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The Role of Saponin as Feed Additive for Sustainable Poultry Production

(Peran Saponin sebagai Pakan Imbuhan dalam Produksi Unggas secara Berkelanjutan)

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ABSTRACT

Poultry, especially broiler is an important protein source for people in developing countries. Under intensive condition, economical and efficient broiler production is disturbed by disease attack due to poor biosecurity management. Feed additives (coccidiostat and antibiotic growth promoter/AGP) that added to feed for disease prevention and growth improvement, have been banned by many countries including Indonesia. Antibiotic growth promoter can be substituted by bioactive compounds in certain plants which can improve poultry production. One of bioactive compounds is saponin which is a glycoside. This paper provides information about source and function of saponin as feed additive for poultry production. Saponin extracts or saponin containing plants have several functions, such as anticoccidia, immunostimulant, antibacteria and antifungi. Sustainable poultry production requires continues supply of feed ingredients and additive with consistent in quality. High saponin-containing plants need to be planted widely and supported by stakeholders for their avability and sustainability.

Key words: Saponin, poultry, function, feed additive

ABSTRAK

Unggas terutama ayam pedaging merupakan sumber protein penting bagi banyak orang di negara berkembang. Dalam kondisi intensif, produksi ayam pedaging yang ekonomis dan efisien dapat terganggu oleh serangan penyakit karena biosekuriti yang kurang baik. Bahan imbuhan pakan (*coccidiostat* dan *antibiotic growth promoter*/AGP) yang ditambahkan ke dalam pakan untuk mencegah penyakit dan meningkatkan pertumbuhan telah dilarang oleh banyak negara termasuk Indonesia. *Antibiotic growth promoter* dapat digantikan oleh senyawa bioaktif dari tanaman tertentu yang berfungsi untuk meningkatkan produksi unggas. Salah satu senyawa bioaktif tersebut adalah saponin yang merupakan senyawa glikosidik. Makalah ini memberikan beberapa informasi tentang sumber dan fungsi saponin sebagai imbuhan pakan untuk produksi unggas. Ekstrak saponin atau tanaman yang mengandung saponin memiliki beberapa fungsi, diantaranya sebagai *anticoccidia*, immunostimulan, antibakteri dan antijamur. Produksi unggas yang berkelanjutan membutuhkan suplai bahan dan imbuhan pakan yang konsisten kualitasnya dan terjamin ketersediaannya. Tanaman yang mengandung saponin tinggi perlu dikembangkan secara luas dengan dukungan para pihak terkait untuk menjamin ketersediaannya secara berkelanjutan.

Kata kunci: Saponin, unggas, fungsi, imbuhan pakan

INTRODUCTION

Demand on animal protein for human consumption has been growing significantly in Asian countries in the last decade following an increase of income and change of lifestyle to consume more convenience food. Most of animal protein especially meat would be derived from swine and poultry production especially broiler. Increase in poultry production, however, faces several challenges including diseases and environment especially high temperature and humidity in tropical countries. In order to achieve optimum production and efficiency, poultry diseases have to be controlled or prevented through vaccination, medical treatment and feed additives. Poultry farming, raised in unhygienic condition is exposed to microbial challenges from the environment, including litter and water. It is a common practice in broiler industries that feed additives have been used to control coccidiosis and other possible diseases such as necrotic enteritis or colibacillosis. Feed additives in the form of coccidiostat and antibiotic growth promoter (AGP) have been added in the broiler feed to maintain intestinal integrity and hence improve efficiency of nutrient utilization and finally improve animal health (Page 2005). It has been well demonstrated that addition of coccidiostat and AGP would result in economic benefit for poultry production and improve animal health including immunity of animal.

The use of antibiotics in animal feeding, however, put several concerns especially in relation to food safety (Paganini 2005). Concern on possible residue of antibiotics in animal products and may cause resistance to antibiotic use in human, resulted in request by many consumers to the government to review the use of AGP in animal feed. In 2006, European Union started to ban the use of AGP in animal feed, however, antibiotics are used only for disease treatment under prescription. Several meat exporting countries to Europe have also been requested not to use AGP during poultry production. Many countries start to review the use AGP in animal production and many food chain companies in US also start to request broiler meat to be free of antibiotic. Several countries, especially Scandinavian, have strict regulation on the use of antibiotics as feed additives in animal feed since the concept of clean, green and ethical animal production is being promoted (Kostadinovic et al. 2015). Indonesia has also a regulation on the ban of AGP use since 2009 and it is now implemented in the new law (UU 41, 2014 on Livestock and Animal Health). Sustainable poultry production urgently needs the alternatives of AGP to be added in poultry feed. Many researchs have been directed towards finding alternative AGP substances that have similar ability to improve gut health and integrity. AGP replacement may be achieved by using probiotics, enzymes, acidifiers, plant extracts etc.

Several plants have been discovered to contain chemical substances that are able to inhibit bacterial growth in the intestine and may be used as replacement to AGP (Windisch et al. 2008). These potential plants should also be available abundantly to provide sustainable supply for the users. Certain plants can synthesize secondary components during their growth and those substances are used as “self defense” against pathogen. Many medicinal plants have been used historically for medicinal purposes for human medicine and those substances found in the plants may be able to inhibit pathogen or bacterial growth in living organism. Natural substances from plants have been considered “safer” rather than antibiotic.

There are thousands of secondary components found in nature especially in the tropics and many of the components have been used for different purposes of human but little information has been found for animal application especially for AGP replacement. Secondary components that can be used for animal medicine include tannin, free amino acids, flavonoids, phenolics, alkaloids, essential oil, enzyme inhibitors, etc (Rochfort et al. 2008; Windisch et al. 2008; Magdalena et al. 2013). Among the secondary components found in tropical plants, one of them is saponin that has been reported to have different functions for animal production (Cheeke 2009; Wina 2012). Several reviews have been published on the role of secondary metabolites as antimicrobia, antivirus (Liu & Du 2012; Pistelli & Giorgi 2012) in poultry (Kostadinovic et al. 2015) or ruminants (Patra & Saxena 2009; Wina 2012). This paper describes latest information on mechanism and application of saponin or saponin containing plants or plant parts to enhance poultry production.

STRUCTURE AND SOURCE SAPONIN

Saponins are diverse family of plant secondary metabolites. Basically, saponin consists of an aglycone (sapogenin) attached with one or more sugar moieties. Based on the structure of its aglycone, saponin is classified into triterpene saponin and steroidal saponin. Considering the diversity of saponin, Vincken et al. (2007) suggested a new classification of saponin consisting of 11 main classes. Diversity also occurs in one location of the plants. Several saponins that have been found in one plant usually have the same aglycone with different sugar moieties. Asao et al. (2009) isolated 20 types of saponin in *Sapindus rarak* fruit pericarp which has the same aglycone, hederagenin.

The word “sapon” means “foam”, which indicates that saponin exhibits foaming property. The foam was formed when the hydrophilic part of saponin was pulled into the water layer causing a reduced surface tension and later form bubbles. Table 1 shows several plants that found in Indonesia contain saponins. The saponin was detected in different parts of plant that have foaming property and exhibit haemolytic activity. The highest foam volume indicates the high saponin content in the plant part or plant materials. Eventhough it is not linier relationship between foam volume and haemolysis activity, there is an indication that the higher the foam volume, the higher activity of saponin causing blood haemolysis. It is very limited data on saponin isolation and identification from plants or plant part found in Indonesia. The presence of saponin mainly came from the qualitatively test for saponin and the quantitative measurement was only for total saponin content. Table 2 shows that each plant or plant part has different name of saponin; the aglycone (the core) of saponin can be in steroid or triterpene structure with different type and number of sugar attached to the aglycone. The highest concentration of saponin was found in tea leaves which has been extracted and semipurified. Saponin concentration varied in *S. rarak* fruit pericarp depended on the particle size of dried *S. rarak* fruit pericarp. The highest saponin concentration was found in the smallest particle size of *S. rarak* fruit pericarp, as reported by Pasaribu et al. (2014a).

Table 1. Foam volume and minimum concentration of plant samples that caused haemolysis

Plant name	Local Indonesian name	Plant part	Foam volume (ml)*	Minimum concentration of saponin extract causing haemolysis (µg/ml)
Albizia saponaria	*Langir/Merbuan*	Leaves	0.83	57.36
Albizia saponaria	*Langir/Merbuan*	Bark	22.33	1.51
Averrhoa bilimbi	*Belimbing wuluh*	Leaves	2.25	39.02
Dioscorea bulbifera	*Gembolo*	Fruit	1.67	27.50
Enterolobium cyclocarpum	*Sengon buto*	Leaves	8.83	1.10
Enterolobium cyclocarpum	*Sengon buto*	Bark	17.50	0.42
Garcinia mangostana	*Manggis*	Pericarp	1.25	13.16
Malvavicus arboreus	*Kembang wera*	Leaves	1.17	50.37
Morinda citrifolia	*Mengkudu*	Fruit	2.42	118.60
Sapindus rarak	*Lerak*	Fruit pericarp	29.17	0.57

*The weight of sample in water was 0.5 g in 10 ml of water

Source: Wina (unpublished)

Table 2. Type and concentration of saponin in some plants

Name of plants	Plant part	Type of saponin	Concentration of total saponin	Reference
Sapindus rarak	Fruit pericarp	Hederagenin (aglycone), rarak saponin, mukurozi-saponin	21.70-43.52%	Asao et al. (2009); Pasaribu et al. (2014a)
Camellia sinensis	Seed, leaf	Theasaponin/camelliasaponin	72% from semi purified tea extract	Matsui et al. (2009)
Morinda citrifolia	Fruit	Triterpene saponin	0.69%	Rahmani et al. (2014)
Moringa oleifera	Leaf	Saponin	2.46-3.42%	Stevens et al. (2015)

SAPONIN EFFECT ON POULTRY PRODUCTION

Saponin in the plant material has several functions when fed to poultry. These functions will be beneficial for chicken itself or for human who consume the meat.

Saponin as anticoccidial agent

One of the common diseases occur in poultry is coccidiosis. It is caused by protozoa belongs to the genus *Eimeria* which can infect in various sites of intestine and caecum. There are many *Eimeria* have been identified in different poultry and present in different part of intestine. The most pathogenic strains that found in chicken are *Eimeria tenella* and *Eimeria necatrix*. It has been reported by many authors that several bioactive containing plants exhibit anticoccidial properties (Kostadinovic et al. 2015). Saponin as one of bioactive compounds reduced significantly protozoa in rumen (Wina 2005), therefore, it is expected that saponin may also reduce *Eimeria* sp in poultry digestive tract. The preliminary information on the effect of saponin containing plants found in Indonesia on *Eimeria* spoocytst in the *in vitro* test was reported by Rahmani et al. (2014). Table 3 shows the effectiveness of some plants extract to damage *E. tenella* oocyte may be related to their total saponin content. When saponin content was high, the percentage of oocyte damaged was also higher. More work is required to explore more saponin containing plants that consistently effective to destroy the oocyte of *E. tenella*.

In vitro experiment showed that the aqueous extract of *S. rarak* fruit pericarp (≥1 mg/ml) caused 90% of sporocysts become abnormal, hence, oocyst became inactive and cannot spread the disease of coccidiosis (Pasaribu et al. 2014a). Further *in vivo* experiment showed that the oocysts shed per gram faeces (OPG) of *Eimeria* sp infected chicken fed with *S. rarak* fruit pericarp were also reduced (Pasaribu et al. 2014b). Saponin containing extract of *M. citrifolia* leaves fed (200 mg/kg BW) to *Eimeria* sp infected chicken, reduced the incidence of coccidiosis, indicated by reduced haemorraghic in the caecum and lower OPG (Karimy et al. 2013). Lower oocysts shed per gram faeces of *Eimeria* sp was previously reported by Hassan et al. (2008) when fed 5% saponin containing guar meal to broiler challenged with *E. tenella*.

Table 3. Total saponin content in some plants extract and its activity on oocytes of *E. tenella*

Leaf extract (1 mg/ml water)	Indonesian name	Total saponin	Oocyte *E. tenella* damage
		------- % -------	
Anredera cordifolia	*Binahong*	3.24	36
Morinda citrifolia	*Mengkudu*	0.69	13
Malvaviscus arboreus	*Wera*	0.73	14

Source: Rahmani et al. (2014)

The mechanism of saponin reducing the number of oocysts may be different from its mechanism to reduce protozoa in the rumen. Saponin lysed the ruminal protozoa by directly binding the outer membrane of protozoa. However, *E. tenella* oocyst's wall consists of two layers that cannot be destroyed by saponin. The inner layer is mainly composed by β-1,3-glucan (Bushkin et al. 2013) while the outer layer contains 90% protein with high level of dityrosine bonds (Mai et al. 2009) and acid fast-lipid (Bushkin et al. 2013). The oocyst's wall is very strong and resistant due to its acid fast-lipid. Oocyst has a micropyle cap that presents at the polar end of oocyst and gap that exists between cap and oocyst's wall as reported by Wiedmer et al. (2011). It was assumed that saponin did not destroy the oocyst's wall but entered the wall through the micropyle cap or gap and directly disturbed the sporocyst (Pasaribu et al 2014a).

Saponin as immunostimulant

One of the significant roles of saponin is its use as adjuvant in vaccines (Sun et al. 2009). Saponin has been used in several vaccines as adjuvant to increase immunogenicity of an antigen resulting in increased antibodies and cytotoxic of T-lymphocytes, hence improved the efficacy of vaccine. Saponin based adjuvants that were isolated and purified from *Quillaja saponaria* Molina bark was Quil A and its derivatives QS21 (Sun et al. 2009). However, the use of Quil A as adjuvant for vaccine that injected intramuscularly may be limited because it has high haemolytic activity. Therefore, efforts to look for low haemolytic saponin have been emphasized for its use as adjuvant. Saponin extracted from the seed of *Momordica cochinchinensis* (Lour) *Spreng* (ECMS) has low haemolytic activity and its inclusion into New Castle disease (ND) vaccine enhanced the serum IgG responses without showing any adverse effect on growth of chicken (Xiao et al. 2009).

The response of enhancing immune response was also reported when saponin from stem-leaf ginseng added to drinking water (5 mg/kg BW) and given to ND vaccinated chicken (Zhai et al. 2009). Hemagglutination inhibition (HI) titers, immunoglobulin A^+ cells and intraepithelial lymphocytes in the intestinal mucosal increased due to saponin addition (Zhai et al. 2009). Not only to ND vaccinated chicken, Zhai et al. (2014) also gave the same saponin to young chicken one week before vaccinated with live infectious bursal disease (IBD) vaccine. As a result, IBDV-specific antibody titer, the number of IgA^+ cells and intestinal intraepithelial lymphocytes in all parts of intestine were significantly higher than control without saponin administration. Immunostimulant of saponin was also shown by the increase of IgG and IgY when ginseng leaf and stem and *S. rarak* fruit pericarp were given to chicken, respectively (Pasaribu et al. 2014b; Park et al. 2015). In the form of complex with antigen and lipid and called Immunostimulating complex (ISCOM), saponin induced antibody responses or protective immunity in several animals (Rajput et al. 2007).

Saponin effect on poultry performance

Recently, several reports on the effect of saponin on body weight gain of poultry have been published (Alfaro et al. 2007; Cheeke 2009; Park et al. 2015).

Table 4 shows that saponin or saponin containing plant materials can provide higher growth rate than control, but when the birds were challenged with *Eimeria* sp, the positive effect of saponin did not appear. Cheeke (2009) explained that the improvements of chicken performance may be due to the increased villus height and crypt depth in the chicken fed saponin containing *Yucca schidigera* extract. A mixture of saponin contaning plants (yucca and quillaja) enriched with quillaja polyphenol caused improvement in body weight gain, feed conversion ratio that were similar to those treated with antibiotic growth promoter suggesting that this saponin mixture can serve as a replacement for antibiotic growth promoter (Cheeke 2009). Different type of saponin makes difficult to determine the best level of inclusion in the feed, therefore, the maximum level of inclusion of each saponin given to chicken should be carefully tested since the higher inclusion may give adverse effect on chicken performance.

Table 4. Effect of saponin or saponin containing plant material on poultry performance

Source of saponin	Level in the diet	Poultry	Effect	Reference
Tea saponin	0; 25; 50; and 75 mg/kg feed	Male broiler	Weight gain increased in dose dependent. Weight gain of chicken fed 75 mg/kg feed increased 25.9% than control	Miah et al. (2004)
Ginseng leaves and stem	0.40%	Broiler chicken	16.6% higher growth rate than control treatment	Park et al. (2015)
Yucca schidigera extract	0.01%	Broiler	Improved daily gain and FCR	Alfaro et al. (2007)
Quillaja saponaria saponin	0.01%	Broiler challenged with *Eimeria* mixture	Body weight gain is similar between treatment with control	Scheurer et al. (2013)
Sapindus rarak fruit pericarp	0.125-0.25%	Broiler challenged with *E. tenella*	Body weight gain was not significantly different	Pasaribu et al. (2014b)

Saponin as triglyceride and cholesterol reducing agent

Triglyceride and cholesterol are two compounds that become the major concern related to human health, and many people will avoid consuming high content of triglyceride (fat) and cholesterol in their food including meat. Saponin has been reported to reduce cholesterol in the meat or blood. The use of *S. rarak* fruit pericarp that contains high saponin content reduced triglyceride and cholesterol in chicken blood plasma in dose dependent manner (125-500 mg/100 g diet) (Pasaribu et al. 2014b). Not only total cholesterol, the more harmful LDL-cholesterol in blood plasma was also reduced. Park & Kim (2016) also reported reduced total cholesterol and LDL-cholesterol was observed in broiler fed 0.1% fenugreek (*Trigonella foenum* graecum L) seed extract which contained steroidal saponin. Addition of Karaya (*Sterculia urens*) saponin was reported to reduce not only cholesterol in serum and liver but also in egg yolk. Bile acid concentration in the liver and concentrations of cholesterol, tricaylglycerol and bile acid in faeces increased with the addition of karaya saponin into layer diet (75 mg/kg diet) (Afrose et al. 2010).

The mechanism of saponin to reduce cholesterol has been published and recently, Shi et al. (2014) reported the mechanism of saponin extract lowering cholesterol at the gene level. Shi et al. (2014) showed that alfalfa saponin extract (ASE) administration inhibited gene expression of two key enzymes of cholesterol biosynthesis, 3-Hydroxy-3-methylglutaryl CoA reductase (Hmgcr), acyl-CoA: Cholesterol O-acyltransferase 2 (Acat2) suggesting that saponin inhibited cholesterol biosynthesis. Besides, ASE increased the cholesterol 7-alpha-hydroxylase (CYP7A1) expression, which enhanced the catabolic pathway of cholesterol. It also increased low-density lipoprotein receptor (LDLR) expression or activity, resulting in the reduction of serum LDL cholesterol level. Shi et al. (2014) concluded that saponin could regulate cholesterol metabolism by inhibiting the synthesis pathway of cholesterol, enhancing the catabolic pathway of cholesterol and enhancing the uptake of LDL-cholesterol.

Saponin as antifungal agent

In humid tropical countries, mycotoxin producing fungi grow very easily in food or feed ingredient. Several mycotoxins such as aflatoxin, fumonisin, zearalenone cause negative effect on animal production. Antifungal agent is usually added in the animal feed to prevent the growth of mycotoxin producing fungi. Several steroidal saponins isolated from *Capsicum frutescens* can inhibit the growth of germinated *Aspergillus flavus*, *A. fumigatus*, and *A. niger* but not *Fusarium moniliforme* and *F. oxysporum* (De Lucca et al. 2006). The mechanism of saponin inhibit the fungi was through its binding with steroids that present in the fungal membrane and these saponin-steroid complexes caused a loss of cytoplasmic constituents, leading to cell death (De Lucca et al. 2006; Cho et al. 2013). However, its antifungal activity was determined by the sugar moiety. When saponin lost one glucose moiety, its activity against fungi would be significantly reduced. The lost of glucose can occur when saponin is hydrolyzed by enzyme excreted by microbes. Jiang et al. (2015) observed a different mechanism of saponin inhibited fungal growth by inhibiting the mycelial growth of fungi *Rhizopus stolonifer*. Saponin increase H_2O_2 production and resulted in membrane lipid peroxidation, hence increase cell membrane permeability and cause leakage in the cell membrane, hence inhibit fungal growth.

Saponin as antibacterial agent

Not only it has antifungal property, saponin also shows antibacterial activity. It may be interact in some ways with bacterial cell wall and caused disruption of bacterial cell membrane (Ray et al. 2013). It was assumed the aglycone part of the saponin is the important factor for antibacterial property. Saponin in the 100% methanol fraction of guar meal was reported to be most effective against *Staphylococcus aureus*, *Salmonella typhimurium* and *Escherichia coli* bacteria (Hassan et al. 2008). Saponin rich fractions isolated from *Gysophila* were also reported to have antioxidant activity based on free radical scavenging activity (Arslan & Çelik 2013). *Y. schidigera* extract displayed high effectiveness in reducing invasion of *E. coli* O157:H7 adhesion to HeLa cells. The presence of extract in some way modified the cell membrane function to prevent bacterial entry. Saponin in the extract possibly interacted with the cholesterol molecules in the cell membrane and consequently disturbed the cell membrane organization and interrupt the interaction of membrane receptors and pathogens (Johson 2013).

Saponin as antioxidant

Feed rich in fat especially in highly unsaturated fatty acids may go autoxidation and become rancid. The rancidity may cause refusal to poultry to consume feed and or reduce nutrient content and cause detrimental to poultry. Many feedmills may use an antioxidant as feed additive to prevent autoxidation of feed and the antioxidant are usually derived from chemicals such as ethoxyquin, Butylated Hydroxy Anisole or Butylated Hydroxy Toluene. Many phenolic compounds from plant materials were reported to have antioxidant activity including some saponins. Tritepenoid saponin extracted from *Xanthoceras sorbifolia* nutshell inhibited tyrosinase activity, had higher scavenging effect than vitamin C suggesting that this saponin extract had high antioxidant activity (Zhang & Zhou 2013). Addition of alfalfa saponin extract to the diet (5-15 g/kg diet) increased Glutathione peroxidase, Superoxide dismutase activities in serum, liver and muscle in dose dependent mannner suggesting that saponin promoted antioxidant activity in the body to scavenge free radicals and prevent the action of lipid peroxidation (Shi et al. 2014).

SAPONIN CONTAINING FEED ADDITIVE FOR INDONESIAN POULTRY PRODUCTION

Sustainable poultry production in Indonesia requires consistent quality and continuous supply of feed and feed additives. The implementation of law to prohibit the use of AGP in animal diet in Indonesia enforces feed industries to use plant sources as feed additive in animal diet. Many potential saponin containing plants such as *S. rarak*, *M. citrifolia* are found in Indonesia, however, the availability of these plants in large amount is limited. Therefore, it requires a good collaboration among farmers, local government and private industries to allocate a special land for growing and developing those potential saponin containing plants as locally natural feed additives. Research on combination of different plants or plant extracts as natural feed additives should be supported so that Indonesia can be self sufficient in feed additives for poultry industries.

CONCLUSION

Experiments have shown that saponin extracts or saponin containing plants can be used as alternative feed additives especially coccidiostat and antibiotic growth promoter (AGP) in poultry production. Beside the function as coccidiostat and AGP, saponins have also different functions in animal production including immunostimulant for animal health, inhibit the fungus growth and have antioxidant properties. However, it has to bear in mind that efficacy of saponin would depend on the level of application to the feed. Higher application may cause negative effect while lower level may reduce its efficacy. Further research is warranted if saponin would be used as feed additive in commercial scale to provide economic benefit to poultry and may be to other animal productions.

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