THE USE OF *ALBIZIA FALCATARIA* WITH CONDENSED TANNIN CONTENT ON IN VITRO GAS PRODUCTION AND RUMINAL FERMENTATION

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ABSTRACT

This study was aimed to evaluate *Albizia falcataria* with condensed tannin content in the diets on gas production and ruminal fermentation parameters. A completely randomized design (CRD) with fours treatment and five replications had applied in this study. The treatment in this study was fours combined of *Brachiaria mutica* and *Albizia falcataria* namely; R0: 60% *Brachiaria mutica* (BM) + 0% *Albizia falcataria* (AF) + 40% concentrate (CON), R1: 50% BM + 10% AF + 40% CON, R2: 40% BM + 20% AF + 40% CON and R3: 30% BM + 30% AF + 40% CON. The concentrate formulated using feed ingredients consists of 58% rice bran, 15% soybean cake meal, 30% corn, 1% salt, and 1% multi-vitamin minerals. The Diets samples were incubated at 39°C. The volume of gas and CH_4 were recorded manually at 3, 6, 9, 12, 24, and 48 h post-incubation. Dry matter digestibility (DMD), organic matter digestibility (OMD), pH, N-ammonia (N-NH_3), protozoa count, and microbial protein production (MP) were measured at 48 h post-incubation. The study showed that increased use of AF decreases (P<0.05) total gas, CH_4, percentage of CH_4, and increases the potency reduction of CH_4. There is a negative correlation (R^2= 0.81) between tannin condensed levels and CH_4 production. Concentration N-NH_3 and protozoa count was significantly (P<0.05) lower with the use of AF in treatment of R1, R2, R3 than R0 (without AF). As well as significantly (P<0.05) increased MP production. The study had concluded that the use of AF in the diet of R3 (30% BM : 30% AF) as a forage source reduced methane gas and N-NH_3 by 62.31% and 25.73%, increasing MP, and without retarded the activity of rumen microbes.

Key words: gas methane, diet digestibility, N-ammonia, microbial protein, total protozoa

استعمال ALBIZIA FALCATARIA مع محتوى التسمير المكثف في إنتاج الغازات الحيوية والتخمير

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المستخلص

هدفت هذه الدراسة إلى تقييم ألبيزيا فالكتاريا بمحتوى التانين المكثف في العلائق على معاملات إنتاج الغاز والتخمير الكرش. تم تطبيق التصميم العشوائي الكامل (CRD) مع أربعة معالجة وخمسة مكررات في هذه الدراسة. كان العلاج في هذه الدراسة مكونًا من أربعة أنواع من Brachiaria mutica و Albizia falcataria وهما: R0: 60٪ Brachiaria mutica (BM) + 0 ٪Albizia falcataria (AF) + 40 ٪Concentration، R1: 50 ٪BM + 10 ٪AF + 40 ٪CON، R2: 40 ٪BM + 20 ٪AF + 40 ٪CON و R3: 30 ٪BM + 30 ٪AF + 40 ٪CON. تتكون التركيبة المركزة باستعمال مكونات العلف من 58٪ نخالة الأرز ، و 15٪ كسب فول الصويا ، و 30٪ ذرة ، و 1٪ ملح ، و 1٪ معادن متعددة الفيتامينات. تم تحضين عينات العلف عند 39 درجة مئوية. تم تسجيل حجم الغاز والميثان يدويًا عند 3 و 6 و 9 و 12 و 24 و 48 ساعة بعد الحضانة. تم قياس قابلية هضم المادة الجافة (DMD) ، وهضم المادة العضوية (OMD) ، ودرجة الحموضة ، و N– الأمونيا (N–NH3) ، وعدد البروتوزوا ، وإنتاج البروتين الميكروبي (MP) في 48 ساعة بعد الحضانة. أوضحت الدراسة أن زيادة استخدام الرجفان الأذيني ينقص (P <0.05) إجمالي الغاز ، والميثان ، ونسبة الميثان ، ويزيد من فاعلية تقليل الميثان. يوجد ارتباط سلبي (R2 = 0.81) بين مستويات التانين المكثف وإنتاج الميثان. كان تركيز N–NH3 وعدد البروتوزوا أقل معنوياً (P <0.05) مع استخدام AF في علاج R1، R2، R3 من R0 (بدون AF). وكذلك زيادة معنوية (P <0.05) في إنتاج MP. خلصت الدراسة إلى أن استخدام الرجفان الأذيني في النظام الغذائي (R3 30٪ BM: 30٪ AF) كمصدر علفي قلل من غاز الميثان و N–NH3 بنسبة 62.31٪ و 25.73٪ ، وزاد MP ، وبدون إعاقة نشاط ميكروبات الكرش.

الكلمات المفتاحية: غاز الميثان ، هضم النظام الغذائي ، N– الأمونيا ، البروتين الجرثومي ، البروتوزوا

Received:23/7/2021, Accepted:17/10/2021

INTRODUCTION

Under normal condition, methane gas is producing during the feed fermentation process by ruminal microbial activity. Methano-bacteria in the rumen reducing H_2 and CO_2 to CH_4 that cause represent about 2-15% loss of feed energy (35). Methane (CH_4) from enteric fermentation in the rumen is the largest source of GHGs and contributes about 40% of the total GHG produced in the agricultural sector. In Indonesia and other developing countries, GHGs production tends to increase was affected by the growth of the livestock population and the expansion of the agricultural sectors (26, 28, 33). The increase of CH_4 production in Indonesia is up to 5.53% per year and estimating to reach 1508,43 Gg CH_4 per year in 2020 (3). Therefore, it is necessary to mitigate enteric methane gas from ruminants in order to obtaining economic and environmental benefits (31). So, improvements in the digestion process in ruminants by nutritional strategies are more likely to be adopted to reduce methane gas production from ruminant livestock rearing system (12,28). However, strategy option are not usually suitable for the available feed condition, especially in developing countries with tropical climates like Indonesia, where ruminant livestock rearing systems with low-quality input. On other hand, the application technology of feed additives in small farmers is not suitable (22). The change in nutrition is more easily adopted for farmers using different plants such as tree foliage with condensed tannin content (CT). Patra et al. (24), report that using plants with CT content can reduce CH_4 emission in ruminants. *Albizia falcataria* (AF) is tree foliage planted to supply the industry of plywood, paper, and paper tissue. Laboratory analysis has found that *Albizia falcataria* leaves contain a high polyphenol compound with a CT content of 8.84% dry matter (DM). The aimed of this study was to determine the using of *Albizia falcataria* as forage source with CT content on methane production and ruminal fermentation.

MATERIALS AND METHODS

Sampling and diets preparation

Sample of *Albizia falcataria* (AF) and *Brachiaria mutica* (BM) were harvested from the farm garden of the Faculty of Animal Husbandry, University of Jambi. The sample of AF and BM were sun-dried followed by oven drying at 60^{o}C for 24 h, and ground to pass of a 1 mm sieve before proximate analysis, mixed with concentrate and *in vitro* incubation. The experimental feed mixture was with a forage : concentrate ratio of 60:40%, with 13% crude protein and 65% TDN content. The formulation of concentrate diet consists of 58% rice brand, 25% milled corn, 6% soybean meal, 9% coconut meal, 1% mineral mix, and 1% salt. The evaluated diet consists of 4 types namely; R0: *Brachiaria mutica* (BM) 60% + *Albizia falcataria* (AF) 0% + Concentrate (CON) 40% (control), R1: BM 50% + AF 10% + CON 40%, R2: BM 40% + AF 20% + CON 40%, R3: BM 30% + AF 30% + CON 40%. Chemical composition of forage, concentrate and diets are presented in Tabel 1.

Table 1. Chemical composition of forage, concentrate and experimental diets

Chemical composition	BM	AF	CON	R0	R1	R2	R3
Dry matter, %	92.25	92.01	91.48	91.94	91.92	91.90	91.87
Crude protein, %	12.94	14.07	12.47	12.75	12.87	12.98	13.09
Ether extract, %	1.37	3.61	3.64	4.35	4.57	4.79	5.02
Crude fiber, %	27.67	14.64	8.81	18.06	16.76	15.45	14.15
Nitrogen free extract, %	50.13	63.21	68.74	57.57	58.88	60.19	61.50
Neutral ditergent fiber, %	71.98	51,22	50.85	63.53	61.45	59.38	57.30
Acid ditergent fiber, %	34.65	25.86	14.78	26.70	25.82	24.94	24.07
Ash, %	7.89	4.47	6.34	7.27	6.93	7.38	7.82
Total digestible nutrients,%	52.76	72.41	78.32	64.98	65.85	66.71	68.58
Condensed tannin, g/kg	ND	88.4	ND	0	8.84	17.68	26.52

BM: *Brachiaria mutica*, AF: *Albizia falcataria*, CON: concentrate, ND: non detected

In vitro gas production

The rumen fluid was taken from the slaughterhouse with 2 male bali cows as donors of rumen fluid. The cows were fed twice daily with a diet containing a 60:40 forage to concentrate ratio as long as three days before slaughter. The collection of rumen fluids is based on the Bioscreen Technologies

(5). The rumen fluid is put into a plastic bottle and placed immediately in a box filled with warm water (39^{o}C), transported to the laboratory, and strained through four layer of cheesecloth and mixed with buffer McDougall (1:4 v/v) according to Tilley and Terry (32). A total of 2000 ml of rumen fluid-buffer mixture was put in a dark bottle with capacity of 2500 ml and saturated with CO_2, then installed an automatic dispenser pipette. Aproximately 500 mg of each experimental diet were weighed into a serum bottle, pre-warmed in the incubator at 39^{o}C over night, and then adding 40 ml of rumen fluid-buffer mixture, sealed with the rubber stopper and crimp seal cups. A total of 43 serum bottles consisting of four treatments, five replication, two experimental units, and three blanks (rumen fluid-buffer mixture only) were incubated in an incubator at 39^{o}C for 48 h. Each serum bottle was injected with the syringe trough rubber stopper to wash out small gas as the start point of incubation. Gas production was determined manually at incubation times of 3, 6, 9, 12, 24, and 48 h with injected needle of a 10 ml piston glass syringe (***Fortune***®) from the headspace of each bottle serum and looked at via the displacement of piston syringe, then gas obtained to be used to measure of methane production. The methane produced was measured by method of Fieves et al. (8), in which a syringe that contains gas is inserting into the inlet of the gas washing capacity of 500 ml ((**Duran** ™) flow through a 4 M NaOH to absorbs CO_2 and then the outlet connected with another syringe to determine the CH_4 produced. Total gas and CH_4 production are determined after substracted by blanks. The concentration of CH_4 and the potency reduction of CH_4 (PRM) of the sample were determined using the equation as follows :

$$\textbf{CH}_4\textbf{ concentartion (\%)} = \frac{\textbf{total metan production (ml)}}{\textbf{total gas production (ml)}}\ \boldsymbol{x}\ \mathbf{100\%}$$

$$\textbf{PRM (\%)} = \frac{\textbf{Total metan production (control)} - \textbf{Total metan production (using AF)}}{\textbf{Total metan production (control)}}\ x\ 100$$

The energy metabolism (ME, MJ/kg DM) was determined using equation of ME (MJ/kg DM) = 2.2 +0.136 x GP_{24} + 0.057CP + 0.0029 CP2 (19). and total volatile fatty acid (TVFA) was calculated by applying the equation as follows (9);

ME (MJ/kg DM) = 2.2 + 0.136 x GP_{24} + 0.057 CP + 0.0029 CP2

TVFA (µmol L^{-1}) = 0.0239 GP_{24} – 0.00425.

Where :

CP and GP are percentage of crude protein and gas production after 24 h incubation. At the end of incubation (48 h), a sample was then centrifugated at a rate of 3,000 g for 15 minutes. The supernatant was used for analyzing the total of protozoa, pH, and N-Ammonia, and then filtrate centrifuged at a rate of 10,000 g for 10 minutes was used to analyze the microbial protein production.

Protozoa population

Protozoa population was calculated using the method according to Ogimoto (21). A total of 4.5 ml of fixation solution (Methyl green formal saline/MFS) was put into a test tube and mixed with 0.5 ml of rumen fluid. Placed the coverglass on the hemocytometer and dropped 0.1 ml of the sample through the side of the cover glass. Protozoa count using microscope with magnification of 40x. The total of protozoa per ml of rumen fluid were calculated by the following formula; Protozoa population/ml = (1/0.1 x 0.065 x 5 x 16) x n x d, where n= number of protozoa and d= dilution factors.

Microbial protein production

Microbial protein production (MP) was measured using the Lowry method (29). The solutions used are Lowry A and Lowry B. Lowry A is made with foline:aquades in a ratio of 1: 1, while Lowry B consists of Lowry B1 (2% Na_2CO_3 in 0.1 N NaOH): Lowry B2 (1% $CuSO_4.5H_2O$) and Lowry B3 (2% K-Na Tartrate) with a ratio of 100:1:1. As standard protein used bovine serum albumin (BSA). A total of 0.5 ml of sample was put into test tube and added with 2.5 ml of Lowry B solution, the homogenized and allowed to stand for 10 minutes. After that, added 0.25 ml of Lowry A solution, shake, and be allowed to rest in the dark for 30 minutes to color development. Then put into the cuvet until the mark and read using a spectrophotometer at λ 750 nm.

Dry matter and organic matter digestibility

In vitro dry matter (DMD) and organic matter digestibility (OMD) at 48 h post incubation determined by recovering the fermentation

residues by filtration through whatman paper 41 under vacuum. The residues was dried at 105^{o}C as overnight and then incerted in a muffle furnace at 550^{o}C for 12 h. Loss in weight after dried and incineration was used to determine of DMD and OMD. Determined of DMD and OMD was used the formula as follows:

$$D$$

$$\boldsymbol{MD} = \frac{\textbf{weight of DM sampel,(g)} - \textbf{(weight of DM residue,(g)} - \textbf{weight of DM blank,(g)}}{\textbf{weight of DM sample (g)}} \; x\; 100\%$$

$$\boldsymbol{OMD} = \frac{\textbf{weight of OM sample,(g)} - \textbf{(weight of OM residue,(g)} - \textbf{weight of OM blank,(g)}}{\textbf{weight of OM sample (g)}} \; \textbf{x 100\%=}$$

Statistical Analysis

The experiment was done using a completely randomized design (CRD) consisting of four treatments and five replication which each replication contained two experimental units. Data obtained from the experiment were analyzed using a one-way analysis of variance (ANOVA) completed with standard error of means. Duncan's multiple range test was used to determine the difference between means with confidence level of P<0.05. The data were analysis using SPSS statistical analysis programe version 17.

RESULTS AND DISCUSSION

Total gas and methane production

The amount of gas and methane (CH_4) produced up to 48 hours of *in vitro* incubation the use of *Albizia falcataria* (AF) with condensed tannin content as forage source are presented in Table 2.

Table.2. The use of *Albizia falcataria* (AF) with condensed tannin (CT) content as forage source on total gas and methane production

	Treatments					
Parameters	**R0**	**R1**	**R2**	**R3**	**SEM**	**P**
Total gas production, ml	**67.63^{a}**	**66.37^{a}**	**57.93^{b}**	**53.51^{c}**	**1.10**	**<0.001**
Methane, ml	**21.75^{b}**	**11.32^{a}**	**8.96^{a}**	**7.61^{a}**	**1.74**	**<0.001**
Methane, %	**32.19^{b}**	**17.07^{a}**	**15.36^{a}**	**14.21^{a}**	**2.63**	**0.001**
Metan reduce potency, %	**0**	**45.71**	**55.83**	**62.31**		

SEM: standard error of means, P: probability. Mean followed by the same letter within same row are not siqnificantly different

The results (Table 2) showed that the total gas production (TGP), CH_4 and the percentage of CH_4 from real TGP was decreased (P<0.05) with the increasing use of AF as forage source in the diets. The present result indicates that a decrease in TGP, CH_4 and the percentage of CH_4 obtained in this study is closely related to the presence of CT in AF, and consintent with Cieslak et al. (6), where supplementation of tannin derived from *Vaccinum vitis idea* and *Quercus cortex* decreased CH_4 production. Similar result were also reported by Bhatta et al. (4), Hariadi and Santoso (11), and Jayanegara et al. (14) reported there was a negative correlation between tannin concentration and TGP and CH_4 production. Figure 1 shows the relationship of different levels of CT from AF on CH_4 production. The CH_4 production (Figure 1) linierly decrease with increasing CTs in the diets, the present study are in line with those obtained by Getachew et al. (10), Hariadi and Santoso (11) there are siqnificant relationship (r: -0.60 and r: -0.70) among levels tannin and CH_4 production. The role of tannins in reducing methane production in the rumen has at least two mechanisms; 1) indirect effect through a decrease in the fiber digestion which resulting the decrease of H_2 production, 2) direct effect through the inhibition of the growth of methanogenic bacteria (7,11,15). The use of AF in the diets at levels of 10% (R1), 20% (R2), 30% (R3) as forage sources (Tabel 2) decreased CH_4 up to 45.71, 55.83, and 62.31%, respectively. This indicates that CTs in the AF is able to reduce CH_4 production. These results are accordance with those reported by several researcher, where tannin found in the diets can suppress CH_4 production both in vitro and in vivo (7,11,17,20)

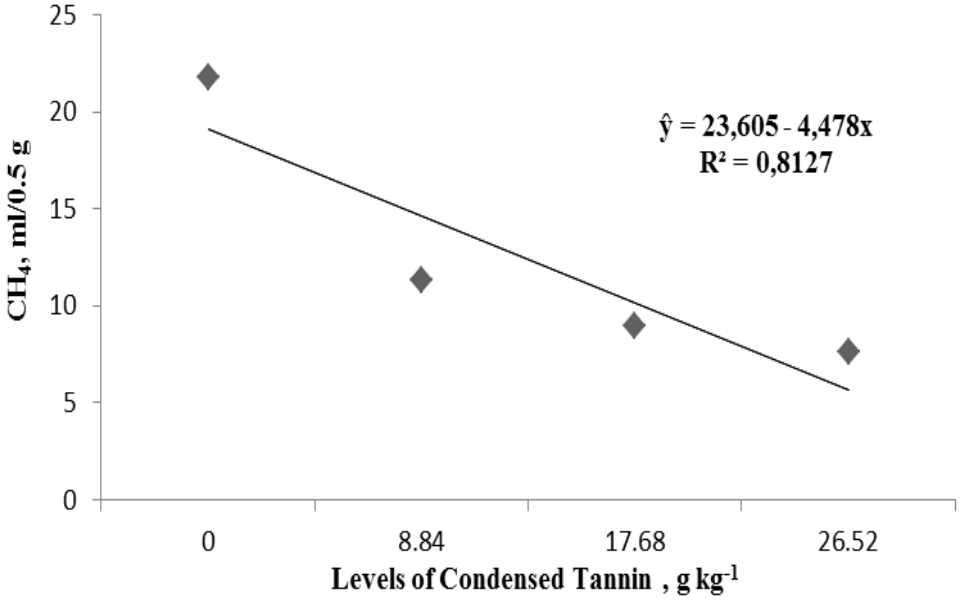

Figure.1. Relationship between condensed tannin (g $^{kg-1}$) from *Albizia falcatarian* on methane production

Diets digestibility

The use of AF in diets with different levels and CT content as a forage source on DMD, OMD, TVFA, and ME are presented in Table 3.

Table.3. The use of *Albizia falcataria* (AF) with condensed tannin (CT) content as forage source on diets fermentability

	Treatments					
Parameters	**R0**	**R1**	**R2**	**R3**	**SEM**	**P**
Dry matter digestibility, %	**63.81**	**66.80**	**68.91**	**70.16**	**2.663**	**NS**
Organic matter digestibility, %	**57.67^{b}**	**60.32^{ab}.**	**65.41^{a}**	**61.76^{a}**	**1.992**	**NS**
Total volatile fatty acid, mM/g	**2.53^{a}**	**2.08^{c}**	**2.07^{c}**	**2.24^{b}**	**0.044**	**<0.001**
Metabolizable energy, MJ/kg DM	**9.38^{a}**	**8.09^{c}**	**8.06^{c}**	**8.54^{b}**	**0.122**	**<0.01**

SEM, standard error of mean; P, probability. NS: non-siqnificant.

Mean followed by the same letter within same row are not siqnificantly different

The results (Table 3) showed that the DMD and OMD were not influenced (P>0.05) by the levels of AF in the diets. However, there was a tendency to increase the OMD (P=0.086) with increasing use of AF in the diets. The heigher protein (14.70% vs 12.94%) and lower NDF and ADF (51.22%, 25.68% vs 71.98, 34.65%) in AF than BM are possible cause. Uslu et al. (34), that there is a negative correlation among NDF and ADF content on digestibility. In addition, that the use of AF up to 30% (R3) with a CTs content of 26.52 g/kg DM has not caused disruption of rumen microbial activity to digesting diets. The study cunducted by Jouany and Morgavi (16) shows that tannin concentration of less than 50 g/kg DM have no effects on the rumen ecology. Meanwhile, Cieslak et al. (7), reported that the use of tannin extracts from *Sangoissorba officinale* (SOTE) up to 40 mg has no implication effects on digestibility. The effect of increasing the use of AF in the diets tended to decrease (P<0.05) the production of TVFA and ME (Table 3) in the rumen compared to without AF (R0). It is due to CTs in AF can form complexes with structural and non-structural carbohydrates and protect from ruminal enzyme activities. This condition has a positive impact in encouraging mainly the utilization of non-structutal carbohydrates (strach) digestion in duodenum, and implies greater energetic efficiency than occurs in the rumen, due to reduced CH_4 production, fermentation heat losses, and higher efficiency of metabolizable energy utilization (13).

Ruminal Fermentation

Ruminal fermentation measured is pH, N-ammonia, protozoa population and microbial protein production presented in Table 4.

Table.4. The use of *Albizia falcataria* (AF) with condensed tannin (CT) content as forage source on ruminal fermentation

Parameters	Treatments R0	R1	R2	R3	SEM	P
pH	6.96	7.05	7.08	7.00	0.37	NS
N-NH_3, mg/dl	12.59^{c}	10.90^{b}	10.21^{ab}	9.35^{a}	0.415	<0.001
Protozoa, 10^4	13.38^{b}	10.46^{a}	11.50^{a}	11.56^{a}	0.384	<0.001
Microbial protein production, mg/ml	0.01^{c}	0.03^{b}	0.04^{b}	0.25^{a}	0.009	<0.001

SEM, standard error of mean; P, probability. NS: non-siqnificant. Mean followed by the same letter within same row are not siqnificantly different

The use of AF was not affected (P>0.05) on ruminal pH value. These result corroborated with Hariadi and Santoso (11), who evaluated several trp[ical plants containing tannin did not produce difference in pH obtained. The rumen pH obtained in this study ranged from 6.96 to 7.08, which is still sufficient to maintain ruminal microbial activity as reported by Abdela (1), where pH value in 6 to 7 favorable to fibrolytic population. N-NH_3 production is a balance between protein degradation, and the utilize N-NH_3 for microbial protein synthesis. The use of AF with CT content was siqnificant (P<0.05) decrease N-NH_3 concentration up to 25.73% at using 30% AF in the diets (R3) compared to 0% AF (R0). The result is in line with Jolazadeh dan Muhammadabadi (15), where the addition of pistachio extract concentrate (PEC) contains tannin in sunflower meal (AFM) reduce the concentration of N-NH_3. It indicated that CT in AF can protect protein and reduce protein degradability in the rumen and could be advantageous to by-pass protein, lower NH_3 emmision, and improve ruminant performance (2). In this study (Tabel 4), the number of protozoa decreased (P<0.05) with the use of AF in the diets, although there was no difference between R1, R2, and R3 (P>0.05). The result shows that CT in AF was able to decrease the number of protozoa up to 16.52% compared to R0 (without AF). The anti protozoa effect of CTs cause disruption of protozoa membrane, inactivation of protozoal enzyme, deprivation of protozoal from substrates and metal ions, which are essential for metabolism (25). CTs with differing molecular weight could reduce the number and impact to alter the protozoa population in the rumen in vitro (30). The presence of secondary metabolites in ruminant feed like condensed tannin has implication for the rumen microorganisme population. The results showed (Tabel 4) that the use of AF containing CT siqnificantly (P<0.05) increasing production of microbial protein. The use of AF at level 0% (R0) siqnificantly (P<0.05) lowest production of microbial protein than R1, R2 and R3. Similary, Cieslak et al. (7), where administration of *Quercus cortex*, *Vaccinium vitis idea*, and their combination has ability to increase the population of rumen bacteria compared to control. The increase of the bacteria population obtained was closely related to the decreased number of protozoa effected by CTs. As is knows, protozoa are predators of rumen bacteria. The number and composition of bacteria will be effected by presence of protozoa (23) A"bacteria-sparing" effect arising from reduced protozoal numbers results in decreased predation of bacteria (18).

CONCLUSION

The study concluded that the use of AF in the diet of R3 (30% BM : 30% AF) as forage source reduced methane gas and N-NH_3 by 62.31% and 25.73%, respectively, increasing microbial protein production (MP) and without retarded the activity of rumen microbes.

ACKNOWLEDGEMENTS

The study had funded by Jambi University through an applied research scheme (contract No: **374/UN21.18/PG/SPK/2020**). The authors wish to thank our under graduate students and the laboratory technician of the Faculty of Animal Science, Jambi University for their cooperation and assistances in this research.

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