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Research Article

Effect of Dietary Supplementation Based on Ammoniated Palm Frond with *Saccharomyces cerevisiae* and Gambier Leaves Waste on Nutrient Intake and Digestibility, Daily Gain and Methane Production of Simmental Cattle

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Abstract | The objective of this study were to evaluate the effect of supplementation of *Saccharomyces cerevisiae* (SC) and Gambier Leaves Waste (GLW) in the diet of ammoniated palm frond (APF) based on nutrient digestibility, growth performance and methane production of Simmental cattle. Experimental design used is a Latin Square Design (LSD) with four treatments and four periods. This experiment used 175-200 kg male Simmental cattle. Cattle were fed a basal diet containing (dry matter basis) 40% APF and 60% concentrate. The treatments were (A) 40% APF + 60% concentrate + mineral S and P, (B) A + SC, (C) A + GLW, and (D) A + SC + GLW. The results showed that the DM, OM, ADF, NDF, and cellulose digestibility of D were significantly (P<0.05) higher than A. There had no significantly (P>0.05) difference among treatments on CP digestibility, nutrient intake, and urine allantoin. Supplementation of SC + GLW was able to improve body weight gain (1.22 kg/head) and reduce methane gas production up to 57 % compared to control. It can be concluded that supplementation with SC + GLW generates the best result in nutrient digestibility, daily gain, and reduce methane gas production of Simmental cattle.

Keywords | Ammoniated Palm Frond, *Saccharomyces cerevisiae*, Gambier leaves waste, Methane, Daily gain Simmental

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INTRODUCTION

Palm frond (*Elaeis guineensis*) is agriculture by product that potential to be used as fiber feed for ruminants (Zain et al., 2008; Suryani et al., 2017). Optimizing the utilization of palm frond need to be followed to attempt to maximize rumen microbial growth with additional treatment. One approach that can be used is the addition of microbial supplements (Zain et al., 2011, Herawaty et al., 2013). *Saccharomyces cerevisiae* (SC) is one of the fungal direct-fed microbes that can increase fiber degradation by providing an optimal rumen environment through oxygen reduction and lactic acid accumulation and stimulating the growth of endogenous fungal rumen. (Suryani et al., 2015). It was reported that the supplementation of fungal culture was able to increase total VFA and the digestibility of fiber fractions in hay or forage basal diets (Plata et al., 1994). Therefore, supplementation of *S. cerevisiae* can to increase performance of ruminants.

The utilization of palm frond oil as a basal diet of is constrained by high lignocellulose content in plant vegetative tissue. High fiber fraction affects a longer rate of passage rumen and does not fulfill daily metabolic energy requirements for ruminants (Ginting et al., 2018). Ammoniation and probiotic fungal supplementation provide favorable results on agricultural waste (Zahari et al., 2003; Zain et al., 2011; Chanjula et al., 2018). High fiber content on tropical feedstuff not only lowers feed efficiency but also increases the production of enteric methane (CH_4) in the ruminant. Several studies providing a variety of diets from natural tannins including gambier leaves on *in vitro*, *in sacco*, and *in vivo* evaluation have had a positive influence on performance and quality of ruminant products (Alam et al., 2007; Garcia et al., 2019; Natalello et al., 2019). The combination of these methods is expected to provide optimum animal performance.

Potential sources of natural tannins that are abundantly available in the West Sumatra region are those derived from squeezed residues from gambier leaves (*Uncaria gambir* Roxb.) or called Gambier Leaves Waste (GLW). Polyphenol fractions detected in gambier leaves were mostly catechin acid (40-61%), tannin (16-25%), and a small portion of pyrocatechol, gambirin and quercetin (Yeni et al., 2014; Nurdin et al., 2018). *In vitro* studies show that supplementation of 15% GLW (as a source of tannin) with basal feed in the form of ammoniated oil palm frond can reduce the production of methane gas up to 53% and increase the digestibility of feed nutrients (Ningrat et al., 2017). Tannins have the capability as an anti-methanogenic agent on rumen methanogenic microbes (Makkar et al., 2007).

This shows satisfactory results at the *in vitro* level of SC and GLR which can stabilize rumen fermentation process while reducing the production of methane gas. However, the impact of synergy between the use of probiotics and botanical extracts simultaneously on increasing fiber digestibility, nutrient utilization, and ruminant growth performance still requires further study at an *in vivo* level (Arowolo et al., 2018). We hypotized that supplementation combination of *S. cerevisiae* and gambier leaves waste could increase Simmental cattle performance as well as decrease methane gas production.

MATERIALS AND METHODS

The oil palm fronds that have been chopped are prepared for the ammoniation process using urea at a level of 4% and chicken manure at 15% from dry matter content of oil palm fronds. After chicken manure is stirred evenly on substrate. Then urea is dissolved in water (1:1) and sprayed thoroughly on palm fronds. The ammonia process takes place under anaerobic conditions using a plastic container for 1 week. after the ammonia process is complete, the plastic seal is opened and palm fronds are dried to remove odor. Palm fronds are ready for consumption by livestock and further nutritional analysis. Pure yeast culture of *S. cerevisiae* (4×10^8 live organisms/g) was obtained from the Biotechnology Laboratory of Gajah Mada University, Yogyakarta Indonesia collection, strain Meyen ex Hansen). The methods of preparation for the growth medium of *S. cerevisiae* following procedure Zain et al. (2016). The addition of probiotic *S. cerevisiae* to an experimental diet was given at a level of 1% of the DM ration. GLW is obtained from gambier plantations in Painan and Payakumbuh in the province of West Sumatera. Gambier residue is a by-product of gambier extraction (leaves and stems) through conventional methods that consisted of boiling for 1.5 hours and squeezing plant materials (Anggraini et al., 2011).

The animal trial was arranged with four male Simmental cattle with 175-200 kg BW equipped were randomly distributed in a 4 x 4 Latin square design. The treatments were (A) 40% APF + 60% concentrate + mineral S and P (B) A + SC (C) A + GLW and (D) A + SC + GLW. The basal diet contained (dry basis) 40% ammoniated palm frond and 60% concentrate. The Composition and nutrient contents of feeds including concentrate are shown in Table 1. Digestibility trials conducted using four animals for each treatment which were separated in individual pens. Cattles were fed *ad libitum* during the adaptation period (15 days) and then restricted during the collection period (6 days) at 90% of the intake feed that was offered at 7:00 and 16:00 h. In the last 15 days of each preliminary periods, animals were equipped with bags fitted to the animals with harness for total collection. Live weight gain was measured every two weeks. During the collection period, accurate records were kept for individual feed intake. Feed intake measured using the difference between the weight of given feed (kg) and the weight of remaining feed (kg) for one day. Determination of feed intake for nutritional attributes of DM, OM, and CP follows the formula: Feed consumption (kg. day^{-1}) ×% DM × % nutritional attributes.

Total fecal excretion was collected once daily and 10% representative samples were dried at 60°C overnight and kept in sealed bags until further analysis. Feed and fecal were ground to pass through a 1-mm screen and composite. Dry matter, organic matter and nitrogen were analyzed by standard methods (AOAC, 1990). Neutral Detergent Fiber (NDF), Acids Detergent Fiber (ADF), cellulose were determined by the procedures outlined by Goering and Van Soest (1970). Digestibility of nutritional items are determined by reducing the amount of feed nutrients consumed (kg/head/day) and contained in feces by the following formula:

Digestibility = [(Feed nutrition consumption - feces) / nutrient consumption of feed] × 100%.

Table 1: Composition and nutritional content of experimental diet (% DM)

Items	Diet (% DM)			
	A	**B**	**C**	**D**
Ammoniated palm frond	40	40	40	40
Rice bran	15	15	15	15
Palm Kernel Cake	33	33	33	33
Corn	10	10	10	10
Mineral	1	1	1	1
Salt	0.5	0.5	0.5	0.5
Sulphur (S)	0.3	0.3	0.3	0.3
Phosphorus (P)	0.2	0.2	0.2	0.2
Total	100	100	100	100
Suplementation				
S. cerevisiae (SC)	-	1	-	1
Gambier leaf waste (GLW)	-	-	15	15
Nutrient Contents				
Crude Protein	12.98	12.98	12.72	12.72
Fat	4.25	4.25	3.99	3.99
TDN	63.28	63.28	62.30	62.30
NDF	56.04	56.04	55.86	55.86
ADF	42.03	42.03	42.18	42.18

The accumulated urine was stored in a glass container with mixture of 10% H_2SO_4 for further allantoin analysis. The resulting methane was measured using Jentsch's equation (Jentsch et al., 2007).

Methane production (MJ/day) = 1.62 dCP-0.38 dCfat+3.78 dCF+149 dNFE+1142 kg/day

Where:
dCP = Digestible crude protein
dCfat = Digestible crude fat
dCF = Digestible crude fiber
dNFE = Digestible nitrogen free extract

Statisical Analysis

Analysis of variance (ANOVA) was performed to test for correlations between the data parameters. Any correlations were tested further for statistical significance using the Tukey test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Effect of Treatments on Nutrient Digestibility

Data of digestibility nutrient could be seen in Table 2. Supplementation of single *S. cerevisiae* could increase digestibility of DM, OM, NDF, ADF and cellulose, but supplementation combination of SC + GLW significantly increase (P<0.05) digestibility of nutrient. Studies conducted by Rivera-Mendez et al. (2017) showed that condensed, hydrolyzable, and tannin combination in Holstein steers gave an increase in dry matter intake by 4% -7.1%. The supplementation of *S. cerevisiae* in diet was able to stimulate the growth of microbes in the rumen and improve the digestibility of feed on ruminants (Zain, et al., 2011). Supplementation of SC could stimulate cellulolytic bacteria growth, can also inhibit the work of pathogenic bacteria (Denev et al., 2007). Reduced activity of pathogenic bacteria such as protozoa in rumen will stimulating population of beneficial rumen microbes. Increasing the population of rumen microbes, it can increase the activity of degrading the organic matter thereby increasing the absorption of organic substances. This is supported by Kamel et al. (2004), which states that there is an increase OM digestibility with the supplementation of SC compared to the control.

Supplementation of SC + GLW more improves nutrient digestibility over the treatments. Besides the SC role, this is related to tannin content in the gambier leaves waste which has a positive effect on increasing efficiency of nutrition absorption in animal. Prasetiyono et al. (2018) reported that the addition of tannins from gambier to protect soybean meal protein resulted DM, OM, and rumen undegradable protein of 86.20%, 84.40%, 69.20%, respectively. Tannins produce favorable conditions for rumen microflora and suppress protozoa populations (Goel et al., 2008). The increased population of bacteria results in producing higher activity of extracellular enzymes that contribute to the digestion of nutrient compounds in feed. This is in agreement with our previous study (Ningrat et al., 2017; Ningrat et al., 2019) which found tannins func

Table 2: Digestibility nutrient of Simmental cattle with experimental diet

Nutrient digestibility (%)	Treatment				S.E.
	A	B	C	D	
Dry matter	57,75[c]	61,55[b]	62,82[b]	65,07[a]	0.41
Organic matter	61.78[c]	65.45[b]	67.52[b]	71.52[a]	0.30
Crude protein	72.50	75.16	75.41	77.41	0.47
Acid detergent fiber	46.10[c]	52.14[b]	51.40[b]	55.40[a]	0.65
Neutral detergent fiber	48.06[b]	55.31[a]	54.18[a]	55.18[a]	0.68
Cellulose	47.83[b]	54.25[a]	53.18[a]	56.18[a]	0.76

Description: Different letters (a,b,c) in the same rows indicate significant differences (P<0.05); S.E.: standard error

tioned as defaunation agents capable of supressing the protozoa population up to 60%-70%. McLeod (1974) states that tannin molecules can disrupt the integrity of the cell wall and membrane of the protozoa, hence destroying the structure of the protozoa. Doležal et al. (2005) reported that the supplementation of SC to dairy cows at a dose of 10 grams/cow/day gave a trend of increasing protozoa populations 27% higher than control treatments. *In vivo* studies on buffalo bulls show a significantly increased effect of SC supplementation on protozoa populations and total bacterial rumen (Kumar et al., 2013). In high-fiber feed, protozoa prey on rumen bacteria reducing their number and level of fiber degradation. By reducing protozoa population, the bacteria population increases as indicated by the higher accumulation of allantoin levels in the urine of animals fed gambier leaves waste.

SC could modify the rumen ecosystem would be better to degrade fiber (Suryani et al., 2016). The study conducted by Zhu et al. (2017) showed that SC fermentation products in the range of 60-180 g/d with a low-quality forage diet could increase population of several species of ruminal cellulolytic bacteria, namely *Ruminococcus albus*, *R. flavefaciens*, and *Fibrobacter succinogenes* and a number of lactic acid bacteria (*Selenomonas ruminantium* and *Megasphaera elsdenii*). This further emphasizes the benefits of SC inclusion in low-quality forage rations to promote better digestion efficiency.

Supplementation of SC + GLW significantly (P<0.05) could increase fiber digestibility. There are strong evidence of synergic effect between yeast culture and plant extract to improve animal production. *S. cerevisiae* supplementation could improve digestibility of NDF, ADF and cellulose. This is in accordance with the results of research Whitley et al. (2009) suggest that there is an increase in NDF and ADF compared with controls. This is supported by Kamel et al. (2004) and Zain et al., 2011 stated that ADF digestibility increases with the addition of SC better than controls. The effect of supplementation yeast culture to ADF digestibility was also described by Chaucheyras-Durand et al. (2012), which states that yeast culture (SC) can stimulate the growth of rumen bacteria, especially cellulolytic bacteria and lactic acid bacteria. This is indicate that supplementation GLW could increase digestibility of NDF and ADF. Supplementation GLW increased digestibility of NDF and ADF at a level 15% of DM. This was due to the role of tannin in degradation of nutrient. Supplementation of GLW 25% tend to decrease digestibility of NDF and ADF. Increasing doses of tannin more than 15% reduced NDF and ADF digestibility (Ningrat et al., 2017). The low ADF digestibility due to high amounts of tannin could inhibit bacterial digestion and decrease performance of animals, especially in feed intake and nutrient digestibility (Smith et al., 2005).

Effect of Treatments on Cattle Performance

Data of DMI, OMI, ADG, methane production and allantoin urine of the experimmental rations with and without SC and GLW supplements are summarized in Table 3. Supplementation of SC and GLW had no significantly different (P>0.05) on DMI, OMI, and urine allanttoin. Combination of SC + GLW significantly (P<0.05) increase ADG and decrease methane gas production compared to control and treatment B, C. The highest mean value of ADG is treatment D (1.22 kg day[1]) and the lowest value is treatment A (0.75 kg day[1]). Supplementation of *S. cerevisiae* on diet were able to produce microorganism balance that beneficial on nutrient degradation in rumen (Williams and Newbold, 1990). Previous studies showed supplementation of SC on cattle diet could improved rumen fermentation characteristics, organic matter and protein digestibility as well as live weight gain in ruminant (Zain et al., 2011; Herawaty et al., 2013; Kamal et al., 2013) According to (Makkar, 2003) a decrease in animal's feed intake has been observed only when the inclusion of tannin in diets is greater than 3% of DM. In this study, supplementation of *S. cerevisiae* and GLW as tannins source influencing on DMI, OMI and protein intake. These results are in accordance Alves et al. (2011) found no effect of tannins supplementation in the diets on DM intake.

Although there was no significant difference between treatments on nutrient intake the table showed that nutri

Table 3: Feed intake, average daily gain, methane gas production and allantoin urine of Simmental cattle with experimental diet.

Items	**Treatment**				**S.E.**
	A	**B**	**C**	**D**	
Dry matter intake ($kg.day^{-1}$)	5.75[a]	5.96[a]	5.87[a]	5.55[a]	0.06
Organic matter intake ($kg.day^{-1}$)	5.13[a]	5.35[a]	5.67[a]	5.06[a]	0.05
Crude protein intake ($kg.day^{-1}$)	0.67[a]	0.70[a]	0.72[a]	0.74[a]	0.01
Urine allantoin (L/d)	195.03[a]	225.68[a]	202.25[a]	206.40[a]	27.52
Average daily gain (kg)	0.75[c]	0.90[b]	0.92[b]	1.22[a]	0.02
Methane gas production ($MJ.day^{-1}$)	2.36[a]	1.42[b]	1.35[b]	1.02[c]	0.05

Description: Different letters (a,b,c) in the same rows indicate significant differences (P<0.05); S.E.: standard error

ent intake were best result in treatment D. This indicated that supplementation of SC + GLW in complete feed of ammoniated palm frond could improve the digestibility of the treatment ration. This is presumably because SC can better modify rumen ecosystems for the development of rumen microbes in fiber digesting (Zain et al., 2011). SC was reported as a potential probiotic for ruminants through improved rumen fermentability by increasing total volatile fatty acids, iso-valerate acid, and reduction of acetate: propionate ratio (Riyanti et al., 2016).

Supplementation of SC + GLW on urine alantoin level could be seen in Table 3. Urine alantoin level can describe microbial protein synthesis. The higher content of urine alantoin microbial protein synthesis is increasing. SC + GLW supplementation was significant different (P <0.05) to increase urinary alantoin levels compared with controls. There were no significant differences between treatments B, C and D. These results are in accordance with studies conducted by Zhao et al. (2019) which combines tannins and cellulases in sheep thus providing a significant increase in nutrient digestibility, liver protein synthesis, and growth performance.

The increase in ADG is shown in Table 3 which indicates there is a statistically significant higher ADG in cattle feed the SC + GLW compared to other treatment. This is further evidence of an increase in feed digestibility possibly because the influence of feed with tannin content reduces rumen methanogenesis activity. Carulla et al. (2005) and Tan et al. (2011) state that natural tannins from tropical legumes produce more available nutrient content for animal production. Another hypothesis indicated that tannins from GLW produce less rumen degradable protein and more rumen undegradable protein and resulted in higher flows of essential amino acids available for absorption into the lower digestive system (Barry and McNabb, 1999; Min et al., 2003). Increase of ADG by *S. cerevisiae*, caused a DM intake, protein increased, also higher nitrogen retention (Hau et al., 2004). *Saccharomyces* sp. could stimulate growth of rumen bacteria especially cellulolytic bacteria which influence feed intake and digestibility of as influence the ADG (Callaway and Martin, 1997). There is a synergic effect when *S. cerevisiae* combine with tannins.

Effect of SC + GLW on methane gas production could be seen in Table 3. Supplementation of SC + GLW was significantly (P<0.05) could decrease methane gas 57% production compared to control. This is because tannins might be suppressing population of protozoa in rumen ecology (Patra and Saxena, 2010). The previous of *in vitro* studies showed that 0.4% tannin extracts from gambier leaves can protect extruded soy flour from rumen degradation, reduce methane gas production up to 25% and total gas production by 19% (Sajati et al., 2012). *In vivo* evaluation on *Bos taurus* × *Bos indicus* crossbreed shows the effect of 80% of *Leucaena* sp. (21% condensed tannins) in ration composition was able to reduce methane emissions by 61.3% without affecting dry matter intake, organic matter intake, and VFA production (Piñeiro-Vázquez et al., 2018).

The mechanism of inhibition of methane production in ruminants by tannin compounds has been initiated (Tavendale et al., 2005) i.e. (1) indirectly via inhibition of fiber digestion which reduces the production of H_2, and (2) directly inhibits the growth and activity of methanogens. This indicates that the tannin content in the GLW is affecting the ruminal methanogenesis pathway by suppressing protozoa numbers (Goel et al., 2008) and has had a toxic effect on methanogenic bacteria (Carulla et al., 2005). At lower doses (5% of DM), reported tannin extracts from gambier leaves were reported not to show significant anti-methanogenic activity besides only affecting ammonia formation *in vitro* (Sinz et al., 2019). Besides that, SC had potential to reduce methane production, SC was able stimulate acetogens to compete with methanogen bacteria (Chaucheyras-Durand et al., 1995).

CONCLUSION

The combination supplementation of 1% *S.cerevisiae* and 15% gambier leaves waste in ration based on ammoniated oil palm frond was able to improve the nutrient digestibility, daily gain, and decrease methane production of Simmental cattle.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest

AUTHORS CONTRIBUTION

Rusmana Wijaya Setia Ningrat, Mardiati Zain, and Elihasridas formulated the experimental design and did experimental work. Malik Makmur, Ezi Masdia Putri, and Yesi Chwenta Sari analyzing the data and drafted manuscript. All the authors read and approved the final version of the manuscript.

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