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To cite this article: Y N Anggraeny *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **648** 012191

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# Evaluation of the use of plant organic components and probiotics on ruminal characteristics and as a decrease of methane

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**Abstract.** The reduction of CH<sub>4</sub> from the digestive tract of ruminants can be done through the use of organic components of plants such as tannins and saponins and the use of probiotics. This study aims to evaluate the addition of organic components and probiotics to the characteristics of rumen fluid and its ability to reduce CH<sub>4</sub> in Ongole Cross Breed (PO) cattle. Sengon (*Paraserianthes falcataria*) leaf meal and Trembesi (*Samanea saman*) leaf meal are used as organic components due to their tannin and saponin content. Probiotics contain *Acetoanaerobium notarae* and *Saccharomyces cerevisiae*. This research used total mixed ration as a feed. A total of 24 heads PO cattle were divided into 4 treatments, ie T1 = control treatment; T2 = T1 + organic components, T3 = T1 + Probiotics and T4 = T1 + organic components + probiotics. The research design was a randomized block design. The combination treatment of the addition of organic components and probiotic caused a decrease in the ratio of acetic acid to propionic acid (C<sub>2</sub>:C<sub>3</sub>), percentage of acetic acid, the concentration of CO<sub>2</sub> and CH<sub>4</sub>, but increases the percentage of propionate acid. The combination of organic components and probiotics is the greatest decrease in CH<sub>4</sub> production from enteric fermentation.

## 1. Introduction

Animal production activities are estimated to contribute 12% to total greenhouse gas emissions [1,2]. The production of methane gas in ruminants is formed from the degradation of organic macromolecules of feed material through anaerobic digestion processes [3]. The methanogenic process or the formation of CH<sub>4</sub> gas is a natural process in the digestive tract of ruminants, especially in the rumen by methanogenic archaea. Methane is a part of the digested energy, which is 15% to 18%, but methane cannot be used as energy for livestock production, so it is a waste of energy [4], whereas Cottle *et al.* [5] stated that around 8% to 14% of the total digested energy was lost in the form of CH<sub>4</sub>, while Jayanegara *et al.* [6] stated that 6 to 10% of the gross energy feed consumed by ruminants was lost as CH<sub>4</sub>.

Some efforts to reduce CH<sub>4</sub> gas emissions in ruminants are through concentrate supplementation [7], the addition of fat [8], organic acids [9] the use of methane inhibitors such as antibiotic compounds including monensin and rumensin [10] and the addition of natural compounds such as tannins and saponins [6,11] and manipulation of rumen microbes [12].

Saponins can be used to reduce protozoa but are selective against microbial inhibition [11]. Tannins can inhibit the growth of methanogenesis bacteria through the availability of hydrogen but tannins can bind to simple molecules in feed, especially in feed-in tropical areas which have low protein and dissolved organic matter content, so the use of tannins as a methane-lowering agent must be limited [13].



Microbial manipulation in the rumen is through the use of probiotics which can inhibit the growth of methanogenic bacteria [12]. *Acetoanaerobium notarae* is homo acetogenic bacteria. It is users of hydrogen and carbon dioxide synthesized into acetic acid, competitive in the use of growing media with methanogenic bacteria that use hydrogen and carbon dioxide to be synthesized into methane gas [14]. The presence of yeast cells could stimulate the use of hydrogen by acetogens and enhance acetogenesis in an experiment utilizing a co-culture of acetogen and methanogen [15]. The addition of yeast culture to sheep feed was able to reduce methane gas production in sheep [16]. *Saccharomyces* is a yeast culture which is commonly used as a probiotic in feed and food. *Saccharomyces cerevisiae* is a probiotic microorganism which rich in enzymes, vitamins and other important cofactors [17]. Chaucheyras-Durand *et al.* [15] and Yang *et al.* [18] showed that yeast cells could increase the utilization of hydrogen by acetogenic bacteria and increase acetogenesis. This is thought to be because yeast cells produce essential nutrients and vitamins needed by rumen microbes.

Based on the combination of the use of plant organic components in the form of saponins and tannins and the use of methane-lowering probiotic microorganisms, it is necessary to do so that methane production can be maximally reduced. Information on the effect of using methane-lowering agents in the form of tannins-saponins and probiotics together in cattle has not been widely informed so it is necessary to research to obtain feed supplements that can reduce the production of natural methane gas based on organic components and probiotics, especially in the feed based on local feed. This study was to examine the effects of the addition of organic components (saponins and tannin) from sengon (*Paraserianthes falcataria*) [1] leaf meal and trembesi (*Samanea saman*) leaf meal and probiotics with the microorganism *A notarae* and *S cerevisiae* on rumen fermentation of Ongole Crossed cattle.

## 2. Materials and methods

### 2.1. Production of sengon leaf meal and trembesi leaf meal

Fresh *P falcataria* and *S saman* leaves were oven-dried at 40°C for 48 to 72 hours. The dried Sengon and Trembesi leaves are then milled using a Willey mill with a size of 0.75 mm to be used as part of the organic components and used for the analysis of saponins, tannins and nutritional content.

### 2.2. Production of dry probiotics

Pure isolate *A notarae* was obtained from the Indonesian Research Institute for Animal Production (IRIAP) in Ciawi, West Java. Production of probiotic *A notarae* using commercial media of coconut water and rumen fluid was 85% (v / v) and 10% (v / v), respectively. Coconut water and rumen fluid are sterilized at 121°C for 15 minutes using an autoclave. After reaching a temperature of 39°C, sterile coconut water and rumen fluid are put into a sterile plastic gallon as an incubation site, then bubbling CO<sub>2</sub> in a sterile manner to create anaerobic conditions for 30 minutes. As much as 5% (v / v) of *A notarae* inoculum was added to sterile commercial media and then incubated at 39°C for 14 days. The preparation of dry probiotic *A notarae* was done by adding 40% (w / v) of cassava flour that has been sterilized using an oven at 80°C for 24 hours. *A notarae* probiotic drying was carried out using a vacuum oven for 48 to 72 hours at a temperature of 50°C. After drying, the probiotic *A notarae* was ground and then stored. in the refrigerator before use.

Pure isolate *Saccharomyces cerevisiae* was obtained from Gadjah Mada University. Making *Saccharomyces cerevisiae* probiotic using commercial media of steamed cassava. Steam cassava with a temperature of 28°C, then inoculated using *Saccharomyces cerevisiae* 5% (w / v) and incubated at room temperature (28°C) for 48 hours. Dry *Saccharomyces cerevisiae* probiotic is prepared by drying at 50°C for 48 to 72 hours. After drying, the probiotic *Saccharomyces cerevisiae* was ground and store in the refrigerator before use.

### 2.3. Animals and feed

This study was used 24 male Ongole crossbred cows aged 12 to 18 months which were divided into 4 treatments. The treatment consists of:

P1 = (control);  
P2 = (P1 + Organic components);  
P3 = (P1 + Probiotic)  
P4 = (P1 + Organic components + Probiotics).

The control treatment was feeding in the form of a total mixed ration (TMR). The total mixed ration has been given as much as 3.5% of the dry matter requirement based on body weight. The ratio of concentrate and forage in the TMR was 50% : 50%. The nutritional value of TMR was 22.48% of crude fibre (CF), 10.25% of crude protein (CP), 51.02% of Total Digestible Nutrient (TDN), 0.92% of EE and 14.83% of ash.

The organic components used in P2 are sengon leaf meal and trembesi leaf meal with a ratio of 1:1. The addition of organic components was 4% of the dry matter requirement based on body weight. The probiotics used in P3 were dry *A notarae* probiotics and *S cerevisiae* probiotics with a ratio of 1:1. The addition of probiotics was 4% of the dry matter requirement based on the cow's body weight. Organic components and probiotics in P4 were organic components and probiotic that was used in P2 and P3 respectively. The organic component added to P4 was 2% of the dry matter requirement based on body weight. The probiotic added to P4 was 2% of the dry matter requirement based on the body weight.

A total mixed ration was given in the morning at 07.30 and at 15.00. TMR has been placed in the available feed bin in each pen. Drinking water was given ad libitum and placed in a water container in individual cages. The feed and water containers were cleaned in the morning. The feed trial was carried out for 14 weeks, consisting of two weeks of adaptation period and 12 weeks of data collection.

#### 2.4. Parameter observed

The parameters that have been observed in this study were the content of tannins and saponins in trembesi leaf meal and sengon leaf meal also rumen characteristic of cattle on every treatments. The rumen characteristics include pH, individual volatile fatty acid (VFA) concentrations (acetic acid, propionic acid and butyric acid), total VFA, NH<sub>3</sub> concentration, ratio acetic acid to propionic acid, individual VFA percentage (acetic acid, propionic acid, butyric acid), CO<sub>2</sub> concentration, CH<sub>4</sub> concentration and microbial protein production. Rumen fluid is taken from the cattle sophagus after 4 hours of morning feeding.

The content of tannins and saponins was analysed using the spectrophotometric test method. pH was observed using a pH meter. Individual VFA was measured using high performance chromatography (HPLC). Total VFA was the sum of the concentrations of acetic acid, propionic acid and butyric acid. Rumen ammonia was measured using a spectrophotometric test at a wavelength of 630 nm. Individual VFA percentage is the percentage of each VFA to total VFA. The CO<sub>2</sub> concentration and the CH<sub>4</sub> concentration were measured according to the method used by Van Soest [19]. Rumen fluid microbial protein estimation was observed using a spectrophotometer at a wavelength of 750 nm.

#### 2.5. Experimental design

This study used a randomized block design. Groups based on body weight and groups were replicated for each treatment. Data were analyzed using analysis of variance and if there was an influence from the treatment, the Duncan Multiple Range Test (DMRT) was performed.

### 3. Results and discussion

#### 3.1. Content of tannins and saponins in trembesi leaf meal and sengon leaf meal

Saponin and tannin content in trembesi leaf meal and sengon leaf meal is shown in table 1.

**Table 1.** Saponin and tannin content in sengon leaf meal and trembesi leaf meal.

Name of Plant	Tannin (g 100 g <sup>-1</sup> )	Saponin (g 100 g <sup>-1</sup> )
Trembesi	2.31	10.72
Sengon	3.78	9.34

The tannin content in trembesi leaf meal in this study was 2.31% while in sengon leaf was 3.78%. The saponin content in trembesi leaf meal in this study was 10.72% while in sengon leaf was 9.34%. Based on the content of tannins and saponins in the two-leaf meal, the organic components in the study contained more saponins. One of the benefits of saponins in ruminants is that they can interfere with the development of protozoa by bonding between saponins and sterols on the surface of the protozoan cell membrane, resulting in protozoan cell membranes rupture so that it undergoes lysis of cells and dies. A decrease in the protozoan population can increase the flow of Rumen bacterial N to the duodenum, because predation of protozoa against bacteria decreased sharply. As much as 9% to 25% of methanogenesis bacteria are symbiotic by sticking to the protozoan surface, so that a decrease in the protozoan population in the rumen will reduce CH<sub>4</sub> [11]. Tannins can inhibit the growth of methanogenesis bacteria through the availability of hydrogen but the use of tannins as methane-lowering agents must be limited because tannins can bind to simple molecules in feed, especially in feed-in tropical areas which contain low protein and dissolved organic matter thus reducing the benefits of feed for livestock [20].

### 3.2. Effect of treatment on rumen characteristic

The effect of organic components (saponins and tannins), probiotics and a combination of organic components and probiotic on rumen characteristics were shown in table 2.

**Table 2.** Characteristics of rumen fermentation

Rumen Characteristic	P1	P2	P3	P4
pH	6.61±0.15	6.95±0.16	6.82±0.49	6.85±0.35
Acetic acid/C <sub>2</sub> (mMol)	54.00±10.35	55.98±18.36	58.26±21.23	55.33±20.50
Propionic acid/C <sub>3</sub> (mMol)	11.33±2.04	12.04±3.88	13.13±4.49	14.95±4.36
Butyric acid/C <sub>4</sub> (mMol)	3.38±1.10	3.17±1.65	4.05±1.71	3.22±1.46
Total VFA (mMol)	68.71±13.05	71.19±23.73	75.44±27.19	73.51±26.13
C <sub>2</sub> :C <sub>3</sub>	4.78±0.59 <sup>b</sup>	4.68±0.46 <sup>b</sup>	4.45±0.33 <sup>b</sup>	3.65±0.36 <sup>a</sup>
Acetic acid (%)	78.58±2.04 <sup>b</sup>	78.78±1.94 <sup>b</sup>	77.31±1.60 <sup>b</sup>	75.02±1.72 <sup>a</sup>
Propionic acid (%)	16.58±1.46 <sup>a</sup>	16.95±1.21 <sup>a</sup>	17.44±1.00 <sup>a</sup>	20.69±1.56 <sup>b</sup>
Butyric acid (%)	4.85±0.90	4.27±0.94	5.25±1.01	4.29±0.73
CO <sub>2</sub> (mMol)	50.70±0.80 <sup>b</sup>	50.04±0.78 <sup>ab</sup>	50.89±0.98 <sup>b</sup>	49.12±0.83 <sup>a</sup>
CH <sub>4</sub> (mMol)	37.57±1.09 <sup>b</sup>	37.29±0.91 <sup>b</sup>	36.92±0.75 <sup>b</sup>	34.48±1.17 <sup>a</sup>
CH <sub>4</sub> (ppm kg <sup>-1</sup> DMI)	11.77	8.49	8.07	6.50
NH <sub>3</sub> (mg 100 ml <sup>-1</sup> )	12.55±0.79	14.68±2.03	13.35±1.68	13.31±1.22
Protein of Microbe	18.31±1.96	18.87±2.39	18.47±2.06	18.82±2.50

The addition of organic components, probiotics and a combination of organic and probiotic components had a significant effect on C<sub>2</sub>:C<sub>3</sub>, percentage of acetic acid, percentage of propionate acid, the concentration of CO<sub>2</sub> and CH<sub>4</sub>.

The combination treatment of the addition of organic components and probiotic (P4) resulted in the lowest percentage of acetic acid and the highest percentage of propionate so this causes the value of C<sub>2</sub>:C<sub>3</sub> on P4 was the lowest. It also resulted in low concentrations of CO<sub>2</sub> and CH<sub>4</sub> in P4. Ungerfeld *et*

al. [21] reported that CH<sub>4</sub> production was influenced by individual VFA profiles. Furthermore, Moss *et al.* [22] stated that increasing the concentration of propionic acid can be a guide and considered as a competitive pathway for CH<sub>4</sub> production.

#### 4. Conclusions

The combination of organic components (saponins and tannins) and probiotics (a mixture of *Acetoanaerobium notarae* and *Saccharomyces cerevisiae*) can be an alternative way to reduce methane production from enteric fermentation.

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