <sup>e</sup>

*abcde* Superscript on the same line showed a highly significant effect ( $P < 0.01$ ); \*Percentage of RDA based on energy needs 2,150 kcal; P0 = 0%, P1 = 5%, P2 = 10%, P3 = 15%, and P4 = 20% substitution edamame flour from full filler

The serving size for meatballs is 50 g per serving (BPOM 2011; BPOM 2016; BPOM 2019) so that with an intake of 50 g per serving of control culled duck meatballs, it is equivalent to calories from fat 77.22 kcal, total fat 8.58 g, total protein 5.31 g, and 0.06 g fiber (Table 2). The substitution of edamame flour filler level 20% with an amount of 50 g per serving showed the highest nutritional content of all treatments, namely calories from fat 89.37 kcal, total fat 9.93 g, total protein 8.07 g, and fiber 1.12 g. This value indicates that culled duck meatballs substituted with edamame flour are included in good processed food because they meet the requirements for intake per serving for processed food, which is no more than 13 g of total fat, 4 g of saturated fat, 60 mg of cholesterol, and 480 mg sodium (BPOM 2011).

The intake of fat, protein, and fiber per serving of rejected laying duck meatballs substituted with edamame flour filler at the 20% level was the highest compared to control meatballs and other substitution level treatments (Table 3). This is because it was influenced by the chemical content of edamame, namely 40.02% protein, 18.43% fat (Safitri et al. 2017), and 3.27% fiber (Widiyawati & Susindra 2018). In addition, fat, protein, and fiber content of meatballs substituted with edamame flour filler at the level of 20% was the highest than other treatments, respectively 19.86, 16.13, and 2.24% (Prayitno & Rahman 2020). Culled duck meatballs have higher nutritional value and intake per serving than beef meatballs. Beef meatballs with a dose of 75 g per serving can meet the daily needs of 13.20% fat and 7.53% protein. The intake of beef meatballs per serving is equivalent to calories from fat 46.17 kcal, 5.13 g of fat, and 5.65 g of protein (Sari & Widjanarko 2015).

## CONCLUSION

Culled duck meatballs substituted with up to 20% edamame flour had a higher content of total fat, total protein, and fiber than control meatballs.

## AUTHOR CONTRIBUTIONS

Agus Hadi Prayitno, Taufik Hainur Rahman, Gilang Setyo Wibisono, Arfiana Rafika Rahmi were contributed equally to this work.

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## Effect of Fermented Corn Straw and Elephant Grass Silage on Performance of Bali Cattle

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### ABSTRACT

The research objective was to determine the effect of fermented corn straw and elephant grass silage on improving the performance of Bali cattle. The research has been carried out at the Gowa Experimental Garden, South Sulawesi Agricultural Technology Research Institute in Bajeng District, Gowa Regency. Using 20 Bali cattle divided into 4 feed treatments with 5 repetitions each, namely: P1 = 50% fermented corn stalks + 50% fresh elephant grass + 2 kg/head/day concentrate, P2 = 50% fermented corn stalks + 50% silage of elephant grass + 2 kg/head/day concentrate, P3 = 50% fermented corn stalks + 50% combined silage (elephant grass + Indigofera legume) + 2 kg/head concentrate, P4 = Control (grass and corn straw without fermentation). Parameters measured were nutritional content of feed, performance of beef cattle, consumption and conversion of feed, and farm analysis. The study was conducted for four months. Data were analyzed using a completely randomized design, further testing with the Duncan method. The results showed that the P3 feed contained crude protein, which was higher than other treatments by 11.98% and crude fiber the lowest was 22.51%. Bali cattle' average daily body weight gain at P3 was the highest compared to other treatments at 0.39 kg/head/day. The consumption of dry matter and organic matter in P2 treatment was the highest compared to other treatments, namely 5,282.9 g/head/day and 4,046.9 g/head/day. The lowest conversion in the P3 treatment was 13.42. The conclusion that can be drawn is that the provision of fermented maize stalks, combined silage of grass and Indigofera and concentrate gives Bali cattle better performance than other treatments.

**Keywords:** Fermentation, Corn straw, Silage, Performance, Bali cattle

### INTRODUCTION

The population of beef cattle in South Sulawesi Province continues to increase every year, where in 2020 there were 1,431,533 heads. Based on BPS data the beef cattle population in South Sulawesi is 8% of the total population in Indonesia and occupies the fourth position with the largest population after East Java, Central Java, and West Nusa Tenggara (Badan Pusat Statistik 2020). The high population of beef cattle in South Sulawesi is not proportional to the

level of distribution in each area which is not balanced and the location of the source of seeds is only found in a few areas (Yusuf et al. 2010). To meet the needs of beef cattle between regions, one of the efforts made is to program an increase in beef cattle productivity. Efforts to increase productivity that can be done are by providing adequate and quality feed sources.

The feed given to beef cattle is generally in accordance with the ability of the breeder, not according to the needs of the livestock. Therefore, the feed provided must also be of economic value and provide benefits to farmers. Feed ingredients are anything that can be eaten and can be partially or completely digested without disturbing the health of the livestock that eat it. The quality of feed ingredients is determined by the nutrient content or chemical composition, as well as the high and low levels of anti-nutritional substances contained in it. Nutrient needs are influenced by several things, including: growth rate, body size of livestock, environment, offspring, diseases, parasites, and types of livestock (Umiyasih & Anggraeny 2007). Efforts that can be made to improve feed quality include processing feed by fermentation or silage.

Corn straw has potential as animal feed because of its abundant availability and most of it is not utilized. However, the use of straw as animal feed has weaknesses, especially in digestibility and low nutritional value. This is due to the high content of lignocellulose, lignin and silica, lack of energy, protein, minerals and vitamins (Sarnklong et al. 2010; Yanuartono et al. 2017). To overcome these weaknesses, corn straw needs to be processed in the form of fermentation. Fermentation is the process of overhauling complex compounds contained in feed ingredients into simpler compounds with the help of enzymes that takes place in a controlled atmosphere (Eafianto 2009).

Forage is a common feed given to livestock, but its availability decreases during the dry season. Efforts to preserve forage with silage are expected to be a solution to the shortage of forage during the dry season. In addition, the manufacture of silage is intended to maintain quality and even improve forage quality. The principle of making silage is forage fermentation by microbes to produce lactic acid which can reduce the growth of spoilage microorganisms (Hanafi 2006). The purpose of this study was to determine the effect of fermented corn straw and forage silage on improving the performance of Bali cattle.

## **MATERIALS AND METHODS**

The research was conducted at the Gowa Experimental Garden, The Assessment Institute of Agriculture Technology South Sulawesi, Gowa,

Indonesia. Using 20 Bali cattle which were divided into 4 feed treatments with 5 replications each, namely:

P1 = 50% fermented corn straw + 50% fresh elephant grass + 2 kg/head/day concentrate

P2 = 50% fermented corn straw + 50% elephant grass silage + 2 kg/head/day concentrate

P3 = 50% fermented corn straw + 50% combined silage (elephant grass + indigofera legume) + 2 kg/head/day concentrate

P4 = Control (unfermented grass and corn straw)

The corn straw fermentation process is: dry field corn straw consisting of stems and leaves chopped 2-3 cm long and collected in the provided place. Corn straw that has been chopped is stacked with a thickness of 20 cm then sprinkled with urea and starbio and passed on to the next layer of straw heap and so on until it reaches 1-2 m. The amount of urea and starbio is 6 kg each for every 1 ton of corn straw. The samples were left for 21 days so that the fermentation process could take place perfectly.

The process of making silage is fresh elephant grass and *Indigofera* sp. (60 : 40%) chopped with a size of 2-3 cm. Then added a starter, namely rice bran and molasses by 5% of the total weight of the material and then all the ingredients were homogenized and put gradually into a plastic size of 60 × 100 cm until it become solid in anaerobic condition, silage process was carried for 21 days.

Forage feed is generally given as much as 10-12% and concentrate feed 1-2% of internal body weight. Concentrate is given before giving forage in the morning (Syafrial et al. 2007). The recommended feed standard for fattening cattle is the consumption of BK 3.5% BW; PK content 8%, TDN 58%, LK 6%, SK 17%, and ash 10% (Sinar Tani 2012).

Giving forage 10% of body weight carried out for 3 months. Feeding is done 2 times a day, in the morning at 07.00 WITA given fermented corn straw and concentrate, in the afternoon at 16.00 WITA silage is given, drinking water is given freely. Weighing is done every month for four months.

Data were designed using a completely randomized design. The data obtained were analyzed by analysis of variance (ANOVA), using the General Linear Model procedure according to SPSS instructions (version 23). If the results of the analysis of diversity show a significant effect, it will be continued with Duncan's multiple-distance test (Kaps & Lamberson 2004), farm analysis with R/C and B/C ratio.

**Table 1.** Concentrated feed formulation

Material	Percentage (%)
Rice bran	50.00
Coconut meal	15.50
Ground corn	19.00
Fish meal	15.00
Salt	0.25
Mineral mix	0.25
Total	100.00

## RESULTS AND DISCUSSION

### Feed nutrient content

The main obstacle to the use of corn straw as feed is the low protein content and high crude fiber so it needs to be treated to increase its nutritional value (Trisnadewi et al. 2017). The results of the analysis of the nutritional content of feed in the form of fermented corn straw, forage silage given to beef cattle can be seen in Table 2 below.

**Table 2.** Nutrient content of feed for beef cattle

Treatment	Content					
	Dry matter	Crude protein	Crude Fat	Crude Fiber	NFE	Ash
Fresh corn straw **	32.66	5.56	1.25	33.58	52.32	7.28
Fermented corn straw ***	94.11	16.10	2.42	29.52	39.24	13.10
Fresh elephant grass *	18.43	6.30	1.73	26.98		
Elephant grass silage ***	92.98	8.03	3.63	25.69	38.48	14.39
Elephant grass silage + Indigofera ***	92.12	11.98	3.44	22.51	34.81	16.16
Concentrate ***	87.54	15.21	10.79	6.14	59.39	8.46

**Source:** \*Animal Feed Nutrition Laboratory, Faculty of Animal Husbandry, Hasanuddin University, 2013; \*\*Sariubang et al. (2015); \*\*\*Animal Feed Nutrition Laboratory, Faculty of Animal Husbandry, Hasanuddin University (2018)

Table 2 shows that fresh corn straw has a crude protein content of 5.56% with a crude fiber content of 33.58%. These results indicate that in fact the crude protein content of straw is very low when compared to forages such as grasses and legumes (Yanuartono et al. 2017). One method of processing straw

as animal feed that is simple, inexpensive and can be done is fermentation (Yanuartono et al. 2019). After fermentation, the crude protein content of corn straw increased to 16.10% and the crude fiber content decreased to 29.52%. According to (Syamsu & Abdullah 2008) that the use of starbio probiotics in straw fermentation was significantly able to increase crude protein levels. Starbio (starter microbes) consists of microbes from the rumen of ruminants that are cultured in natural materials, such as soil, grass roots and rotting leaves. Starbio functions to ferment high-fiber feed ingredients, such as straw. In addition, straw fermentation using Starbio can reduce the crude fiber of straw (Antonius 2009). The increase in crude protein content is also due to the addition of urea as a mixture in the fermentation process, causing N fixation into rice straw tissue and this fixed nitrogen will later be measured as crude protein (Amin et al. 2015).

Elephant grass after making silage increased its crude protein content from 6.03 to 8.03% and crude fiber content decreased by 25.69% from 26.98%. Silage of elephant grass and indigofera with a ratio of 60:40, has a higher crude protein content of 11.98% and a lower crude fiber content of 22.51%. The crude protein content of combined elephant grass and indigofera silage is higher than that of elephant grass silage because indigofera has a high protein content, so that when mixed with elephant grass silage, the crude protein content of silage increases. According to (Muzzazinah 2016), the protein value of Indigofera leaves varies from 25-28% and can even be up to 31%.

The concentrate feed, as shown in Table 2, was of appropriate quality for lactating beef cattle. The concentrate given has a crude protein content of 15.21%. Giving concentrate to livestock aims to increase the value of feed and increase energy.

## **Performance of Bali cattle**

Body weight gain is an indicator to determine the rate of livestock growth, especially for fattening cattle and the efficiency of using feed served. According to (Huyen et al. 2011), performance of cattle is not only influenced by breed, but also strongly influenced by feeding management. Good quality feed and given in sufficient quantities will increase livestock productivity.

The results of analysis of variance showed that the feed treatment for Bali Cattle had a significant effect ( $P < 0.05$ ) on average daily gain of Bali Cattle. Duncan's test showed that the P3 treatment was significantly higher than the other treatments, and the P4 treatment was significantly.

The highest weight gain was in treatment P3 (0.37 kg/head/day) followed by treatment P2 (0.34 kg/head/day), P1 (0.28 kg/head/day) and the lowest was P4 (0.19 kg/head/day). These results indicate that the feed in P3 treatment, namely

**Table 3.** Average body weight and daily gain of Bali cattle fed with fermented corn straw and forage silage

Parameter	Feed treatment			
	P1	P2	P3	P4
Initial weight (kg)	170.1	170.8	169.4	170.4
Final weight (kg)	194.6	200	201.6	186.6
Weight gain (kg)	24.5	29.2	32.3	16.2
Average daily gain (kg/day)	0.28±0.019 <sup>b</sup>	0.34±0.019 <sup>c</sup>	0.37±0.008 <sup>d</sup>	0.19±0.013 <sup>a</sup>

Superscripts following the same values on the same line showed significant differences ( $P<0.05$ ); P1 = 50% fermented corn straw + 50% fresh elephant grass + 2 kg/head/day concentrate; P2 = 50% fermented corn straw + 50% elephant grass silage + 2 kg/head/day concentrate; P3 = 50% fermented corn straw + 50% combined silage (elephant grass + indigofera legume) + 2 kg/head/day concentrate; P4 = Control (unfermented grass and corn straw)

fermented corn straw plus elephant grass, indigofera silage and concentrate was able to increase the daily body weight of Bali Cattle, as well as P2 treatment, namely fermented corn straw and elephant grass silage, which could increase the daily body weight of livestock. This shows that the provision of fermented corn straw and forage silage (elephant grass and Indigofera) is better able to meet the nutritional needs of livestock because it has good nutritional content compared to only given fermented corn straw and fresh forage or only given unfermented corn straw and fresh forage. According to (Nadhifah et al. 2012), fermented feed ingredients produced good physical quality and high palatability compared to those that were not fermented. In addition to fermented corn straw, the high palatability of livestock is thought to be due to the dense texture of forage silage and a distinctive aroma (lactic acid) that can affect consumption and is highly favored by livestock and the brownish green color which is generally consumed by ruminants (Tahuk & Bira 2019). The quality of feed for cattle must be strictly controlled. The goal is that feeding is more economical, livestock can choose their own feed they like, and train muscles and maintain body condition. Good quality and quantity of feed to maintain the condition of the livestock body so that they are not too fat or too thin because if the cow is too thin it will produce calves who are weak due to lack of nutrition. Meanwhile, cattle who are too fat will have difficulty when giving birth (Fikar & Ruhyadi 2010).

### Consumption and conversion

Maximum feed consumption is highly dependent on the balance of nutrients in digestion. This is because nutritional needs are the main stimulus to be delivered to the hypothalamus as a hunger center. Furthermore, (FAO

2012) stated that the imbalance of feed nutrients will affect feed consumption. The average consumption of nutrient feed treatment during the study is listed in Table 4.

**Table 4.** Average consumption of dry and organic ingredients

Parameter	Feed treatment (g/head/day)			
	P1	P2	P3	P4
Dry matter	4,917.17±19.96 <sup>b</sup>	5,282.93±28.16 <sup>d</sup>	5,177.20±63/75 <sup>c</sup>	3,210.09±11.92 <sup>a</sup>
Organic ingredients	2,999.00±4.43 <sup>b</sup>	4,046.95±22.37 <sup>d</sup>	3,974.21±50.38 <sup>c</sup>	1,714.88±7.05 <sup>a</sup>
Crude protein	1,587.29±21.15 <sup>c</sup>	1,446.71±13.43 <sup>b</sup>	1,726.64±23.17 <sup>d</sup>	1,020.39±2.5 <sup>a</sup>
Conversion	19.61±6.7 <sup>b</sup>	16.10±0.9 <sup>b</sup>	13.42±1.3 <sup>a</sup>	21.04±1.7 <sup>b</sup>

*Superscripts following the same values on the same line showed significant differences (P<0.05); P1 = 50% fermented corn straw + 50% fresh elephant grass + 2 kg/head/day concentrate; P2 = 50% fermented corn straw + 50% elephant grass silage + 2 kg/head/day concentrate; P3 = 50% fermented corn straw + 50% combined silage (elephant grass + indigofera legume) + 2 kg/head/day concentrate; P4 = Control (unfermented grass and corn straw)*

Analysis of variance showed that the application of fermented corn straw and forage silage had a significant effect ( $P<0.05$ ) on dry matter consumption, organic matter consumption and conversion. Duncan's test showed that the dry matter consumption of P2 (5,282.93 g/head/day) was significantly higher than other treatments, then P3 (3,974.21 g/head/day), P1 (4,917.17 g/head/day) and P4 (3,210.09 g/head/day) was significantly lower than other treatments. Organic matter is part of dry matter, so if dry matter increases it will cause an increase in organic matter content in the same material (Wahyuni et al. 2014). Analysis of variance showed that the application of fermented corn straw and forage silage had a significant effect ( $P<0.05$ ) on the consumption of organic matter. Duncan's test showed that the consumption of organic matter in treatment P2 (4,046.95 g/head/day) was significantly higher than other treatments, respectively P3 (3,974.21 g/head/day), P1 (2,999.00 g/head/day) and P4 (1,714.88 g/head/day) was significantly lower than the other treatments. The high consumption of organic matter in P2 and P3 was due to the high consumption of dry matter in both treatments. This is in accordance with what was stated by Noviani et al. (2014) that the high and low consumption of organic matter will be influenced by the high and low consumption of dry matter. This is because most of the dry matter components consist of organic components, the difference between the two lies in the ash content.

Analysis of variance showed that fermented corn straw and forage silage had a significant effect ( $P<0.05$ ) on crude protein consumption. Duncan's test

showed that the crude protein consumption of treatment P3 (1726.64 g/head/day) was significantly higher than the other treatments. Next, respectively, P1 (1587.29 g/head/day), P2 (1446.71 g/head/day) and P4 (1020.39 g/head/day) was significantly lower than other treatments. The high consumption of protein in the P3 treatment was because in addition to cattle fed fermented corn straw, they were also given a combination of elephant grass and indigofera silage which had a high crude protein content. The high crude protein content in elephant grass and Indigofera silage is due to Indigofera containing high crude protein. According to (Muzzazinah 2016), the protein value of Indigofera leaves varies from 25-28% and can even be up to 31%. In accordance with the opinion of (Miller 2002) that protein consumption is influenced by the amount of BK consumed by livestock. The more BK consumed, the higher protein consumption.

Analysis of variance showed that the administration of fermented corn straw and forage silage had a significant effect ( $P < 0.05$ ) on feed conversion. Duncan's test showed that the conversion of P3 (13.42) was significantly lower than the other treatments, P2 (16.10), P1 (19.61) and P4 (21.04) were significantly higher than the other treatments respectively. These results indicate the P3 treatment is the most efficient compared to other treatments. There are indications that the provision of fermented corn straw and forage silage makes feed more efficient for consumption so that the conversion value is lower. The conversion value of the research results is in accordance with the opinion of (Siregar 2008), which states that the conversion of feed for cattle is 8.56-13.29. A high feed conversion rate indicates that the process of utilizing the feed provided is less efficient. Feed conversion is influenced by the availability of nutrients in the ration and animal health. (Mide 2007) also suggests that the smaller the feed conversion ratio means the more efficient the feed is to produce body weight gain.

## **Farm analysis**

Display of reproducibility, the data used as the basis for calculating the value of farming analysis is daily body weight gain of livestock. The calculated input value is the price of feed while the output value is the price value of daily body weight gain. In detail the analysis of farming is shown in Table 5. Table 5 shows, P3 provides the greatest profit of Rp 554,067. The next were P2 (Rp 417,744), P1 (Rp 205,814) and the lowest was P4 (Rp -24,012). The gains obtained in the P3 treatment were higher than other treatments because the gain in body weight obtained was also higher. According to (Zakiatulyaqin et al. 2017), the factors that influence the calculation of farming are body weight gain during fattening, feed consumption and feed prices. However, high body

weight gain does not necessarily guarantee high profits, but low feed costs followed by good growth and feed efficiency will produce maximum profits.

**Table 5.** Farming business analysis of Bali cattle fed with fermented corn straw and forage silage

Description	Treatment			
	P1	P2	P3	P4
Input				
Feed cost	5,713	6,358	6,344	3,767
Drug costs	1,000	1,000	1,000	1,000
Labor costs	5,000	5,000	5,000	5,000
Number of inputs (Rp/head)	11,713	12,358	12,344	9,767
Output				
Average daily gain	0.28	0.34	0.37	0.19
Selling price /kg	50,000	50,000	50,000	50,000
Number of Outputs (Rp/head/day)	14,000	17,000	18,500	9,500
Profit (Rp/head/day)	2,287	4,642	6,156	(267)
Profit (Rp/head/90 days)	205,814	417,744	554,067	(24,012)
R/C Ratio	1.20	1.38	1.50	0.97
B/C Ratio	5.12	2.66	2.01	(36.61)

*Note: Price of corn straw: Rp 150/kg, Fermented corn straw Rp 300/kg, elephant grass Rp 150/kg, forage silage: Rp 400/kg, concentrate Rp 2282.5/kg; P1 = 50% fermented corn straw + 50% fresh elephant grass + 2 kg/head/day concentrate; P2 = 50% fermented corn straw + 50% elephant grass silage + 2 kg/head/day concentrate; P3 = 50% fermented corn straw + 50% combined silage (elephant grass + indigofera legume) + 2 kg/head/day concentrate; P4 = Control (unfermented grass and corn straw)*

The value of the R/C ratio is calculated by comparing the amount of revenue with the total costs incurred (Hariance et al. 2018). The highest R/C ratio was at P3 (1.50), P2 (1.38), P1 (1.20) and P4 (0.97) respectively. Treatments P1, P2 and P3 have a value of more than one, these results indicate that the effort to improve the performance of cattle with fermented corn straw and forage silage feed treatments is profitable while P4 values below one indicates the business is not profitable. The highest B/C ratio obtained was P1 (5.12), followed by P2 (2.66), P3 (2.01) and P4 (-36.61). Treatments P1, P2 and P4 have a value of more than one, this result shows that efforts to improve the performance of cattle with fermented corn straw and forage silage feed treatments are feasible to be developed, while P4 has a value below one indicating the business is not feasible to be developed.

## CONCLUSION

Based on the results and discussion, it can be concluded that the application of fermented corn straw and forage silage (elephant grass and Indigofera) plus concentrate can improve the performance of Bali cattle (0.37 kg/head/day), increase dry matter consumption (5,177.20 g/head/day), consumption of organic matter (3,974.21 g/head/day) and reduced feed conversion value (13.42). The results of farming analysis showed that the provision of fermented corn straw and forage silage (elephant grass and Indigofera) gave the highest profit (Rp. 554,067).

## AUTHOR CONTRIBUTIONS

All authors were contributed equally to this work

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## **Stress Responses and Live Weight Loss of Anpera Goat as Affected by Supplement of Bioport during Transportation**

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### **ABSTRACT**

The objective of this study was to evaluate the live weight shrunk and stress response in Anpera goat as affected by feeding bioport during transportation. Anpera goat is a dairy type, a cross between Anglo Nubian and Ettawa grade. Twenty-five goats with an average body weight of  $20.29 \pm 6.15$  kg (8-22 months) were divided into two groups, consisted of control ( $n=13$ ) and bioport supplementation ( $n=12$ , level 50 g/d). The supplementation was given during and after transportation. All goats were blood sampled, observed physiological responses (respiration rate, heart rate, rectal temperature) and weighed just before loading onto a truck and after loading to assess shrinkage (72 hours transportation). A blood sample was taken to investigate the level of glucose, creatinine, blood urea nitrogen, and hematology. The result showed that bioport supplementation did not have a significant effect on physiological responses and weight loss, but tended to reduce 1% loss of body weight during transportation. The heart rate of the goat is decreased ( $122.37 \pm 8.98$  vs  $76.32 \pm 10.58$ ) after loading while the respiratory rate ( $41.57 \pm 9.15$  vs  $50.72 \pm 3.95$ ) was increased. The average rectal temperature did not show any difference during transportation ( $39.23 \pm 0.34$  vs  $39.29 \pm 0.20$ ). The results indicate that the stress responses of goats due to 72 hours of transportation may increase stress responses and bring about metabolic changes. It was concluded that supplementation bioport was not sufficient to prevent the impact of the stress response of the Anpera goat.

**Keywords:** Transport time, Stress, Crossbreed goat, Serum, Biochemical parameters

### **INTRODUCTION**

Transportation of live animals, as an inevitable husbandry practice, has been recognized as one of the main causes of stress (Saeb et al. 2010). Handling, loading, fasting, confinement, vibrations, centrifugal forces, rapidly changing light conditions, poor air quality and mixing of unfamiliar groups are some of the potential stressors during transport (Saeb et al. 2010; Zhong et

al. 2011). Road transportation of livestock is a stress-inducing situation that may lead to psychological and physiological insults. Several studies have reported the responses of different physiological parameters to the effect of road transportation stress in other species of livestock (Odore et al. 2004; Saeb et al. 2010; Ayo et al. 2009). Transportation stress has considerable physiological effects such as increased adrenal cortical activity, decreased immunity, increased morbidity and mortality due to infectious diseases, decrease in meat quality and weight loss (Saeb et al. 2010; Maejima et al. 2005). These stressors can also result in oxidative stress, which further depresses animal health, feed conversion efficiency, and meat quality (Delters & Hansen 2020). As a result, transportation stress has both economic and animal welfare concerns, and its decrement, to fewer side effects, has attracted considerable attention in recent years. The indicators of welfare and stress during transport are assessed in terms of huddling behavior, respiration rate, neutrophil, eosinophil, and blood electrolytes and cortisol concentration to predict the degree of stress (Ayo et al. 2009). Although clinical, biochemical, hormonal, and immunological effects of transportation stress in farm animals have been evaluated, it is well established that the different animal species and even different breeds may have different responses to the same stressor (Saeb et al. 2010).

Bioport is an adaptation or recovery feed produced by the Animal Research Center (Balitnak) with the aim of reducing the stress experienced during the trip and also speeding up the recovery process to less than six days. Bioport contains probiotics and electrolyte ingredients to replace the livestock's body ions lost during travel. The use of Bioport in cattle can reduce body weight loss due to transportation from NTT to Jakarta from 13 to 7% (Meroukh 2002 in Winugroho & Widiawati 2004), as well as it can reduce sheep body weight loss during transportation from 8.3 to 3.8% (Kusumawardhani 2000 Unpublished data in Winugroho & Widiawati 2004).

Goats, as economically important producers of meat, hair, and milk, have a high economic value in many countries. Anpera goat is a dairy type, crossing between Anglo Nubian and Ettawa grade. There is little information regarding the Anpera goat in general and to the best of our knowledge, there is no previous study regarding the physiological effects of transportation stress in this valuable breed. This study was undertaken to evaluate the influence of this stress on physiological responses (respiratory rate, heart rate and rectal temperature), some serum biochemical parameters, and blood hemoglobin. In addition, this study was aimed to evaluate supplementation bioport to overcome transportation stress.

## **MATERIALS AND METHODS**

This study was performed in April 2021 on 21 clinically healthy Anpera goats (6 male and 19 non-lactating non-pregnant female goats, average body weight of  $20.29 \pm 6.15$  kg, age range: 8-22 month). The animals were managed under the intensive system at the Goat Research Unit at Research Institute for Animal Production, Bogor. Livestock transportation was carried out at night (April 21<sup>st</sup> 2021 at  $\pm 19:00$ ), and arrived at the location at night (April 24<sup>th</sup> 2021 at  $\pm 19:00$ ), which was 72 hours of traveling. The distance traveled was  $\pm 1,942.5$  km.

### **Experimental design**

Twentyfive goats were divided into two group, consisted of control (n=13) and bioport supplementation (n=12, level 50 g/d). The supplementation was given during and after transportation. All goats were blood sampled, observed physiological responses (respiration rate, heart rate, rectal temperature) and weighed just before loading onto a truck and after loading to assess shrinkage (72 hours transportation). Blood sample was taken for investigation level of glucose, creatinine, blood urea nitrogen, and hematology.

### **Physiological measurement**

Physiological components measured were rectal temperature, respiratory rate, and heart rate. The respiration rate of goats was measured based on the flank movement (x/minute), heart rate (x/minute) was measured using stetoskop, and rectal temperature (°C) was recorded using a rectal thermometer by inserting the thermometer about 1 inch into the rectum until stable.

### **Blood sample collection**

Blood samples were taken during the day (2 hours after eating), or 24 hours before loading. Meanwhile, blood sampling (after transportation) was carried out after 1-2 hours arrived at the location (last feeding time at 16.00). After ensuring proper restraint, a 21-gauge needle and syringe was used for blood collection through the jugular vein and sequel to swabbing with methylated spirit and cotton wool to ensure asepsis. An amount 5 ml of blood were collected in separate vacutainer plain and containing EDTA for biochemical and hematological analyses, respectively. The blood sample in a plain vacutube was centrifuged for 10 minutes at 3000 rpm to obtain a serum sample. Serum was then transferred into a 1.5 ml Eppendorf tube and stored at  $-20^{\circ}\text{C}$ .

## **Hematological and biochemical serum analysis**

Blood samples were analyzed at Primate Research Center (PSSP), IPB University. The parameters hematological determined were red blood cell count (RBC), white blood cell count (WBC), platelets, haemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), Red cell distribution width (RDW), mean platelet volume (MPV), platelet distribution width (PDW). The parameters serum biochemical determined were glucose levels (mg dl<sup>-1</sup>), creatinine (mg dl<sup>-1</sup>) and blood urea nitrogen (BUN) (mg dl<sup>-1</sup>).

## **Statistical analysis**

The data were subjected to statistical analysis by ANOVA to test mean significance between and among groups. Statistical analysis was performed using IBM SPSS Statistics 22.

# **RESULTS AND DISCUSSION**

## **Physiological response and weight loss**

Indicators of physiological response and body weight showed differences in goats after transportation but were still within normal limits (Table 1). There was not significant effect of supplementation bioport during transportation on shrinkage and physiological response. The mean shrinkage were  $2.28 \pm 1.03$  (11.7%) and  $2.03 \pm 0.45$  (10.1%) in control and with supplementation groups, respectively. Many results shows that stress transportation significantly on shrinkage, but in our study it was not affected by transportation ( $P > 0.05$ ). The mean body weight before and after transportation were  $20.1 \pm 5.97$  and  $17.94 \pm 5.43$ , respectively. Gibran et al (2015) shows that body weight loss is higher than 7 hours transport time with mileage 300 km, young goats experienced a decrease in body weight of 6.67% and 4.92% in adult goats. Ginting (2006) reported that the main cause of weight loss during transportation is stress. Livestock that are transported will be exhausted because discomfort during transportation makes the movement of cattle in the truck bigger so that the level of fatigue is even greater. These factors lead goat stress because of goat lose a lot of body fluids and decrease body weight. Delters & Hansen (2020) added that goat stress exposure was due to handling, transportation, restrictions on feed and drinking required energy to repair or degrade oxidatively damaged molecules and to support production of cellular antioxidants (Delters & Hansen 2020). Bioport supplementation had an indication that it could reduce shrinkage, this ability was due to bioport

consisting of electrolyte compounds containing 10 vitamins (55%), probiotics (25%), and ingredients containing immunoglobulins derived from pure colostrum powder (20%). In our study, there are indication that supplementation bioport was not sufficient to prevent the impact of oxidative stress due to exposure to psychological stress during transportation which can lead to weight loss. Triutama et al. (2016) conducted a study that the administration of 1,500 mg of vitamin C could reduce weight loss by 0.63% during the transportation of cattle from Lampung to Palembang. Thus, the role of vitamin C is a non-enzymatic, water-soluble protector (antioxidant) that can counteract various free radicals, helping absorption iron, and restore the body's condition due to the oxidation reaction of various harmful compounds.

**Table 1.** Shrinkage and physiological response of treated Anpera goats

Parameter	Before transportation		After transportation		Variation	
	R0	R1	R0	R1	R0	R1
BW (kg)	19.86±2.98	20.37±2.98	17.58±7.17	18.33±2.81	2.28±1.03	2.03±0.45
RT (°C)	39.17±0.39	39.29±0.29	39.32±0.17	39.26±0.24	0.15±0.42	-0.03±0.31
RR (×/min.)	41.95±8.26 <sup>b</sup>	41.17±10.39 <sup>b</sup>	49.85±3.51 <sup>a</sup>	51.67±4.33 <sup>a</sup>	7.90±9.97	10.50±10.27
HR (×/min.)	124.41±6.96 <sup>a</sup>	120.17±10.63 <sup>a</sup>	78.77±11.12 <sup>b</sup>	73.67±9.72 <sup>b</sup>	-45.64±8.05	-46.50±17.89

R0 = control; R1 = bioport supplementation (50 g/d). Different subscripts on the same line indicate a significant difference ( $P < 0.05$ ); BW = Body weight; RT = Rectal temperature; RR = Respiratory rate; HR = Heart rate

In generals our results indicated a slight influence of transportation on physiological parameters related to stress, but in this study no significant effect was found on heart rate, respiratory rate, and rectal temperature due to the addition of bioport. Anpera goats increased heart rate to try to restore homeostatic zone (condition before stress occurs). The average rectal temperature before and after transportation were  $39.23 \pm 0.34$  and  $39.29 \pm 0.20$ , respectively. The average heart rate after transportation was  $122.37 \pm 8.98$  and  $76.32 \pm 10.58$ , respectively. An increase in heart rate is a fear response of livestock to handling and transportation. Moreover, a habituation effect (adaptation to handling) could reduce the reduce the stress response after transportation in proper conditions (Gradin 2000). In this study, there was no increase in pulse frequency, probably due to good handling, especially during unloading. There was also a difference in heart due to microclimate and facilities at loading, transportation, and unloading. The average respiratory rate before and after transportation were  $41.97 \pm 9.15$  and  $50.72 \pm 3.95$ , respectively. An increase in respiratory rate is one of the livestock's efforts to

reduce body heat to keep it in normal condition. In addition, the physiological status of livestock also affects the frequency of breath. Young cattle tend to adapt more quickly to their environment (Nelvita et al. 2018). If livestock are transported in a transport room by maintaining good and comfortable air temperature conditions for livestock, livestock will not experience an increase in rectal temperature (Lendrawati et al. 2019). Changes in physiological conditions during transportation are the response of livestock to environmental conditions. Changes in environmental temperature will stimulate thermoreceptors in the hypothalamus, in response the hypothalamus will release the hormone cortisol which will dilate blood vessels to accelerate blood flow throughout the body to release heat (Ramadhan et al. 2017).

### **Hematological and biochemical serum**

Hematological indicators and blood metabolites showed changes in goats after transportation but were still within normal limits (Table 2). Bioport supplementation had no significant effect on changes in serum biochemical concentrations but had a significant effect on hematological parameters. In our study, after transportation caused an increase in hemoglobin in Anpera goats. The mean hemoglobin level before and after transport were  $8.86 \pm 0.81$  gdl<sup>-1</sup> and  $10.10 \pm 0.90$ , respectively. It seems that the need for oxygen increases when the livestock is under stress which has an impact on increasing the hemoglobin content. An increase in oxygen demand when cattle are under stress is necessary for the continuity of the energy intensive metabolic process at that time. While the number of erythrocytes and platelets, hematocrit, MPV, PDW, RBW there was a significant difference ( $P < 0.05$ ) between the control group and the bioport supplementation group. In general the stress response by catecholamines increase red blood cells, thus haematocrit and haemoglobin increase. There was a trend for this response be accentuated after the long trips (6 h).

Transportation stress has been reported to cause an elevation in serum glucose, creatinin and BUN concentrations, but in our case, we did not observe significant increases as a result of the transport. The increase in plasma glucose is associated indirect with stress as a consequence of the catechiominergic action and glucocorticoids (Lendrawati et al. 2020). Almost the same thing was also expressed by Anton et al. (2016) which states that when livestock suffer from transportation stress and lack of feed, the central nervous system works actively and triggers hormone work to release glucose, resulting in an increase in blood glucose levels due to glycogenolysis associated with an increase in the catecholamine and cortisol hormones which are below sympathetic

nervous control released by stress. The values of glucose serum Anpera goats in this study were around 51-60 mg/ml. Normal goat and sheep blood glucose levels around 34-84 mg dl<sup>-1</sup> (Panousis et al. 2012). When there is a lack of nutritional intake, blood glucose content will decrease, then glucagon will be secreted into the blood from pancreatic alpha cells, the impact of high levels of glucagon and epinephrine in the blood will cause gluconeogenesis in the liver and glycogenolysis in muscles (Astuti et al. 2014) urea is the result of end of gluconeogenesis proteins that are not utilized by the body of livestock. In this study, the level of BUN Anpera goats were arround 15-46 mg/ml. Normal blood urea levels in healthy goats and sheep range from 15.0 sampai 36.0 mg dl<sup>-1</sup> (Bendryman et al. 2000), 29.91-35.87 mg dl<sup>-1</sup> (Rahayu et al. 2017). The concentration of creatine kinase (CK) in plasma can be associated with muscle damage and physical stress, or poore welfare (Lendrawati et al. 2019). Lendrawati et al. (2020) also reported that creatinine is the result of the breakdown of creatinine phosphate which is used as an energy source when cattle are under stress, so that creatinine levels will increase when cattle are stressed. The level of creatine Anpera goats were arround 0.6-1.1 mg/ml.

**Table 2.** Hematological and biochemical serum of treated Anperla goats

Parameter	Before transportation		After transportation		Variation	
	R0	R1	R0	R1	R0	R1
Leukosit/WBC (10 <sup>3</sup> /μl)	16.55±3.38	17.89±4.35	20.15±6.44	18.46±5.70	3.60±4.78	0.57±5.29
Eritrosit/RBC (10 <sup>6</sup> /μl)	12.08±1.37 <sup>b</sup>	11.82±1.34 <sup>b</sup>	13.26±1.48 <sup>a</sup>	12.31±1.30 <sup>b</sup>	1.18±0.85	0.49±0.65
Hemoglobin (g/dl)	8.62±0.92 <sup>b</sup>	9.12±0.63 <sup>b</sup>	10.15±1.00 <sup>a</sup>	10.04±0.81 <sup>a</sup>	1.52±0.84	0.93±0.61
Hematokrit (%)	21.58±2.58 <sup>b</sup>	21.02±2.41 <sup>ab</sup>	24.26±3.39 <sup>a</sup>	20.43±6.24 <sup>b</sup>	2.68±1.89	-0.59±6.25
MCV (fl)	17.80±0.32	17.78±0.19	18.00±0.17	17.80±0.32	-2.57±6.90	-1.47±5.18
MCH (pg)	7.18±0.73	7.79±0.73	7.81±0.74	8.25±0.74	0.57±2.66	-0.23±2.10
MCHC (g/dl)	40.24±4.28	43.83±3.92	42.25±4.65	44.25±3.28	2.01±3.63	-6.78±19.83
Tombosit/PBC (10 <sup>3</sup> /μl)	721.31±372.38 <sup>b</sup>	1074.50±452.35 <sup>a</sup>	938.15±424.08 <sup>ab</sup>	1138.50±421.20 <sup>a</sup>	216.85±262.10	61.00±90.09
RDW (%)	20.62±2.19 <sup>ab</sup>	19.39±5.61 <sup>b</sup>	23.42±4.96 <sup>a</sup>	22.23±4.01 <sup>ab</sup>	2.79±3.14	2.84±5.73
MPV (fl)	3.64±1.10 <sup>a</sup>	2.28±2.02 <sup>bc</sup>	3.24±1.44 <sup>ab</sup>	1.91±2.00 <sup>c</sup>	-0.40±1.03	-0.38±1.11
PDW (%)	10.65±1.48 <sup>b</sup>	12.43±2.22 <sup>a</sup>	12.10±2.10 <sup>ab</sup>	13.42±2.33 <sup>a</sup>	1.45±1.70	0.99±1.02
BUN (mg dl-1)	31.85±11.05	27.39±5.20	34.72±8.72	28.62±8.88	2.88±12.13	1.23±7.42
Creatinin (mg/dl)	0.75±0.25 <sup>b</sup>	0.70±0.17 <sup>b</sup>	0.92±0.20 <sup>a</sup>	0.93±0.18 <sup>a</sup>	0.17±0.19	0.22±0.18
Glukose (mg/dl)	60.08±9.25	51.42±13.30	58.67±15.58	56.08±23.30	-0.17±22.40	4.67±30.59

-R0 = control; R1 = bioport supplementation (50 g/d). Different subscripts on the same line indicate a significant difference ( $P < 0.05$ )

- MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = Red cell distribution width; MPV = mean platelet volume; PDW = platelet distribution width (PDW); BUN = blood urea nitrogen

Livestock are exposed to a variety of psychological stressors during transport including handling during loading and unloading, unfamiliar noises and environments, as well as commingling. The study of Detres and Hansen (2020), the body responds to stress by activating 2 major hormonal axes. In the short term, activation of the sympathetic adrenal medullary axis initiates the release of catecholamines including epinephrine (*i.e.*, adrenaline) and norepinephrine. These hormones support the fight-or-flight response by increasing heart rate, blood pressure, and glucose availability via stimulation of hepatic glycogenolysis. The long-term stress response is maintained by the hypothalamic pituitary adrenal axis. Briefly, corticotrophic releasing hormone and vasopressin released from the hypothalamus stimulate the release of adrenocorticotrophic hormone from the pituitary, which ultimately signals the release of glucocorticoids (primarily cortisol in mammals) from the adrenal cortex (Charmandari et al. 2005). Glucocorticoids stimulate the release of glycerol and fatty acids from adipose tissue as well as AA from muscle, directing these nutrients toward the liver for enzyme synthesis and gluconeogenesis (Baxter & Forsham 1972). The uptake and aerobic metabolism of these substrates by other tissues increases mitochondrial-derived ROS simply as a result of greater electron flow through the electron transport chain and electron leakage to oxygen. In our study, there were indication that supplementation bioport was not sufficient to prevent the impact of oxidative stress due to exposure to psychological stress during transportation. Vitamins E and C have direct antioxidant activity due to their ability to scavenge free radicals, making them essential components of the cellular antioxidant defense system. Supplemental VE recommendations for newly received calves (400 to 500 IU/d or 1.6 to 2.0 IU/kg of BW) were established based on previous evidence that increased concentrations of VE during periods of high stress may promote animal health (NASEM 2016). Furthermore, it has been demonstrated that VC can mitigate the negative effects of glucocorticoids (such as dexamethasone) on bovine neutrophil function (Roth & Kaeberle 1985).

## CONCLUSION

The results indicate that the stress responses of goats due to long journey (72 hours transportation) might increase stress responses and bring about metabolic changes. It was concluded that supplementation bioport during transportation (3 days at level 50 g/day) was not sufficient to prevent the impact of the stress response of the Anpera goats. It is necessary to further study the effect of the time of giving bioport to livestock (before, medium, and

after transportation), as well as to identify the right dose of bioport at different transportation distances (short, medium, and long journey).

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## **Physiological Response of Bali Cattle with Improved Feed through Palm Frond Substitution on Dry Land in Central Kalimantan**

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### **ABSTRACT**

Central Kalimantan is one of the Indonesian provinces with great potential for a beef cattle centre in the future. It's supported by the availability of potential land and feed supply. By products of oil palm processing are potential for cattle feeding in these provinces. Central Kalimantan was recognized as third largest oil palm land plantations area in Indonesia with total areas of 2.02 million hectares. Oil palm plants can produce 18-25 fronds/tree/year or about 10 tons/ha/year dry materials. This research was conducted in January - December 2019 and located in the Jaya Makmur farmer group, Kotawaringin Timur Regency, Central Kalimantan Province. A total of 16 Bali cattle aged 24-36 months were used and grouped based on a palm frond fermentation treatment made from palm fronds (T0, T1, T2 and T3). The research was statistically analyzed using a completely randomized design (CRD). The environmental conditions during the study obtained the temperature of the cage ranging from 29-32°C. The use of palm frond fermentation has been shown the daily body weight of cattle by 320±75 grams/head/day. The treatment of palm fronds on rations had a significant effect ( $P<0.05$ ) on the daily weight gain for Bali cattle and as a result, it shown that T1 is the best result significantly. It was also able to increase the BCS value and conception rate due to improved feed ration formula, calculated and adjusted to the cattle needs.

**Keywords:** Physiological, Bali cattle, Palm fronds, Fermentation

### **INTRODUCTION**

Central Kalimantan Beef cattle population in 2017 was ± 73,424 heads (BPS 2017). Central Kalimantan was need around ±9,000 heads/year of cattle to fulfill their meat needs and only about 50% could be met. Shortage of beef cattle in this province is about ±4,500 heads/year, was met from outside of Central Kalimantan (Prokal. 2017). The results of the 2017 cattle census show that there are several districts in Central Kalimantan that have a cattle

population of more than 7.000 heads, namely the districts of West Kotawaringin, East Kotawaringin, Seruyan, Pulang Pisau and Katingan. The west region of central Kalimantan (East Kotawaringin, West Kotawaringin, Lamandau, and Seruyan Regencies) is a very potential district as a beef cattle center for the future.

Feed is the main component in beef cattle farming. One of the dominant factors in livestock development is the source of feed, including its quality and quantity. Traditional farmers use natural grass with low protein and energy content to feed their cattle. This condition will not only impact on body weight gain, but also on livestock reproduction. When daily body weight gain of livestock is low and body condition score is below standard, it does not guarantee a high level of pregnancy, but usually results in low fertility. In addition, it also has an impact on low birth weight, slow growth, long first calving age, low live weight or slaughter weight of adult cattle. Another problem of this condition is the high child mortality rate, due to nutritional deficiencies.

Oil palm plantations by-products are potential to be an alternative source of feed in livestock businesses. Central Kalimantan Province is third largest oil palm plantation area in Indonesia. This province is potential for livestock development with the support of abundant natural resources in the form of land and feed resources that have not been used optimally. These potentials include extensive land, forage, agricultural waste and plantation waste. Productive oil palm areas in Central Kalimantan was around 1,495,605.89 ha and produce 4,454,675.65 tons of oil palm in 2016 (BPS 2017). The vast land has great potential as a source of animal feed in the form of grass in oil palm plantation areas and also processing factories and palm oil fields by-products. Oil palm plants can produce 18-25 fronds/tree/year (Lubis 1992) or about 10 tons/ha/year dry mater (Purba and Ginting 1997). Oil palm plantations by-products utilization is an alternative source of feed for beef cattle in Central Kalimantan. The aim of the study was to determine the physiological response of Bali cattle to feed based on palm fronds.

## **MATERIALS AND METHODS**

### **Research time and place**

This research was carried out in January - December 2019. Research location was in the Jaya Makmur Tani Farmers Group, East Kotawaringin Regency, Central Kalimantan Province.

## Research procedure

Bali cattle were adapted to feed changes (pre-eliminary) for two weeks, before being given treatment. Preparation for its maintenance includes cleaning the cattle pen and preparing feed. Fronds chopped using a chopper before being given to livestock. The cattle used were 16 bali cattle with an estimated age of 24-36 months in the People's Farm. Cattle were given concentrate feed and palm fronds with different concentrations. The research used a completely randomized design (CRD) design, with feed made from palm fronds on 16 cattle.

The feed ingredients were mixed according to the ration composition of the treatment with adding EM4 for bacterial source. Mixed feed then fermented by inserting it into the sacks that had been layered with plastic so to get unerobic environment. Fermented feed can be used after keeping it for 21 days. Feed adaptation was carried out for two weeks (14 days) and then continued for data collection. Cattle were fed as much as  $\pm 10\%$  of their live weight. Nutritional requirements of feed calculation refers to the guidelines for the Nutrient Requirements of Cattle (NRC 2001). Feed was given twice a day at 08.00 and 16.00 WIT. The observed variables consisted of body weight gain, body condition score and physiological response. Environmental factors measured include air temperature and humidity.

Obtained data will be analyzed quantitatively with the Analysis of Variance (ANOVA), if there is a significant difference, then proceed with Duncan's Multiple Range Test at 5% level with the Statistical Product and Service Solution application tool (SPSS ver 23.0).

**Table 1.** Feed ration treatment formula made from palm fronds

Feed Ingredients	T0	T1	T2	T3
	%			
Palm fronds	65.65	67.02	67.69	68.34
Palm kernel cake	7.57	6.33	5.72	5.12
Solid decanter	23.70	23.28	23.08	22.88
Rice bran	2.25	2.21	2.19	2.17
Fish meal	0.00	0.35	0.52	0.69
Mineral	0.20	0.20	0.20	0.19
Calcium	0.20	0.20	0.20	0.19
Salt	0.20	0.20	0.20	0.19
Molasses	0.23	0.23	0.22	0.22
Total (%)	100.00	100.00	100.00	100.00

*T0 = 0% fish meal; T1 = 0.35% fish meal; T2 = 0.52% fish meal; T3 = 0.69% fish meal*

## **RESULTS AND DISCUSSION**

### **Curent condition**

The results of microclimate conditions observations in the study site showed that pen temperature at 07.30 and 17.30 west indonesian time ranged from 29-32°C. This condition is in accordance with the climate type in Central Kalimantan in the form of a humid and hot tropical with an average air temperature of 29°C and a maximum of 33°C.

Livestock rearing at the activity site is still done traditionally. In general, the amount of feed given is not in accordance with the needs of cattle, both in quality and quantity. Farmers have not provided additional feed to complete the nutritional deficiency. The amount and quality of feed given is not in accordance with the physiological status of livestock, even in the dry season there tends to be grass difficulties so that farmers have to tie grass to neighbouring villages with ten km distance from the location. Problems found at the activity site related to reproduction, namely the availability of males, technical problems with Artificial Insemination and mating timing.

### **Palm oil waste-based feed technology innovation**

Palm oil plantation and industrial waste has not been optimally utilized as animal feed, although it is very abundant and available at low prices. Utilization of waste from oil palm plantations, both managed by the company and plasma plantations/people's plantations in the form of pruning leaves and fronds, while the palm oil mill will produce solid, palm kernel cake, fruit feeling fiber and empty bunches. According to Elisabeth (2003), for ruminants, palm fronds can be used as a substitute for grass, while mud and palm kernel cake can be used as protein sources with protein content of 14.5 and 16.3%, respectively. Waste in the form of palm fronds, palm kernel cake and solids will be processed as cattle feed in the form of complete or concentrated feed. Feed produced is used to meet the needs of cattle belonging to members of the farmer group and if it possible sold to meet the needs of consumers outside the farmer group.

Feed ingredients that found in large quantities, continuous supply and good nutritional value are a by-product of oil palm processing. Palm kernel cake contains 91.83% dry matter, 16.33% crude protein, 6.49% crude fat, 35.68% crude fiber, 0.56% calcium, 0.84% phosphorus, energy 5.175 kcal/kg (Widjaja 2005). Nutrients content of midrib and palm leaves are dry matter 48.78%, crude protein 5.3%, hemicellulose 21.1%, cellulose 27.9%, crude fiber 31.09%, ash 4.48%, NFE 51.87%, lignin 16.9% and silica 0.6% (Imsya 2007).

According to research by Utomo (2004), solid contains 81.56% dry matter, 12.63% crude protein, 9.98% crude fiber, 7.12% crude fat, 0.03% calcium, 0.003% phosphorus, and 154 cal/100 g energy. Treatment of palm fronds on the ration had a significant effect ( $P<0.05$ ) on the increase in body weight (PBB) of bali cattle.

**Table 2.** Effect of palm fronds feeding on Bali cattle

Treatment	Body weight (kg)	
	Before	After
T0	167.17	182.71
T0	98.95	112.60
T0	203.94	216.87
T0	208.94	222.87
T1	249.08	270.40
T1	188.93	214.02
T1	211.55	233.42
T1	224.17	246.79
T2	215.05	236.17
T2	183.37	213.27
T2	184.43	206.11
T2	186.24	214.24
T3	170.98	190.00
T3	194.41	214.60
T3	179.20	198.96
T3	187.00	206.50

*T0 = 0% fish meal; T1 = 0.35% fish meal; T2 = 0.52% fish meal; T3 = 0.69% fish meal*

**Table 3.** Effect of palm fronds feeding on Bali cattle

Treatment	Average body weight gain
T0	0.22±0.017 <sup>a</sup>
T1	0.36±0.026 <sup>bc</sup>
T2	0.39±0.067 <sup>c</sup>
T3	0.31±0.008 <sup>b</sup>
Average	0.32±0.075

*Superscript significantly different; T0 = 0% fish meal; T1 = 0.35% fish meal; T2 = 0.52% fish meal; T3 = 0.69% fish meal*

The results showed that the addition of palm fronds (T0) resulted in daily body weight gain (DBEG) was lower than T1, T2 and T3 ( $P < 0.05$ ). The highest daily body weight gain was achieved at T2 which was significantly higher than the other treatments. Meanwhile, between T1 and T3 did not show a significant difference in DBWG. This condition is in accordance with the research of Purba et al. (2012) providing complete feed based on oil palm showed a positive response to the increase in the weight of cattle. Based on research by Utomo (2004), *ad libitum* administration of solid in the form of fresh palm Fronds to PO bulls gave daily body weight gain (DBWG) of 770 g/head/day.

Based on the body measurements approach, namely Live Weight (LW), and Body Condition Score (BCS), Bali cattle can show a fairly good growth performance in oil palm waste-based feed experiments. This is different from the traditional rearing pattern where the amount of forage feeding is limited and only relies on the available feed on site. Complete feed made from palm by-products is able to have a positive impact on the significant increase in growth performance, development, and reproductive potential of Bali cattle. Thus, the performance of Bali cattle can be increased by adding rations made from palm by-products.

This good performance will greatly determine the success of female Bali cattle to get pregnant, so that Bali cattle breeding efforts can run optimally. Efforts to improve the quality of Bali cattle can be done by considering the factors that affect production and reproduction nature (Suranjaya et al. 2010). The results of Pasambe et al. (2000), that the use of local feed with a composition of 50% field grass, 50% fermented straw and 1% of body weight concentrate was able to improve the performance of Bali cattle. Meanwhile, the results of research by Hafid & Rugayah (2010), that the type of ration with the addition of local ingredients cocoa pod by 35% and sago dregs by 5% has an impact on body weight gain and the efficiency of feed use in cattle. Meanwhile, according to Sugama and Budiari (2012) that feeding forage and fermented rice straw combined with rice bran and probiotics can increase the productivity of Bali cattle.

In semi-intensive rearing, cattle are grazed during the day and return to the pen in the afternoon. Additional feed based on palm oil waste was given in the morning and evening. According to Purwantari et al. (2014), based on several studies, it shows that the integration of oil palm-cow with a grazing system is economically feasible (feasible). Meanwhile, at night the cattle are kept in cages and given concentrate feed in an amount according to the needs of the cattle.

Bali cattle began to show an increase in performance along with the completion of the feed adaptation period. According to Purba et al. (2012)

providing complete feed based on oil palm showed a positive response to the increase in the weight of cattle and peanut goats. According to Gustiani et al. (2014) cattle that were treated with additional feed had a better effect on birth weight, livestock weight gain, and post partum estrus.

The increase in the good BCS value was due to an improvement in the feed ration formula which was calculated and adjusted to the cattle needs. This ration improvement was carried out with the aim of improving the BCS value of Bali cattle so that the value could approach 3. Better BCS value indicates that the performance of bali cattle both in terms of growth and development as well as reproductive potential has increased. According to Utomo et al. (2011) feed improvement in cattle can have a positive effect on increasing the BCS value. Furthermore, according to Budiawan et al. (2015), the BCS score has a positive relationship to service per conception and calving distance.

Efforts to increase the BCS value are carried out by compiling rations by combining feed ingredients from fiber sources and energy sources to serve as complete feeds and supplementary feeds. Efforts to utilize complete feed based on plantation agroecosystems and PKS can increase the productivity of ruminants (Maluyu 2012). The main source of fiber comes from palm fronds, while the source of energy source for strengthening feed comes from solid palm oil and palm kernel cake. Provision of palm fronds and leaves as a substitute for forage in beef cattle feed up to a level of 60% can increase the weight of beef cattle compared to using only forage (Nurhayu et al. 2014).

Utilization of oil palm by-products for beef cattle feed is proven according to the needs of farmers in general (location-specific). Improvements in management are carried out in feeding, disease, pen and reproduction management. Mechanization facilities that are not utilized by farmers can be activated so that their use can be of direct benefit to farmers and members of other groups.

**Table 4.** Effect of palm fronds feeding on body condition score and conception rate

Treatment	BCS increase	Conception rate (%)
T0	0.25	75
T1	0.38	100
T2	0.38	100
T3	0.25	75
Average	0.31	88

*T0 = 0% fish meal; T1 = 0.35% fish meal; T2 = 0.52% fish meal; T3 = 0.69% fish meal; BCS = body condition score*

## CONCLUSION

The treatment of palm fronds on rations had a significant effect ( $P < 0.05$ ) on the daily weight gain for Bali cattle and as a result, it shown that T1 and T2 are better than others. It was also able to increase the BCS value and conception rate due to improved feed ration formula, calculated and adjusted to the cattle needs.

## AUTHOR CONTRIBUTIONS

All authors were contributed equally to this work.

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## **Production, Nutritional Quality and *In Vitro* Digestibility of the Whole Corn Plant as Forage for Ruminant in Two Seasons**

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### **ABSTRACT**

Corn crop biomass is potential to be used as ruminant's feed. This research aimed to study production, nutritional quality, and in-vitro digestibility of whole of corn plants planted in a different season as forage for ruminant feed. Planting maize seeds was carried out with a spacing of 25×50 cm with a planting depth of 3 to 5 cm. Corn crop biomass is harvested at 50 to 60 days after planting at 10 cm above the ground. This research was conducted in a randomized block design, consisting of two groups of season planting (dry and rainy season). Each group consisted of 5 replications with an experimental plot area of 400 m<sup>2</sup> for each replication. The variables observed were production, nutrient content, and in-vitro digestibility. Production data were analyzed using a t-test. There was no significant difference between fresh and dry matter productions planted in the dry and rainy seasons. Fresh productions were 19.35 and 17.36 t/ha while DM productions were 6.35 and 6.33 t/ha for the rainy and dry season, respectively. The nutritional quality and in-vitro digestibility were almost similar both in the rainy and dry seasons. Crude protein contents were 9.83 and 9.04% for the rainy and dry seasons, respectively. The ADF content was 33.41 and 36.60% for the rainy and dry season, respectively, while NDF content was 56.89 and 58.63%, respectively. The IVDMD and IVOMD were 49.35 to 51.78% and 48.42 to 50.96%, respectively. It can be concluded that the whole corn plant can be used as a forage source with good quality as ruminant feed.

**Keywords:** Corn crop, Forage feed, Ruminant

### **INTRODUCTION**

Corn is a cereal crop that can be grown in tropical, subtropical, and temperate climate zones. The main use of corn is for food but also used for feed. According to Sariubang & Herniwati (2011), from 1989 to 2002 national corn production was able to meet 50% of domestic demand. Thus feed industry is an important downstream agribusiness activity in corn commodities. The availability of good quality forage, especially during the dry

season is one of the obstacles in raising ruminants. Corn crop biomass (leaf, stem, and baby corn) is the potential to be used as goat's feed.

Morphologically, the maize plant consisted of seeds (kernels), husks and hair (silk), root, rod (stalk), leaves, and flowers (tassel). There are many types corn biomass potential used as ruminants feed: (a) Biomass available after harvesting the ears, consisted of corn stalk. This stalk containing stem, leaves, and young corn. This biomass usually chopped and given to livestock directly, (b) Biomass available after the cobs collected from the stalks after left dry in the fields, the biomass by products was called Corn straw, (c) Corn husk, is the part of corn ear and usually thrown away, and (d) Corn cob, which is part of the corn ears after the grains are collected as described by Kumalaningsing et al. (2009). Corn plants is all parts of the plant including stems, leaves and young fruit which are generally harvested at the age of 45-65 (Soeharsono & Sudaryanto 2006). This corn plants contained 12.06% crude protein, 25.2% crude fiber, calcium 0.28% and phosphorus 0.23% (Erna & Sarjiman 2007).

The purpose of this research is to study production, nutritional quality, and in-vitro digestibility of the whole corn plants those planted at different season as forage for feed ruminant which harvested at the age of 50 to 60 days after planting.

## **MATERIALS AND METHODS**

### **Place and time**

This research was carried out in the experimental yield of the Sei Putih Goat Research Station, Deli Serdang, North Sumatra. This activity was done in 2016.

### **Research material**

The materials used in this experiment were: corn seeds, herbicides and organic-fertilizer. The maize seeds planted were the Srikandi variety obtained from the Cereal Crops Research Institute, South Sulawesi. The tools used are: tractor, sprayer, hoe, and scale.

### **Research methods**

#### ***Planting and harvesting corn***

The first stage of this research activity was cultivation of a 0.5 ha of land area which includes ground grafting and double rotating. Planting maize seeds was carried out with a spacing of 25×50 cm (one seed per hole) with a

planting depth about 3 to 5 cm. Planting time was done twice representing dry and rainy seasons, planting in March for dry season and, in May for rainy season. The plants were treated in the form of weeding, spraying weeds with herbicides and fertilization. The first fertilization was done seven days after planting while the second fertilization was done four weeks after planting corn. The maize was harvested at 50 to 60 days old of plant age. Harvesting was done by cutting the plant by hand at 10 cm above ground.

### ***Experimental design***

The research was conducted in a randomized block design, consisting of group planting in the dry season and in the rainy season. Each group consisted of 5 replications with an experimental plot area of 400 m<sup>2</sup> for each replication. The total area of the experimental plot was 4,000 m<sup>2</sup> (10×400 m<sup>2</sup>). The total land area for research including the border on each experimental plot is 5,000 m<sup>2</sup> (equivalent to 0.5 hectare). Production data were analyzed using t-test (SAS 1998).

### ***Observed variables***

The variables observed were biomass fresh production, dry matter production, nutrient content (dry matter, organic matter, ash, crude protein, crude fat, neutral detergent fiber, acid detergent fiber, crude energy) and in-vitro digestibility (dry matter and organic matter).

### ***Biomass production***

Biomass fresh production was obtained by cutting the plants at about 10 cm above the soil surface then weighed fresh for each experimental plot. The fresh biomass then sub sample for chemical analysis purposes. The sub sample were taken from three consecutive plants from the centre rows randomly selected within each plot replication on both group of the dry season and the rainy season. Those plants were cut by hand at about 10 cm above ground and weighed. These samples were chopped and composite for five replications, mixed evenly and took a sample of 500 g. The sample was put into a paper bag, dried in an oven at 60°C for 48 hours and then the sample was weighed to determine the moisture content. Dry matter production was obtained by multiplying fresh production by dry matter content.

### ***Nutritional content***

The whole-corn plants were analyzed to determine dry matter, organic matter, ash, crude protein, neutral detergent fiber, acid detergent fiber and

crude energy content. Determination of dry matter of the samples were done by drying the sample in an oven at 135°C for 2 hours, then weighed. The dried samples then ground for chemical analysis. Crude protein analysis was carried out by measuring the total nitrogen content of the sample using macro-Kjedhal and ash content determined followed the method of AOAC (2005). NDF and ADF analysis was done using the Goering and Van Soest method (Goering & Van Soest 1970).

### ***In-vitro digestibility***

In vitro digestibility was carried out at the Ciawi Animal Research Institute, Bogor West Java. The method used in the measurement of in-vitro digestibility was two-stage technique for the in vitro digestion according to Tilly & Terry (1963) method.

## **RESULTS AND DISCUSSION**

### **Production of the whole corn plant in different seasons**

Production of the whole corn crop (fresh and dry matter productions) in both rainy and dry seasons is presented in Table 1. There were no significant differences of neither fresh production nor dry matter production between rainy and dry seasons. No effect of seasons on biomass production presumably because corns that were planted in the dry season at that time still some rain during one month of May (Table 2).

**Table 1.** The fresh and DM production of the whole corn plant in different seasons

Replication	Fresh production (kg/400m <sup>2</sup> )		Dry matter production (kg/400m <sup>2</sup> )	
	Rainy season	Dry season	Rainy season	Dry season
R-1	768	735	252	268
R-2	753	687	247	250
R-3	789	674	259	246
R-4	776	683	255	249
R-5	783	693	257	253
Average	773.8	694.4	254.0	253.1
Sd	14.0	23.7	4.6	8.6

**Table 2.** Rainfall data in the lowlands of Sei Putih, Deli Serdang, North Sumatra in 2016

Month	Rain falls (mm)	Rainy days (days)
January	84	4
February	205	11
March	70.5	7
April	12	2
May	297	11
June	174	11
July	274	7
Agust	177	10
September	218	12
October	143.5	7
November	45	5
December	202.5	11
Total	1,902.5	98
Average	158.5	8.2

*Source: Indonesian Goat Research Station (2017)*

The total fresh production of crop and weighted cob results from the research of Noviarini et al. (2017) at several fertilization treatments that harvested at 75 days after planting ranged from 4.99 to 9.73 kg/5m<sup>2</sup> or an average of 7.35 kg/5m<sup>2</sup>. Fresh productions on this study both in rainy and dry season (9.67 kg/5m<sup>2</sup> and 8.68 kg/5m<sup>2</sup>, respectively) were higher than the results reported by Noviarini et al. (2017) although harvested at younger age (50 to 60 days). On the other hand, the average dry matter production in this study was 6.35 t/ha in the rainy season and 6.33 t/ha in dry season, lower than the yield obtained in Mexico [10] with dry matter production reaching 6.8 to 10.5 t/ha at harvest age 68 to 73 days.

### **Chemical composition of the whole corn plant in different seasons**

Chemical composition of the whole corn plant at both rainy and dry seasons are presented at Table 3. Overall, the chemical composition of maize stover in the rainy season was higher than in the dry season, except for DM, NDF and ADF contents. Crude protein content of whole plant crop was almost similar to the research results reported by the following researchers: 8.6 to 9.6% (Moreno-Resendez et al. 2017), 7.1 to 9.7 (Balseca-Guzman 2018), 7.7 to 8.8% (Ferreira et al. 2014), 7.5 to 9.7% (Balseca-Guzman 2018), and 8.89% (Seleiman et al. 2017). While ash content of corn crop was lower than reported by Seleiman et al. (2017) which was 8.89%.

Chemical analysis of whole corn crop reported by Tulung et al. (2020) as follow: 10.90% crude protein; NDF 69.81%; ADF 40.20% and crude energy 3,791 kcal kg<sup>-1</sup>. Those results is relatively different from the results presented in Table 2. These differences could be due to differences in location and time of planting the maize. Tulung et al. (2020) research was conducted in North Sulawesi in 2015. The NDF and ADF content of maize forage that fertilized with organic in Mexico were 53.5 and 35.9%, respectively (Moreno-Resendez et al. 2017). These results are relatively the similar to the recent study presented in Table 3. Zamora-Villa (2016) stated that low quality forages had higher ADF (35%) and NDF (60%). Based on this statement, the whole corn crop in the current study can be classified as forage with good quality.

**Table 3.** Chemical composition of the whole corn plant in different seasons

Item	Chemical composition	
	Rainy season	Dry season
Dry matter (%)	32.83	36.45
Organic matter (%)	88.93	89.82
Ash (%)	5.87	6.21
Crude protein (%)	9.83	9.04
Crude fat (%)	2.71	1.93
Neutral detergent fiber (%)	56.89	58.63
Acid detergent fiber (%)	33.41	36.60
Crude energy (kcal kg <sup>-1</sup> )	4,312	4,261

### ***In-vitro* digestibility of the whole corn plant in different seasons**

In-vitro digestibility (dry matter and organic matter) of the whole corn plant at both rainy and dry seasons are presented at Table 4. Both the in-vitro digestibility of dry matter and organic matter in the rainy season were slightly higher than in the dry season. IVDMD of the whole corn plant on this research was lower than the results of the study that reported by Balseca-Guzman et al. (2018) and Seleiman et al. (2017) with an IVDMD value were 52.1% and 68.6%, respectively.

**Table 4.** *In-vitro* digestibility of the whole corn plant in different seasons

In-vitro digestibility	Seasons	
	Rainy season	Dry season
Dry matter (%)	51.78	49.35
Organic matter (%)	50.96	48.42

The results of research by Nasriya et al. (2016) on the use of corn stover for feed male PO calves showed an average of apparent dry matter digestibility in the range of 56.27-59.48% and when the whole corn crop was offered as single feed (100%) the digestibility value was higher than when the whole corn crop was offered in mixture king grass. Furthermore, it was also reported that the digestibility of organic matter reached 88.71% (Nasriya et al. 2016) much higher than the results of this study which were only 48.42-50.96%.

Ayasan et al. (2020) had reported that in-vitro digestibility of corn plant in Turkey, were 51.57 and 48.64% for IVDMD and IVOMD respectively. These results were almost the similar with data presented in Table 4.

## **CONCLUSION**

Biomass production, chemical composition, and in-vitro digestibility of the whole corn plant were not different in both rainy and dry season at Sei Putih, North Sumatra and potential to be used as ruminant feed.

## **AUTHOR CONTRIBUTIONS**

Sirait J and Simanihuruk K were contributed equally to this work

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**Appendix B.** The results of testing soil samples from the lowlands of Sei Putih, Deli Serdang, North Sumatra

Type of analysis	Value	Method
C-organic (%)	1.09	Spectrophotometry
N-total (%)	0.13	Kjehdal
P-Brayl (ppm)	1.57	Spectrophotometry
K-dd (me/100g)	0.15	AAS
Mg (me/100g)	0.39	AAS
pH (H <sub>2</sub> O)	4.10	Elektrometry
Texture:		
Sand (%)	25.89	Hydrometer
Dust (%)	14.80	Hydrometer
Clay (%)	59.31	Hydrometer

**Source:** AIAT North Sumatra Laboratory (2013)

## Protected Fatty Acid-Amino Acid as Energy Source for Fattening Ongole Crossbred Cattle

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### ABSTRACT

Providing feed to the ruminants often has obstacles especially energy and protein because of seasonal changes. A supplement containing high-density energy and amino acid will be useful for cattle especially when insufficient energy occurs during the dry season. This study aimed to evaluate the efficacy of a protected fatty acid-amino acid supplement for fattening cattle. A complete randomized block design was applied, with different level of protected fatty acid-amino acid addition as treatments A: *null*; B: 0.3 g/kg LW and C: 0.6 g/kg LW with 10 heads in each group. Thirty Ongole crossbred bulls were used with the initial liveweight (LW)  $\pm$  sem (478.2  $\pm$  13.5) kg. The bulls offered *ad libitum* concentrate containing 16% crude protein and 65% total digestible nutrient and elephant grass. The supplement was prepared by reacting palm fatty acid distillate with CaO in a heating process, lysine monohydrochloride was added as much as 20% of the total supplement produced. The results showed that the LWGs were A. 0.99 kg/d B. 1.00 kg/d and C. 1.09 kg/d. The body condition score gains were A: 0.54, B: 0.54; and C: 0.61. The supplement effect did not reach a maximum result although a high LWG was reached. There were no differences in leptin, creatinine, total cholesterol, glucose, total protein, and blood urea nitrogen concentrations affected by the supplement. Besides, there were no differences in rumen characteristics affected by the supplement. To conclude, all groups reached high LWG although the efficacy of the supplement was not optimum.

**Keywords:** Fatty acid, Lysin, Energy, Fattening, Ongole

### INTRODUCTION

Feedstuffs containing energy such as corn and wheat pollard often cannot meet the energy requirement for ruminants due to quality and quantity issues. Nutrients containing a high density of energy such as fat and free fatty acid can be used as energy when they are metabolized in the body (Wina & Susana 2013). More importantly, fat provides double energy content in comparison with carbohydrates (NRC 2001). However, feed containing >5% of fat may be toxic for rumen microbes that cause metabolism imbalance such as in fiber

digestibility (Wina & Susana 2013). Moreover, fat covers the fiber in the rumen and is harder for rumen microbes to digest the fiber (Palmquist & Jenkins 2017). An effort was done to avoid metabolism issues of feeding fat to the ruminants with bypass calcium salts of fatty acid (Jenkins & Palmquist 1984). Therefore, the bypass fatty acid may reduce the negative effect of fat in the digestibility of carbohydrates and the rumen microbes, and more importantly, it can suppress the fat hydrogenation in the rumen.

Calcium salts of fatty acid were used in the feed of ruminants. For example, dairy cattle offered calcium salts of fatty acid at lactation period showed improvement in milk yield and fat content and the reproductive performances (de Souza et al. 2017; Jolazadeh et al. 2018) and it was reported that the use of calcium salts of fatty acid also increases the daily gain of ewes, improving palmitic and oleic acid content of the milk (Wina & Tangenjaya 1996). Calcium salts of fatty acid were also used in fattening cattle, that increased DM intake, liveweight gain (LWG), feed efficiency and fatty acid intake, and hot carcass weight of Nellore bulls (Nascimento et al. 2020).

Improving meat quality is very important as it affects human health. This is because the meat that we eat contains saturated fatty acid causing cardiovascular problems and other degenerative diseases (Nestel et al. 1973). The meat quality can be measured physically (Honikel 1998), chemically (Tansawat et al. 2013) as well as the concentration of lipid in the plasma such as cholesterol and triglyceride (O'Kelly 1968) to predict the meat quality that is very important for customer satisfaction and health.

The protected fatty acid-amino acid supplement was prepared by reacting palm fatty acid distillate with CaO in a heating process, lysine monohydrochloride was added as much as 20% of the total supplement produced. This supplement can be used as a source of energy and is expected to improve the productive performance of fattening Ongole crossbred cattle. Amino acid inadequacy limits the growth of animals. The two first limiting amino acids are lysine and methionine (Silva et al. 2021). A high fat content might be toxic to rumen microorganism, the protection of fat was done using CaO to prevent the negative effect of fat. This study aimed to test the efficacy of protected fatty acid-amino acid supplement for fattening cattle.

## **MATERIALS AND METHODS**

The following experiment was conducted under the guidelines of the Indonesian Code of Practice for the Care and Use of Animals for Scientific Purposes and was approved by the Indonesian Ministry of Agriculture Animal Ethics Committee (Balitbangtan/Lolitsapi/Rm/08/2020).

## Experimental design

The experiment consisted of a 14-day pre-experimental period followed by a 112-day experimental period. The experiment commenced in early Mei 2020 and was completed in mid of August 2020. Before the start of the experiment, 30 Ongole crossbred bulls of approximately 400 kg LW were selected from the herd of the population in Indonesian Beef Cattle Research Station (BCRS) at Grati, East Java, Indonesia, and were weighed and measured (LW and BCS), and treated with anthelmintic before the experiment was started.

A 2-week pre-experimental period consisted of two-week feeding in individual pens, with 1 bull/pen. Bulls were fed fresh elephant grass (*Pennisetum purpureum*; 893 g OM, 70 g CP, 712 g NDF /kg DM) with unrestricted access to fresh drinking water throughout the pre-experimental period.

The experimental design was a randomized complete design with three different supplement treatments, with ten (10) bulls in each treatment group. The supplement treatments were provided in Table 1.

All bulls were fed elephant grass (EG) and concentrate diet (CONS) diet at 3% LW on a DM basis, with the ratio of EG: CONS diet was 30: 70. Prior to the commencement of the experiment, the 30 bulls ( $478 \pm 70$  kg LW, mean  $\pm$  SD) were ranked and blocked on LW. Bulls were allotted to individual pens with one bull in each pen. Within each block, bulls were randomly allocated to each of the supplement treatments Table 1.

The supplement was prepared by reacting palm fatty acid distillate with CaO (Strohmaier et al. 2003) and lysine monohydrochloride was added (20%) and mixed thoroughly until yellowish granules were formed. The supplement then dried and grounded for easier handling.

**Table 1.** Experimental design

Treatments	Number of bulls	Feeding
A	10	Elephant grass + Concentrate diet (Control)
B	10	Control + supplement at 0.3 g/kg liveweight
C	10	Control + supplement at 0.6 g/kg liveweight

**Table 2.** Feed composition and nutrient content of concentrate diet and elephant grass

Ingredients	%	
Wheat pollard	30	
Cassava powder	20	
Kopra meal	20	
DDGS	15	
Soya bean meal	13	
Mineral mix	1	
Salt	1	
Nutritional content (% dry matter)		
	Concentrate diet	Elephant grass
Crude protein	16.0	9.7
Crude fat	4.0	1.8
Crude fiber	16.0	30.7
Ash	7.4	15.0
Total digestible nutrient	70.0	48.8

## Measurements

### *Feeding and feed intake*

The feed was offered at 07.00 AM each morning after refusals from the previous day were collected and weighed. Feed intake was measured every day during the experimental period by subtracting the amount of feed refused from the weight of feed offered. Sub-samples of feed offered were collected each day, bulked for a week mixed thoroughly, and duplicate sub-samples were collected for proximate analysis (DM, CP, organic matter (OM), crude fiber (CF), and total digestible nutrient (TDN) (AOAC 2005).

### *Liveweight gain, body condition score and blood collection*

Liveweight was measured before feeding every fortnight. Body condition score (BCS) was assessed by the same person at the start and at the end of the experimental period in a 1 to 5 score system (score 1 was emaciated and score 5 was obese) (Wildman et al. 1982; Edmonson et al. 1989) which is based on visual assessments of body condition at specific points in the body of the cow, ranging through a scale of 1 to 5, regardless of the bull weight or frame size.

Blood samples were collected before feeding at the end of the study for the leptin, creatinine, and blood metabolite analyses.

### ***Rumen characteristics***

The concentration of NH<sub>3</sub>, acetic acid, propionic acid, butyric acid, and the pH from the rumen liquor was measured on week 7 by collecting rumen fluid at 4 hours after feeding.

### ***Blood metabolites and hormones***

Blood metabolites (the concentrations of plasma glucose, total cholesterol, LDL and HDL) were analysed in Sejahtera laboratory in Pasuruan East Java. The blood hormones (leptin and creatinine) were analysed in Faculty of Medicine, The University of Brawijaya, Malang, East Java using Bovine leptin and creatinine ELISA Kits (BT Lab, Bioassay Technology Laboratory, Zhejiang, China).

### ***Statistical analysis***

The SPSS software program (SPSS Statistic, IBM, New York) version 23 with the General Linear Model was used to analyse animal measurement data. The data presented in the bar chart were least-square means. The 5% level significance was used to consider the difference between means.

## **RESULTS AND DISCUSSION**

### **Dry matter intake, dry matter digestibility, liveweight gain, and body condition score**

Supplement treatments did not affect DM intake. This is because the bulls ate the same concentrate diet and EG at the same ratio. The inclusion of supplement in the diet could not increase DM intake. This also occurred in DM digestibility. A study in dairy cattle fed isoenergetic and isonitrogenous diet and was supplemented with protein and fat did not increase DM intake (Nichols et al. 2019). Therefore, the main factor contributing to DM intake is the basal diet fed to the cattle. Dry matter digestibility did not differ between treatments. This was probably because of the same basal diet that they ate. The DMDs were high and indicated an efficient diet that was digested efficiently.

**Table 3.** Dry matter (DM) intake, DM digestibility, liveweight gain, and body condition score (BCS) gain of bulls offered a protected fatty acid-amino acid supplement at *null* 0 g/kg; B 0.3 g/kg and C: 0.6 g/kg liveweight

Parameters	A	B	C	sem	P
DM intake (% LW)	3.03	3.09	3.03	0.04	0.77
DM digestibility (%)	74.45	76.40	75.65	0.46	0.23
Liveweight gain (kg/d)	0.99	1.00	1.09	0.05	0.75
BCS gain	0.54	0.53	0.61	0.76	0.12

A = Elephant grass + Concentrate diet (Control); B = Control + supplement at 0.3 g/kg liveweight; C = Control + supplement at 0.6 g/kg liveweight

Although all bulls reached a high LWG (Table 3) the supplement did not affect the LWG. This was in contrast to a study in Nellore bulls fed the same kind of supplement that could increase the LWG (Fiorentini et al. 2014) and other carcass traits (Nascimento et al. 2020; Silva et al. 2020; Andrade et al. 2014). The difference probably because all bulls ate the same amount of concentrate and grass, the inclusion of a small amount of supplement could not increase the LWG, the diet fed to the bulls had already contained high-quality fractions such as crude protein and total digestible nutrients (Table 2). Similarly, the increase of BCS did not show a significant difference between treatments, although bulls in the C group tended to have a high increase of BCS. The results in bull performance were not affected by the supplement treatment, the supplement would probably affect a better bull performance in the low-quality diet. It is suspected that there was an optimum growth limit where the bulls could not make any improvement because of their growth capacity in the high-quality diet offered. In a low input system, the high energy density probably will improve nutritional content hence improve the productive performance.

### Endocrine and metabolite changes during the experimental period

Table 4 showed the plasma metabolite profiles of glucose, total protein, and urea concentrations, as well as cholesterol, creatinine, and leptin concentrations.

The protected fatty acid-amino acid supplement did not affect plasma glucose, total protein, and urea concentrations. Although, all bulls were in a good condition with the concentration of those blood metabolites were in normal ranges (45-100 mg/dl for glucose; 5.7-8.1 g/dl (Radostits et al. 2006) for total protein, 8-24 mg/dl for blood urea nitrogen and <150 mg/dl for cholesterol). A study in fattening bulls fed the whole corn or grounded corn

resulted in high glucose concentration 89-91 mg/dl (Freitas et al. 2019). Thus, feeding a high-energy diet resulted in a high blood glucose concentration. Unlike in non-ruminants, feeding forages to the ruminants, the absorption of glucose is very low in ruminants although glucose is needed for maintenance, growth, and production such as in lactating period. The requirement of glucose in ruminants is met from glucose synthesis in the liver. It uses glucose precursor that is absorbed following fermentation and feed digestion (Reynolds 2005). Therefore, glucose metabolism in ruminants producing meat represents the balance between glucose requirement for muscle production and the supply of carbon glucose that is integrated by the liver.

All bulls had a normal range of total protein. Our result was comparable to another study with Holstein bulls offered a different level of protein, the increase of protein level did not result in a significant difference in total protein concentration (Xia et al. 2018). However, the concentrations of total protein in our bulls were slightly lower in comparison with other studies in fattening cattle kept in a pasture that had total protein concentrations 6.79-6.97 g/dl (Doornenbal et al. 1988) and 7.56 g/dl (Mitruka & Rawnsley 1981). The difference was probably because of the feeding system between the two studies, we fed the bulls with concentrate diet that had high protein content and the percentage of concentrate diet that was high (70%) vs grass (30%). The concentration of total protein in the plasma indicated two fractions, such as albumin that is produced in the liver and is important for muscle growth and globulin that comes from a variety of protein components such as alpha, beta, and gamma types produced in the liver and immune system. Some globulins bond with hemoglobin, and others had transport function such as in zinc and a function in an immune system.

The concentration of leptin and creatinine were not affected by the supplement treatment. All bulls had high leptin concentrations (>15 ng/ml) (Table 4). Our results were comparable with a study in *Bos indicus* crossbred steers fed high energy and protein diet (CP 16% and ME 11.6 MJ/kg LW), the leptin concentration was 11-14 ng/ml (Antari 2018). Leptin is an adipocytokine that is anti-satiety and a hormone that was produced from adipose tissue (Münzberg et al. 2014). This hormone is very important in providing information about energy depot in the peripheral tissue to the brain (Frederich et al. 1995), is also associated with appetite, anti-obesity, reproduction, and bone mass (Karsenty 2006). Leptin concentration decreased concurrently with the decline of liveweight during feed restriction, although the concentration increased 12 h after refeeding, and feed intake did not affect leptin concentration (Weigle et al. 1997). Thus, leptin concentration is more likely affected by the adipose tissue mass than feed intake and energy source in the diet.

**Table 4.** Hormonal and metabolite profiles of bulls offered protected fatty acid-amino acid supplement at null 0 g/kg; B 0.3 g/kg and C: 0.6 g/kg liveweight

Parameters	A	B	C	sem	P
Glucose (mg/dl)	93.9	92.5	97.4	1.89	0.57
Total protein (g/dl)	7.9	8.0	8.1	0.03	0.29
Urea (mg/dl)	20.9	19.5	19.9	0.31	0.16
Cholesterol (mg/dl)	112.4	113.5	106.9	2.03	0.38
Leptin (ng/ml)	21.4	19.6	23.9	1.74	0.6
Creatinine (µmol/ml)	208.4	243.0	301.0	29.9	0.4

A = Elephant grass + Concentrate diet (Control); B = Control + supplement at 0.3 g/kg liveweight; C = Control + supplement at 0.6 g/kg liveweight

The concentration of creatinine was associated with muscle metabolism, the concentration of creatinine on our bulls was not different between treatments. This was because the LWG was not different between treatment as well as the BCS. This was in contrast to a study in steers and European cattle (Fries Holstein, Brown Swiss, and Simmental) suffering from ketosis (Issi et al. 2016) having a significant increase in creatinine concentration. Moreover, a study reported that the concentration of creatinine increased linearly with liveweight increase so that the relationship between creatinine concentration and liveweight followed the same relationship between muscle and protein concentration and liveweight (de Lana Ferreira et al. 2020). Creatinine is the end product of muscle metabolism and is excreted through the kidney. Therefore, there was not probably a relationship between liveweight and creatinine concentration in Ongole crossbred bulls fed the supplement.

### Rumen characteristics

The supplement treatment did not result in a significant difference in NH<sub>3</sub>, acetic, propionic, and butyric acids and pH (Table 5).

**Table 5.** The concentration of NH<sub>3</sub> and volatile fatty acid in the rumen liquor

Parameters	A	B	C	sem	P
NH <sub>3</sub> (mg/100 ml)	17.5	15.5	17.5	0.19	0.90
Acetic acid (mmol)	119.2	115.0	135.3	0.52	12.70
Propionic acid (mmol)	40.5	39.0	47.0	0.50	4.80
Butyric acid (mmol)	34.3	32.7	38.0	0.71	45.00
pH	6.5	6.6	6.6	0.03	0.06

Treatment of protected fatty acid-amino acid: A = 0 g/kg; B = 0.3 g/kg; and C = 0.6 g/kg liveweight

The bulls consumed a diet containing high energy and protein. The carbohydrate kinetic degradation should have the same pace as protein degradation affecting microbial protein synthesis (Widyobroto et al. 1998). Thus, a high concentration of  $\text{NH}_3$  in the rumen liquor is needed for the digestibility process. The acetic acid and propionic acid ratio were  $>2.3$  that was slightly higher than the previous study in Madura and Ongole crossbred cattle kept in an intensive management system, about 1.85 and 1.69 respectively (Umar et al. 2011). The increased percentage of concentrate diet improved non-structural carbohydrate digestibility and decreased fiber digestibility so it lowered the ratio between acetic and propionic acid (Walsh et al. 2009). Although the pH level was not different between treatments, the bulls have a normal range of pH (Purbowati et al. 2014).

## CONCLUSION

Treatment with protected fatty acid-amino acid supplement was not able to improve the productive performance of Ongole crossbred cattle because of probably the maximum growth limit in this study, although high LWG has been achieved. The supplement probably provides a significant effect when it is used in a low-quality diet.

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## AUTHOR CONTRIBUTIONS

Risa Antari: as a senior author, working on literature collection, data collection, and analyses, writing the original manuscripts.  
Mariyono, YN Anggraeny, NH Krishna, AS Putri, Aryogi, and E Wina contributed equally in reviewing and editing the manuscript.

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## **Amino Acid Profile and Proximate Composition of Black Soldier Fly Larvae (*Hermetia illucens*) with Two Drying Methods**

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### **ABSTRACT**

One of the determinants of animal feed quality is expressed in terms of crude protein content and essential amino acids profile based on dry feed ingredients. Black Soldier Fly Larvae (BSFL) is an alternative feed protein source that cannot be stored in the fresh form for a long time without drying. Improper drying can reduce the protein and amino acid content of the feed. This study aimed to find the best method for drying BSFL. Two methods of BSFL drying (stove oven and microwave drying) were evaluated. Fifteen days old larvae of BSF were dried either using the stove oven drying method for 75 minutes or using 800 Watts microwave drying for 25 minutes. The analysis of the nutrient composition of the samples was carried out by proximate and amino acids analysis. The results showed that the protein content of BSF larvae, were not significantly different between drying treatments, while the amino acid content was higher in the microwave drying than the stove oven drying method. This study concludes that the two drying methods can be used in preserving BSF larvae as a source of dietary protein for farm animals.

**Keywords:** Black soldier fly larvae, Drying, Amino acid, Nutrient

### **INTRODUCTION**

The availability of quality animal feed is one of factors determines the success of the livestock business, in addition, feed is the largest maintenance cost factor, which is 50-70% (Katayene et al. 2014). Sources of protein in animal feed play an important role in forming body tissues and vital metabolism such as enzymes, hormones, antibodies, and so on (Beski et al. 2015). Raw materials for protein sources currently used in the feed industry in Indonesia include fish meal, soybean meal, and meat bone meal, which are relatively expensive compared to other feed ingredients (Wu et al. 2014).

The use of insects as a source of protein has been extensively studied and discussed around the world. Protein from insects is known to be more economical, environmentally friendly, easy for mass production, and has high

feed conversion efficiency (Van Huis 2013). They're also part of the natural feed for poultry (Makkar et al. 2014). Insect cultivation can reduce organic waste that has potential environmental pollution. In addition, Indonesia's tropical climate conditions are ideal for BSF cultivation (Li et al. 2011). Another beneficial factor is that insect-based protein sources do not compete with humans' consumption, so they are very suitable for use as animal feed ingredients, particularly for poultry and fish (Veldkamp et al. 2012). As a source of feed raw materials, insect-based products must also be safe from chemical contaminants.

Insects are animals that can be used as feed and have a high protein content (Jayanegara et al. 2017) with a good and balanced amino acid profile (Sánchez-Muros et al. 2014). The use of insects as a source of feed is one solution that has the potential to overcome the limited supply of feed, especially as a source of protein. One of the insect species that has the potential to be used as feed is black soldier fly larvae (BSF, *Hermetia illucens*) due to its easy production system, fast growth rate and protein content of around 40% (Liland et al. 2017). The body of BSF also has good microbes that can fight pathogenic microbes so that the use of BSF feed is safe from pathogenic microbes that can harm livestock (Van Huis 2013).

The protein content in larvae of BSF is used as animal feed. Larvae are living materials that, if not treated, will continue to grow into adult flies. In addition, if stored in a dead condition, the larval product will rot because it has a high-water content. Drying is an easy and inexpensive method to extend the shelf life of the product. Drying is the process of hydrating or removing water from a material (Hasibuan 2005). The selection of the drying method and the appropriate drying time will facilitate the process of chemical analysis, storage, and preservation of feed. The purpose of drying is to increase durability, reduce packaging costs, reduce transport weight, improve the taste of the ingredients, and maintain the nutritional content of the ingredients (Achanta & Okos 2000). Therefore, BSF larvae need proper processing strategies to maintain nutrient content in ingredients, to be able to extend a longer shelf life and, make it easier to be used in formulations of feed ration. The purpose of this study was to determine the nutrient content based on the results of proximate and amino acid testing of 15-day-old BSF larvae based on two different drying methods.

## MATERIALS AND METHODS

### Drying method

BSF larvae 15-day-old about 300 grams were dried using two methods: (a) Was heated using a stove oven for 75 minutes; and (b) Dehydration by heating method using an 800 watt microwave for 25 minutes.

## Proximate analysis and amino acid analysis

Chemical Properties Dried BSF larvae were analyzed in duplicate for moisture content, crude protein, crude fat, crude fiber, and ash. Moisture, ash, crude protein content, were determined as describe in AOAC methods (AOAC, 2005). The moisture content was determined by drying in the oven at  $105^{\circ}\text{C} \pm 5^{\circ}\text{C}$  for 12 hours and ash content was estimated by burning of BSFL at  $550^{\circ}\text{C}$  for 6 hours in muffle furnace. The nitrogen (N) was analyzed by the Kjedahl method, and the value was multiplied by protein conversion factor of 6.25. Fat content was analyzed by Soxhlet method and crude fiber content was analyzed by Eter Extract. Measurement of amino acids using High Performance Liquid Chromatography (HPLC). The wavelength of the detector was 254 nm. Standards of the different amino acids were supplied by Sigma Chemical Co. All samples and standards were injected into the column at least in duplicate.

## Statistical analysis

Data were analyzed using MS. Excel 365. The effect of different drying method was tested on BSFL proximate composition and amino acid profile presented descriptively.

## RESULTS AND DISCUSSION

Black Soldier Fly (BSF) (*Hermetia illucens*) is one of the insects whose characteristics and nutritional content are being studied a lot. This fly originated in America and subsequently spread to subtropical and tropical regions of the world (Cicková et al. 2015). Antibacterial studies in Korea showed that methanol extract from BSF larvae had antibiotic properties against gram-negative bacteria, but was not effective against gram-positive bacteria (Choi et al. 2012; Lalander et al. 2013). BSF larvae were also reported to be able to reduce virus survival (Lalander et al. 2014).

Black soldier fly larvae are a high value food source, rich in protein and fat. They contain about 40-53% crude protein (CP) (Surendra et al. 2020). The amount of fat varies widely and depends on the type of diet: reported values are 25-36% for larvae fed on poultry manure, 36% on pig manure, 35% in cow dung (Wang et al. 2020), 35-37% in food waste (Salomone et al. 2017) and 26–31% in vegetable and fruit waste (Gianetto et al. 2015). They tend to contain less CP and more lipids than house fly maggots (*Musca domestica*). The ash content is relatively high but varies, from 11-28% dry matter (DM). Larvae are rich in Ca (1-30% DM) and P (4-11% DM) (Sprangher et al. 2017; Liland et al. 2017). The

DM content of fresh larvae is quite high, in the range of 35-45%, which makes it easier and cheaper to dehydrate than other fresh by-products. The fatty acid composition of larvae depends on the fatty acid composition of the diet. The lipid acid value contained 21% lauric acid, 16% palmitic acid, 32% oleic acid and 0.2% omega-3 fatty acids for larvae fed cow dung (Gold et al. 2020).

BSFL in the present study had a body size of 18-25 mm with a brownish yellow color and chewy texture. According to Wardhana (2016), newly hatched larvae from eggs are approximately 2 mm in size, then grow to 5 mm. After molting, the larvae develop and grow larger with a body length of 20-25 mm. Results from drying showed that the stove-oven method produced dried BSFL with a darker color and firm texture, while the microwave method showed the dried BSFL with a golden yellow color and crispy texture (Figure 1.). The weight loss of larvae during the drying period reached 63% (Purnamasari et al. 2019). Blackening of the dried BSFL in oven methode is due to the formation of iron-polyphenol complexes (Janssen et al. 2019) and due to the maillard reaction (Ruhnke et al. 2018 because the dried temperature more than 90°C for 75 minutes.

**Table 1.** The proximate composition of BSFL with two drying method

Nutritional content (DM)	Oven method (%)	Microwave method (%)	Methods
Dry matter	93.55	95.62	SNI 01-2891 - 1992, point 5 .1
Ash	9.36	8.61	SNI 01-2891-1992, 6.1
Crude protein (CP)	37.77	37.71	18-8-31/MU/SMM - SIG, Kjeltec
Crude fat (CF)	25.62	36.00	18-8-5/MU/SMM - SIG, Weilbull

Foodstuffs produced from agricultural products generally contain water content. The water content if not removed can affect the physical condition of the food. Some fresh feed ingredients contain 70% or more water. Food and feed contain two types of water, namely free water and bound water. Free water is water that is easily removed through evaporation, while bound water is water that is difficult to remove even by drying (Winarno 2002). The proximate test of BSFL using two different drying methods, it shows microwave method have different nutrient content in dry matter, ash, and crude fat.



**Figure 1.** Fresh and dried black soldier fly larvae

CP results from microwave and oven drying ranged from 37.71-37.77 which was slightly lower than the research by Spranghers et al. (2017) which was in the range of 39 to 43% of BSFL maintained in various organic waste media. Nguyen et al. (2015) also reported CP content of BSFL reared on fruit and vegetable waste was 39%. Purnamasari et al (2019) showed that BSFL can be dried in a temperature range of 55-75°C for 18-24 hours with protein content ranges from 41.99 to 51.49%. The CP in the preent study was 37.77% for oven method drying and 37.71% for microwave method drying of BSFL, this CP content was lower than the previous study. According to Shumo et al. (2019) the nutrient composition of BSFL fed on kitchen waste were DM 86.7-88.7%, CP 33-34%; ash 8-11,2% and CF 33.9-34.7%. This nutrient content was lower than other protein source such as soybean meal, CP 43-46%, ash 5-6.5% and CF 5-8% (Sheikhhasan et al. 2020) and fish meal, CP 48-65%, ash 7-15.5% and CF 1.8-8% (Xiokang et al. 2019).

The total nitrogen content in insects in general and BSF, in particular, contains nitrogen derived from protein and non-protein sources such as chitin.

Therefore, non-protein separation from protein nitrogen is necessary to obtain accurate crude protein content and to avoid estimation of values previously reported using standard conversion factors (Janssen et al. 2017; Caligiani 2018). The previous study reported that maintenance substrate affected on CF content of BSF. Nguyen et al. 2015 reported higher CF content for larvae reared on fish and liver compared to chicken feed. However, in our study CF was affected by drying method on the CF content of BSFL. Crude fat was higher in the microwave method because in this method did not extracted more oil from larva's body, while in the oven method, a lot of oil came out.

The chemical test of feed is one of the tests carried out in addition to the physical test. Measuring the quality of a feed ingredient should not only be up to macronutrient limits, but also micronutrients, one of which is amino acids (Table 2).

**Table 2.** The amino acids profile of BSFL with two drying methods

Amino acids	Oven method (mg/kg)	Microwave method (mg/kg)
L-Methionin	910.62	2822.81
L-Threonin	3230.69	12777.77
L-Lisin	4463.30	16983.76
L-Arginin	2856.22	18348.27
L-Fenilalanin	2605.15	14653.63
L-Triptofan	4335.19	3767.29
L-Isoleusin	3267.60	12932.81
L-Histidin	1653.49	10571.85
L-Leusin	5035.95	20084.95
L-Valin	4493.37	19060.71

Amino acids are essential to produce quality livestock, especially their ability to break down other proteins and to produce energy (Henchion et al. 2017). Even though the amino acid profile of soybean meal generally has better quality than that of other plant-based feeds, it still lacks lysine, methionine, threonine, and valine when compared to animal protein sources (Henchion et al. 2017). Previous studies determined that the amino acid profile of some edible insects including yellow mealworm, common house fly, and BSF was comparable to that of soybean meal with methionine or methionine and cysteine and sometimes arginine which is as the most limiting essential amino acid for pig and broiler farming (Veldkamp & Bosch 2015). BSFL are also a rich source of bioavailable arginine (Belghit et al. 2019), which is often a limiting amino acid in plant-based protein. BSFL dried by the microwave method has

a higher amino acid content than the oven method. However, this figure still has a higher quality amino acid profile than the 2004 FAO standard amino acid profile reported for soybean and sunflower. The BSFL methionine level in our study even surpassed that of fish meal reported by FAO 2004 and the correlation was responded to within the recommended range for broilers according to the 1994 National Research Council (NRC) standard. The amino acid profile in BSF biomass is not greatly influenced by the amino acid profile of the substrate (Spranghers et al. 2017). Microwave drying also produce the higher digestible protein and amino acid profile than conventional drying at temperature 60°C). Thus, drying BSFL by the oven and microwave method resulted in amino acid profiles in a source of high-quality protein that in this respect is almost equivalent to fish meal and surpasses many vegetables protein sources in animal feed.

## **CONCLUSION**

Black soldier fly larvae are a high value feed source, rich in protein, fat and essential amino acids. Both drying methods can support the shelf life of the BSFL and can be used in processing BSF larvae into raw material for feed protein source without reducing its nutrient content.

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## **AUTHOR CONTRIBUTIONS**

Listya Purnamasari, Nurul Pratiwi, Wildan Muhlisson, and Irwanto Sucipto conceived and designed the experiments. Listya Purnamasari, Desy Cahya Widyaningrum, and Melinda Erdy Krismaputri contributed reagents, materials, analysis tools, and performed the experiments. Nurul Pratiwi analyzed the data. Listya Purnamasari drafted the manuscript. All authors read and approved the final version of the manuscript.

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## The Effect of Tannin Extracted from Sorghum Seed to Rumen Fermentation Characteristics and Methane Production

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### ABSTRACT

The suppression of methane gas production is often directly related to the decrease in dry matter consumption or digestibility. This phenomenon is closely related to the process of formation of methane as a result of degradation of fibers that generate  $H_2$  in the rumen, thereby utilized by methanogenic bacteria to be converted into  $CH_4$ . Efforts made in the suppression of methane gas should not be followed by a decrease but an increase or maintain the quality of digestibility of feed ingredients. The current study was aimed to study the effect of supplementation sorghum grain and tannin extracted from sorghum grain on dry matter digestibility, total gas and methane production in the cattle diet evaluated using in vitro technique. In vitro experiment with 3 diet treatments and 4 replications were applied on this study. The treatments were P0 (Napier grass), P1 (Napier grass + 0.15% sorghum seed flour), P2 (Napier grass + 0.15% tannin extracted from sorghum seed flour). Observed parameters were dry matter digestibility,  $N-NH_3$  concentration, VFA total, gas production and methane production. Supplementation of sorghum grain and tannin extracted from sorghum can decrease the cumulative of methane emission by 24.86% and enhanced the quality of dry matter digestibility.

**Keywords:** Greenhouse gas, Methane mitigation, Tannin, Sorghum

### INTRODUCTION

The most important issue and attracts the world's attention today is global warming, which generated from increasing concentration of greenhouse gases in the atmosphere. Ruminants contributed to global warming through the emission  $CO_2$  and methane ( $CH_4$ ) resulting from the process feed fermentation processes in the rumen. The production of methane from ruminant livestock contributes to 95% of the total methane emissions produced by livestock and humans, and about 18% of the total greenhouse gases in the atmosphere (FAO Report 2006). The methane produced from anaerobic fermentation of structural and nonstructural carbohydrates by *methanogenic archaea* in the rumen emitted to the air through the process eructation.

Methane emissions are considered to reflect loss of energy which cannot be used for production (Jayanegara et al. 2009a). About 6-10% of the ruminants' gross feed is lost as methane. The suppression of methane gas production is often directly proportional to the decrease in consumption of dry matter or dry matter digestibility. This phenomenon is closely related to the process of formation of methane as a result of degradation of fibers that generate  $H_2$  in the rumen, thereby utilized by methanogenic microbes to be converted into  $CH_4$ . Efforts to mitigate methane should not be followed by a decrease but an increase or maintain the quality of digestibility of feed ingredients.

One of the efforts that have been made to suppress the production of methane is the addition of an organic acid compounds such as tannins. Tannin is a polyphenolic compound present in plants and is highly prospective in reducing the production of methane gas produced by ruminants. Several previous studies such as supplementation of forage containing tannin such as *Salix alba* and *Rhustyphina* (Jayanegara et al. 2009a), chestnut (*Castanea* sp.), *Mimosa* (*Mimosa* sp.), *Quebracho* (*Schinopsis* sp.), and sumach (*Rhustyphina*) (Jayanegara et al. 2009b), leaves of oak (*Quercuspyrenaica*) (Frutos et al. 2007), tannin extract of *Swietenia mahagony* (Juliana et al. 2014) can reduce the production of methane gas. The mechanism of inhibition of methane production in ruminants is described by Tavendale et al. (2005), which is indirectly by inhibiting the digestion of fiber that reduces the production of  $H_2$  and directly that inhibits the growth and activity of methanogenic. It also inhibits the growth of protozoa which is the main host of methanogens (Goel et al. 2008).

Sorghum is a cereal crop that is quite easy to develop in Indonesia and has a fairly high nutritional content as animal feed ingredients. Sorghum grain is one of sorghum yield besides leaves and stems. The nutritional content of sorghum grain are water 11.99-12.62%, ash 1.42-1.88%, protein 1.45-1.95%, crude fiber 7.95-8.12%, fat 1.59-2.04%, carbohydrates 75.40-76.90% and tannins 0.30-10.60% (Suarni & Firmansyah 2005).

The addition of tannins at high concentrations can cause toxic to ruminants, while at medium and low concentrations, the addition of tannins can be used a source of *bypass protein* that can be absorb in the small intestine. Mendez et al. (2017) reported that the addition of tannins up at 0.6% of dry matter basis, did not affect the consumption of dry matter. Nevertheless, Aguerre et al. (2016) reported that the addition of 0.45% tannins in dry matter basis decreased the concentration of N-ammonia. Barman & Rai (2008) reported that analysis of tannin content of Total Mixed Ration feed with different tannin compositions resulting in different condensed tannin concentrations as well, moreover, up to a level of 0.31% dry matter, tannin

may lower gas production by 7.15%. However, it is followed by reducing digestibility of dry matter by 8.96%. Based on the description, this study determined the effect of tannin extract from sorghum seed at lower concentration from previous research.

## MATERIALS AND METHODS

Tannin was extracted from sorghum grains by using evaporation method as described by Sulastrri (2009). Sorghum grains were dried into the oven at temperature 30-50°C to remove the water content. White Sorghum seeds are ground and sifted with a size of 45 mesh, then weighed 50 grams and then put into distillation flask. Into the distillation flask was added 96% ethanol solvent of 250 ml, then refluxed.

The distillation process lasts for 2-3 hours with temperature outside the distillation flask ranges at 75-85°C. After the solvent distilled, tannins that have been formed are washed with hexane and then evaporated or dried for qualitative and determination of tannins concentration as previously describe by Sulastrri (2009). The tannin extract and the napier grass (*Pennisetum purpureum*) were used as substrate for in vitro experiment.

The *in vitro* experiment was done as previously describe by Abrar et al. (2015). Rumen fluid taken from rumen cattle in Slaughterhouse wer filtered through four layers of gauze, then diluted (1:2) with a buffer solution *McDougall* while bubbled with CO<sub>2</sub> gas and kept *shakerwaterbath* with a temperature at 39°C for 10 minutes. Diluted rumen fluid was used as an inoculum. After Incubation for 24 hours at a temperature of 39°C. The samples are then transferred into the 50 mL tubes then centrifuged at 2500 rpm for 10 minutes to separate the substrate and the supernatant.

The supernatant was used to determine the content of N-NH<sub>3</sub> and the concentration of total VFA. While the substrate is used to determine the dry matter digestibility. The residue was dried in the oven with a temperature of 70°C for 72 hours. Loss of mass after the incubation is calculated as a percentage of dry matter *in vitro* digestibility.

Measurement of N-NH<sub>3</sub> concentrations was determined by micro-diffusion Conway technique. A total of 1 mL of the supernatant was placed on one side of the cup of Conway and the other side is placed 1 mL of Na<sub>2</sub>CO<sub>3</sub>. Conway's cup position is placed in such a way that the two liquids do not mix before the cup is closed. In the center of the Conway plate filled with 1 mL of indicator boric acid solution. The cup is sealed with vaseline. The supernatant and a solution of Na<sub>2</sub>CO<sub>3</sub> are mixed by shaking the cup. Ammonium released from the reaction of both materials will be captured by boric acid and indicated by color change in boric acid solution. After 24 hours, ammonium

borate titrated with  $\text{H}_2\text{SO}_4$  0.005 N solution until the color changes back into the original color of boric acid.

Measurement of the total production of VFA (Volatile Fatty Acid) is done with *steam distillation* methods. The five mL supernatant were put into a distillation tube. Add 5 mL of  $\text{H}_2\text{SO}_4$  15% then closed with a rubber cap and connect with cooling flask. Insert the distillation tube into the distillation flask containing boiling water. Hot water vapor will push the VFA and will condense in the cooling flask. The formed liquid is then accommodated in an erlenmeyer containing 5 mL of NaOH 0.5 N up to volume 250 mL. Then added PP 2-3 drops, then titrated with HCl 0.5 N until the color change from pink to clear.

Gas production were observed at 0, 4, 8, 12, 16, 20 and 24 hours of incubation by inserting the needle of 50 mL glass syringe. Volume of gas produced were determined by the scale of piston moved by the pressure of gas from in vitro fermentation. Samples of methane ( $\text{CH}_4$ ) was taken using the 1 mL syringe glass. The gas was transferred into a vacuum tube for determination of methane gas concentration. Methane gas concentration were analyzed by using Gas Chromatography technique as described by Santoso & Harianto (2007)

The study was conducted in completely randomized design with 3 treatments and 4 replications. The treatments were described as follows P0 = Napier grass (control); P1 = Napier grass + 0.15% Sorghum Seed Flour; P2 = napier grass + 0.15% tannin extract from Sorghum Seed Flour. Each experiment was performed in 4 weeks consecutively. Data were analysed of variance for CRD, significant different between means treatment was tested using DNMRT (Duncan's Multiple Range Test) (Steel & Torrie 1991).

## RESULTS AND DISCUSSION

The effect of tannins extracted from sorghum on dry matter digestibility presented in Table 1. The results of analysis of variance indicates that the average percentage of dry matter digestibility (DMD) was not significantly different ( $P>0.05$ ) among treatments.

The low level of tannins supplementation may cause the adaptation of rumen microbes with the presence of the tannins. Gonzalez et al. (1990) states that ruminants is able to tolerate polyphenolic substances (such as tannins) at a low level. Moreover, the effect of bioactive compounds is also influenced by the composition of the ration (Cieslak et al. 2016). Differences in the composition of rations in this study is the addition of tannins both with and without the extraction. The basic components (such as crude protein, crude fiber) can interact with phytochemicals (such as tannins) and causes its availability is

**Table 1.** Dry matter digestibility, volatile fatty acid, and N-ammonia of Napier grass with supplementation of tannin

Parameters	Treatment		
	P0	P1	P2
Dry matter digestibility (%)	47.44±0.83	50.22±1.63	54.51±1.98
Volatile fatty acid (mM)	142±0.35 <sup>a</sup>	132±0.17 <sup>a</sup>	167±0.82 <sup>b</sup>
N-NH <sub>3</sub> (mM)	4.22±0.28 <sup>b</sup>	3.96±0.91 <sup>b</sup>	2.59±0.84 <sup>a</sup>

P0 = Napier grass; P1 = Napier grass+0.15% Sorghum seed flour; P2 = Napier grass+0.15% tannin extract from sorghum seed flour; Column means with different superscript(s) differ significantly at  $P<0.05$

reduced (Cieslak et al. 2014). Reduced availability of crude fiber can improve the digestibility of a feed material.

Previous studies showed the different reports on the addition of tannin extract and plant containing tannin which can lower digestibility of the feed, although still within the normal range (Patra et al. 2006; Bhatta et al. 2009). Furthermore, Barman & Rai (2008) also reported that the addition of tannins to a level of 0.31% can reduce dry matter digestibility by 8.96%. This difference is closely related to the nature of tannins as anti-microbial agent. Anti-microbial properties of tannins are thought to have only an impact on methanogens so as not to interfere with other bacterial activity (Cieslak et al. 2014). This study proves that the addition of tannins at the right doses does not have a negative effect on digestion.

The energy availability indicator for livestock is determined by the total production of VFA during the fermentation process. Suherman et al. (2013) states that the total production of VFA in the rumen can be used as a benchmark of the efficiency of the fermentation process of feed in the rumen. Treatment of tannin addition was significantly ( $P<0.05$ ) did not interfere fermentation activity by microorganisms in the rumen (Table 1). This result was opposite to other study (Jayanegara et al. 2009b) which reported that the addition of various type of tannin reducing total VFA in rumen fermentation. This study showed that the total VFA concentration of tannin extracted from sorghum seed had a higher concentration than control treatment. Factors affecting the total production of VFA is a type of microbes, absorption and fermentability of the carbohydrates feed (Hindratiningrum et al. 2011).

N-ammonia concentration is the result of feed protein fermentation in the rumen. The effect of tannin treatment on N-ammonia can be seen in Table 1. The treatment of P2 was significantly different ( $P<0.05$ ) from the treatment

**Table 2.** Cumulative of gas production at the time incubation to 24 hours

Treatment	Mean $\pm$ SD (ml)
P0 (Napier grass)	11.00 $\pm$ 0.82 <sup>b</sup>
P1 (Napier grass + 0.15% sorghum seed flour)	10.50 $\pm$ 1.00 <sup>ab</sup>
P2 (Napier grass + 0.15% tannin extract from sorghum seed flour)	9.25 $\pm$ 0.50 <sup>a</sup>

*Column means with different superscript(s) differ significantly at  $P < 0.05$*

of P0 and P1. The treatment of P1 was not significantly different from P0 but significantly different from P2.

This decrease is caused by the tannin activity in P2 treatment. Tannins can form complex bonds with proteins thereby inhibiting protein degradation in the rumen. A decrease in protein degradation in the rumen is directly proportional to the increase in by-pass protein. Protein by pass can increase the supply of protein into the intestine or post rumen to be absorbed and utilized by the body. Makkar (2003) also explains that the addition of tannins to the ration can reduce the ingestion of digestible nutrients, resulting in decreased concentrations of N-ammonia.

Factors affecting N-Ammonia concentrations include levels of protein content and levels of protein degradability of feed ingredients, protein solubility, the source and proportion of soluble carbohydrates and proteins that are resistant to rumen microbial degradation. Gas production represent of feed fermentation process by rumen microbes. The gas formed from the fermentation process consisting of CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub> and O<sub>2</sub>.

The effect of treatment on cumulative gas production presented in Table 2. average cumulative gas production of P0 was significantly different ( $P < 0.05$ ) to P2. Treatment with the addition of tannins in both the form of sorghum seed powder (SGF) and tannin extract from sorghum seed flour indicated a decreasing gas production. It is related to the interaction of tannins with the components that contribute to the production of gases such as protein and fiber. This is also similar with the study of Silanikove et al. (2001) which states that the tannins have the ability to bind macromolecules (proteins, structural carbohydrates and starches) and lowers its availability to be digested. Inhibition of protein and carbohydrate degradation can reduce gas production

The mechanism of decreasing cumulative gas production by the addition of tannin is further described by Sugoro (2004) which states that tannins can bind proteins thereby inhibiting rumen microbial activity by inactivating enzymes, decreasing protein degradation, binding to amino acids and minerals and binding to cell walls causing a decrease in nutrient transport. This has led to inhibition of rumen microbial activity and reduced gas

production. Several previous studies have also reported that the addition of tannin from *officinalis* with concentrations of 20, 40 and 100 mg (Cieslak et al. 2016), the addition of *Acacia mearnsii* (mimosa) and *Quebracho schinopsis* (quebracho) with a concentration of 0.5, 0.75 and 1 mg / ml (Jayanegara et al. 2010), and the addition of some plants pure tannin (Jayanegara et al. 2009b) can lower gas production. The similar results also reported by Barman & Rai (2008) the addition of condensed tannins at 0.31% can reduce gas production by 7.15%.

Jouany & Morgavi (2007) reported that tannin concentrations below 50 g/kg of dry matter ration did not cause negative effects on rumen ecosystems. The use of tannins in this study either with or without extraction is still relatively low at 0.15%, although there is a decrease in gas production but the rumen ecosystem is not disturbed. The effects of bioactive compounds also depend on the composition of the ration. Differences in feed composition on treatment P1 and P2 affect the gas production of each treatment. Treatment of P1 with the addition of non extracted tannins had higher gas production than treated with extracted tannins. This due to beside addition to tannin there were also carbohydrates addition in sorghum seed. Carbohydrates, especially solubles carbohydrates was fastly changed in the form of gas and increase the total gas production as well as the blocking of tannins from methanogenesis (Cieslak et al. 2016). This also in accordance with Kurniawati (2004) which reported that the addition of carbohydrates in the diet can increase protein degradation by microbes so that it can be used for microbial growth where the increase in microbial growth is characterized by increased gas production.

In general, methane formation in the rumen occurs through the reduction of CO<sub>2</sub> by H<sub>2</sub> catalyzed by enzymes produced by rumen methanogenic *archaea*. The effect of tannin supplementation to methane concentration can be seen in Table 3. The results indicate that the average of gross methane production is not significantly different ( $P > 0.05$ ) among treatments.

The non significant effect ( $P < 0.05$ ) on methane production in this study because the use of tannin concentration was still in a low level so it has not been able to reduce methane emissions. Gonzalez et al. (1990) suggest that ruminants were able to tolerate alkaloid compounds (such as tannins) at low levels. Increased production of methane gas concentration on treatment P1 and P2 compared to the control was in contrast to some previous studies such as the addition of tannin extract on different green feed (Yogianto 2014) as well as the addition of condensed tannins (Animut et al. 2008) were able to reduce emissions of methane.

**Table 3.** Methane concentration (ppm) of Napier grass with supplementation of tannin

Treatment	Mean $\pm$ SD (ppm)
P0 (Napier grass)	66.89 $\pm$ 30.27
P1 (Napier grass + 0.15% sorghum seed flour)	59.56 $\pm$ 24.72
P2 (Napier grass + 0.15% tannin extract from sorghum seed flour)	50.67 $\pm$ 17.27

*Column means with different superscript(s) differ significantly at  $P < 0.05$*

The difference with the previous study was thought to be due to the fraction of polyphenol compounds that significantly reduced methane emissions were total phenol (tannin phenol and non-tannin phenol) and total tannins, while the tannins as in this study had not been shown to reduce methane emissions. This data is consistent with the results reported by Jayanegara et al. (2009b) and Juliana et al. (2014) that condensed tannins have not been able to reduce methane emissions. Oliveira et al. (2007) also reported that there was no effect of tannin levels from rations containing sorghum silage with low and high tannin concentration on methane production. Similar data were reported by Beauchemin et al. (2007) that rations containing tannin extract from quebracho up to 20 g/kg of dry matter have not been able to reduce methane emissions.

The results from current study shows that the addition of condensed tannin compounds is still not consistent in reducing methane emissions. The non significant effect of tannin addition to methane production in the present study could be due to the high variability of the data obtained in this study (Table 3) which standard deviation was about almost 50% from the mean values in P0. Therefore, more study need to be done to see the real affect of extracted tannin addition from sorghum.

## CONCLUSION

It can be concluded that the treatment with the addition of sorghum seed flour and tannin extract can reduce cumulative gas production and maintain the quality of digestibility but was not able to reduce methane emissions from rumen fermentation.

## AUTHOR CONTRIBUTIONS

Arfan Abrar, Langgeng Priyanto and Riswandi were contributed equally to this work

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## Performance of Three Broiler Ducks Lines with Added Two Different Types of Feed Formulation for Eight Weeks of Age

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### ABSTRACT

The process of optimal duck growth, efficient feed utilization, and low mortality are the main goals for duck breeders. The purpose of this study was to test the effectiveness of two types of ration formulas with different nutritional content (lysine and energy). As many as 240 ducks since day-old ducks were reared for 8 weeks and allocated to 6 treatments, in 4 replications, and each replication consisted of 10 ducks. The research was design as a completely randomized design (CRD) in a 3x2 factorial pattern. The first factor was 3 broiler duck lines (Pekin, E-PMp, and PMp), the second factor was 2 types of ration formulas i.e: A-Ration and B-Ration. The variables observed included: feed consumption, live body weight, weight gain, FCR, and mortality. The results showed that the average feed intake, live body weight, and FCR ducks were not significantly ( $P>0.05$ ) affected by ration formulas, but were significantly ( $P<0.05$ ) affected by strains. Pekin duck lines were better compared with E-PMp and PMp duck lines. Types of ration formulas and duck lines significantly affected ( $P<0.05$ ) body weight gain, but no interaction between rations and duck lines was detected ( $P>0.05$ ). There was no mortality observed in all treatments. It can be concluded that the effectiveness of A-Ration was relatively similar to B-Ration in producing duck performance. The Pekin duck line was better than the other two ducks, especially the average final body weight, body weight gain, and FCR during the 8 weeks of observation.

**Keywords:** Feed formula, Lysine, Energy, Broiler ducks, Performance

### INTRODUCTION

Market opportunities for duck meat and egg products are growing up in line with the increasing needs of the community. The Directorate General of PKH (2020) reported that egg consumption during 2020 in Indonesia increased by 36.9% from 2019. The average community consumption of commercial laying hens is 5.3 kg/capita/year while the consumption of broiler meat is 2.4 kg/capita/year (Aryani et al. 2018). Recently, the role of broiler ducks also seems to be increasing because in a relatively short maintenance period they

can be harvested. Furthermore, the harvest is processed into meat or carcass and can be used as consumption material or sold to earn money. The Covid-19 pandemic, which is still ongoing now, has also caused the demand for duck production in the form of eggs and meat to increase. To maintain exposure to the corona virus, middle to upper economic class people generally need food derived from animal products such as eggs and poultry meat (Ilham & Haryono 2020). This is due to public awareness that recently it is very important to consume eggs and poultry meat which contain high nutrition (REPUBLIKA 2020). In addition to maintaining a healthy body, eating eggs can increase the body's immunity, can be a shield to protect the corona virus (Ilham & Haryono 2020; Hidanah 2021). The increasing demand for duck production in the form of eggs and meat abroad is also increasing so it must be followed by an adequate amount of production (Fouad et al. 2018). For the Asian region, China is the country with the highest egg and meat production (Huang et al. 2012).

There were four types of poultry that have high potential as meat producers, namely: Pekin duck, *Chairina moscata*, mule duck, and Mallard duck (Adeola 2006). Until now, the Pekin duck is the most popular meat producer. At first, Pekin ducks came to Indonesia to be kept and then breed well until they have high adaptability and have adapted to the natural and environmental conditions in Indonesia. One of the superior characteristics of Pekin ducks is their large body size and weight, fast growth, high carcass weight, and quality. The average final body weight of Pekin ducks selected at 7 weeks of age was 3,287 g/bird and the percentage of breast meat weight was 13% (Pingel 2011). The average body weight of Pekin ducks at the age of 42 days was able to reach 3.4 kg, body weight gain was 102.6 g/day with an average carcass weight of 76.2% (Xie et al. 2014).

Besides Pekin ducks, Balitnak Ciawi-Bogor as one of the research institutes in Indonesia has also conducted research on several types of ducks that act as meat-producing ducks, namely PMp and E-PMp ducks. The PMp duck is the result of a cross between a male Pekin duck with a white female Mojosari duck and it's a new line produced by Balitnak and has been commercialized. Besides being able to function as a meat producer, PMp ducks also have a high potential for egg production. The average egg production can be reached from 67-70% per year (Purba et al. 2015). Other broiler ducks that are also researched and developed by Balitnak are E-PMp ducks. The E-PMp ducks were the result of a cross between a male *Chairina moscata* with a female PMp duck. Biologically, E-PMp ducks have (inherited) the characteristics of 3 genotypes of their parents, namely: *Chairina moscata*, Pekin ducks, and Mojosari white ducks. The superior characteristics of E-PMp ducks include: fast growth, large body size and have thick muscles or flesh,

varied feather color (a mixture of the white and black fur), high carcass weight, and pure white carcass color (Purba & Prasetyo 2014).

Information on the nutritional requirements for local broiler ducks in Indonesia already exists but information on the nutritional requirement of Pekin ducks reared in Indonesia is still limited (Purba et al. 2017). Information on the nutritional requirements for Pekin ducks, especially for the breeders at the farmer or community level, is also relatively limited (Adeola 2006; Liu & Niu 2008; Choo et al. 2014). Until now, as a reference for nutritional needs for ducks, both in research activities and in commercial duck cultivation, generally refer to the recommendations of NRC (1994) and Chen (1996). The NRC's (1994) recommendation, although it has been 27 years since its publication, is still used as a reference today. The same thing has also been described by Fouad et al. (2018). Nutritional requirements in the form of protein and energy of Pekin ducks aged 0-2 weeks were 22% and 2900 kcal/kg, respectively, while at 2-7 weeks of age they were 16% and 3000 kcal/kg, respectively (NRC 1994). The FCR of ducks as both broiler and laying types is still relatively high. The average FCR value of the ducks in the starter period is 2.69, while the finisher period ranges from 4.15 to 4.29 (Ketaren et al. 2011). The high FCR value in broiler ducks is one of the main problems considering the feed factor can reach 65-70% of the total production cost of raising ducks.

The best energy and amino acid lysine levels for body weight gain and feed conversion ratio of E-PMp broiler ducks in the starter phase were 2900 kcal/kg and 1.15%, while the finisher phase was 2700 kcal/kg and 0.80% respectively (Ketaren et al. 2011). Nutrient content in the form of protein and energy in feed plays a very important role in stimulating maximum growth and production in ducks. The protein and energy requirements of poultry are determined based on the age, type, and body size of the livestock. NRC (1994) recommended that the nutritional requirements of Pekin ducks aged 0-2 weeks are 22% (protein) and 2900 kcal/kg (energy) in feed. The nutritional requirement of Pekin ducks at the age of 2-7 weeks was 16% (protein) and energy was 3,000 kcal/kg. Other researchers provided information that the nutritional requirement in the form of the amino acid lysine for white-feathered Pekin ducks at the age of 0-3 weeks was 1.06%, while at the age of 3-7 weeks it was 1.02% (Bon et al. 2002). Xie et al. (2009) reported that to produce body weight gain and increase feed efficiency in white-feathered Pekin ducks at the age of 7-21 days, the total lysine content required was 0.84; 0.90 and 0.98%. The lysine amino acid requirement for Korean local ducks after hatching was 0.70 and 1.01% (Wikramasurya et al. 2016). When viewed from these descriptions, it was clear that the amino acid lysine requirement was closely related to age.

The treatment rations tested in this study were the amino acid content of lysine and the energy of the ration. Lysine is one of the second essential amino acids after methionine for poultry, its role is very important to stimulate growth. The amino acid requirement for lysine in Pekin ducks aged 0-2 weeks was 0.90%, while at the age of 2-7 weeks it was 0.65% (NRC 1994). Lysine deficiency in poultry rations can inhibit growth, consumption, and high feed efficiency and can reduce quality and carcass weight. In addition to the amino acid lysine requirement, the energy requirements in the diet for poultry are very important. Energy deficiency in the ration can result in decreased growth, livestock production and result in increased ration consumption so that it is not efficient in the use of rations. The amount of consumption of poultry will increase if the nutritional content in the form of protein and energy in the ratio is low (Leeson et al. 1996; Hernandez et al. 2004). From an economic point of view, any increase in energy demand in the ration can result in an increase in production costs. Therefore, the role of nutritional adequacy, especially the amino acid lysine and balanced energy in the ratio is very important.

The optimum level of lysine and energy for local broiler ducks still needs to be considered and studied to reduce feed consumption and FCR in the three duck genotypes studied. Therefore, it is necessary to do research on the nutritional requirement of broiler ducks that have been carried out by Balitnak Ciawi and tested on Pekin ducks and compare with the nutritional requirement of Pekin ducks according to the NRC recommendation (1994). This paper aims to test and evaluate the effectiveness of the two types of treatment ration formulas on the performance of Pekin ducks, E-PMp and PMp at 8 weeks of age.

## **MATERIALS AND METHODS**

The materials used in this study were three genotypes of broiler ducks, namely: Pekin, PMp, and E-PMp ducks. The research was design with a completely randomized design (CRD) with a 3x2 factorial pattern. The first factor was 3 (three) genotypes of ducks, and the second factor was 2 (two) types of ration formula. The two types of ration formulas used were: first, the ration formulation according to the recommendations of the NRC (1994) as A-Ration, and the second was the best ration formulation based on the ileal amino acid digestibility of the research conducted by Ketaren et al. (2011) which was referred to as B-Ration. The formula and nutritional content of the ratios used in this study are listed in Table 1.

**Table 1.** Ingredients, formulation and nutritional content of treatment rations

Ingredients	A-Ration (starter)	B-Ration (starter)	A-Ration (grower- finisher)	B-Ration (grower- finisher)
	kg	kg	kg	kg
Rice brand corn	18.39	22.20	29.90	35.52
Soybean meal	37.00	43.80	43.03	47.11
Fish meal	29.00	22.00	13.50	14.10
MBM	6.70	3.00	4.00	2.00
Methionine	2.00	2.00	2.00	2.00
Lysine	0.18	0.15	0.14	0.17
Premix	0.00	0.57	0.10	0.32
CPO	0.20	0.25	0.20	0.25
DCP	5.00	4.50	5.60	0.00
Soybean meal	0.74	0.59	0.74	0.74
CaCO <sub>3</sub>	0.59	0.74	0.59	0.59
Salt	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00
Nutritional content (calculation result)				
Protein (%)	22.00	19.00	16.00	16.32
Lysine (%)	0.90	1.15	0.65	0.80
Energy (kcal/kg)	2,913.00	2,917.00	3,000.00	2,705.00
Crude fiber (%)	3.93	4.32	5.17	5.55
Calcium (%)	1.33	1.04	1.11	0.98
Phosfor (%)	0.80	0.72	0.77	0.75

*A-Ration (NRC 1994); B-Ration (Ketaren et al. 2011); MBM=Meat bone meal; CPO = Crude palm oil; DCP=Dicalcium posphat*

The total lysine for A and B-Ration for the starter period was 0.90 and 1.15%, respectively. Total lysine for B-Ration was higher compared to A-Ration. The energy levels for the A and B-Ration were relatively the same at 2913 and 2917 kcal/kg, respectively. The total lysine for A and B-Ration for the grower-finisher period were 0.65 and 0.80%, respectively. The total lysine for B-Ration was higher than A-Ration, while the energy levels for the A-Ration and B-Ration were 3000 and 2703 kcal/kg, respectively. The energy level for B-Ration was lower than A-Ration.

Each treatment ratio had 4 replications, and each replication consisted of 10 ducks. The total number of ducks was 240 ducks. Ducks were reared from

0 to 8 weeks of age in accordance with standard operational duck maintenance procedures imposed by Balitnak. At the age of 0 to 4 weeks, all ducks were kept in brooder cages equipped with heating lights, feeding, and drinking places. When the ducks were 4 weeks old, the ducks were transferred to a pen-shaped cage with an acrylic floor. Places for feed and drinking water are also provided in each pen. Feed was given twice a day, in the morning and evening, while drinking water was given *ad libitum*. The starter feed was given from 0 to 4 weeks of age, while the grower-finisher feed was given after the ducks were 4 weeks of age.

Ducks were weighed every week to obtain body weight, the remaining feed was also weighed every week to obtain data of feed consumption and feed conversion ratio (FCR). The data obtained were analyzed using the General Linear Model (GLM) procedure with the help of the Statistical Analysis System program (SAS ver. 6.12 1997). If there were data that shows a significant difference, it will be continued with the Duncan test.

## RESULTS AND DISCUSSION

### Feed consumption

The average feed consumption of the three genotypes of broiler ducks by giving two types of treatment ration formulas (A-Ration and B-Ration) for 8 weeks is presented in Table 2. The results of statistical analysis have shown that the administration of the two types of ration formulas did not significantly affect ( $P>0.05$ ) feed consumption, but feed consumption was significantly influenced ( $P<0.05$ ) by genotype.

**Table 2.** The average feed consumption of the three genotypes of broiler ducks by giving two types of treatment ration formula for 8 weeks of age (g/duck)

Genotype	A-Ration (g/duck $\pm$ SE)	B-Ration (g/duck $\pm$ SE)	Average (g/duck $\pm$ SE)
Pekin	8,368 $\pm$ 7.80 <sup>a</sup>	8,357 $\pm$ 13.79 <sup>a</sup>	8,362 $\pm$ 40.24 <sup>a</sup>
E-PMp	7,410 $\pm$ 42.93 <sup>b</sup>	7,535 $\pm$ 65.92 <sup>b</sup>	7,472 $\pm$ 40.24 <sup>b</sup>
PMp	6,564 $\pm$ 92.08 <sup>c</sup>	6,292 $\pm$ 67.21 <sup>d</sup>	6,478 $\pm$ 40.24 <sup>c</sup>
Average	7,447 $\pm$ 47.60	7,395 $\pm$ 48.97	

Different super script letters in each row and column show a significant difference ( $P<0,05$ ); A-Ration (NRC 1994); B-Ration (Ketaren et al. 2011); SE=Standard error

For the next stages of analysis, it has also been shown that the interaction between the two types of treatment ration formulas with the duck genotype did not significantly affect ( $P>0.05$ ) feed consumption for 8 weeks of age. Table 2 explained that the highest average of feed consumption was achieved by

Pekin ducks and the lowest or the best feed consumption produced by PMp ducks. The percentage of feed consumption achieved by PMp ducks compared to Pekin and E-PMp ducks was 22.53% and 10.64%, respectively, while the difference in feed consumption of PMp ducks compared to E-PMp ducks was 13.30%. Table 2 also shows that the administration of the two types of treatment rations, namely A and B-ration for the Pekin and E-PMp lines did not significantly affect ( $P>0.05$ ) feed consumption, but significantly ( $P<0.05$ ) affected the PMp duck line. Giving by B-Ration was lower or better compared to giving by A-Ration for PMp duck line at 8 weeks of age. The average feed consumption of Pekin ducks was significantly ( $P<0.05$ ) higher than the other genotypes of ducks. It can be explained that Pekin ducks have better genetic characteristics, including the utilization of nutrients in the feed during the digestion and absorption process by the ducks. The size and body weight of Pekin ducks are genetically larger than the other genotypes of ducks. Larger body sizes and weights generally require more feed, mainly to balance the growth and maintenance of the duck's body.

The best average feed consumption in this study was achieved by PMp ducks which were 6,478 g/head and the highest was achieved by Pekin ducks (8,362 g/head). This study illustrated that the role of nutritional content, especially lysine, is very influential on feed consumption. The total lysine content in the duck ration in the starter and grower-finisher periods in the B-ration was higher than the B-ration (Table 1).

Feed consumption can be influenced by various factors, one important factor is the quality of the ration, namely the nutritional content in the ration (Hernandez et al. 2004; Ghaffari et al. 2007; Fan et al. 2008; Fouad et al. 2018; Purba & Kostaman 2020). The nutritional content in the form of lysine and energy derived from the consumed ration is thought to be sufficient for the growth process and for the maintenance of ducks. Increasing the optimal energy content in the ratio can reduce feed consumption, on the contrary, if the energy content is low (not sufficient) it will lead to an increase in feed consumption and even tend to lead to fat accumulation in the abdominal tissue including in the meat/carcass (Grobas et al. 1999; Shelton et al. 2003; Perez-Bonilla et al. 2012; Fouad et al. 2018).

Lysine is one of the essential amino acids. Its role is very important to stimulate growth, reduce feed consumption and increase ration efficiency in poultry. Provision of low rations with nutritional content, namely the amino acid lysine, can result in decreased performance in poultry (Shelton et al. 2003; Garcia & Batal 2006; Dozier et al. 2008). Nutritional requirements for local ducks generally refer to the recommendations of the NRC (1994) and Chen (1996). The amino acid requirement of lysine for Pekin ducks at the age of 0-2 weeks is 0.90%, while at the age of 2-7 weeks it is 0.65% (NRC 1994). The

results of this study showed that the average feed consumption of ducks produced from both types of rations for PMp ducks for 8 weeks showed that B-ration was better than A-ration, but the average feed consumption of Pekin and E-PMp duck lines was relatively similar.

The energy content of the ration in the grower-finisher period can be reduced by 3,000 kcal/kg (A-ration) to 2,705 kcal/kg (B-ration) but the amino acid lysine level must be considered and should not be less than the required amount, which is 0, 80% (B-ration). The results of this study showed that in the starter period (0-4 weeks) the protein content of feed of 19% and lysine of 1.15% was considered sufficient to support the growth of ducks. Furthermore, in the grower-finisher period, the decrease in the energy content of the ration from 3,000 kcal/kg to 2,705 kcal with a lysine content of 0.80% also fulfilled enough to produce feed consumption for ducks at the age of 8 weeks. This study have illustrated that the role of nutritional content, mainly the addition of lysine content in the ration was very influential in reducing feed consumption in ducks at 8 weeks of age.

### Live body weight

The average live body weight of three genotypes of ducks at the age of 8 weeks with the provision of two types of treatment ratio formulas is described in Table 3. The administration of both types of ration formulas, namely A and B-Ration did not significantly affect ( $P>0.05$ ) the average live body weight of ducks but was significantly influenced ( $P<0.05$ ) by strain. Based on the results of the statistical test, it was explained that the interaction between the two types of treatment ratio formulas with the duck genotype did not significantly affect ( $P>0.05$ ) the average live body weight of ducks at 8 weeks of age.

**Table 3.** The average live body weight of the three genotypes of ducks by giving two types of treatment ration formulas for 8 weeks of age (g/head)

Genotype	A-Ration (g/duck $\pm$ SE)	B-Ration (g/duck $\pm$ SE)	Average (g/duck $\pm$ SE)
Pekin	2,380 $\pm$ 55.32 <sup>a</sup>	2,500 $\pm$ 15.14 <sup>b</sup>	2,429 $\pm$ 30.79 <sup>a</sup>
E-PMp	1,738 $\pm$ 53.44 <sup>c</sup>	1,766 $\pm$ 37.38 <sup>c</sup>	1,752 $\pm$ 30.79 <sup>c</sup>
PMp	1,650 $\pm$ 53.27 <sup>d</sup>	1,531 $\pm$ 31.52 <sup>e</sup>	1,591 $\pm$ 30.79 <sup>e</sup>
Average	1,922 $\pm$ 54.01	1,933 $\pm$ 28.01	

Different super script letters in each row and column show a significant difference ( $P<0.05$ ); A-Ration (NRC 1994); B-Ration (Ketaren et al. 2011); SE=Standard error

Table 3 shows that the treatment ratio for Pekin and PMp ducks significantly affect ( $P>0.05$ ) the average live body weight of ducks, but did not significantly affect ( $P<0.05$ ) the E-PMp ducks. The average live body weight of

Pekin ducks given by the B-ration was significantly higher ( $P < 0.05$ ) compared to the A-ration, but in the PMp duck line, given the A-ration was significantly higher ( $P < 0.05$ ) compared to the B-ration. The difference in the live body weight of Pekin ducks with the provision of B-ration compared to the A-ration was 5.68%, while the difference in the live body weight percentage of PMp ducks with the provision of A-ration compared to B-ration was 7.21%. Based on the results showed that the response of Pekin ducks given by B-ration was better compared to the A-ration. Table 3 explained that the average live body weight of Pekin ducks was able to reach for body weight of 2.5 kg at the age of 8 weeks. It was different for PMp duck lines that the provision of A-ration resulted was better performance when compared to the provision of B-ration.

The higher average live body weight given the B-ration to Pekin ducks showed that the role of the total lysine content, as well as the energy and protein content of the diet, was optimal enough to stimulate growth and achieve live body weight at 8 weeks of age. Unlike the case in PMp ducks, although the total lysine content in the ratio was increased, the average live body weight achieved was not optimal. This also shows that the duck strain factor can give a different response to the increase in live body weight for 8 weeks. Based on the results of the analysis of variance, the interaction of giving the two types of rations with the strains had no significant effect ( $P > 0.05$ ). This also explains that the two types of ration formulas with duck lines do not have any influence on the achievement of body weight of ducks.

The average live body weight of Pekin ducks was higher compared to the PMp and E-PMp ducks. Genetically, the growth of Pekin ducks is relatively fast, with a larger body size when compared to the other two types of ducks. The average live body weight of Pekin ducks with given by B-ration was higher compared to the A-ration. The difference on live body weight values was estimated as the effect of increasing lysine content in the ration and triggering to increase in live body weight gain of ducks. Xie et al. (2009) stated that the increasing lysine content in the ration could increase and trigger the growth rate of Pekin ducks reared from the age of 7-21 days. The increase in live body weight of ducks in line with the addition of total lysine in the ration in this study is also in line with the opinion of Bon et al. (2002).

The relatively fast growth rate of Pekin ducks has also been reported by (Adeola 2006; Pingel 2011; Wang et al. 2012). It was explained that the genetic traits possessed by Pekin ducks cannot be separated from an intensive and targeted selection process so that the quality of the resulting product also increases. In line with Adeola (2006), another researcher, namely Pingel (2011), also strengthened that in addition to genetic factors, management factors and appropriate feed nutritional content greatly affected performance even on the carcass quality of Pekin ducks (Dozier et al. 2008; Xie et al. 2009; Xie et al. 2014).

The study also illustrates that the effectiveness of the B-ration formula to produce optimal body weight in Pekin ducks was better than A-ration, but in PMp ducks, A-ration was better than B-ration.

### Body weight gain

The average body weight gain of the three genotypes of ducks by giving two types of treatment ratio formulas for 8 weeks is described in Table 4. The provision of both types of ration formulas significantly affects ( $P < 0.05$ ) on body weight gain of ducks. Table 4 shows that the mean body weight gain of ducks given by B-ration was significantly higher (better) compared to the A-ration. The average body weight gain of the three duck lines with the giving A-ration was 1866 g/duck while giving the B-ration was 1,950 g/duck (Table 4). When viewed from the aspect of the strain, giving both types of rations at the age of 8 weeks also significantly affected ( $P < 0.05$ ) on body weight gain of ducks. Table 4 shows that the average body weight gain on Pekin ducks was higher compared to the other two duck lines. The lower body weight gain was achieved by PMp ducks. The difference in body weight gain produced in this study was caused by the genetic characteristics possessed by each duck, mainly in terms of body weight and body posture. Genetically, Pekin ducks have heavier body weight and posture compared to the other two duck strains. PMp ducks are categorized as medium type broiler ducks.

**Table 4.** The average body weight gain of three genotypes of ducks given two types of treatment ration formulas for 8 weeks of age (g/head)

Genotype	A-Ration (g/duck $\pm$ SE)	B-Ration (g/duck $\pm$ SE)	Average (g/duck $\pm$ SE)
Pekin	2,306 $\pm$ 55.21 <sup>a</sup>	2,449 $\pm$ 15.65 <sup>b</sup>	2,378 $\pm$ 30.91 <sup>a</sup>
E-PMp	1,690 $\pm$ 54.15 <sup>c</sup>	1,718 $\pm$ 37.86 <sup>c</sup>	1,704 $\pm$ 30.91 <sup>c</sup>
PMp	1,602 $\pm$ 52.99 <sup>cd</sup>	1,682 $\pm$ 31.58 <sup>d</sup>	1,642 $\pm$ 30.91 <sup>d</sup>
Average	1,866 $\pm$ 54.12 <sup>e</sup>	1,950 $\pm$ 28.36 <sup>f</sup>	

*Different super script letters in each row and column show a significant difference ( $P < 0.05$ ); A-Ration (NRC 1994); B-Ration (Ketaren et al. 2011); SE=Standard error*

The average body weight gain of Pekin ducks with treatment rations A-ration and B-ration at the age of 8 weeks was significantly different ( $P < 0.05$ ). Based on the results of statistical analysis, the average body weight gain of Pekin ducks with B-ration was significantly different ( $P < 0.05$ ) when compared to A-rations. The difference in body weight gain between B-ration and A-ration was 5.8%. Unlike the case for E-PMp ducks and PMp ducks, the provision of A-ration and B-ration as well as between E-PMp and PMp ducks was also not significantly different ( $P > 0.05$ ). Based on the results of subsequent

statistical analysis, the interaction between the administration of two types of treatment ration formulas with the duck genotype did not significantly ( $P>0.05$ ) affect the body weight gain of ducks at 8 weeks of age. From the results of the study, it can be explained that between the two types of ration formulas with duck lines, there was no effect on each other to produce a body weight gain of ducks for 8 weeks of maintenance.

The increase in body weight in the three duck lines with the provision of B-ration was also largely determined by the nutritional content, especially the lysine and energy content. The administration of total lysine of 0.80% and energy content of 2,700 kcal/kg in the grower-finisher period was effective enough to increase body weight gain in ducks. The results of this study also indicated that the provision of B-ration seemed better when compared to the A-ration to produce body weight gain of ducks for 8 weeks. The role of the amino acid lysine and sufficient energy content in the treatment ratio can affect the increase in the body weight gain of ducks. The results of this study are in line with the opinion of Xie et al. (2009) who reported that the body weight gain of Pekin ducks reared from the age of 7-21 days increased at the level of lysine contained in the ratio starting from the level of 0.65; 0.80; 0.90; 1.10; and 1.20%.

The quality of feed including the nutritional content in it is a major factor in increasing body weight gain in poultry (Hernandez et al. 2004). Other researchers have also reported that normal growth in ducks can be achieved if the diet consumed contains sufficient energy, protein (amino acids), vitamins, and minerals (Shelton et al. 2003; Adeola 2006; Kamran et al. 2008). Low energy rations if consumed sustainably can cause slow growth in ducks. Slow growth in ducks can have an impact on low egg and meat production (Shelton et al. 2003).

The study illustrates that the body weight gain of Pekin ducks with given B-ration was higher and better than A-ration. The increase in body weight in E-PMp and PMp ducks with the B-ration seemed higher than the A-ration, but not significantly different, this illustrates that the effectiveness of the two types of rations given was relatively almost the same to produce body weight gain in the three. duck strain for 8 weeks of age.

### **Feed conversion ratio (FCR)**

The average feed conversion ratio (FCR) of the three genotypes of ducks by giving two types of treatment ratio formulas for 8 weeks of age is described in Table 5. The provision both of feed treatments (A-Ration and B-Ration) did not significantly affect ( $P>0.05$ ) the FCR of ducks for 8 weeks. The FCR value of Pekin ducks was lower (better) compared to the other two duck lines. This

indicates that the Pekin duck line is more efficient in utilizing the nutritional content derived from the consumed rations. The FCR value achieved of Pekin ducks by giving B-ration seemed to be decreasing compared to the FCR value in A-ration. Although the decrease was a relatively small amount, it could explain that the decrease was related to the increasing content of lysine in the B-ration. The total lysine content for the grower-finisher period in the A-ration was 0.65%, while the B-ration was 0.80%.

**Table 5.** The average feed conversion ratio (FCR) of three genotypes of ducks given two types of treatment ration formulas for 8 weeks of age (g/head)

Genotype	A-Ration (g/duck $\pm$ SE)	B-Ration (g/duck $\pm$ SE)	Average (g/duck $\pm$ SE)
Pekin	3.64 $\pm$ 0.08 <sup>a</sup>	3.42 $\pm$ 0.02 <sup>a</sup>	3.53 $\pm$ 0.06 <sup>a</sup>
PMp	4.39 $\pm$ 0.12 <sup>b</sup>	4.40 $\pm$ 0.09 <sup>b</sup>	4.40 $\pm$ 0.06 <sup>b</sup>
E-PMp	4.11 $\pm$ 0.12 <sup>b</sup>	4.25 $\pm$ 0.06 <sup>b</sup>	4.18 $\pm$ 0.06 <sup>b</sup>
Average	4.05 $\pm$ 0.11	4.10 $\pm$ 0.06	

*Different super script letters in each row show a significant difference ( $P < 0.05$ ); A-Ration (NRC 1994); B-Ration (Ketaren et al. 2011); SE= Standard error*

The average FCR of ducks from the three strains of ducks given the A-ration was 4.05, while the B-ration was 4.10 (Table 5). Based on the duck strains, it appeared that the average FCR of ducks significantly affected ( $P < 0.05$ ) the FCR produced at 8 weeks of age. Based on the results it was shown that the average FCR of Pekin ducks was lower (better) when compared to the FCR of the other two duck lines. In addition to the nutritional content factor, the influence of the strain also has a significant effect on the FCR achieved. It is possible that research in the fields of genetics, nutrition, and cultivation of Pekin ducks, mainly in the Asian region such as Taiwan, China, and Korea, takes place intensively, continuously, and is supported by adequate funds. The study results also increasingly contribute to providing information about science and technology. The study also provided information that according to the results of statistical tests, it was shown that the interaction between the two types of treatment ratio formulas with the duck genotype did not significantly affect ( $P > 0.05$ ) the FCR of ducks for 8 weeks of age.

The FCR value of Pekin ducks was lower (better) when compared to the other two duck lines. This indicates that the Pekin duck line is more efficient in utilizing the nutritional content derived from the consumed rations. The FCR value achieved by Pekin ducks by giving B-ration seemed to be decreasing compared to the FCR value in A-ration. Although the decrease was seen in a relatively small amount, it could explain that the decrease was related to the increasing content of lysine in the B-ration. The total lysine content in the A-ration was 0.60%, while the B-ration was 0.90%. This pattern of

decreasing FCR is in line with the opinion of Xie et al. (2009) who reported that the ration efficiency of Pekin ducks reared from the age of 7-21 days became more efficient as the lysine content in the diet increased.

## **CONCLUSION**

Giving two types of treatment ration formulas had different effects on the performance of three duck lines. Giving B-Ration was better compared A-Ration to produce final body weight and body weight gain of Pekin ducks for 8 weeks of age, while for EPMp and PMp ducks were similar. Feed consumption and feed conversion ratio (FCR) did not affect by the two treatment rations but were influenced by strain. The best FCR was achieved by Pekin ducks at 8 weeks of age. The provision rations with lysine and energy levels of 1.15% and 2,917 kcal/kg respectively for the starter period and then lysine and energy content of 0.80% and 2,705 kcal/kg respectively for grower-finisher periods, was quite optimum for the performance of Pekin ducks, EPMp and PMp at 8 weeks of age. During the study, there was no mortality, which explained that the quality of the duck lines, the nutritional quality of the rations, and the maintenance of the ducks were in line with expectations.

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## Evaluation of Commercial Multienzyme on Performance of Laying Hens Fed Palm Kernel Cake

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### ABSTRACT

Palm kernel cake (PKC) has been used in poultry diets with limited amounts due to its high anti-nutritional factors such as the non-starch polysaccharide. An experiment was designed to study the effect of the addition of a commercial multi-enzyme (DGDP) into diets consist of PKC on the performance of laying hens. A control diet without PKC and diet with 7.5% and 15% PKC was formulated to meet the requirement of laying hens 5 dietary treatments were formulated. DGDP enzymes were added into diets consist of PKC. Each diet was fed to 7 replicates of 4 laying hens, and the performances were measured from 20 to 36 weeks of age. Results showed that the inclusion of 7.5 or 15% PKC in the diet did not affect feed intake, the egg production significantly, and the feed conversion ratio. The addition of enzymes into the diet with 7.5% PKC improved 3% egg production and 1.0% FCR but improved only 1.3% egg production and 0.6% FCR on diet with 15% PKC, these improvements, however, were not significantly different. The inclusion of PKC did not reduce the dry matter digestibility, and true metabolizable energy (TME) but reduced significantly protein-digestibility of the feed. The addition of the enzyme slightly improved the dry matter digestibility, and the TME increased 42 kcal/kg and 34 kcal/kg for the feed with 7.5% and 15% PKC, respectively. It is concluded that inclusion of PKC in laying hens diet did not reduce the egg production or impair the FCR, and supplementation of DGDP enzyme didnot improve the egg production or FCR.

**Keywords:** Palm kernel cake, Multienzyme, Egg production

### INTRODUCTION

The poultry industry in Indonesia is growing due to the increasing demand of animal protein. From 2016 to 2018, the population for broiler and layer chickens increased 7.75% and 6.2%/year, respectively, and the consumption of poultry meat and eggs increased 13.6% and 6.9%/year, respectively (Ditjen PKH 2018). These were followed by the increase of poultry feed production 7.8%/year and import of feed ingredients such as meat and bone meal (MBM), soybean meal, and other ingredients. Import of feed

ingredients in large quantities tends to increase the feed price and make the local poultry farmers less competitive.

Indonesia produces palm kernel cake (PKC) in large quantities. The PKC production in 2019 was 5.860 million ton and tend to increase every year, but most of them (about 87.9%) are exported (Index Mundi 2020). Some research have been conducted to prove that the PKC could be used in poultry feed despite some limiting factors such as high fiber, low ME, and low amino acids digestibility in the PKC (Sinurat et al. 2012). Therefore, some efforts have been reported to eliminate limiting factors in the use of PKC as poultry feed, through reduction of shell contaminant, fermentation process, or supplementation of enzymes (Sinurat et al. 2013; Sinurat et al. 2016; Sharmila et al. 2014; Oladokun et al. 2016).

Enzymes are one of the feed additives widely included in the poultry diet, especially after the ban of antibiotic growth promoters as feed additives in many countries. Enzymes break down complex chemical structures such as crude fibre in feed ingredients to a much simple structure which results in increases digestibility of nutrients in a feedstuff (Ojha et al. 2019). In vitro study showed that cocktail enzymes improved dry matter, and crude fiber digestibility of feed (Jimoh 2018). Supplementation of enzyme in the feed contains high fiber ingredient such as PKC is expected to increase the utilization of such ingredient in poultry feed.

There are many enzymes available for use in poultry feed. Some are composed of a single enzyme or multi-enzymes. A new multi-enzyme called "Dige Grain Delta Plus" or DGDP has been registered and ready to market. The enzyme contains amylase 800,000 U/kg, Mannanase 2,500,000 U/kg, xylanase 600,000 U/kg, cellulase 3,000,000 U/kg, pectinase 1,000,000 U/kg, glucanase 500,000 U/kg, protease 1,000,000 U/kg dan phytase 1,000,000 U/kg. The effectivity of an enzyme could not be measured by the (*in vitro*) enzyme activities alone but should be determined by its biological activity. Therefore, an experiment was designed to study the effectiveness of the DGDP enzyme on improving the digestibility of nutrients of diets containing palm kernel cake and its effect on the performance of laying hens.

## MATERIALS AND METHODS

All procedures in this experiment regarding the use of live animals were carried out according to the approval of The Animal Welfare Committee at the Indonesian Agency for Agricultural Research and Development: Balitbangtan/Balitnak/A/05/2020.

## **Animals and management**

Lohman pullets aged 15 weeks were purchased from a farmer in Majalaya, West Java and then reared in a poultry house in Balitnak, Ciawi–Bogor. The birds were weighed on arrival and allocated into individual wire cages with a size of 30×37×30 cm (length × wide × height), and fed a commercial grower diet until the experiment started. Only birds with bodyweight closed to the average were used in this experiment. All birds were free access to feed and drinking water provided in front of the cage. Every 4 birds were provided with 1 feeder and therefore regarded as 1 experimental unit. A total of 140 birds were used in this trial. The birds were reared according to normal laying hen management practices.

## **Experimental diets**

All diets used in this experiment were formulated to have similar nutrient content, and meet the requirement of laying hens. Five experimental diets were formulated, *i.e.*, 1. Control diet without PKC, 2. Diet with 7.5% PKC without enzyme supplement, 3. Diet with 7.5% PKC with 500 g/ton DGDP enzyme supplement. 4. Diet with 15% PKC without enzyme supplement, and 5. Diet with 15% PKC with 500 g/ton DGDP enzyme supplement. The composition and nutrient contents of the diet are presented in Table 1. Each diet was fed to 28 birds (7 replicates of 4 birds per replicate) from 19 weeks of age to 33 weeks of age, and the production measurement was started after one week of feeding the experimental diet or from 20 to 33 weeks of age.

## **Parameters measured and data analyses**

During the trial, feed intake was measured weekly and egg production (hen-day) and egg weight were measured daily. Egg quality (yolk color score, eggshell thickness, and albumin height) was measured when birds aged 30 weeks old. Egg quality of all eggs produced at the day of measurement, *i.e.*, 3 or 4 eggs/replicate were measured. At the end of the experiment, 6 birds from each treatment were selected randomly to measure the nutrient digestibility of the feed, *i.e.*, dry matter digestibility, protein digestibility, and true metabolizable energy (TME) of the diet.

The digestibility study was performed by the total collection method as described by Sinurat et al. (2013). In brief, tested diets were pelleted and all birds were trained to feed tested diets 110 g/d in 2 hours for 3 consecutive days to avoid feed spillage followed by fasting for 24 h. Then, each bird was fed with 110 g/bird/d of test diet for 2 h, and a tray was placed underneath the

cage to collect the excreta. Feed intake was measured, and the excreta were collected and dried in an oven (70°C) every day. These procedures were repeated for 4 consecutive days. Dried excreta during 4 days collection from each bird were pooled and weighed. The dry matter, crude protein, and gross energy of the excreta and feeds were measured in the laboratory. Endogenous excreta were obtained by placing 6 hens in wire cages, fasted, and the excreta were collected 24 h after fasted. The excreta were dried, weighed, and regarded as endogenous excreta or excreta of fasted birds. The dry matter, protein digestibility, and the true metabolizable energy (TME) were calculated following the formula:

$$\text{Dry matter digestibility (\%)} = 100\% \times [(DMf \times FI) - ((DMexc \times Exc) - (DMend \times End))] / (DMf \times FI)$$

$$\text{Protein digestibility (\%)} = 100\% \times [(CPf \times FI) - ((CPexc \times Exc) - (CPend \times End))] / (CPf \times FI)$$

$$\text{True Metabolizable Energy (kcal/kg)} = [(GEf \times FI) - ((GEexc \times Exc) - (GEend \times End))] / FI$$

Where, DMf = dry matter of feed; DMexc = dry matter of excreta; DMend = drymatter of excreta from fasted birds; FI = total feed intake; End = weight of excreta from fasted birds; GEf = gross energy of feed (kcal/g); CPf = crude protein in feed (%); CPexc = crude protein in excreta (%); GEexc = gross energy in excreta (kcal/g); GEend = gross energy in excreta of fasted birds (kcal/g).

All performance and egg quality data were subjected to analyses of variance (ANOVA) in a completely randomized design with 5 treatments and 7 replicates. The digestibility data were analyzed with 5 treatments and 6 replicates. Further analyses to evaluate differences between treatments were carried out by Duncan's multiple range test when the ANOVA showed significance at  $P < 0.05$ .

**Table 1.** Ingredient's composition and nutrient contents of the experimental diets

Feed ingredients	Control (0% PKC)	7.5% PKC	15% PKC
Corn	50.96	44.88	44.56
Wheat pollard	10.00	8.73	1.52
Palm kernel cake (PKC)	0.00	7.50	15.00
Soybean meal	24.85	24.37	24.73
Palm oil	1.33	2.50	2.50
Meat bone meal (MBM)	1.69	1.28	0.84
Limestone	9.21	8.71	8.69
Dicalcium phosphate	1.25	1.34	1.50
Methionine	0.18	0.16	0.13

Feed ingredients	Control (0% PKC)	7.5% PKC	15% PKC
Salt	0.200	0.200	0.200
Vitamin-mineral premixes	0.310	0.310	0.310
Total	100.000	100.000	100.000
Nutrient content			
Dry matter, %	88.600	89.200	89.800
Crude fibre, %	3.430	4.100	4.700
Metabolisable energy, kcal/kg	2700	2700	2700
Crude protein, %	17.990	18.140	18.360
Ether extract, %	5.100	6.300	7.500
Calcium, %	4.000	4.000	4.000
Available phosphorus, %	0.340	0.340	0.340
Lysine, %	0.904	0.901	0.902
Methionine, %	0.454	0.461	0.468
Methionine + Cystine, %	0.720	0.727	0.734
Tryptophan, %	0.208	0.208	0.208
Threonine, %	0.663	0.663	0.666

PKC = *Palm kernel cake*

## RESULTS AND DISCUSSION

### Productive performance of laying hens

The production performance of the laying hens is presented in Table 2. Results showed that treatments did not significantly ( $P>0.05$ ) affect feed intake, hen day egg production, FCR, and egg weight. The feed intake tends to increase as the PKC was included in the diet. The inclusion of 7.5% PKC and 15% PKC in the diet increased 2.5, and 4.4 g/b/d, respectively. Chong et al. (2008) reported an increase in feed intake when 12.5% or 25% PKC was included in layers feed. Zanu et al. (2012) showed no significant effect of inclusion of PKC from 5 to 15% on the feed intake of laying hens. However, Sinurat et al. (2011) reported that inclusion of PKC up to 20% in laying hens diet decreased the feed intake by about 2.5 g/b/d. Supplementation of the DGDP enzyme did not decrease the feed intake. Effect of multi-enzymes supplementation in laying hen's diet on the feed intake is varied. Khan et al. (2011), Lee et al. (2014), and Rasteh et al. (2016) showed a reduction in feed intake between 3 – 4.6% due to multi-enzyme supplementation. However, Resende et al. (2017), Khempaka et al. (2018), Narashima et al. (2013) showed an increase in feed intake between 2.0 – 5.4%, and some (Munir & Maqsood

2013; Sinurat et al. 2011; Cufadar & Ceylan 2018) also reported no significant effect of enzyme supplementation on feed intake.

Improvement of nutrient digestibility is expected either to reduce feed intake or to increase egg production which result in improvement of the FCR. Sinurat et al. (2011) showed no significant effect on the feed intake due to enzyme supplementation in diets with 10 or 20% PKC, but the egg production increased, and the FCR was improved.

The HD egg production slightly decreased from 91.6% to 89.8% and 90.3 % when PKC was included in the diet 7.5% and 15%, respectively. Sinurat et al. (2011) showed no reduction in HD egg production when PKC included 10% but the HD egg production decreased from 83.8% to 79.1% when the PKC included 20%. Zanu et al. (2012) also showed an improvement in the HD egg production when PKC included 5 or 10% in the diet but the egg production decreased when 15% PKC was included in the diet. Nutrient compositions of the control diet and the PKC- diets in this experiment, Zanu et al. (2012), and Sinurat et al. (2011) were made similar. Therefore, different results obtained in different reports may contribute to different quality of the PKC used in the trial. In general, PKC could be included up to 10% in the diet of laying hens without adverse effect on the egg production, and impair the egg production when higher (15%) levels of PKC included in the diet (Azizi et al. 2021).

Supplementation of DDGP enzyme into PKC-diet tend to improve the egg production 3%, i.e., from 89.8% to 92.5%, and 1%, i.e., from 90.3% to 91.5% on birds fed 7.5% PKC and 15% PKC, respectively. Sinurat et al. (2011) showed a significant increase in HD egg production from 83.2% to 87.1% when enzymes were supplemented in diets containing 10% PKC, but no improvement when 20% PKC was included in the diet. These results indicated that less improvement in HD egg production due to enzyme supplements when higher level of PKC included in the diet.

The FCR was also slightly impaired when the PKC was included in the diet. The FCR increased 6.5% (from 2.354 to 2.506), and 5.2% (from 2.354 to 2.476) when 7.5% and 15% PKC included in the diet, respectively. Sinurat et al. (2011) reported that inclusion of 5 and 10% PKC in laying hen's diet improved the FCR 7.6% and 3.8%, respectively. However, the FCR were impaired 1.7% when 20% PKC included in the diet.

Supplementation of DDGP enzymes in the PKC diets only slightly improved the FCR, i.e., 1% and 0.6% on layers fed 7.5 and 15% PKC, respectively. Chong et al. (2008) showed an improvement of 2.9% and 1.9% on FCR of layers fed 12.5 and 25% PKC diets, respectively. Sinurat et al. (2011) also reported that multi enzymes improved the FCR 3.9%, 5.7%, and 7.9% on layers fed 5%, 10%, and 15% PKC, respectively.

**Table 2.** Performance of laying hens fed with palm kernel cake and enzyme supplement at 20 - 33 weeks of age

Dietary treatments	Feed intake (g/d)	Egg production (% HD)	FCR (g feed/g egg)	Egg weight (g/egg)
Control (0% PKC – enzyme)	112.3±4.3	91.6±5.4	2.354±0.145	55.6±2.4
7.5% PKC - enzyme	114.8±5.4	89.8±3.5	2.506±0.222	56.0±1.9
7.5% PKC + enzyme	118.3±2.5	92.5±3.2	2.481±0.164	55.7±1.2
15% PKC - enzyme	116.7±5.5	90.3±4.3	2.476±0.154	56.0±1.2
15% PKC + enzyme	116.9±3.5	91.5±2.3	2.461±0.136	56.7±1.5
P- value	0.13	0.72	0.49	0.69

PKC = palm kernel cake; FCR = feed conversion ratio; HD = hen day

Many reports have been published on the effect of enzyme supplementation on the performance of laying hens. The results showed that enzyme supplementation could improve the performance of laying hens by increasing egg production, and or reducing feed intake which results in improvement of the FCR (Khan et al. 2011; Solminski 2011; Khajali et al. 2007; Sinurat et al. 2011; Jia et al. 2008; Rasteh et al. 2016). However, some studies also showed that supplementation of enzymes in diets did not improve the performance of laying hens (Munir & Maqsood 2013; Resende et al. 2017; Khempaka et al. 2018; Ceylan & Cufadar 2018). Many factors affect the effectiveness of enzyme supplements in improving the performance of chickens such as the dose or activity of the enzyme (Attia et al. 2020), type, and nutrient composition of feed ingredients used in the diet (Cowieson & Klunter 2019).

### Egg quality

Data on the egg quality is presented in Table 3. Albumin height and eggshell thickness were not significantly ( $P>0.05$ ) affected by treatments. Zanu et al. (2012) also reported that the inclusion of PKC in layers diet did not affect the eggshell thickness and haugh unit (HU). However, the yolk color score was significantly ( $P<0.05$ ) affected by treatments. Increasing the PKC inclusion in the diet increased the yolk color score. The yolk color score depends on the presence of feedstuffs with pigment in the diet. Corn is well known as a source of egg yolk color. Therefore, a higher level of corn in the diet will result in a higher egg yolk score. However, in this trial, the egg yolk color score produced by layers fed with PKC was higher than the control, although the percentage of corn in the diet was lower (Table 1). This result indicated that PKC may contain some pigments which could influence the egg yolk color.

**Table 3.** Egg quality of hens fed with palm kernel cake and enzyme supplement

Dietary treatments	Albumin height (mm)	Yolk colour score	Egg shell thickness (mm)
Control (0% PKC – enzyme)	8.9±0.4	7.46±1.0 <sup>c</sup>	0.13±0.08
7.5% PKC - enzyme	9.0±0.3	7.52±0.9 <sup>bc</sup>	0.14±0.07
7.5% PKC + enzyme	9.0±0.4	7.50±0.7 <sup>bc</sup>	0.15±0.07
15% PKC - enzyme	8.9±0.4	8.0±0.8 <sup>ab</sup>	0.12±0.04
15% PKC + enzyme	9.0±0.4	8.0±0.7 <sup>a</sup>	0.12±0.03
P- value	0.95	0.03	0.29

*Different superscript at the same column shows significantly different (P<0.05); PKC = palm kernel cake*

### Nutrient digestibility

Dry matter of excreta, dry matter digestibility of feed, and true metabolizable energy (TME) of feed were not significantly ( $P>0.05$ ) affected by treatments as shown in Table 4. Excreta dry matter of layers increased 50% (from 10.3% to 15.5%), and 86.4% (from 10.3% to 19.2%) when PKC included in the diet 7.5% and 15%, respectively. A higher dry matter of excreta is normally preferred by farmers since it will produce a better quality of the litter. Although high improvement was found due to PKC inclusion, it was not significant due to high variation within the treatment (coefficient of variation between 21.0-47.6%). Supplementation of DGDP enzymes reduced the dry matter of excreta 6.9% (from 15.5 to 14.5%) and 18.5% (from 19.2 to 16.2%) of birds fed the diet with 7.5 and 15% PKC, respectively. Ivarsson & Wall (2017) reported that no effect of enzymes supplements on the dry matter content of excreta in broiler chickens.

The dry matter digestibility of feed was decreased 6.2 and 6.6% when 7.5 and 15% PKC was included in the diet, respectively. Since PKC contains high crude fiber, the inclusion of the PKC in the diet increased the crude fiber content of the diet (Table 1). An increasing percentage of crude fiber in broiler's cockerel diet has been reported to increase dry matter excreted but reduced the dry matter digestibility of the diet (Georgieva et al. 2014). Chong et al. (2008) reported a decrease of 13.5 and 25.9% dry matter digestibility of feed when included 12.5 and 25.0% PKC in layers diet, respectively. Supplementation of the DGDP enzymes into the diet with 7.5 and 15% PKC increased dry matter digestibility 2.6 and 1.0%, respectively.

True metabolizable energy (TME) of the feed was decreased by 95 kcal/kg (3.2%) and 56 kcal/kg (1.9%) when the 7.5% PKC and 15% were included in the diet, respectively. Chong et al. (2008) also showed a reduction of TME 156 kcal/

kg (4.6%) and 142 kcal/kg (4.2%) when 12.5% and 25% PKC were included in the layers diet, respectively. Supplementation of DDGP enzymes into diet contained 7.5% PKC increased the TME 42 kcal/kg (1.5%), and supplementation of the enzymes into diet contained 15% PKC increased the TME 34 kcal/kg (1.2%).

As shown in Table 4, true crude protein (CP) digestibility of the feed was significantly ( $P<0.05$ ) affected by treatment. The inclusion of 7.5% PKC in the diet reduced the CP digestibility by 9.2% and the inclusion of 15% PKC reduced the CP digestibility by 29.8%. Supplementation of DDGP enzyme into the diet with 7.5% did not improve the true CP digestibility, but the DDGP enzymes increased the true CP digestibility significantly (from 58.7% to 64.3%) in diet with 15% PKC. Improvement in the crude protein digestibility may be related to the presence of 1,000,000 U/kg protease in the DGDP as stated earlier. Although it is not clear why the improvement occurred on 15% PK diet but not on the 7.5% PKC diet.

In general, the results of this experiment showed that the inclusion of PKC in the diet of laying hens reduced nutrient digestibilities of the diet. Supplementation of DGDP enzymes into the diet with PKC showed a small improvement in nutrients digestibility. This indicates that the enzymes used in this experiment may not be the optimum dose. More trial is required to prove this hypothesis.

**Table 4.** Dry matter of excreta, dry matter and crude protein digestibilities and true metabolizable energy of diets included with palm kernel cake and enzyme supplement

Dietary treatments	Dry matter of excreta (%)	True dry matter digestibility (%)	True metabolizable energy (kcal/kg)	True crude protein digestibility (%)
Control (0% PKC – Enzyme)	10.3±4.9	74.1±2.1	2,954±63	76.2±3.5 <sup>a</sup>
7.5% PKC - Enzyme	15.5±5.1	69.8±3.6	2,859±116	69.8±6.4 <sup>ab</sup>
7.5% PKC + Enzyme	14.5±4.0	71.6±2.5	2,901±105	67.6±7.2 <sup>abc</sup>
15% PKC - Enzyme	19.2±5.5	69.4±3.4	2,898±102	58.7±7.4 <sup>c</sup>
15% PKC + Enzyme	16.2±5.6	70.1±2.4	2,932±90	64.3±9.7 <sup>bc</sup>
P- value	0.09	0.07	0.57	0.01

Note: Different superscript at the same column show significant different ( $P<0.05$ ); PKC = palm kernel cake

## CONCLUSION

The inclusion of 7.5 and 15% PKC in laying hens diet did not reduce significantly the egg production or impair the FCR, DM digestibility, and true metabolizable energy of the diet. But it reduced significantly CP digestibility, the egg quality, especially the yolk color score was improved by the inclusion of PKC in the diet. Supplementation of DGDP enzyme at 500 g/ton feed did not improve significantly the performance of the laying hens and nutrient digestibility of feed with PKC. The small insignificant improvement obtained due to enzyme supplementation may indicate that the dose of enzyme used in this experiment was not optimum.

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## AUTHOR CONTRIBUTIONS

Writing-Original draft preparation. Haryati T, Herliatika A, Ishak ABL: Analysing data and editing the manuscript. Subeni I: Funding acquisition

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## Encapsulation of Gambir Extract: Yield, Total Phenol, Encapsulation Efficiency, and Solubility

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### ABSTRACT

Gambir (*Uncaria gambir*) extract contains high level of catechin, which was reported beneficial for improving muscle growth. Catechin, however, is easily degraded in the rumen. Therefore, this experiment aimed to get the best method to protect gambir extract using calcium palm fatty acid distillate. The experiment was arranged in a completely randomized with factorial design. The 1st factor was the encapsulation method (consisted of one and two stages) and the 2nd factor was level of gambir (consisted of 1, 3, 5, 10%). The yield of encapsulated gambir (EG), total phenol (TP) content and encapsulation efficiency were measured in four replicates. The solubility experiment was done using EG 10% in pH 2 and pH 7.9 solution in 5 replicates. The yield was significantly higher using one than two stages method ( $P < 0.05$ ) (98.50% vs 93.58%) but no effect of gambir level on yield. TP content was affected by method, level and its interaction. The highest TP was at 10% EG and produced by two stages method or 70% acetone (1.63%), followed by 50% methanol (1.29%). Encapsulation efficiency (EE) was significantly higher using 1 stage than 2 stages method (94.84% vs 47.79%) but not significantly different with different gambir level. Solubility of 10% EG was higher at pH 2 pH 7.9 solution. In conclusion, the best method to encapsulate gambir was one stage method and 10% gambir in EG could be protected 70% at pH 2 solution.

**Keywords:** Gambir, Encapsulation, Yield, Total phenol, Solubility

### INTRODUCTION

Livestock performance is influenced by nutritional adequacy and the balance of metabolic system. There are many strategies that are developed including genetic, reproduction, nutrition, management etc. In the nutrition aspects, beside weight gain, meat quality also become the target to obtain good quality and healthy meat. At present, monensin which is classified as antibiotic growth promoter (AGP) has been banned in Indonesia. Many research works are looking for substances from natural resources as the alternatives of AGP. Gambir is a traditional product that has been used as traditional medicine (Mat

Saad et al. 2020). It was extracted from the leaves of *Uncaria gambir*, which is a climbing shrub, native to Southeast Asia. Gambir is also an important export ingredient. Gambir waste has been used for animal feed, however, the use of gambir extract for animal feed or feed additives is still limited. Gambir extract was found in the market as pale brown dried cubes. It contains flavonoids and catechin was the major compound. Catechin mainly was reported in green tea, and cacao. It was reported to improve muscle and inhibited myostatin activity (Li et al. 2020). Double muscle formation was caused by inhibiting myostatin gene. The potential of catechin has not been applied in the ruminant. It was reported that catechin was easily degraded in the rumen (Wein et al. 2016). Therefore, technology is required to protect catechin from degradation in the rumen. The catechin that is released after passing the rumen, may be absorbed into the blood and it is expected to give an effect on the activity of myostatin. This experiment aimed to obtain the best method to protect catechin by calcium-fatty acid and measured its encapsulation efficiency based on total phenol and its solubility in pH 2 HCl solution and buffer solution (pH 7.9) that often mixed with rumen liquor for in vitro tests.

## MATERIALS AND METHODS

### Materials:

Gambir extract was bought directly from the small producer in Payakumbuh, West Sumatra. The products were in the form of dried powder with light brown color. Palm fatty acid distillate was bought from a trader and Calcium oxide was bought from a shop.

### Encapsulation of gambir extract

#### *One stage method*

Palm fatty acid distillate (PFAD) was heated and mixed continuously with Calcium oxide Gambir extract and different levels (1,3,5 and 10%) of gambir extract. Water was added and mixed until it formed a hard solid. These encapsulated products which contained a different level of gambir extract (EG1) was dried in the oven and later was ground. This work was done in duplicates.

#### *Two stages method*

Palm fatty acid distillate (PFAD) was heated and mixed continuously with sodium hydroxide solution until it became brown paste. While heated, calcium

chloride solution and gambir extract at different levels (1, 3, 5 and 10%) of gambir extract were added and mixed until it formed granulated product. Then, the granulated product was separated from the mixture by filtration. Water was added to wash the product twice. The encapsulated products which contained different level of gambir extract (EG) was dried in the oven and later was ground for further analysis. This work was done in duplicates..

### **Analysis of total phenol**

Analysis of total phenol was conducted by extracting EG with 50% methanol or 70% acetone and done in four replicates. Tannic acid was used as the standard (0-10 ug/ml). The analysis was conducted according to Makkar (2003).

### **Solubility of encapsulated gambir**

Two types of solution with different pH were prepared. One buffer solution of sodium bicarbonate and ammonium carbonate at pH 7.9 was prepared according to the method for in vitro fermentation before flushing with CO<sub>2</sub> gas (Makkar 2003). The second solution was prepared using 0.1N HCl at pH 2. Two hundred milligrams of EG were incubated at 39°C in 40 ml of solution with two different pH and replicated 5 times. Sample of buffer solution was taken at 0, 3, 6, 24 hours of incubation.

### **Design experiment and statistical analysis**

The experiment was arranged in completely randomized factorial design. The first factor was encapsulation method (consisted of 1 stage and 2 stages) and the second factor was the inclusion level of gambir extract (consisted of 1, 3, 5 and 10%). The yield of encapsulated gambir (EG), total phenol (TP) content and encapsulation efficiency were measured in four replicates. The solubility experiment was done using EG 10% in pH 2 and pH 7.9 solution, done in 5 replicates. The analysis was done on the yield of encapsulated gambir (EG), total phenol (TP) content in 50% methanol, total phenol content in 70% acetone and encapsulation efficiency based on total phenol. The statistical analyses were performed using SPSS16. If significant, further analysis was done using Duncan's test.

## **RESULTS AND DISCUSSION**

Encapsulation of gambir extract was aimed to protect gambir extract from the degradation by rumen microbes. Wein et al. (2016) reported that when

catechin was fed intraruminally or orally administered, catechins were intensively metabolized by ruminal microorganisms. When administered directly to duodenum, catechins were detected in bovine plasma indicating that catechins were absorbed into the body. Two methods of encapsulation were conducted using the same material PFAD. In the two stages method, there were two reactions occurred, *i.e.* first, the reaction of PFAD with sodium hydroxide solution produced sodium-PFAD salt as a brown paste, and second, the reaction of sodium-PFAD with calcium chloride solution produced calcium-PFAD salt as granule yellowish products. However, when gambir extract was added to the second reaction, it formed encapsulated gambir in calcium-PFAD (EG) as dark red granules. After being milled, EG color changed from dark red became light brownish. The two stages method was developed earlier in Balitnak to protect fatty acid from its degradation in the rumen and the protected fatty acid was very beneficial as a source of energy for dairy cow and calves (Wina et al. 2015; Wina et al. 2018). Recently there was a simpler and cheaper method has been developed by Handojo et al. (2018a) and it has been modified in Balitnak.

Table 1 shows that one stage method has significantly higher percentage of EG yield than two stages method (98.50 vs 93.58%) and the increasing level of gambir extract did not give any effect on the yield percentage of EG. The yield percentage of EG only depended on the method of production. In the two stages method, there was a washing process to wash away sodium chloride from the EG product. Sodium chloride in encapsulated gambir was formed from the reaction of sodium fatty acid salt with calcium chloride solution. Sodium chloride in EG caused EG could not dry completely and looked wet even though EG has been dried in the oven for several days. Therefore, sodium chloride had to be washed away by water which some of encapsulated gambir might also escape and caused slightly lower yield of EG in two stages method. While in one stage method, there was no formation of sodium chloride during the reaction, so washing process was not required.

In one stage method, water was added at the last step of the procedure. The reaction of CaO and H<sub>2</sub>O was a carbonation reaction and exothermic, which causing strong heat and raised the temperature sharply Calcium hydroxide

**Table 1.** Effect of method and level of gambir extract on the yield of encapsulated gambir

Method		P value	Level (%)				P value
One stage	Two stages		1	3	5	10	
98.50 <sup>a</sup>	93.58 <sup>b</sup>	0.001	96.20	97.76	95.86	94.35	0.14

*P*<0.01 = highly significant; *P*<0.05 = significant; *P*>0.05 = non significant

( $\text{Ca}(\text{OH})_2$ ), (Yuan et al. 2018). This Calcium hydroxide reacted quickly with fatty acid and straightaway formed solid product. The quality of calcium oxide is very important. Handoyo et al (2018a) used titration method to check the quality of calcium oxide. They dissolved calcium oxide after in heated 50% sucrose solution and titrated with sulphuric acid solution. Quality of calcium oxide may vary due to several factors, *i.e.*: the original source of calcium, the production process, the method of storage or transportation. The improper long storage of calcium oxide would cause calcium oxide absorb carbon dioxide from the air and formed calcium carbonate. The reaction of fatty acid with calcium carbonate would form a greasy product. Besides using calcium oxide (Handojo et al. 2018b) also used calcium hydroxide as another calcium source to protect fatty acid and the mole ratio for the reaction between  $\text{Ca}(\text{OH})_2$  and fatty acid was 1:1.5.

Gambir extract in the form of a cube and used in this experiment, was produced traditionally by the pressing process after steam treatment of gambir leaves, then, the extract was dried under the sun. The dried cube gambir extract was soluble in hot water. Total phenol content of gambir extract extracted by 50% methanol was lower than by 70% acetone (80.78% vs 97.72%). This result showed that the solubility of phenolic compounds in gambir extract was higher in 70% acetone than in 50% methanol suggesting that the phenolic compounds in gambir extract were less polar. Kassim et al. (2013) reported that phenolic compounds in gambir extract were higher in ethyl acetate fraction than methanol or hot water fraction, indicating that phenolic compounds in gambir extract were also less polar compounds and more readily dissolved in less polar solvent. There were many compounds reported in gambir and catechin was the main compound in gambir extract as reported by Yeni et al. (2014); (Duangyod et al. 2014). However, the content of catechin in gambir extract from different areas in Indonesia varied from 7-76% depended on the method of production. Beside catechin, other compounds in gambir that were analysed by HPLC were epicatechin, procyanidins, gambirinin, polymeric flavans, quinic acid, quecetin and kaempferol (Taniguchi et al. 2007; Sazwi et al. 2013).

Table 2 (by 50% methanol) and Table 3 (by 70% acetone) showed total phenol contents in EG extracted by two aqueous solvents. Both tables show that there were significant effects of method and inclusion level of gambir extract, and there was also an interaction effect of both method and inclusion level. At one stage method, the total phenol content was very low in both solvent extraction, ranging from 0.02-0.79% (extracted by 50% methanol) and 0.02-0.74% (extracted by 70% acetone). At 2 stages method, the total phenol content was higher than one stage method ranging from 0.17-1.79% (extracted by 50% methanol) and 0.21-2.52% (extracted by 70% acetone). Not only in gambir extract, total phenol content in encapsulated gambir was also higher

**Table 2.** Effect of method and level of gambir extract on total phenol in encapsulated gambir extracted by 50% methanol

Method	Level (%)				Average
	1	3	5	10	
One stage	0.02 <sup>s</sup>	0.05 <sup>s</sup>	0.07 <sup>s</sup>	0.79 <sup>q</sup>	0.23 <sup>b</sup>
Two stages	0.17 <sup>s</sup>	0.44 <sup>r</sup>	0.64 <sup>q</sup>	1.79 <sup>p</sup>	0.26 <sup>a</sup>
Average	0.10 <sup>c</sup>	0.24 <sup>bc</sup>	0.35 <sup>b</sup>	1.29 <sup>a</sup>	P<0.001

*P*<0.01 = highly significant; *P*<0.05 = significant; *P*>0.05 = non significant

**Table 3.** Effect of treatment and level on total phenol in encapsulated gambir extracted by 70% acetone

Method	Level (%)				Average
	1	3	5	10	
One stage	0.06 <sup>s</sup>	0.08 <sup>s</sup>	0.16 <sup>s</sup>	0.74 <sup>r</sup>	0.26 <sup>b</sup>
Two stages	0.21 <sup>s</sup>	0.54 <sup>r</sup>	1.19 <sup>q</sup>	2.52 <sup>p</sup>	1.11 <sup>a</sup>
Average	0.14 <sup>c</sup>	0.31 <sup>c</sup>	0.68 <sup>b</sup>	1.63 <sup>a</sup>	P<0.001

*P*<0.01 = highly significant; *P*<0.05 = significant; *P*>0.05 = non significant

in 70% acetone than in 50% methanol. It seems that 70% acetone can extract more phenolic compounds from EG than 50% methanol. The highest total phenol content was extracted from the encapsulated gambir at 10% inclusion level of gambir and produced by two stages method either by 50% methanol (1.29%) or 70% acetone (1.63%). The higher the level of gambir inclusion, the higher the total phenol content in EG. It seems that gambir in EG produced by second stages method was not completely protected by Ca-Fatty acid so that some phenolic compounds can easily released when extracted by 70% acetone. Calcium hydroxide could also react with phenolic compounds in gambir, therefore the ratio of calcium and fatty acid in the reaction should be further studied in the presence of phenolic compounds to prevent the reaction between phenolic compounds and calcium and later the occurrence of a complex of calcium-phenolic and its solubility in the rumen also requires further study.

Table 4 shows the encapsulation efficiency based on total phenol content was affected by treatment and level. Method of encapsulation and inclusion level of gambir extract significantly affected the encapsulation efficiency (EE). Based on the method, EE by one stage method (94.88% and 93.87%) was two times higher than EE by two stages method (47.95% and 48.90%). There were two reasons that caused low EE in two stages method, *i.e.* 1) the washing procedure in two stages method caused some loss of total phenol content from EG and 2) the protection of gambir from calcium-fatty acid salt may be weak

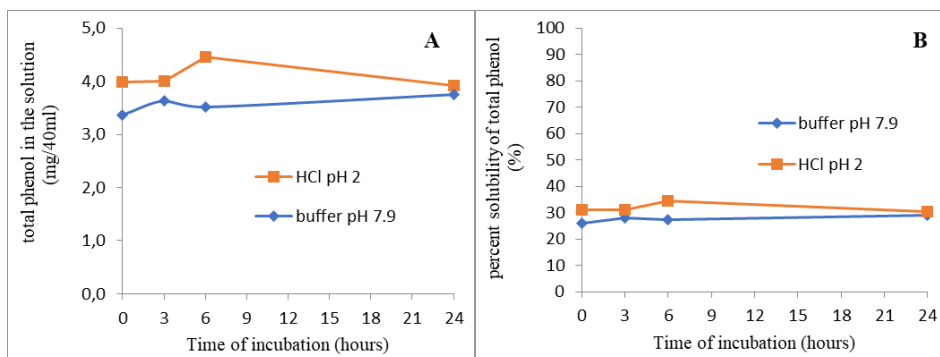
**Table 4.** Effect of treatment and level on encapsulation efficiency

Solvent	Method		P value	Level (%)				P value
	one stage	two stages		1	3	5	10	
50% Methanol	94.88 <sup>a</sup>	47.95 <sup>b</sup>	<0.001	73.04 <sup>x</sup>	74.83 <sup>x</sup>	73.94 <sup>x</sup>	63.85 <sup>y</sup>	0.003
70% Acetone	93.87 <sup>A</sup>	48.90 <sup>B</sup>	<0.001	72.89 <sup>xy</sup>	76.44 <sup>x</sup>	70.48 <sup>yz</sup>	65.74 <sup>z</sup>	0.004

*P*<0.01 = highly significant; *P*<0.05 = significant; *P*>0.05 = non significant

and caused some phenolic compounds easily extracted by aqueous solution. The encapsulation efficiency was the lowest at the highest level of gambir inclusion (63.85 and 65.74%). It seems that the inclusion level of gambir at 10% was too much to be mixed properly by hand during reaction with Calcium oxide and fatty acid. Handoyo et al. (2018b) showed that an acid value can become an indicator of good formation of Calcium-fatty acid. High acid value of unreacted fatty acid was expected but after reaction with calcium oxide, the acid value should drastically reduce.

Figure 1 shows the solubility pattern of EG in buffer solution pH 7.9 and HCl solution pH 2.0 and Figure 1A shows the amount of total phenol that is soluble in the solution. The higher the total phenol in the solution, the higher the solubility of EG into the solution. In the pH 2 solution, higher solubility of total phenol occurred rather than in the pH 7.9 solution. At pH 2 solution, the solubility EG slightly increased after 6 hours of incubation, while at pH 7.9 solution, the solubility slightly increased at 3 hours of incubation. However, a longer time of incubation at 39°C without shaking did not increase the solubility of EG in both solutions. In terms of percent solubility of total phenol, it was only 30% of total phenol in EG that soluble during incubation in the buffer (Figure 1B). This result indicated that gambir extract was well protected or encapsulated by Ca-fatty acid salt. It was also reported that alkaline solution might affect the stability of some phenolic compounds in gambir, hence, reduced total phenol content significantly in the liquid (Sazwi et al. 2014). However, catechin which was a major phenolic compound in gambir extract was stable at pH 3-11 since its multiring aromatic structure was more resistant to degradation by pH compared to the monocyclic polyphenolic compounds (Friedman & Jurgens 2000). Encapsulated gambir needs to be evaluated in the *in vitro* rumen fermentation followed by pepsin-HCl incubation. Rumen microbial activity may partly degrade the encapsulated gambir by Ca-fatty acid salt and release some phenolic compounds in the rumen and some phenolic compounds especially catechin may be released after passing the rumen. It is expected that catechin in the intestine may be absorbed and give a beneficial effect on muscle development as it was reported that catechin indirectly enhancing skeletal muscle development by inhibiting the activity of Myostatin (Li et al. 2020).



**Figure 1.** Solubility of encapsulated gambir measured as total phenol (A) and percent solubility of total phenol (B) in different pH solution

## CONCLUSION

Encapsulation of gambir extract was better conducted using Calcium - fatty acid by one stage method than two stages method due to high encapsulation efficiency and low solubility in low or high pH solution.

## ACKNOWLEDGEMENT

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## Nutrient Requirement for Pogasi Heifers

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### ABSTRACT

This study aimed to observe the nutritive standard for Pogasi heifers. Forty-eight heifers were randomized and housed in individual pens. The heifers were allotted to one of four crude protein (CP) levels (A.140; B.155; C.170 and D.185% of the NRC 1996 for growing cattle with the targeted live weight gains (LWGs) were 0,68 kg/d) with six replicates. The duration of the study was 14 weeks. The feed was 70% concentrate diet and 30% elephant grass on a dry matter (DM) basis. The parameters were LWG, body condition score (BCS), feed intake, volatile fatty acid, rumen characteristics, glucose, and blood urea nitrogen. Live weight was measured fortnightly, the BCS was observed at the start, mid, and end of the study. The results showed that DM intake was different between treatments ( $P<0.05$ ), the DM digestibility did not differ between treatments ( $P>0.05$ ). The LWGs for every treatment were 0.62, 0.58, 0.84 and 0.62 kg/d respectively. The BCS scores increased 1.08-1.42 points. The highest rumen pH was 6.3 in the C group. The glucose level was 48.17-55.50 mg/dl. The blood urea nitrogen concentrations were 46.67-56.33 ng/dl. To conclude, achieving 0.68 LWG in Ongole grade heifers requires 1.7 CP of NRC 1996 recommendation.

**Keywords:** Nutrient, Requirement, Pogasi, Heifer

### INTRODUCTION

The success of the livestock business depends very much on feeding management. Although environmental and genetic factors also contribute to it. Studies reported that the feed cost contributes to the highest production cost, accounting for 70-80%. Feeding management adapted to the age and physiological status of the livestock is a way to save the feed cost.

Beef Cattle Research Station (BCRS) has carried out breeding activity since 2002. It has provided young cattle in the third and fourth generations as a new breed having special characteristics. The new breed is expected to have different characteristics from the common Pogasi cattle. The name of the new breed is Pogasi that has high adaptation to a low-quality diet. The approach and feeding system in BCRS successfully support the breeding.

National Research Council (NRC) is the common feed standard for determining the nutrient requirement. The Standard was made in a North American environment that has a temperate climate and is used for *Bos taurus* cattle that are adapted to the cold weather. In NRC 1996, before the seventh edition, the cattle that were used as reference was Angus cattle that are native Scottish cattle (Vasconcellos et al. 2003). It is believed that the system is not appropriate to be used in Indonesia, including climate and breed differences. Besides, the feed quality that is available in Indonesia is very difficult to meet the nutrient requirements for cattle.

In the nutrient requirement table (NRC 1996), physiological status, production target, and the breed were used for reference in providing the nutritional standard, it indicated that it may not be suitable for all cattle breeds. In NRC (2000), Brahman, Nellore, and Sahiwal cattle represent *Bos indicus* cattle. Whereas the common cattle in Indonesia are Ongole grade (PO), Bali, Madura, Pesisir, etc. that were not calculated appropriately. The existing NRC cannot calculate in detail the nutrient requirement of new breeds that have adapted to the tropical climate. NRC 2000 needs corrective factors such as climate, wind, and humidity that are difficult to be done. Therefore, this study used nutrient requirement standards in NRC (1996).

Mariyono et al. (2012) stated that the feed for weaner Ongole grade can be composted from the agricultural and its industrial byproducts, the formula can meet 9% of crude protein, <6% of crude fat, <17% of crude fiber, and <12% of ash. Weaner Ongole grade bulls that would be the candidate for Pogasi bulls were fed with nutrient requirement standard NRC (2000) at the maintenance level with the additional live weight gain (LWG) 0.25; 0.5; and 0.75 kg, resulted in a low growth rate. The above bulls that were kept for five months and there was no live weight gain (Krishna et al. 2016). Previous research reported that nutrient requirement for heifers kept in a tropical climate was 165% of NRC (1978) standard. This study aimed to obtain a nutrient requirement standard for Pogasi heifers with the target of LWG was >680 g.

## MATERIALS AND METHODS

This study was conducted in experimental pens at BCRS, Grati, Pasuruan, East Java. The study consisted of a 12-week experimental period, and a two-week preliminary period. Twenty-four of Pogasi heifers, at the age of 19 to 21 months, LW 199-298 kg were allotted in individual pens with water and feed troughs and had *ad libitum* access to fresh drinking water. The heifers then were randomly divided into four nutritional treatments. The nutritional standard used was NRC (1996) for *Bos indicus* heifers with the LWG target were 0.68 to 0.91 kg. The level of feed offered was shown in Table 1.

**Table 1.** The level of feed offered for Pogasi heifers with referring to NRC 1996 standard for targeted LWG 1.5 lb

Treatments	The level of feed offered
A	140% of NRC standard
B	155% of NRC standard
C	170% of NRC standard
D	185% of NRC standard

The feed offered with the level 2.75-4.00% live weight on dry matter (DM) basis, the feed refusal was no less than 10% of feed offered. The feeds were elephant grass (EG) 25% and concentrate 75% (DM basis). The feed was offered twice a day at 07.30 AM and 01.30 PM, the ratio of feed offered in the morning vs in the afternoon was 2 : 1. The aim of giving the feed twice a day was to increase feed intake, stabilize the rumen environment, improve digestibility, therefore, improving LWG (Mayulu et al. 2021). The feed ingredients and nutritive value of the concentrate diet were shown in Table 2.

Parameters observed were body weight (BW), Body Condition Score (BCS), feed intake including DM, Organic Matter (OM), CP, TDN, CF, EE, ADF, and NDF, rumen characteristics (pH, acetic acid, propionic acid and butyric acid (Bachruddin 1996), blood glucose and blood urea nitrogen. Liveweight measurement was done every two weeks before feeding in the morning. Live weight gain was calculated from liveweight over a certain period divided by the number of days and followed an equation Cole (1996). The BCS scale adopted 1-9 scale (Rasby et al. 2017) was measured at the same time as LW measurement, at the start, mid, and end of the experimental period. Feed intake was measured every day throughout 12 weeks, by subtracting feed offered with feed refusals. The feed refusals were collected daily, 100 g for the concentrate diet and 300 g for EG. Concentrate diet was bulked weekly and a subsample was collected and analyzed. The EG refusals were collected, dried in the oven at 55°C, and bulked. Concentrate and EG refusals for every pen bulked in the same way as feed offered. The measurements of pH and VFA concentrations in the rumen liquor were done at the end of the study. The rumen liquor was collected per oral at 3 h after eating. This was in accordance to van Soest (1994) who said that the peak of fermentation for concentrate diet is at 2-3 h after eating while the grass was fermented at the peak hour at 4-5 h after eating.

Blood glucose and urea nitrogen were measured by collecting blood samples through the jugular vein at 3 h after eating. Proximate analysis, fiber (NDF and ADF), and rumen pH rumen were carried out at the Nutrition and Feed Laboratory, BCRS. The analysis of rumen VFA and blood urea nitrogen was done

**Table 2.** Feed ingredients and the nutritive value of a concentrate diet

Ingredients	Concentrate composition (% dry matter)	
Wheat pollard	29	
Kopra meal	9	
DDGS	9	
CGF	18	
Commercial feed	33	
Calcium	1	
Salt	1	
Nutrient <sup>1</sup>	Nutritional content (% dry matter)	
	Concentrate	Elephant grass
DM	91.0	22.1
CP	17.6	8.1
EE	3.7	1.6
CF	15.3	22.0
Ash	7.1	7.3
TDN	70.8	52.8
NDF	40.0	60.1
ADF	35.0	38.8

*1 Nutrition and Feed Laboratory, BCRS; CGF = Corn Gluten Fiber; DDGS = Distiller's Dried Grain with Soluble; DM = Dry Matter; CP = Crude Protein; EE = Extract Ether; CF = Crude Fiber; TDN = Total Digestible Nutrient; NDF = Neutral Detergent Fiber; ADF = Acid Detergent Fiber*

in Animal Husbandry Laboratory at Gadjah Mada University, in Yogyakarta, and blood glucose analysis was done in Sejahtera Laboratory, in Pasuruan.

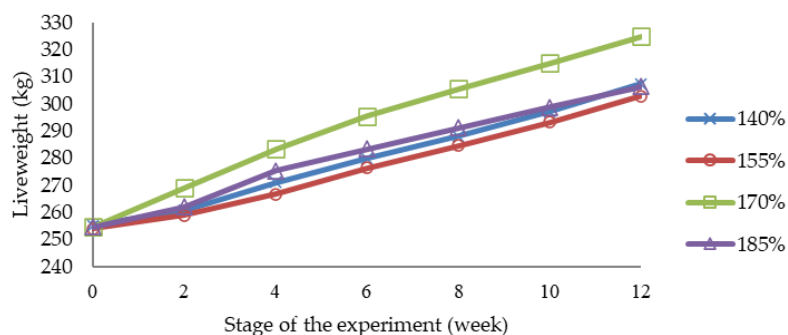
The experimental design was a randomized complete design with four feeding levels and six replicates. One-way ANOVA was performed and when there were differences between treatments, Duncan's Multiple Range Test was tested at 5% of significant level (Steel & Torrie 1995).

## RESULTS AND DISCUSSION

### Average daily gain and body condition score

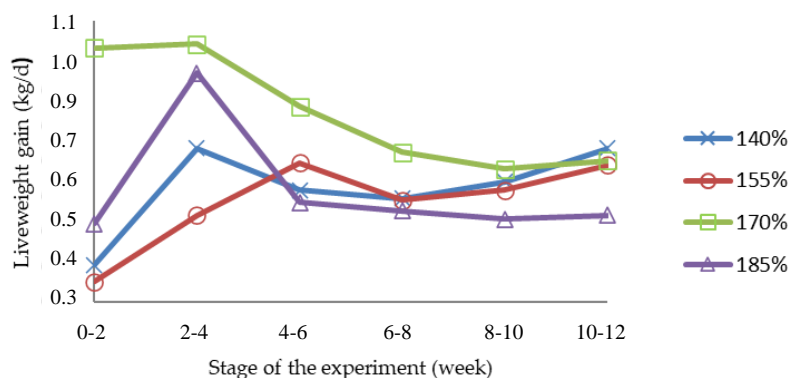
The initial LW of Pogasi heifers at the age of 19 to 21 months was 199 to 288 kg and had a high variety, so at the start of the study, the heifers were randomized based on LW and BCS. It resulted in the average LW at A, B, C, and D groups was 255.2±37.5; 254.0±43.7; 254.7±36.2; and 254.7±33.4 kg respectively. The growth rate of Pogasi heifers was presented in Figure 1. After the initial two weeks of the current study, Pogasi heifers who received 170%

of NRC standard showed a higher LW than those eating 140, 155, and 185% of NRC standard. The growth rate of heifers in A, B, and D were similar.



**Figure 1.** Liveweight of Pogasi heifers that were offered different levels of nutritional standard

Figure 2 showed that the highest LWG in all groups occurred from week-2 to 4. This was a compensatory growth because the heifers ate good quality feed. The LWG on week-4 to 12 tended to decline. Keogh et al. (2016) said that compensatory growth is a phenomenon when there is an accelerated growth after the feed restriction. Jennings (2014) stated that the compensatory growth in cattle is a process of growth at a faster pace as a result of feeding good quality feed. Compensatory growth can be used as an effective management approach to reduce the feed cost and to stimulate puberty in *Bos indicus* Nellore cattle (Misura et al. 2021).



**Figure 2.** Liveweight gain of Pogasi heifers that were offered different levels of nutritional standard

The analysis of variance was reported in Table 3, which showed that the mean of LWG of Pogasi heifers offered feed at 170% of NRC standard was higher than other cohorts ( $P < 0.05$ ). The live weight gain in A, B, and D groups

were not different ( $P>0.05$ ). The increase of feed offered at 185% of the NRC standard was not followed by a high LWG. The LWG of Pogasi heifers was higher than the previous study by Umar et al. (2007) who experimented in 1.5 years old Ongole grade offered EG *ad libitum* and 1.75% of concentrate diet. It resulted LWG 0.6 kg/d, DM intake was 5.7 kg/day or 3.03% LW.

**Table 3.** Liveweight gain and BCS gain of Pogasi heifers that were offered different levels of nutritional standard

Levels of feeding (% NRC)	Liveweight gain (kg/d)	BCS gain
140	0.62±0.2 <sup>a</sup>	1.17±0.4 <sup>a</sup>
155	0.58±0.2 <sup>a</sup>	1.08±0.7 <sup>a</sup>
170	0.84±0.2 <sup>b</sup>	1.42±0.4 <sup>a</sup>
185	0.62±0.2 <sup>a</sup>	1.33±0.4 <sup>a</sup>

<sup>a,b</sup> different superscript within the same column showed significant difference ( $P<0.05$ )

At the start of the study, the BCS was no different between treatments. At the mid-to the end of the experiment, the BCS increased (Table 4). The ration provided enough nutrients to meet the optimum BCS for successful reproduction, at 5 to 6.5. At this BCS level, the heifers did not show the rib bone and cannot be seen barely. The back part is plump and full (Rasby et al 2017). The BCS 5 to 6 is an ideal condition to promote a successful reproduction cycle. Mathis et al. (2002) reported that 92% of cows having BCS 5 to 6 showed estrus post-partum on day 90.

**Table 4.** The body condition score of Pogasi heifers was offered different levels of the nutritional standard at the start, mid, and end of the experimental period

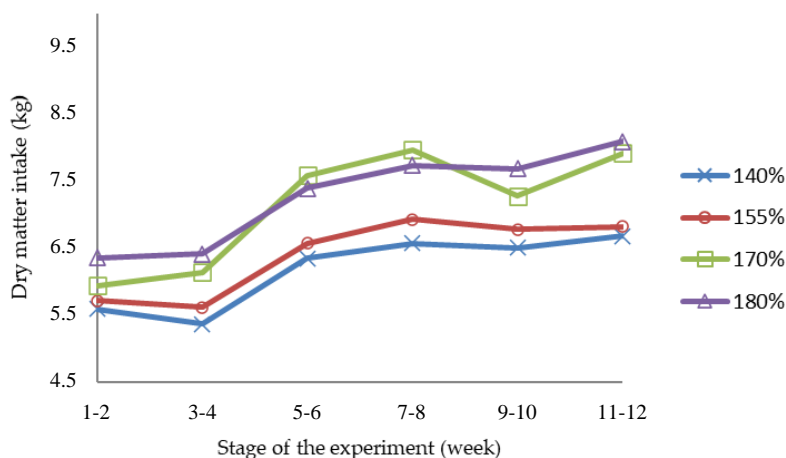
Levels of feeding (% NRC)	Weeks		
	0	6	12
A	4.92±0.2	5.50±0.6	6.08±0.5
B	4.83±0.5	5.42±0.7	5.92±0.7
C	4.92±0.2	5.92±1.0	6.33±0.4
D	4.92±0.2	6.08±0.6	6.25±0.3

<sup>a,b</sup> different superscript within the same column showed significant difference ( $P<0.05$ )

## Feed intake

Feed intake is the amount of feed eaten by the animals when they have *ad libitum* access to it. It can be compared. Feed intake can show the level of palatability. Feed intake of good quality feed is higher than a low-quality feed.

The feed intake increased concurrently with the stage of the experiment and the growth of the heifers. Dry matter intake of Pogasi heifers was presented in Figure 3. The feed for heifers should contain enough nutrition for maintenance, growth, and reproduction. In general, factors affecting feed intake are BCS, sex, age, physiological status, and body size. The age and LW at the maturity stage are affected by the level of nutrition (Bagley 1993).



**Figure 3.** The dry matter intake of Pogasi heifers that were offered different levels of nutritional standard

Table 5 showed that DM intake at 170 and 185% of NRC standards was significantly higher ( $P < 0.05$ ) than at 140%. The 140% and 150% levels were not different, 170% and 185% were also not different. The DM intake of Pogasi heifers at LW 279 to 290 kg was about 6.18 to 7.28 kg/head/day. The DM intake on LW basis at 140% was 2.2%, at 155% was 2.3%; at 170% was 2.5% and at 180% was 2.6%. The DM intake was lower than a research report using Ongole crossbred cows at LW 298.2 kg resulted in DM intake of around 8.2 kg or about 2.75% LW (Periambawe et al. 2016). The DM intake was lower than the nutrient requirement at about 8.97 kg. A study reported that in Tuban, East Java, Ongole crossbred cows with LW 300-350 kg/head, and fed EG, native grass and agricultural byproduct *e.g.* rice straw, corn stover and rice bran was 5.85-5.94 kg/head/d whereas the requirement of feed based on LW was 7.4 to 8.3 kg/head/day (Huda et al. 2018). Umiyasih et al. (2005) reported that one-year-old Ongole crossbred cattle ate 2.69% LW, CP intake 519 g/head, and TDN 2.43 g/head. Liveweight gain was 550 g. A comparative study carried out by Mariyono et al. (2013) showed that the difference of feedstuff that has isonitrogenous and isoenergetic, with the 9.76% CP and 60.02% TDN, resulted in a variety of LWG, from 0.46 kg and 0.59 kg. The feed intake also varied,

about 2.75 and 3.27% LW (Antari 2016). Kearl (1982) reported that cattle at 350 kg LW with 1 kg targeted LWG needed 8.50 kg DM intake (2.4% LW). The difference between the feed intake was affected by feed quality, a study in Periambawe et al. (2016) used a large amount of forage while the current study used 75% of DM of feed offered from the concentrate diet.

**Table 5.** Feed intake of Pogasi heifers that were offered different levels of nutritional standard

Levels of feeding (% NRC)	Feed Intake							
	DM	OM	CP	TDN	CF	EE	ADF	NDF
	----- kg/head/day -----							
140	6.18±0.5 <sup>a</sup>	5.58±0.5 <sup>a</sup>	1.12±0.1 <sup>a</sup>	5.22±0.4 <sup>a</sup>	1.51±0.1 <sup>a</sup>	0.23±0.0 <sup>a</sup>	3.04±0.2 <sup>a</sup>	4.04±0.3 <sup>a</sup>
155	6.41±1.1 <sup>ab</sup>	5.78±1.0 <sup>ab</sup>	1.17±0.2 <sup>ab</sup>	5.47±0.8 <sup>ab</sup>	0.59±0.2 <sup>ab</sup>	0.24±0.0 <sup>ab</sup>	0.19±0.4 <sup>ab</sup>	0.25±0.6 <sup>ab</sup>
170	7.14±0.6 <sup>b</sup>	6.44±0.6 <sup>bc</sup>	1.30±0.1	6.08±0.5 <sup>c</sup>	1.77±0.1 <sup>bc</sup>	0.27±0.0	3.55±0.3 <sup>c</sup>	4.73±0.4 <sup>c</sup>
185	7.28±0.6 <sup>b</sup>	6.56±0.5 <sup>c</sup>	1.32±0.1	6.27±0.5 <sup>c</sup>	1.85±0.1 <sup>c</sup>	0.27±0.0	3.69±0.3 <sup>c</sup>	4.94±0.4 <sup>c</sup>

<sup>a,b</sup> different superscript within the same column showed significant differences ( $P < 0.05$ )

Organic matter, CP, TDN, CF, EE, ADF, and NDF intakes at levels of 170% and 185% were higher than a level of 140% dan 155%. The increase in feed intake did not result in an increase in LWG that occurred at a level of 170%. Probably, at the level of 175% was the optimum level of Pogasi heifers.

### Rumen characteristics

The observation on rumen characteristics was done on pH and VFA parameters that were collected at 3 h after eating provided in Table 6. PH, acetic, propionic, and butyric acids were not affected by the different levels of feeding ( $P > 0.05$ ). This was probably because there were no differences between treatments on nutrient content and the ratio between grass and concentrate offered. The concentration of propionic acid was mostly determined by the level of soluble carbohydrate and crude fiber in the diet, while in the current experiment the fractions were relatively similar. The VFA concentrations in the current experiment were higher than those studied of Pamungkas et al. (2013) that reported Ongole grade bulls fed 30% of forage and 70% of concentrate and the CP and CF contents were 10.4% and 26.3% resulted in the VFA production about  $C_2=28.85$ ,  $C_3=8.35$ , and  $C_4=3.95$  mmol/l.

**Table 6.** pH and rumen VFA of Pogasi heifers that were fed different levels of nutritional standard

Treatments	pH	Acetic acid (mmol)	Propionic acid (mmol)	Butyric acid (mmol)
A	6.48±0.3 <sup>a</sup>	122.55±25.1 <sup>a</sup>	31.79±7.4 <sup>a</sup>	19.35±6.5 <sup>a</sup>
B	6.44±0.4 <sup>a</sup>	107.23±305 <sup>a</sup>	46.96±51.9 <sup>a</sup>	17.07±6.8 <sup>a</sup>
C	6.34±0.3 <sup>a</sup>	123.38±18.6 <sup>a</sup>	28.26±12.1 <sup>a</sup>	20.80±5.1 <sup>a</sup>
D	6.55±0.3 <sup>a</sup>	118.66±30.0 <sup>a</sup>	33.07±7.7 <sup>a</sup>	22.09±4.1 <sup>a</sup>

<sup>a,b</sup> different superscript within the same column showed significant differences ( $P<0.05$ )

### Blood glucose and urea nitrogen

**Table 7.** Blood glucose of Pogasi heifers that were fed different levels of nutritional standard

Levels of feeding (% NRC)	Glucose (mg/dl)	Urea (mg/dl)
140	52.50±9.1 <sup>a</sup>	56.33±7.6 <sup>c</sup>
155	53.00±5.5 <sup>a</sup>	46.67±7.0 <sup>a</sup>
170	48.17±6.1 <sup>a</sup>	54.50±5.5 <sup>ab</sup>
185	55.50±2.9 <sup>a</sup>	52.33±7.7 <sup>ab</sup>

<sup>a,b</sup> different superscript within the same column showed significant differences ( $P<0.05$ )

The analysis of variance in Table 7 showed that blood glucose in all treatments was not different ( $P>0.05$ ). Blood urea nitrogen at a level of 140% was higher than at levels of 155%, 170%, and 185%. There were no differences in blood urea nitrogen concentration at levels of 155, 170 and 185% ( $P>0.05$ ). The concentration of blood glucose in Pogasi heifers was similar to the study conducted by Tuhuk et al. (2011) and Suwasono et al. (2013). Tuhuk et al. (2011) reported that blood glucose and urea nitrogen in Bali cattle were not different before eating (0 h), and at 2, 4, and 6 h after eating. The concentration of blood glucose was 56.94 to 61.20 mg/dl. The concentration of blood urea nitrogen in the current experiment was higher in comparison with Tuhuk et al. (2011), which was 23.66 to 2481 mg/dl. The differences between the concentration of blood urea nitrogen were probably because of the different levels of CP content in the diet containing a high level of CP resulted in high blood urea nitrogen. Suwasono et al. (2013) reported that the concentration of blood glucose of Javanese cattle was not different at 0 h (before feeding) was 53.54 mg/dl, at 3, 6, and 9 h after feeding, at about 51.11 63.61; and 54.93 mg/dl, respectively. The concentration of blood urea nitrogen at 0 h (before feeding) was 29.92 mg/dl, and at 3, 6, and 9 h after feeding 26.49; 26.25 and 48.51 mg/dl respectively.

## CONCLUSION

To conclude, the live weight gain achieved is similar to the nutritional standard shown in the table of NRC. The Pogasi heifers need nutrient supply more than 1.7 times of NRC.

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## Supplementation of Monosodium Glutamate Industry By-products in Beef Cattle Ration

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### ABSTRACT

The monosodium glutamate industry generates liquid by-products which contain lots of nitrogen and have a chance to be used as feed supplements for beef cattle. This study aimed to determine the optimal level of liquid monosodium glutamate by-product (MBP) supplementation in beef cattle fattening rations. Twenty-four male Madura were housed in individual pens for 18 weeks of feeding observation. They were randomly distributed into four feeding treatment groups, each group got proportional age and body weight. Treatment A was a control, they fed elephant grass (EG) and concentrate (C) which ingredient was from agricultural by-products. Treatment B (RG + C + 4% MBP), Treatment C (RG + C + 8% MBP), Treatment D (RG + C + 12% MBP). Parameters observed were, daily weight gain (ADG), dry matter intake (DMI), and feed conversion ratio (FCR). The data were analyzed by covariance analysis with body weight as the covariate, then continued to LSD Test. The highest ADG (mean  $\pm$  SEM) was on treatment C ( $907.40 \pm 141.99$  g/head/day), significantly different from treatment A ( $296.90 \pm 82.17$  g/head/day) but not different from treatment B ( $681.90 \pm 129.0$  g/head/day) and D ( $479.50 \pm 175.52$  g/head/day). The DMI did not differ between treatments, it was  $9.70 \pm 0.89$  kg/head/day (treatment A), ( $9.24 \pm 0.60$  kg/head/day (treatment B), ( $9.53 \pm 0.51$  kg/head/day (treatment C) and ( $9.49 \pm 0.32$  kg /head/day (treatment D). The feed conversion was not different between treatment (11.94-21.48). The completeness of amino acids in MBP was supposed to have a greater role in increasing body weight than the amount of protein contributed to the feed. It was concluded that the optimal MBP supplementation in cattle concentrate feed based on agricultural by-products was at 8%.

**Keywords:** MSG by-product, Beef cattle, Fattening

### INTRODUCTION

MSG is produced as a food flavoring enhancer, it is a microbial fermentation product with molasses/sugar cane as a carbon source and zaetin or ammonia as a nitrogen source (Fitri et al. 2016). The process of MSG production, basically divided into three step, they are fermentation, isolation

and refining. (Gresinta 2015) explained that the raw materials were fermented using the bacteria *Brevibacterium lactofermentum*. In this fermentation process, glutamic acid will be produced, hereafter the mix added with soda (sodium carbonate) to obtain the monosodium glutamate. The process is continued by purification and crystallization into pure crystalline powder which is ready to distributed to market.

The fermentation process produces a by-product in liquid form which is no longer needed in MSG formation (Fitri et al. 2016). The liquid is dark brown and contains high organic and inorganic compounds, hence it is often used to increase soil fertility (Muyassir 2006). Some people called the liquid by-product as sipramin, it is an acronym from "sisiproses fermentasi asam amino (glutamat dan L-lysine)" or "the rest of the amino acid fermentation process (glutamate and L-lysine)". Sipramin is good as fertilizer because it contains some macro N, P, K, Ca, Mg and micro elements such as Cu and Zn; Sipramin is also rich organic matter (8.1-12.7%) therefore it can increase soil organic matter (Utami 2016)

The character of liquid MSG by-product is similar to molasses, the color is blackish-brown, slightly thick, smell sweet-redolent odor, and the energy is quite high. Basically, MSG produced without toxic additives and raw material (food grade), so the by-products also do not contain harmful material, accordingly, the MSG industry by-product can be used and safely supplemented in animal feed, without any adverse effects on feed intake, digestibility, rumen fermentation, milk yield, and compositions (Sato et al. 2019). Moreover, the by-products are considered potential as animal feed supplements due to their high nitrogen content, especially its form as nucleic acids. The product has been used on steer and has a good effect on their performance. (Padunglerk et al 2017) reported that soybean meal able to be replaced MSG liquid by-product by 20-60% in the feed for dairy cows, there was no negative effects on their performances, moreover the feed cost could decrease 2.9-17.3% and increase milk production profit up to 33.3%.

PT Miwon Indonesia as one of the major MSG manufacture considered their liquid by-product to have a good prospect to be used as feed supplements. The laboratory analysis showed their by-product contained 6.45% nitrogen or equivalent to 40.31% crude protein. This product also contains glutamic acid, alanine, proline, and aspartic acid in major amounts compare to other amino acids. Amino acids play a role in cellulolytic bacteria activity (Suryapratama & Suhartati 2005).

Besides amino acids content, liquid MSG by-product also contains many micro minerals, the two largest micro minerals are zinc and manganese while the rest were in a low quantity. (Wina et al. 2019) stated that micro minerals such as Zinc, Manganese, Cobalt, and Copper play an important role in fiber

digestion. The purpose of this activity was to determine the optimal level of monosodium glutamate by-product which generated by PT Miwon Indonesia as beef cattle fattening ratios supplement.

## MATERIALS AND METHODS

Twenty-four male Madura cattle aged 2-3 years with a bodyweight of 300-400 kg were used. The animals were randomly distributed in four groups of feed treatment with six heads as replication. All animals were placed into individual pens completed by feeding and drinking equipment. The basal/control feed was forage and concentrate in a ratio of 35:65 (based on dry matter). Feed was given *ad libitum*.

The forage was elephant grass (EG) which was chopped to about 5 cm length. Concentrate (C) consisted of wheat pollard, palm kernel cake, copra meal, corn gluten fiber (CGF), cassava flour, and rice bran with the composition as shown in Table 1. The treatment was based on the addition of liquid MSG by-product (MBP) to concentrate. The addition is the percentage of MBP to concentrate and calculated at fresh conditions. MBP is mixed well with concentrate before being poured into the feed. The liquid MSG by-product was obtained from PT Miwon, Gresik, Indonesia.

Treatment A: Elephant Grass (EG) + Concentrate (C)

Treatment B: EG + C + 4% MBP

Treatment C: EG + C + 8 % MBP

Treatment D: EG + C + 12 % MBP

The observations were carried out for 18 weeks, including two weeks prior as an adaptation period. The parameters were average daily gain (ADG), dry matter intake (DMI), and feed conversion (FCR). The animal's body weight was weighed every two weeks along the study period. The ADG was obtained by dividing the gain by weighing days interval and the total ADG was an average of ADG every two weeks. The feed was given every morning at 08.00 am and offered for 24 hours a day. The next morning, the feed refusals were weighed to determine feed intake. The FCR was calculated by dividing the amount of dry matter intake by body weight gain. The experimental design used was a completely randomized design (CRD) as the equation model:

$$Y_{ij} = \mu + \beta_i + \epsilon_{ij}$$

$Y_{ij}$  : observation in the jth unit under  $i^{th}$  treatment

$\mu$  : mean population

$\beta_i$  : effect of adding "MBP" by the  $i^{th}$  treatment

$\epsilon_{ij}$  : random error component in the (i,j)<sup>th</sup> unit

**Table 1.** The composition and nutrient value of basal feed (forage and concentrate)

Ingredients	Feed composition (% dry matter)
Forage	35.00*
Elephant grass	100.00
Concentrate	65.00*
Lime stone	1.10
Salt	1.10
wheat pollard	11.80
Palm kernel cake	12.00
Copra	12.00
Corn gluten fibre (CGF)	12.00
Dried cassava flour	30.00
Rice bran	20.00
Nutrient parameter*	Nutrient content (% dry matter)
Dry matter	36.15
Crude protein	10.33
Extract ether	3.93
Crude fiber	16.83
Ash	10.60
TDN	63.48

\*feed nutrient content of Forage: Concentrate = 35 : 65 (dry matter base)

The data were analyzed by covariance analysis with body weight as the covariate. When the prior statistical analysis was significantly different, the analysis continued by the least significant difference (LSD) test. The mean values displayed have been adjusted by body weight as the covariate.

## RESULTS AND DISCUSSION

Along the treatment period, the house monitored the temperature and relative humidity to ensure the animals in good condition. The ambient temperature (mean  $\pm$  standard error of mean) was  $28.37 \pm 0.49^{\circ}\text{C}$  and relative humidity was  $80.87 \pm 1.48\%$ . In that certain environment the animals look comfort, the ambient temperature and relative humidity were conducive to Madura cattle, in fact, the Madura cattle have good adaptability (Pradana et al. 2015) compared Madura cattle in the lowlands to highlands with different average temperatures and humidity, their physiological status was detected

relatively the same. (Suherman et al. 2013), explained the combination of increasing temperature and humidity from the ambient where animals live will increase their heat stress, usually indicated by changing animal physiological statuses such as respiration frequency, pulse, rectal temperature, and skin surface temperature

The observations on the variable weight were calculated as growth rate and served in average daily gain (ADG) as shown in Table 2. At the initiate period, the highest average weight gain was achieved in treatment C (adjusted mean  $\pm$  standard error of mean) of  $907.40 \pm 141.99$  g/head/day, not different from treatment B ( $681 \pm 129.0$  g/head/day) but significantly different from treatment A ( $296.90 \pm 82.17$  g/head/day) and D ( $479.50 \pm 175.52$  g/head/day). In the last period, the ADG pattern was a little bit different, the difference was that treatment D was not different from treatment C. The ADG average along observation had a similar pattern to the last observation.

**Table 2.** The average daily gains of treatments on several MBP levels (g/head/day)

Treatment	Initial ADG	Last ADG	Total ADG
A (MBP 0%)	$296.90 \pm 82.17^a$	$524.10 \pm 104.02$	$446.40 \pm 93.77^a$
B (MBP 4%)	$681.90 \pm 129.0^{ab}$	$681.70 \pm 61.12$	$682.20 \pm 76.78^{ab}$
C (MBP 8%)	$907.40 \pm 141.99^b$	$830.70 \pm 106.12$	$872.20 \pm 114.29^a$
D (MBP 12%)	$479.50 \pm 175.52^{ab}$	$890.10 \pm 90.30$	$694.60 \pm 124.60^{ab}$

*The different superscripts in the same column showed differences between treatments ( $P < 0.05$ )*

The highest ADG during observations was achieved in treatment C with an amount of  $872.20 \pm 114.29$  g/head/day, it was different from treatment A ( $446.40 \pm 93.77$  g/head/day), but not different from treatment B ( $682.20 \pm 76.78$  g/head/day) and treatment D ( $694.60 \pm 124.60$  g/head/day). In Table 2. It can be seen that MBP addition has a significant impact on the cattle's daily weight gain. The gain was quite good compared to (Ngadiyono et al. 2001) observations on Madura cattle that were given coconut meal and "bioplus" supplements, by that supplement, the ADG reached 0.55-0.61 kg/head/day. Meanwhile, (Prabowo et al. 1999) fattened young Bali and Madura cattle for 112 days got 0.82 and 0.86 kg/head/day, respectively.

Considering the ADG characteristics shown in Table 2. Treatment C was quite consistent which had the highest ADG, whereas treatment D which contained the highest MBP, the ADG was under treatment C. This finding implies that adding MBP as much as 8% of concentrate to be considered as the optimal level in those feed characteristics. The 8% MBP as an optimal contribution was due to the amino acid and mineral content, not due to the increasing crude protein content after supplementing.

Soto et al. (1994) stated that in pure culture, peptides and amino acids increased cellulolytic bacteria. Amino acids tend to stimulate the growth rate of cellulolytic bacteria and peptides tend to influence some non-cellulolytic species. (Russell et al 1991) reported the long-chain peptides were used at a slower rate than short ones and the hydrophilic amino acids (glutamate, arginine, lysine, and aspartate) were deaminated more than twice as fast as the hydrophobic peptides. Glutamic acid is a substrate in the bio-production of gamma-aminobutyric acid, by this synthesis, succinate can be produced. Then, propionate and other metabolites can be produced from the succinate (Mamuad & Lee 2021).

Base on laboratory analysis, some amino acids contained in MBP had the same amount as soybean (Sitompul 2004) and corn amino acids (Pasaribu et al. 2009). Soybean is often a reference source of protein with complete amino acids while corn is generally considered as an adequate protein source and the price is affordable concerning feed. MBP is an adequate source of amino acids, especially glutamic acid and alanine.

Liquid MSG by-product also has a fairly complete mineral content and it is close to the basic for ruminants need, including macro minerals (Ca, P, Mg, S, Na, K, Cl, S) and micro minerals (Fe, Mn, Zn, Cu). (Suprijati 2013) stated Zn is an essential micro mineral required by ruminants since it is a component of over 300 enzymes for carbohydrate, protein, and fat metabolisms; Zn supplementation enhanced nutrient digestibility, growth, feed efficiency, and milk production. The body weight and libido of 7 years of age Simmental bulls were in well-maintained condition by supplemented Zn, Mn, and Se in their feed (Kurnia et al. 2020). (Bomko et al. 2018) reported that supplementation mixed Zn, Mn and Co improved digestion rate and milk production.

The average dry matter feed intake in a day was shown in Table 3. The DMI did not differ between treatments, the DMI ranged from 9.24-9.70 kg/head/day. The DMI per body weight also did not differ between treatments, the range was from 2.52-2.58%. Feed conversion figures out the amount of kg of dry matter feed intake needed to yield 1 kg of live weight, it is usually formulated in a ratio known as feed conversion ratio (FCR). The lower value of FCR means better. The FCR was not different between treatments, The FCR treatment A was  $21.48 \pm 4.13$ , B ( $14.52 \pm 1.96$ ), C ( $11.94 \pm 1.57$ ), and D ( $14.99 \pm 2.89$ ). Significant differences in gain between treatments did not able influence the FCR to be different yet, though the DMI between treatments tended to be equal.

For local cattle fattening, it was more effective using high energy feed; the application of low and medium energy feed, the FCR was 11.81-15.79 while using high energy feed was 7.53 (Priyanto et al. 2015). (Ngadiyono et al. 2001) fattening male Madura cattle with an initial body weight of  $136.62 \pm 21.61$  kg,

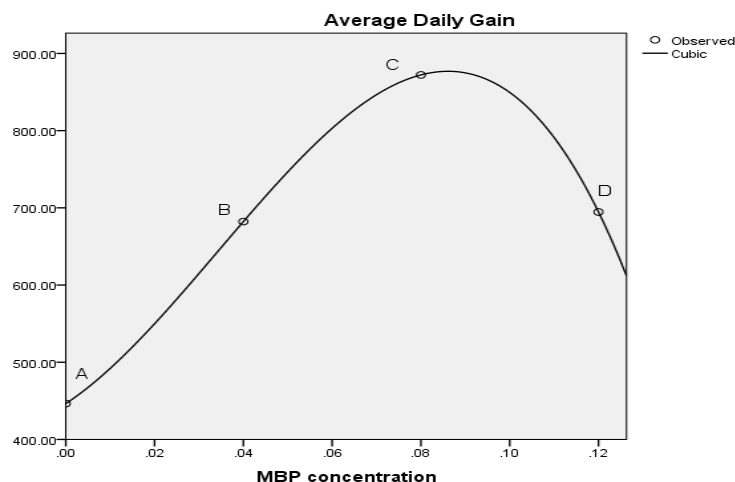
**Table 3.** Dry matter intake and feed conversion ratio

Treatment	DMI (kg/head/day)	DMI/body weight (%)	Feed Conversion Ratio
A (MBP 0%)	9.70 ± 0.89	2.52 ± 0.19	21.48 ± 4.13
B (MBP 4%)	9.24 ± 0.60	2.49 ± 0.08	14.52 ± 1.96
C (MBP 8%)	9.53 ± 0.51	2.58 ± 0.10	11.94 ± 1.57
D (MBP 12%)	9.49 ± 0.32	2.58 ± 0.06	14.99 ± 2.89

DMI = *dr matter intake*

using coconut meal supplements and "Bioplus" obtained FCR of 7.29-10.41. A higher FCR value was obtained by (Priyanto et al. 2015) than Ngadiyono et al. (2001) probably due to the animals were bigger. While (Lestari et al. 2011) reported that the feed conversion of Java cattle; Ongole and Madura cattle were 11.49; 9.21; and 10.21.

Correlation between the MBP concentration and the reached ADG is represented by Figure 1. The distribution of treatment points, then predicted to a proper trend, the most appropriate one was a part of trend modeled by polynomial order 3 (cubic model). The equation was  $f(x) = -838,020.8x^3 + 86,250x^2 + 3,785.8x + 446.4$ ; with  $R^2 = 1$ . By this equation, the maximum value was at (0.086;876.86). it indicated that to get the highest ADG response (876.86 g/head/day), the MBP might be added at the level of 0.086 or 8.61% of the concentrate.



**Figure 1.** Regression the level of MBP to expressed optimum ADG

In case MBP is supplemented to fattening cattle with a bodyweight of 400 kg, with a DMI of 3% body weight and the ratio of forage to concentrate is

35:65, in sum, the consumption of dry matter concentrate is 8.76 kg. With the optimal level of MBP was 8.61%, consequently, MBP might have an optimal effect when it is given as much as 755 g/400 kg cattle per day.

## CONCLUSION

Considering the weight gain and feed conversion of Madura cattle, in the composition of forage to concentrate was 35:65 (dry matter basis), supplementing the liquid monosodium glutamate by-product up to 8.61% of concentrate was an optimal level or 0.19% of body weight.

## ACKNOWLEDGEMENT

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## **Establishment of Ruminant Feed Mill in West Nusa Tenggara: Challenges and Opportunities**

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### **ABSTRACT**

West Nusa Tenggara or Nusa Tenggara Barat (NTB) Province is one of the centres of beef cattle production in Indonesia. This research aimed to analyze the opportunities and challenges of establishing a ruminant feed mill in NTB Province. Primary data collections were carried out through surveys and Focus Group Discussions (FGD) with competent stakeholders at an agency that handles the livestock sector. Secondary data collections were sourced from government and private agencies, reports on the results of existing studies, and other relevant sources. Analysis of the opportunities and challenges of establishing a ruminant feed mill in NTB Province was using a SWOT analysis. The results showed that the opportunities for the establishment of a feed mill in NTB Province were indicated by the high number of ruminant livestock population, the strong political support from the provincial government, the local production system, the more intensive food agriculture system, the willingness of farmers to buy feed produced from the feed mill. The threats for the establishment of a feed mill in NTB Province were the declining tendency of the ruminant livestock population, the increasing livestock and meat importation, lack of the farmer's capital land conversion, and decreased land carrying capacity, lack of group resources, the non-competitive products in quality and quantity with foreign products. The construction of a ruminant animal feed mill in NTB Province is feasible and technically, production and marketing are very prospective because commercial animal feed needs are still imported from outside NTB Province.

**Keywords:** Feed ruminant, Opportunities, Challenges, SWOT analysis

### **INTRODUCTION**

Animal feeds can be defined as raw materials that consumed by livestock to meet energy and nutrients in the ration. The high quality feed contains balanced protein, fat, carbohydrates, minerals, and vitamins. The most important substance in feed is a protein (Umiyasih & Anggraeny 2007). Animal feed ingredients can come from plants and animals. Feed materials can be grouped into feed raw materials as an energy source, feed raw materials

as a protein source (vegetable and animal), feed raw materials as a mineral source, as well as additional and complementary feed raw materials (feed additives and feed supplements). The fluctuation of livestock commodity prices is generally caused by changes in the price of animal feed in the market (Becker 2012). A direct increase in feed prices will generally lead to an increase in the price of livestock commodities, this is because the largest production cost of animal husbandry is the cost of feed. The development of the domestic cattle industry is strongly influenced by three factors, namely the selling price of cattle, the US dollar exchange rate against the rupiah, and the price of feed. If business actors can make efficiency, production costs will decrease so that they can compete with imported products (Liano 2019).

The cattle population in Indonesia is currently about 17.4 million head (BPS 2020), of which 43% is in Java Island, 25% is in the Eastern Islands, and the remaining 32% is on other islands spread around Indonesia. The province of West Nusa Tenggara (NTB) has considerable agricultural potential and a large cattle population. Based on BPS data for 2019, the population of beef cattle in NTB Province is 1,234,640 heads, which is the 4th largest cattle population in Indonesia. Cows are the second largest commodity after chickens in their role as meat providers in Indonesia (Ditjen PKH 2017). The potential for cattle in Indonesia has not been fully managed, which results in the low growth of local cattle business in Indonesia.

NTB is one of the provinces that is of particular concern for livestock development. This is because of NTB has the potential for large ruminants. So far, the Province of NTB has contributed a lot to national livestock, starting from sending meat and sending calves to several regions in Indonesia. In 2017, NTB Province has exported 30,000 cows and 10,000 calves. Data on the cattle population in NTB Province from year to year have increased quite a lot, causing the need for animal feed to increase. The utilization of agricultural waste as a source of animal feed has not been carried out optimally. Besides, the conversion of agricultural land into residential areas in NTB Province is increasing.

The development of a feed mill can be used as a solution for cattle development in NTB by utilizing agricultural products and agricultural waste as good quality cattle feed in the form of finished feed. However, the reality so far shows that the commercial animal feed that meets the local market is from outside the NTB Province, especially from the island of Java. This fact is a challenge as well as an opportunity for business development in the field of animal husbandry by utilizing existing local resources so that the production costs of existing cattle farming businesses can be reduced.

## MATERIALS AND METHODS

This research was conducted in two districts in NTB Province, namely East Lombok Regency and Sumbawa Regency. The variables observed to describe the results of research and assessment of the establishment of ruminant feed factories in NTB Province included: (a) General descriptions of the study sites; (b) The development and number of cattle population in the last year; (c) Forage production and livestock population; (d) Animal feed production; (e) The area of land for feed sources; (f) The development of the number of cattle farmer groups; and (g) Research results related to the establishment of cattle feed mill.

The data collected consisted of secondary and primary data. Primary data collection was carried out through surveys and Focus Group Discussions (FGD) with competent stakeholders at the Service or Agency that handles the livestock sector. Secondary data collection was sourced from government and private agencies, reports on the results of existing studies and other relevant sources.

### Potential yield of agricultural products

Natural forage potential (Tanuwiria et al. 2007):

- Rice field =  $(0.77591 \times \text{land area} \times 0.06 \times 6.083)$  tons of dry matter/year
- Dry land (land) =  $(1.062 \times \text{land area} \times 0.09785 \times 6.083)$  tons of dry matter/year
- Forest land =  $(2,308 \times \text{land area} \times 0.05875 \times 6.083)$  dry matter/year

### Livestock carrying capacity (Animal Unit, AU)

$$\text{Livestock carrying capacity} = \frac{\text{total potential feed available}}{3.32}$$

The feed requirement for each AU is 9.1 kg of dry matter (DM)/day or 3.32 tons DM/year (Ashari et al. 1995).

### Analysis of the opportunities and challenges

Analysis of the opportunities and challenges of establishing a ruminant animal feed mill was conducted using SWOT analysis (Sammut-Bonnici et al. 2015). In principle, SWOT analysis is an analysis of internal and external environmental factors, which consists of factors of strength, weakness, opportunities, and threats. From the identification of these factors, a strategy is developed using the SWOT matrix.

## RESULTS AND DISCUSSION

### Overview of the research area

In 2019, the land area of East Lombok Regency which is used as rice fields is 47,598 hectares, 75,787 hectares of non-rice fields, and 37,169 hectares of non-agricultural land. The area specified by the district is shown in Table 1.

**Table 1.** Area by sub-district in East Lombok Regency in 2019

Sub-district	Area (km <sup>2</sup> )	Area (%)
Keruak	40.49	2.52
Jerowaru	142.78	8.89
Sakra	25.09	1.56
Sakra Barat	32.30	2.01
Sakra Timur	37.04	2.31
Terara	41.41	2.58
Montong Gading	25.66	1.60
Sikur	78.27	4.87
Masbagik	33.17	2.07
Pringgasela	134.26	8.36
Sukamulia	14.49	0.90
Suralaga	27.02	1.68
Selong	31.68	1.97
Labuhan Haji	49.57	3.09
Pringgabaya	136.20	8.48
Suela	115.01	7.16
Aikmel	122.92	7.66
Wanasaba	55.89	3.48
Sembalun	217.08	13.52

*Source: East Lombok Regency in Figures 2020*

Sumbawa Regency consists of 24 sub-districts and 157 villages. The total area is detailed by sub-district as shown in Table 2.

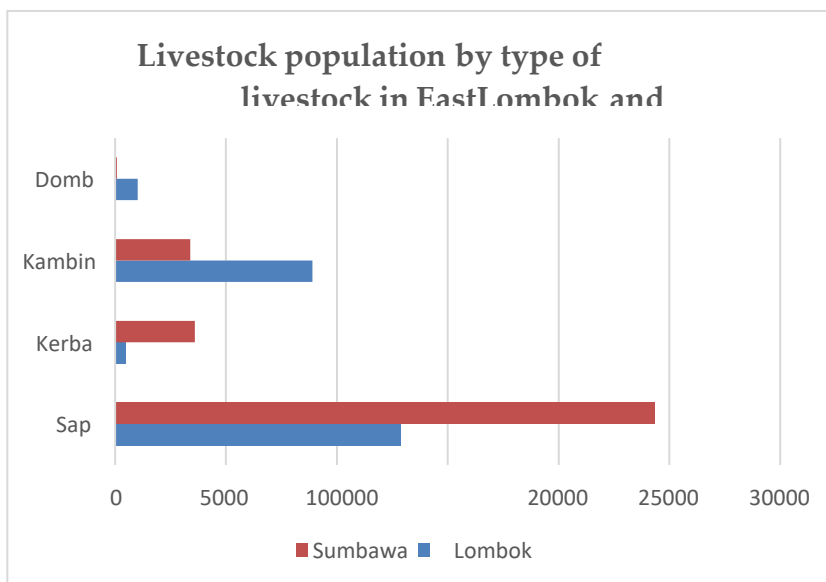
**Table 2.** Area by sub-district in Sumbawa Regency in 2019

Sub-district	Area (km <sup>2</sup> )	Area (%)
Lunyuk	513.74	7.73
Orong Telu	465.97	7.01
Alas	123.04	1.85
Alas Barat	168.88	2.54
Buer	137.01	2.06
Utan	155.42	2.34
Rhee	230.82	3.47
Batulanteh	391.40	5.89
Sumbawa	44.83	0.67
Labuhan Badas	435.89	6.56
Unter Iwes	82.38	1.24
Moyohilir	186.79	2.81
Moyo Utara	90.80	1.37
Moyohulu	311.96	4.70
Ropang	44.48	6.69
Lenangguar	504.32	7.59
Lantung	167.45	2.52
Lape	204.43	3.08
Lopok	155.59	2.34
Plampang	418.69	6.30
Labangka	243.08	3.66
Maronge	274.75	4.14
Empang	558.55	8.41
Tarano	333.71	5.02
Number	6,643.98	100.00

*Source: Sumbawa Regency in Figures 2020*

## Development and number of livestock population

Ruminant farms in the Province of West Nusa Tenggara contribute to beef in Indonesia, reaching 10,961 tons per year or about 2% nationally (Ditjen PKH 2020). The population of ruminants in East Lombok and Sumbawa is shown in Figure 1.



**Figure 1.** The population of ruminants in East Lombok and Sumbawa

In the livestock sub-sector, the largest livestock population in 2019 in East Lombok Regency was cattle, which reached 128,433 heads, followed by buffalo (4,739 heads), goat (89,026 heads), and sheep (10,160 heads). Besides being superior in the production of food crops, Sumbawa Regency is also superior in the livestock sector. Judging from the number of livestock available, in 2019 the largest number of large livestock was in the form of Sumbawa buffalo as many as 35,984 heads, Sumbawa cattle (14,498 heads), goat (33,965 heads), sheep (920 heads).

### **Animal feed production and feed source area**

The land area in East Lombok Regency mostly consists of 75,787 hectares of non-rice fields. Non-rice fields consist of gardens, fields, community forests, grasslands, state forests, and others. In detail, data on land area according to its use in East Lombok Regency is presented in Table 3.

In Table 3, it can be seen that most of the rice fields are irrigated (97%), only about 3% are rainfed. Non-paddy agricultural land used as agricultural land is garden and field covering an area of 23,390 ha. Thus the agricultural land in East Lombok Regency is recorded at around 70,988 ha, consisting of 47,598 ha of rice fields and 18,087 ha of fields/gardens, and 5,303 ha of fields. This agricultural land is a potential land for cattle feed. Apart from agricultural land, a small portion of state forest land, community forests, and plantations can also be used as a source of animal feed.

**Table 3.** Land area according to its use in East Lombok in 2019

Type of land	Land area (ha)
Rice fields	47,598
Irrigation	45,145
Rainfed rice fields	2,453
Non-rice fields	75,787
Garden	18,087
Field	5,303
Farm	3,171
Community forest	1,407
Pasture	679
Not cultivated	14,135
State forest	18,597
Others	14,408
Non-agricultural land	37,169
Total	160,554

Productive land, such as rice fields, gardens, fields, plantations in Sumbawa Regency, covered 155,809 hectares or about 27.49% of this area. Other land consisted of ponds, state forests, and others, reaching 288,323 hectares, about 50.87%. Of all the existing productive land, the area of garden land was 56,351 hectares, exceeding the paddy fields which have an area of 51,588 hectares. The rice field area in 2013 increased by 4,558 hectares compared to 2012 due to the new rice field printing program. The carrying capacity of livestock in an area is largely determined by the availability of feed in that area. The more feed is available in an area, the more livestock can be kept in that area, and vice versa. Animal feed is not only available in the wild such as in fields, forests and mountains, and others but can also be supplied from agricultural land in the form of waste. Agricultural waste can even be a classy and promising source of feed in livestock development. Feed is often an obstacle in itself in Sumbawa Regency, especially related to its availability in the dry season. The relatively dry condition of a small part of the Sumbawa area has resulted in several cows that tend to be thinner due to lack of feed.

The potential of natural forage dry matter in East Lombok Regency in a year can reach 99,486 tons DM/year and Sumbawa Regency of 758,751 tons DM/year. Tubangsa (2018) states that the level of availability of forage fodder in an area is one of the most important factors and also influences population dynamics in the success of livestock development. Livestock capacity is the

Table 4. Land size according to its use in Sumbawa regency

Description	Not planted withrice	Not cultivated	Amount
1. Rice field area	3	1	19,016
a) Technical irrigation	-	-	11,298
b) Semi-technical irrigation	3	3	4,204
c) Simple irrigation	-	-	7,211
d) Village irrigation	-	-	9,859
e) Rainfed	-	-	-
f) Others			
Number of rice fields	6	4	51,588
2. The land area is not rice fields			
a) Garden			60,611
b) Field			17,178
c) Farm			26,496
d) Community forest			88,409
e) Pond			3,029
f) Pool			252
g) Pasture			3,750
h) Not attempted			16,569
i) Others			20,357
Number of non-rice fields			236,651

ability of an administrative area to accommodate the needs of the animal feed at a certain time, such as natural grass danhasil without the processing of agricultural products Tubangsa (2018). The capacity livestock in East Lombok Regency and Sumbawa.

These data illustrate that there is a need for planning in the development of livestock business by adjusting feed resources to the population in each district. Saputra (2016) stated that the addition and development was adjusted to the land ecology and development pattern can be with spatial diversification, namely development on land that already has a designation, among others for food crops and plantations in the form of an integrated pattern, or spatial extensification pattern, namely development on forestry land and weeds.

**Table 5.** Feed production and carryng capacity of East Lombok and Sumbawa

Regency	Feed production (tons BK/year)	Capacity (animal unit)
East Lombok	99,486	29,966
Sumbawa	758,751	228,539

## SWOT analysis

The use of SWOT analysis is intended to take an approach in identifying internal and external factors in planning activities (Rangkuti 2013). This analysis can be used in the establishment of feed ruminants in NTB Province. Internal and external environmental analysis is an analysis of internal and external conditions that affect the efforts to establish a feed ruminant mill in NTB Province. Critical factors from the internal and external analysis can be identified and formulated that come from the research results. Identification of internal factors includes factors of strength and weaknesses, and external factors include factors of opportunities and threats, as described in Table 5.

**Table 5.** SWOT Analysis of establishment of ruminant animal feed mill in NTB

<div>Internal</div> <div>External</div>	<b>S (Strength)</b> <ul style="list-style-type: none"> <li>• Agricultural waste feed resources were large</li> <li>• The distance to the source of feed raw materials is close</li> <li>• Animal husbandry business activities have developed</li> <li>• The availability of food crop wastes was large</li> </ul>	<b>W (Weaknesses)</b> <ul style="list-style-type: none"> <li>• The nutritional quality of food plant waste raw materials is low</li> <li>• Facilities and infrastructure for transportation and storage of raw material for food plant waste are not available</li> <li>• The level of application of food plant waste feed processing technology is low</li> <li>• Production of food crop waste is seasonal</li> </ul>
<b>O (Opportunity)</b> <ul style="list-style-type: none"> <li>• Large number of ruminant livestock population</li> <li>• Support for livestock development in NTB Province</li> <li>• The traditional system of raising livestock is still traditional</li> <li>• Food crop agriculture is getting more intensive</li> <li>• The willingness of the farmer to buy feed produced by the mill</li> </ul>	<b>SO</b> <ul style="list-style-type: none"> <li>• Utilization of animal feed processing technology</li> <li>• Develop an intensive maintenance system</li> <li>• Cooperating with local governments to streamline marketing networks to meet market demand</li> <li>• Optimal use of agricultural waste feed which is abundant</li> </ul>	<b>WO</b> <ul style="list-style-type: none"> <li>• Providing mentoring and training programs to improve the ability of breeders</li> <li>• Introduction of agriculture-based feed management technology by regional conditions</li> <li>• Optimizing self-sufficiency programs</li> <li>• to increase the scale of livestock ownership and increase farmer knowledge</li> </ul>

The farmer agrees to establish a ruminant feed mill Farmers are willing to buy feed produced by the mill.		and increase farmer knowledge
T (Threats)	ST	WT
The ruminant population tends to decline Livestock and meat imports are increasing The ruminant livestock business is still part- time and lacks capital The occurrence of livestock disease and slaughter of productive female livestock Transfer of land status, land conventions, and reduced land carrying capacity	Developing human resource skills to increase the amount of production Establish partnership efforts with several stakeholders	Facilitate the provision of seeds Intensive cattle business development Development of forage land for livestock Monitor livestock diseases and slaughter of productive female livestock

### Opportunities of establishment for feed ruminants

External factors identified as opportunities for the establishment of a feed ruminants in NTB Province are: (1) The number of ruminant livestock populations is high. Data from the BPS of NTB Province in 2019, showed that the total population of beef cattle in NTB Province was 1,242,749 heads, which was the 4th largest cattle population in Indonesia; (2) Support for livestock development in NTB Province. The vision of livestock development in NTB Province is the realization of NTB as a Center for Animal Husbandry Production Towards Industrialization in Improving Community Welfare. Thus, in developing animal husbandry, priority should be optimal use of local resources with minimum dependency on external resources. Food crop wastes are the local feed resources that can be developed as a main sources of animal feeds; (3) The pattern of raising livestock is still traditional. The pattern of raising livestock is still based on the people's livestock business pattern with a traditional maintenance system, which is still relying on the maintenance pattern with the livestock being released, or removing the cage so that the quality of feed obtained by livestock does not allow maximum body weight gain to be achieved; (4) Food crop agriculture is getting more intensive. The increasing intensification of food plants has implications for an increase in the amount of food crop waste products that can be used as feed raw materials;

and (5) The farmer agrees to establish a feed mill and is willing to buy feed produced from the feed mill. This is a big opportunity when setting up a feed mill because the distribution of feed will be purchased by breeders to increase the productivity of the livestock being kept.

### **Threat feed ruminants**

External factors identified as threats posed by the establishment of feed ruminants in NTB Province are: (1) The ruminant population tends to decline. In the last five years (2015 - 2019), the population of buffalo, goats, and sheep in Sumbawa Regency have decreased while the number of cattle population has increased. Meanwhile, in East Lombok Regency, the population of cows, goats, and sheep have decreased. Meanwhile, the buffalo population has increased. The declining population was due to an increase in the number of animals slaughtered; (2) Livestock and meat imports are increasing. To meet the demand for meat, the government issued a policy of importing meat and feeder cattle. This condition shows that there is a limitation in the ability of the pattern of livestock development based on the people's husbandry business to guarantee the availability of meat to meet domestic needs; (3) The ruminant livestock business is still part-time and lacks capital. Animal husbandry is still considered a part-time business so that the time spent for breeders who also work as food crop farmers is reduced. Besides, to increase the business scale by increasing the number of livestock ownership, there are obstacles in terms of capital to buy livestock; (4) The occurrence of livestock disease and slaughter of productive female livestock. The slaughter of productive female animals is still an unsolved problem in the development of ruminants, especially beef cattle; and (5) Changes in land status, land conversions, and decreased land carrying capacity, group resources are still limited, the quality and quantity of production cannot compete with external products.

## **CONCLUSION**

Based on the results of the SWOT analysis, the construction of a ruminant animal feed mill in NTB Province is feasible and technically, production and marketing are very prospective because commercial animal feed are still imported from outside of the NTB Province.

## **AUTHOR CONTRIBUTIONS**

All authors were contributed equally to this work.

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## **Pest Identification and *In Vitro* Control of *Indigofera zollingeriana* Seeds Supports the Development of Forages Crops**

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### **ABSTRACT**

*Indigofera zollingeriana* is a leguminous plant that has high protein content and dry matter digestibility and therefore a potential forage to support more productive ruminants. Effective development and distribution of this nutritious forage is using seeds as planting materials. The objective of this study is to identify types of pests mostly attack on *Indigofera* seeds that inhibit its propagation, and to evaluate the effectiveness of chemical and biological agents as pest control. Identification on pest types was done by trapping the pests, and then observed its type at the zoology laboratory of LIPI Bogor. Test of pest control was conducted in vitro at the Laboratory of Goat Research Station, using completely randomized design consisting of four pest control treatments and five replications. namely: (a) Chemical treatment (active ingredient Deltamethrin), (b) Biological treatment (*Beauveria bassiana* fungus), (c) Biological treatment (*Metarizium anisopliae* fungus), and (d) Water treatment (control). Pests were put in the jars (three heads/jar) and carried out five replicates. Mortality rate was measured for five consecutive days. The results showed that *Nezara viridula*, *Halyomorpha* nr *halys*, and *Tineidae* sp. are the most common pests that attacked *Indigofera zollingeriana* seeds. The highest pest mortality rate (91.67-100%) was due to the chemical treatment on the first day of observation, and the treatment effect on pest mortality was significant. The biological treatment caused more than 50% seed mortality on day three and 100% mortality on the day five. It is concluded that chemical treatment on *Indigofera zollingeriana* seed is more effective to control pests compared to the biological or water treatments.

**Keywords:** *Indigofera zollingeriana*, Seeds, Identification, Pest control, Forage

### **INTRODUCTION**

Livestock is one of the sub-sectors that supports food security through the availability of animal protein. In the livestock business, fodder plants as a source of forage for animal feed are one of the components that determine the success of the livestock sub-sector in Indonesia (Nurhayati 2016). *Indigofera*

*zollingeriana* is one of the feed crops that is highly demanded by farmers. It is a legume plant that has very high nutrients and palatability. The propagation of *Indigofera* is through seeds and one of the active producer of *Indigofera* seeds is the Indonesian Goat Research Station in North Sumatra. Requests are coming from researchers, breeders, entrepreneurs and government agencies and farmers.

The challenge in development of *Indigofera zollingeriana* is the low viability of its seeds as planting materials for propagation. The germination rates in several locations were reported to range from 30 to 50% (Girsang 2012; Hutasoit et al. 2017). This is most likely due to pest attacks on seed pods resulted in seed damaged and rotten. The types of pest that attack the seeds is not clear yet making pest controlling less effective. The pest also could possibly acts as a vector of disease in the newly sprouted seed shoots. Information regarding pests type on *Indigofera* seeds is very limited, therefore, it is important to identify type of pest on *Indigofera* seeds before taking into account for control measure. According to Sophialena (2018), pest control measures of pests can be carried out by chemical and biological controls.

Biological pest control techniques can be carried out using entomopathogenic fungi (Koswanudin & Wahyono 2014). The advantages of biological pest control are having a high production capacity, relatively short life cycle and being able to form spores that are resistant to environmental influences. Biological control can use the fungus *Beauveria bassiana*, which is a control that is cheap, easy to obtain and environmentally friendly. Obtained from entomopathogenic fungi isolated from *B. bassiana* from *Helopeltis* pests. In addition to *B. Bassiana* fungus, *Metarizium anisopliae* is one of the fungi that is also entomopathogenic which can be used to control pests on plants, both insects that attack plants and antagonistic organisms in the soil. This fungus can cause disease when it infects insects, so it can reduce the population of insect pests in an agricultural area (Thungrabeab et al. 2007), these insect pests include uret, pest ladybugs, rice bugs, corn borer, coconut beetle, grasshoppers, leafhoppers. cacao, and many other insect pests, its use is done by spreading fungal spores into insect habitation areas, such as insect breeding areas. The fungus that is spread will then infect the larvae from the mating. This method turns out to be able to produce a high infection rate. This fungus is commonly found in the soil, is saprophytic, and is generally found in various stages of infected insects, growing at a temperature of 18.3-29.5°C and humidity of 30-90%. The pH level for the growth of *Metarhizium anisopliae* ranged from 3.3 to 8.5. Optimal growth occurs at pH 7.

Avoiding *Indigofera* seeds from pests plays an important role in increasing their productivity. There is no many information of pest control in *Indigofera* seeds, so, it needs further research for identifying the types of pests

that attack *Indigofera* seeds, so that they can be controlled by chemical or biological pesticide. The purpose of this study was to identify the types of pests mostly attack *Indigofera zollingeriana* seed and to evaluate the effectiveness of the pest control by chemical and biological treatments.

## MATERIALS AND METHODS

### Study sites

The experiment was carried out at the Indonesian Goat Research Station, Sungai Putih and the Laboratory of the Center for Plant Seed and Plant Protection, Medan, North Sumatra, and the Research Center for Zoological Biology, LIPI, Bogor.

### Experimental design and treatments

#### *Pest preparation*

Pest collection was carried out by trapping the past and by direct catching all pests present in the seed of *Indigofera zollingeriana* planted at the *Indigofera* seed garden area. A pest trap of yellow container filled with soapy water and left for 24 hours was used to trap the insects. The insects were then put into a container containing alcohol solution. Direct catching was performed by taking pests on trees and larvas contained in the seed pods marked by the presence of holes in the pods as an indicator of the presence of caterpillars/pod borers. Once the pods were opened, alive caterpillars were taken and cultured by inserting them into a jar and cover with gauze until they became butterflies. Identification of pest was conducted in the zoology laboratory of LIPI Bogor.

#### *Pest control*

Pest collection was carried out by trapping the past and by direct catching all pests present in the seed of *Indigofera zollingeriana* planted at the *Indigofera* seed garden area. A pest trap of yellow container filled with soapy water and left for 24 hours was used to trap the insects. The insects were then put into a container containing alcohol solution. Direct catching was performed by taking pests on trees and larvas contained in the seed pods marked by the presence of holes in the pods as an indicator of the presence of caterpillars/pod borers. Once the pods were opened, alive caterpillars were taken and cultured by inserting them into a jar and cover with gauze until they became butterflies. Identification of pest was conducted in the zoology laboratory of LIPI Bogor.

## Variable observed

### *Mortality percentage*

Observation of mortality percentage was carried out every day after application. Mortality percentage is calculated using the following formula:

$$MP = \frac{IP}{NO} \times 100\%$$

MP: Mortality percentage

IP: Infected pests

NO: Number of pests observed

## Statistical analysis

Pest identification observations were carried out descriptively. Data were analyzed by making an overview of the collected data. Meanwhile, the pest control treatment used a completely randomized design (CRD) consisting of four pest control treatments, and each treatment consisted of five replications.

The statistical analysis model used is as follows:

$$Y_{ij} = \mu + T_i + E(ij)$$

$Y_{ij}$  = Results of observations / average observations

$\mu$  = Mean value of treatment (pest control)

$T_i$  = Effect of treatment (pest control) tested

$E(ij)$  = Experimental random error, effect of random from - i treatments and replication - j

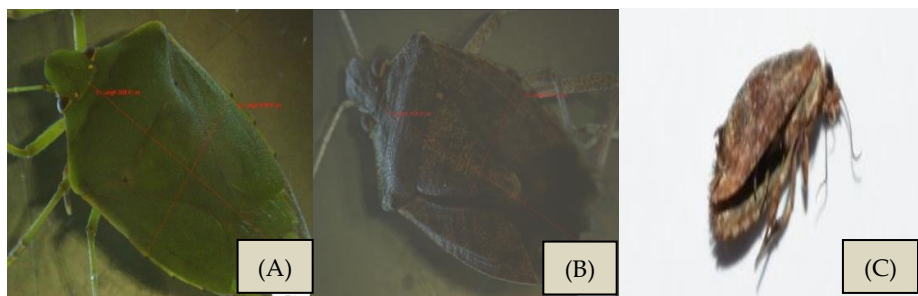
i = 1,2,...i

j = 1,2,3,...j

## RESULTS AND DISCUSSION

### **Pest identification**

Pest profile found in the *Indigofera zollingeriana* seed are shown in Figure 1. The types of pest identified were *Nezara viridula*, *Halyomorpha nr halys*, and *Tineidae* sp. These pests have attacked the seeds by boring the pods and sucking the liquid in the pods, resulting in damaged seeds and decreased production.



(A): *Nezara viridula*; (B): *Halyomorpha nr halys*; (C): *Tineidae* sp.

**Figure 1.** Types of pests on *Indigofera* seeds

### ***Nezara viridula***

The green *Nezara viridula*, also known as the green ladybug, has a pair of toothed antennae. The body shape is flat, has short legs and a head that bends down, has two pairs. The forewings are thickened at the base and at the ends of the membranous. *Nezara viridula* is a pod-sucking pest. Generally, the seeds attack young seeds by sucking the *Indigofera* seed pods causing the pods to dry out so that it interferes with seed development.

### ***Halyomorpha nr halys***

*Halyomorpha nr halys* is a species of brown insect from the *Pteridae* family, also known as the brown ladybug. Irregular light brown spots were scattered all over his body. It is a Hemiptera (insects) with one eye and body length of 16 mm. *Halyomorpha nr halys* is a pod-sucking pest that causes *Indigofera* seed pods to become damaged and dry out.

### ***Tineidae* sp.**

Pest *Tineidae* sp is a pod borer pest that is grayish in color, bent head shape, and a body length of 10 mm. These pests mostly found in caterpillar-shaped pods, eating the entire contents of the pods and a week later turning into flying larvae and laying eggs on the underside of the leaves, causing the seeds in the pods to rot and emit a smelly liquid.

### ***In vitro* pest control**

Effect of several types pest control on the pests of *Nevara viridula*

**Table 1.** Several types of pest control against *Nezara viridula*

Pest control	Observation (days)				
	I	II	III	IV	V
	Mortality (%)				
Deltametrin	91.67 <sup>a</sup>	100.00 <sup>a</sup>	100.00 <sup>a</sup>	100.00 <sup>a</sup>	100.00 <sup>a</sup>
<i>B. bassiana</i>	0.00 <sup>c</sup>	0.00 <sup>d</sup>	25.00 <sup>c</sup>	50.00 <sup>d</sup>	100.00 <sup>a</sup>
<i>M. anisopliae</i>	0.00 <sup>c</sup>	8.33 <sup>c</sup>	66.67 <sup>b</sup>	83.33 <sup>b</sup>	100.00 <sup>a</sup>
Water (control)	11.11 <sup>b</sup>	44.44 <sup>b</sup>	55.56 <sup>b</sup>	55.56 <sup>c</sup>	77.78 <sup>b</sup>

Numbers followed by different letters in the same column are very significantly different at the 1% level according to the DMRT

The effect of treatments on *Nezara viridula* pests is presented in Table 1. The mortality rate of pest was observed for five days. The result showed that there is significantly different on the mortality rate of pest treated with chemical (ingredient Deltamethrin) and biological (*B. bassiana* and *M. anisopliae*) compare to control. The mortality rate of pest treated with both chemical and biological insecticides was 100%, that occurred on the day two and day five of treatment respectively, while the mortality rate of pest of control treatment was 77.78%. The different duration of pest mortality due to *B. bassiana* and *M. anisopliae* fungi needs time to penetrate the insect integument to cause infection and death (Suprayogi et al. 2015; Turnip et al. 2018), while the insecticide Deltamethrin is classified as a contact poison. This result is accordance to the research results conducted by Suprayogi et al. (2015), *M. anisopliae* was more effective than *B. bassiana* in controlling the pest *Nezara viridula* on soybeans with a mortality rate of 94.44% for *Metarhizium* and 77.78% for *Beauveria*.

### *Effect of several types pest control on the pests of Halyomorpha nr halys*

**Table 2.** Several types of pest control against *Halyomorpha nr halys*

Pest control	Observation (days)				
	I	II	III	IV	V
	Mortality (%)				
Deltametrin	100.00 <sup>a</sup>	100.00 <sup>a</sup>	100.00 <sup>a</sup>	100.00 <sup>a</sup>	100.00 <sup>a</sup>
<i>B. bassiana</i>	41.67 <sup>b</sup>	50.00 <sup>b</sup>	58.33 <sup>c</sup>	75.00 <sup>c</sup>	100.00 <sup>a</sup>
<i>M. anisopliae</i>	0.00 <sup>c</sup>	33.33 <sup>c</sup>	83.33 <sup>b</sup>	83.33 <sup>b</sup>	91.67 <sup>b</sup>
Water (control)	0.00 <sup>c</sup>	40.50 <sup>b</sup>	55.56 <sup>c</sup>	60.65 <sup>d</sup>	75.00 <sup>c</sup>

Numbers followed by different letters in the same column are very significantly different at the 1% level according to the DMRT.

Table 2 shows that Deltamethrin was more effective in controlling *Halyomorpha nr halys*. The mortality rate of pest treated by Deltametherin was 100% mortality. This result is significantly different compare to others treatment (*B. bassiana*, *M. anisoliae* and water), those were 41.67, 0, and 0% respectively. According to Adiba (2015) this pesticide is working well if exposed by direct contact with the target. The poison in the pesticide will enter the body tissues of the target organism. Furthermore, there will be disruption of the physiological function of the target organism which results in death. On the fifth day of observation, *B. bassiana* treatment was able to control pests up to 100%, significantly different from *M. anisopliae* treatment and control obtained 91.67 and 75%, respectively. *Halyomorpha nr halys* pest control using the fungus *M. anisopliae* in this study was relatively slow compared to *B. bassiana*. For this type of pesticide it works does not directly kill pests. The pesticide poison after being sprayed will stick to the target and be absorbed into the tissue. Insects can be infected with conidia through the cuticle, or through the gaps between the body segments. The fungus germinates by forming a tube, then enters the host's body and spreads to the haemocoel tissue. (Feng et al. 2004). Furthermore the fungus infects the food tract and respiratory system causing the insects died.

### *Effect of several types pest control on the pests of Tineidae sp.*

**Table 3.** Several types of pest control against *Tineidae* sp.

Pest control	Observation (days)				
	I	II	III	IV	V
	Mortality (%)				
Deltametrin	100.00 <sup>a</sup>	100.00 <sup>a</sup>	100.00 <sup>a</sup>	100.00 <sup>a</sup>	100.00 <sup>a</sup>
<i>B. bassiana</i>	0.00 <sup>b</sup>	0.00 <sup>d</sup>	66.67 <sup>b</sup>	50.00 <sup>c</sup>	91.67 <sup>c</sup>
<i>M. anisopliae</i>	0.00 <sup>b</sup>	41.67 <sup>b</sup>	66.67 <sup>b</sup>	66.67 <sup>b</sup>	91.67 <sup>c</sup>
Water (control)	0.00 <sup>b</sup>	22.22 <sup>c</sup>	66.67 <sup>b</sup>	66.67 <sup>b</sup>	66.67 <sup>b</sup>

Numbers followed by different letters in the same column are very significantly different at the 1% level according to the DMRT

Similar to *Halyomorpha nr halys*, the mortality rate of *Nezara viridula* and *Halyomorpha nr halys* treated with Deltamethrin showed the highest mortality rate. On the first day *Tineidae* sp experienced mortality reaching 100%. While the other treatments were 0%. On the second day of treatment, *M. anisopliae* and water (control) began to show mortality rates of 41.67 and 22.22%, respectively. On the third day the number of uniform deaths in the three treatments (*B. bassiana*, *M. anisopliae* and water) each reached 66.67%. At the end of the fifth day of observation, *B. bassiana* and *M. ansopliae* were able to control pests up to 91.67%,

significantly different from water, which was only able to kill *Tineidae* sp. 66.7%. This indicated that there was no significant difference in mortality rates of *Tineidae* sp. pests due to the administration of *M. anisopliae* and *B. bassiana* fungi. The high mortality in chemical treatment in controlling *Tineidae* sp. pests in this study was most likely due to its broad spectrum which can kill pests in a relatively short time and is very effective at killing various types of insects (Hamim 2015). Although chemical control is more effective, it has been reported to have many negative and adverse effects, because it also suppresses the population of natural enemies of plants and destroys the ecosystem (Pimentel & Burgess 2014). Chemical pest control users should know the chemical and physical properties of pesticides before used. Whereas biologically are slow pesticides, but more friendly to environmental and economic efficiency (Indiati & Marwoto 2017).

## CONCLUSION

*Nezara viridula*, *Halyomorpha nr halys*, and *Tineidae* sp. are the main types of pests identified in *Indigofera zollingeriana* seeds. In vitro study indicated that chemical treatment is more effective to control those types of pests compared to the biological treatments. *M. anisopliae* as biological agent is more effective than *B. bassiana* in controlling the pest.

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Nurul Aini	Dinas Pertanian, Pangan dan Perikanan Kabupaten Sleman
Nurul Hilmiati	Assessment Institute for Agriculture Technology of West Nusa Tenggara
Nurul Hilmiati	Assessment Institute for Agriculture Technology of West Nusa Tenggara
Nurul Huda Aprilianti	Pusat Penelitian dan Pengembangan Perkebunan
Nurul Khaeriyah Amrullah	Airlangga University
Nurul Pratiwi	Indonesian Center for Animal Research
Oktora Dwi Putranti	Universitas Khairun
Oscar Yanuarianto	Faculty of Animal Science Mataram University
Pande Komang Suparyana	University of Mataram
Pitri Indriani	Dinas Pertanian Kabupaten Kuantan Singingi
Pontjo Tri Andajani	Indonesian Centre for Livestock Training Batu
Pradiptya Ayu Harsita	Universitas Jember
Prima Mei Widiyanti	Indonesian Research Center for Veterinary Sciences
Pritha Kartika Sukmasari	Indonesian Beef Cattle Research Station
Priyono	Indonesian Center for Animal Research and Development
Purwaningsih	Universitas Papua
Putri Jelita	Institut Pertanian Bogor

R. Gilar Gautama RA Yeni Widiawati	Ministry of Agriculture Indonesian Research Institute for Animal Production
Rachmad Dharnawan	Assessment Institute for Agricultural Technology of Bali
Rachman Teguh Sukma Julio Rahayu Endang Pujiati	SMP Islam Al Fikri Badan Perencanaan Pembangunan, Penelitian dan Pengembangan Daerah
Rahma Jan Rahmat Hidayat	Universitas Mataram Animal Husbandry, University of Padjadjaran
Rahmawati Elvianora Pulungan	Pusat Penelitian dan Pengembangan Pternakan
Ramlah Fatmawati Letto	Dinas Pertanian, Pternakan dan Perkebunan Kota Palopo
Raphaella Widiastuti	Indonesian Research Center for Veterinary Science
Rapi Wijayatri	Dinas Pertanian dan Ketahanan Pangan DI Yogyakarta
Ratna Ayu Saptati	Indonesian Center for Animal Research and Development
Ratri Retno Ifada Rd. Triana Susanti	BPTP Sulawesi Utara Indonesian Research Institute for Animal Production
Reni Indarwati Reny Debora Tambunan	Balai Besar Pelatihan Pternakan Batu Assessment Institute for Agriculture Technology of Lampung
Reza Mahendra Yudha Permana Rhahma Lukmana	Swasta Dinas Pternakan dan Kesehatan Hewan Kabupaten Lima Puluh Kota Provinsi Sumatra Barat
Rianto Kurniawan	Dinas Perikanan dan Pternakan Kabupaten Garut
Rida Tiffarent	Indonesian Research Center for Veterinary Science
Ridho Kurniawan Rusli Ridwan Winaldi	Universitas Andalas Dinas Perikanan dan Pternakan Kabupaten Bogor
Rina Andriati	Fakultas Pternakan Universitas Mataram

Rini Damayanti	Indonesian Research Centre for Veterinary Science
Rini Nur Hayati	Assessment Institute for Agriculture Technology of Jawa Tengah
Rini Wojayanti	Direktorat Pakan
Rio Adhitya Cesart	Pusat Penelitian dan Pengembangan Pternakan
Risky Chintia Desiane	Balai Pembibitan Ternak Unggul dan Hijauan Pakan Ternak Sembawa
Ristaqul Husna Belgania	Kementerian Pertanian
Risti Ramadhianti	Husbandry Departement Sukabumi District
Rizal Saputra	Personal
Rizka Putri Martani	Faculty of Animal Science Brawijaya University
Rizky Ramadhan	Dinas Perikanan dan Pternakan Kabupaten Bogor
Rizky Wiraswasti Novitasari	Assessment Institute for Agriculture Technology of South Sumatra
Rohmatu Sholikhudin	Dinas Pertanian Kabupaten Tulang Bawang
Roisu Eny Mudawaroch	Universitas Muhammadiyah Purworejo
Romsyah Maryam	Indonesian Research Center for Veterinary Science (IRCVS)
Roy Malindo	Directorate General of Livestock and Animal Health Services MOA
Rr. Agustien Suhardiani	Fakultas Pternakan Universitas Mataram
Rubella Candida Milladiah	Majalah TROBOS Livestock
Ruliansyah	Indonesian Center for Animal Research and Development
Rulita Wijyaningdyah	PT Era Kreasi Agro (eRKA Farm)
Rusli Fidriyanto	Pusat Penelitian Bioteknologi LIPI
Ruth Dameria Haloho	Universitas Quality Berastagi
Ryan Aryadin Putra	University of Mataram
Sabarta Sembiring	Faculty of Animal Husbandry, Nusa Cendana University, Kupang
Saefudin	Pusat Penelitian dan Pengembangan Perkebunan

Saepuloh	Dinas Ketahanan Pangan, Pertanian dan Perikanan
Sahiruddin	Dinas Pertanian Kabupaten Luwu
Saimin	Manunggal Farm
Sajimin	Indonesian Research Institute for Animal production
Salfina Nurdin Ahmad	Assessment Institute for Agriculture Technology of Kepri
Santiananda Arta Asmarasari	Indonesian Research Institute for Animal Production
Sari Suryanah	Universitas Insan Cendekia Mandiri (formerly named Universitas Bandung Raya)
Sari Yanti Hayanti	Assessment Institute for Agriculture Technology of Jambi
Selvie Diana Anis	Faculty of Animal Science, University of Sam Ratulangi
Sepdrian Dwikirana Putra	Indonesian Center for Animal Research and development
Serli Anas	Assessment Institute for Agricultural Technology of Gorontalo
Simon P Ginting	Loka Penelitian Kambing Potong
Sionita Gloriana Gunawan	Assessment Institute for Agriculture Technology of East Kalimantan
Siti Dewi Rindiyani	Indonesian Center for Estate Crops Research and Development
Siti Ma'rifah	Institut Pertanian Bogor
Siti Nadifah Afid	Dinas Pertanian Kabupaten Gresik
Sitti Nurani Sirajuddin	Faculty of Animal Science, Hasanuddin University
Sjaloom Ester Sakul	Fakultas Peternakan Universitas Sam Ratulangi
Slamet Siswoyo	Dinas Ketahanan Pangan dan Pertanian Kabupaten Pekalongan
Sri Darwati	Fakultas Peternakan Institut Pertanian Bogor
Sri Lestari	Dinas Pertanian Kabupaten Gresik
Sri Suryatmiati P	Indonesian Research Center for Veterinary Science
Stanly O.B. Lombogia	Fapet Unsrat
Sudirman	Universitas Samawa

Sugeng Rahayu	Equalindo Farm
Sukarne	University of Mataram
Sukirno	Fakultas Peternakan Universitas Mataram
Sulaiman N Depamede	Faculty of Animal Science, University of Mataram
Sulistiyoningtiyas Irmawanti	Beef Cattle Research Station
Sumiati	Institut Pertanian Bogor
Sumiati	Fakultas Peternakan Unram
Suprayitno	Dinas Tanaman Pangan Hortikultura dan Peternakan Kabupaten Muara Enim Sumatra Selatan
Supriadin	Universitas Teknologi Sumbawa
Supriyadi	Dinas Ketahanan Pangan Perikanan dan Pertanian Kota Tasikmalaya
Suranto	Dinas Pertanian Kabupaten Bandung
Suryo Agung Pranoto	Unit Pelaksana Teknis Dinas Balai Benih Pertanian Provinsi Kepulauan Bangka Belitung
Susan M. Noor	Indonesian Research Center for Veterinary Science
Susana IW Rakhmani	Indonesian Research Institute of Animal Production
Susanti	Indonesian Research Center for Veterinary Science
Sutiastuti Wahyuwardani	Indonesian Research Center for Veterinary Science
Syafaruddin	Indonesian Center for Estate Crops Research and Development
Syahrul Rohman	Dinas Pertanian dan Peternakan Kabupaten Kaur
Syamsuhaidi	Fakultas Peternakan Universitas Mataram
Sylvia Komansilan	Fapet Unsrat Manado
Tahyah Hidjaz	Animal Husbandry Faculty, Mataram University
Tanda Sahat Panjaitan	Assessment Institute for Agriculture Technology of West Nusa Tenggara
Taqiuddin	Faculty of Animal Science, University of Mataram
Tarsono	Universitas Tadulako

Tatang Sopian	Food and Agriculture Service of Purwakarta Regency
Tati Ariyanti	Indonesian Research Center for Veterinary Science
Tedy Dirhamsyah	Indonesian Center for Estate Crops Research and Development
Thaharuddin	Balai Pembibitan Ternak Unggul dan Hijauan Pakan Ternak Indrapuri
Tiara Swastika Putri	Dinas Peternakan Provinsi Jawa Timur
Tike Sartika	Indonesian Research Institute for Animal Production
Tin Tin Ajeng Kartini	Dinas Perikanan dan peternakan Kabupaten Garut
Tiurma Pasaribu	Indonesian Research Institute for Animal Production
Tjeppy D. Soedjana	Indonesian Center for Animal Research and Development
Tjuk Imam Redtiadi	Faculty of Veterinary Medicine Airlangga University
Totok B Julianto	Assessment Institute for Agriculture Technology of West Nusa Tenggara
Totti Tjiptosumirat	Badan Tenaga Nuklir Nasional
Tri Lestari	Bangka Belitung University
Tuti Haryati	Indonesian Research Institute for Animal Production
Ulfi Rahmi	Dinas Peternakan dan Kesehatan Hewan provinsi Sumatra barat
Umar Suryanaga	Balai Uji Terap Tehnik dan Metode Karantina Pertanian
Usman Ali	Ministry of Agriculture RI
Usman Tio Abimanyu	Universitas Brawijaya
Veronica Sri Lestari	Faculty of Animal Science, Hasanuddin University
Wahyu Marmoyojati	IndahFarm
Wahyudi irdas. S. Pt	Dinas Peternakan dan Kesehatan Hewan Provinsi Sumatra Barat
Wandi Gumelar	Sabar Integrated Farming
Wardi	Assessment Institute for Agriculture Technology of Central Sulawesi
Warisnu Anugerahani	PT Petrokimia Gresik

Wayan Wariata	Fakultas Peternakan Universitas Mataram
Wemvi Risyana	Dinas Perikanan dan Peternakan Kabupaten Bogor
Wenny Novita Sari	Universitas Islam Kebangsaan Indonesia (UNIKI)
Widodo	Dislutrannak Kabupaten Batang
Wisri Puastuti	Indonesian Research Institute of Animal Production
Yashanti Berlinda Paradisa	Pusat Penelitian Bioteknologi
Yayi Sumiyati	Dinas Peternakan Kabupaten Sukabumi
Yeni Widyaningrum	Indonesian Beef Cattle Research Station
Yenny Nur Anggraeny	Indonesian Beef Cattle Research Station
Yessy Anastasia	Indonesian Research Center for Veterinary Science
Yohannis L.R Tulung	Sam Ratulangi University
Yuli Eka Setiawan	Universitas Gadjah Mada
Yulinar Firdaus	Indonesian Center for Estate Crops Research and Development
Yulius Duma	Universitas Tabulako
Zaid Al Gifari	Universitas Mataram
Zakia Ulfah	Dinas Peternakan dan Perikanan Kabupaten Magelang
Zikri Maulina Gaznur	Syiah Kuala University
Zul Efendi	Assessment Institute for Agriculture Technology of Bengkulu
Zuratih	Indonesian Center for Animal Research and Development



## Appendix

### Agenda

#### The 4th International Seminar

#### on Livestock Production and Veterinary Technology

Time	Agenda	Speaker	Moderator/ Secretary
Monday, September 6 <sup>th</sup> 2021			
07.00 – 08.30	Room zoom preparation		
08.30 – 09.30	Opening Ceremony		
08.30 – 08.35	Agenda of the opening	MC	Gresy Eva Tresia, S.Pt., M.P.
08.35 – 08.40	The Indonesian National Anthem		
08.40 – 08.45	Praying		Prof. Adji S. Dradjat
08.45 – 09.00	Remarks from Chairman	Director of Indonesian Centre for Animal Research and Development	
	Remarks from Rector of University of Mataram	Rector of University of Mataram	
	Signing of the Cooperation Agreement between Indonesian Centre for Animal Research and Development and Faculty of Animal Science, University of Mataram	Director of Indonesian Centre for Animal Research and Development; and Dean of Faculty of Animal Science, University of Mataram	Hasanatul (Indonesian Centre for Animal Research and Development) Ir. Sumiati, M.P. (University of Mataram)
09.00 – 09.30	Remarks from Director General of IAARD	Director General of Indonesian Agency for Agricultural Research and Development	Director of ICARD
	Keynote speech and Seminar Opening	Minister of Agriculture	DG of IAARD
09.30 – 09.45	<i>Coffee break</i>		
09.45 – 11.15	Plenary Session I		
09.45 – 10.05	Opportunities and Challenges to Increasing Productivity	Prof. Dr. Heather Burrow, University of	Prof. (R) Dr. Ir. Ismeth Inounu, MS / Dr. Susan M Noor

Time	Agenda	Speaker	Moderator/ Secretary
Monday, September 6 <sup>th</sup> 2021			
	in Cattle Farmed by Smallholders in Asia and Africa	New England, Armidale	
10.05 – 10.25	Dietary Mitigation of Enteric Methane Emissions and Animal Production from Ruminants: Plant Tannins Mitigation Options	Dr. Byeng Ryel Min, USDA Agricultural Research Service Conservation and Research Laboratory, Texas, USA	
10.25 – 10.45	Recent advances on our understanding of the pathogenesis of brucellosis	Dr. David O'Callaghan, Universite Montpellier, France	
10.45 – 11.15	Discussion		
11.15 – 13.00	<i>Lunch and Prayer Break</i>		
13.00 – 15.30	Oral Presentation Session I	Committee	
13.00 – 13.10	Breakroom preparation	Committee	
13.10 – 13.15	Opening	Room Moderator	
	<u>Oral Presentation Room 1 (Livestock Production)</u>		
13.15 – 13.25	2789_LP_1	Author	Dr. Atien Priyanti SP, MSc. / Dr. Ir. Dwi Yulistiani, MApp.Sc., Ph.D
13.25 – 13.35	2801_LP_2	Author	
13.35 – 13.45	2790_LP_3	Author	
13.45 – 13.55	2814_LP_4	Author	
13.55 – 14.15	Discussion	Room Moderator	
14.15 – 14.25	2832_LP_5	Author	Dr. Tanda Panjaitan/ Dr. Nurul Hilmianti
14.25 – 14.35	2807_LP_6	Author	
14.35 – 14.45	2805_LP_7	Author	
14.45 – 15.05	Discussion	Room Moderator	
15.05 – 15.10	Housekeeping	Room Moderator	
	<u>Oral Presentation Room 2 (Veterinary Science)</u>		
13.15 – 13.25	2825_VS_1	Author	Dr. Simson Tarigan. MSc. / Dr. Raphaella Widiastuti, BSc.
13.25 – 13.35	2823_VS_2	Author	
13.35 – 13.45	2817_VS_3	Author	
13.45 – 13.55	2783_VS_4	Author	

Time	Agenda	Speaker	Moderator/ Secretary
Monday, September 6 <sup>th</sup> 2021			
13.55 – 14.15	Discussion	Room Moderator	
14.15 – 14.25	2793_VS_5	Author	Prof. Adji S. Dradjat / Dr. Made Sriasih
14.25 – 14.35	2791_VS_6	Author	
14.35 – 14.45	2777_VS_7	Author	
14.45 – 14.55	2840_VS_8	Author	
14.55 – 15.15	Discussion	Room Moderator	
15.15 – 15.20	Housekeeping	Room Moderator	
	<u>Oral Presentation Room 3 (Agricultural Social Economics/Policy)</u>		
13.15 – 13.25	2792_ASE_1	Author	Dr. Yeni Widiawati / Ir. Juniar Sirait, M.Si.
13.25 – 13.35	2770_ASE_2	Author	
13.35 – 13.45	2794_ASE_3	Author	
13.45 – 14.05	Discussion	Room Moderator	
14.05 – 14.15	2798_ASE_4	Author	Dr. Moh. Taquiuddin/ Dwi Kusuma Purnamasari, S.Pt., M.Si.
14.15 – 14.25	2804_ASE_5	Author	
14.25 – 14.35	2765_ASE_6	Author	
14.35 – 14.55	Discussion	Room Moderator	
14.55 – 15.00	Housekeeping	Room Moderator	
	<u>Oral Presentation Room 4 (Livestock Nutrition and Feed Technology)</u>		
13.15 – 13.25	2815_LNF_1	Author	Dr. Bess Tiesnamurti / Dr. Ir. Aryogi, M.P.
13.25 – 13.35	2819_LNF_2	Author	
13.35 – 13.45	2818_LNF_3	Author	
13.45 – 13.55	2774_LNF_4	Author	
13.55 – 14.15	Discussion	Room Moderator	
14.15 – 14.25	2824_LNF_5	Author	Prof. I Ketut Gede Wiryawan/ Sukirno, S.Pt., M.Food.St.
14.25 – 14.35	2782_LNF_6	Author	
14.35 – 14.45	2833_LNF_7	Author	
14.45 – 15.05	Discussion	Room Moderator	
15.05 – 15.10	Housekeeping	Room Moderator	

Tuesday, September 7 <sup>th</sup> 2021			
08.00 - 09.50	Plenary Session II		Prof. Yusuf A Sutaryono / Dr. Made Sriasih
08.00 – 08.20	The Development of Dorper Sheep in Indonesia	Ir. Yudi G. Noor, IPU, Indonesian Sheep and Goat Farmer Association	
08.20 – 08.40	Dairy Production in New Zealand: Production Systems, Marketing and Role of Fonterra	Prof. Stephen Morris, Massey University, New England	
08.40 – 09.00	Moving into More Profitable Beef Production Systems	Prof. Dennis Poppi, University of Queensland, Australia	
09.00 – 09.20	Genomic Resources of Indigenous Chickens Inform the Rapid Improvement of Their Production Efficiency	Prof. Dr. Jianlin Han, Chinese Academy of Agricultural Sciences	
09.20 – 09.50	Discussion		
09.50 – 10.00	<i>Coffee break</i>		
10.00 – 12.10	Oral Presentation Session II	Committee	
10.00 – 10.10	Breakroom preparation	Committee	
10.10 – 10.15	Opening	Room Moderator	
	<u>Oral Presentation Room 1 (Livestock Production)</u>		
10.15 – 10.25	2773_LP_8	Author	Dr. Tike Sartika, MSi. / Lisa Praharani, MSc., Ph.D
10.25 – 10.35	2802_LP_9	Author	
10.35 – 10.45	2800_LP_10	Author	
10.45 – 10.55	2813_LP_11	Author	
10.55 – 11.15	Discussion	Room Moderator	
11.15 – 11.25	2796_LP_12	Author	Prof. Sulaiman Depamede/ Sukirno, S.Pt., M.Food.St.
11.25 – 11.35	2803_LP_13	Author	
11.35 – 11.45	2795_LP_14	Author	
11.45 – 11.55	2882_LP_19	Author	
11.55 – 12.15	Discussion	Room Moderator	
12.15 – 12.20	Closing	Room Moderator	
	<u>Oral Presentation Room 2 (Livestock Nutrition and Feed Technology)</u>		

10.15 – 10.25	2806_LNF_8	Author	Dr. Elizabeth Wina, MSc., Agr. / drh. Rini Damayanti, MSc.
10.25 – 10.35	2767_LNF_9	Author	
10.35 – 10.45	2776_LNF_10	Author	
10.45 – 10.55	2772_LNF_11	Author	
10.55 – 11.15	Discussion	Room Moderator	
11.15 – 11.25	2811_LNF_12	Author	Prof. Adji S. Drajad/ Dwi Kusuma Purnamasari, S.Pt., M.Si.
11.25 – 11.35	2820_LNF_13	Author	
11.35 – 11.45	2799_LNF_14	Author	
11.45 – 11.55	2816_LNF_19	Author	
11.55 – 12.15	Discussion	Room Moderator	
12.15 – 12.20	Closing	Room Moderator	
	<u>Oral Presentation Room 3 (Mix: Livestock Production &amp; Livestock Nutrition and Feed Technology)</u>		
	Session I (Livestock Production)		
10.15 – 10.25	2839_LP_15	Author	Dicky Pamungkas, MSc. / Dr. drh. Eny Martindah, MSc.
10.25 – 10.35	2787_LP_16	Author	
10.35 – 10.45	2852_LP_17	Author	
10.45 – 10.55	2843_LP_18	Author	
10.55 – 11.15	Discussion	Room Moderator	
	Session II (Livestock Nutrition and Feed Technology)		
11.15 – 11.25	2827_LNF_15	Author	Dr. Tanda Panjaitan/Dr. Nurul Hilmiati
11.25 – 11.35	2808_LNF_16	Author	
11.35 – 11.45	2812_LNF_17	Author	
11.45 – 11.55	2844_LNF_18	Author	
11.55 – 12.15	Discussion	Room Moderator	
12.15 – 12.20	Closing	Room Moderator	
12.20 - 13.30	<i>Lunch and Prayer Break</i>		
13.30 - 14.30	Closing Ceremony	Committee	
	Concluding remarks	Chief Editor	
	Best Presenter	Chairman	
	Closing	Director of ICARD / Dean of Faculty of Animal Science, University of Mataram	

# The 4<sup>th</sup> International Seminar on Livestock Production and Veterinary Technology

“TECHNOLOGY INNOVATIONS AND COLLABORATIONS IN LIVESTOCK PRODUCTION FOR SUSTAINABLE FOOD SYSTEMS”

This proceeding contains papers presented at the National Seminar on Animal Husbandry and Veterinary Technology on September 6-7, 2021. The seminar's theme is "Technology Innovations and Collaborations in Livestock Production for Sustainable Food Systems", held by the Indonesian Center for Animal Research and Development and the Faculty of Animal Science, University of Mataram. During this seminar, several new topics were presented and discussed which will contribute to livestock development in the future. This seminar's various issues are divided into four topics: Livestock Production, Veterinary Science, Livestock Nutrition & Feed Technology, and Agricultural Social Economics & Policy.

In the field of Agricultural Social Economics and Policy, topics are presented on: Improving the quality of agricultural, human resources that can increase work productivity and welfare; Farmer cooperative group which is an institutional model of farmer group economic cooperation with an agribusiness orientation through land consolidation into one stretch; The application of digitalization to improve the position of farmers; food security is achieved by increasing the efficiency of livestock and industrial businesses in line with the increasing global population. Climate change requires livestock in the tropics to tolerate some of the challenges of increasing their productivity and reducing greenhouse gas emissions; Reducing CH<sub>4</sub> emissions will reduce greenhouse gas emissions and increase the efficiency of animal production. Strategies for dietary feeding modification include a tannin-rich diet, the dairy industry largely under a pastoral-based production system and cooperative industrial structure, the development of reliable genomic markers to monitor the impact and efficiency of genetic improvement for native chickens.

In the topic of Veterinary Science, the control of human brucellosis depends on controlling its source: animal disease; Inventions in animal husbandry and animal health which are intended to support the food system, especially from the livestock sub-sector that will improve the farmer welfare.

The livestock Production topic discussed crossbreeding between Dorper sheep and local Garut sheep in Livestock Production. At the same time, in the topic of Livestock Nutrition and feed Technology discussed the lowest cost ration formulation system (LCR) seeks a way out of poverty for small cattle farmers in Indonesia



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