

ORIGINAL ARTICLE

Effect of different feeding management on the respiratory methane emission and feces-derived methane yield of goat

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ABSTRACT

Objective: This study aimed to evaluate the respiratory methane emission and ultimate methane yield (B_0) of goat feces that fed roughage consisted of *Pennisetum purpureum* and *Gliricidia* and fed roughage and concentrate with different protein source in the ration (fish meal and soybean meal).

Materials and Methods: Fifteen Kacang bucks were allocated to the control group (T0): goats were fed roughage only, T1: goats were fed roughage and concentrate with fish meal as protein sources, and T2: goats were fed roughage and concentrate and the protein source in the ration was soybean meal.

Results: The protein content of feces from T0 was significantly lower ($p < 0.05$) than that from the other treatments. The same phenomenon was also found in the respiratory methane emission in terms of l/head/d, l/kg digestible dry matter, and l/kg body weight. However, there was no significant effect ($p > 0.05$) of different ration composition on the ultimate methane yield (B_0) of goat feces. This study found that B_0 of goat feces from treatment T0, T1, and T2 was 17.40%, 25.78%, and 61.29%, respectively, higher than that from the international default value for developing countries.

Conclusion: Feeding grass and legume can reduce methane respiration emission in goat. B_0 of feces in the present study was higher than that in the international default value; therefore, the potential emission of goat manure in tropical developing countries could be higher than that in the present estimation.

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Introduction

Methane (CH_4) emission from enteric livestock fermentation is well recognized as the major contributor of greenhouse gases (GHGs) emission from the agriculture sector. The livestock sector is responsible for 18% CH_4 and 9% CO_2 of GHGs emissions [1]. Methane is produced during the fermentation of organic material in humid and anaerobic environments. Therefore, among other animals, ruminants are the main methane producers since the rumen is very large and has a continuous fermentation system [2].

In total, small ruminants contribute 12.25% of the total ruminant GHGs emission in the form of CH_4 from enteric fermentation, manure storage, and its application and N_2O from manure management. This is equivalent to 9.45 kg

CO_2 per kg body weight (BW) [3]. In recent years, GHGs emission has gained more attention since it is expected to cause increased average global temperature with the main effect of extreme weather changes. Furthermore, it can have an impact on the crop yield and productivity, food supplies and prices, animal metabolism and health, reproduction and productivity [3,4].

Among other small ruminants, goats have an important role as a source of animal protein in Indonesia. The demand for goat usually increases during the Muslims Holy Sacrifice Day and the goat population in this country is expected to increase annually. For instance, the goat population in Indonesia in 2014 was 18,640,000 heads and increased by about 2% (19,013,000 heads) in 2015 [5]. This trend

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is predicted to continue in the future along with increasing income and population in Indonesia and the fact that increasing prosperity will be followed by a dietary shift from carbohydrate towards protein sources. Even though the goat population in Indonesia is expected to increase, farm conditions in the country are mainly smallholdings and only a few farms are considered large-scale. In smallholder farms, farmers are usually raising their animals with the traditional management system. Farmers usually feed their goats mainly with grass, legumes, or agricultural by-products without concentrate.

Other than the enteric fermentation process, methane is also produced by anaerobic degradation of organic material in the manure during storage and application to the field [6]. Protocols to estimate methane emission from manure management include the data for volatile solid (VS) excreted by the animals, CH₄ conversion factor (MCF), and ultimate methane yield (B₀). Moreover, a more precise B₀ documentation will provide useful information for dimensioning, projecting, and economic budgeting of new biogas plants based on that particular animal manure [7].

Although previous studies have measured enteric methane emission in semiarid region [8], the effect of different forage: concentrate ratio on enteric methane emissions in goat [9,10], none, to our knowledge, has measured respiratory methane emissions and methane production of goat feces from different feeding management in tropical developing country. Therefore, the objectives of this current study were to evaluate the respiratory methane emission and ultimate methane yield (B₀) of goat feces from different feeding systems, i.e., feeding with roughage (*Pennisetum purpureum*) and (*Gliricidia*) only and feeding with roughage and concentrate with different protein source in the ration (fish flour in the second treatment and soybean meal in the third group). Thus, this paper provides comprehensive information regarding respiratory methane emission and feces-derived methane yield of goats due to different feeding management in a tropical developing country.

Materials and Methods

Ethical approval

Experimental protocol was approved by the Animal Care and Use Committee, Faculty of Animal and Agricultural Sciences, Diponegoro University. Certificate No. 3078/UN7.5.5/KP/2017 (20/05/2017).

Animal and diets

Fifteen Kacang bucks (17.05 ± 1.51 kg) were randomly divided into three groups (each group consisted of five replications). In the first treatment, goats were fed grass (*P. purpureum*) and legume (*Gliricidia*) only (T0), which

represents the smallholder farms that are common in Indonesia. For the second and third treatments, goats were fed roughage and concentrate with different protein sources in the ration, representing intensive farming. Fish meal was the protein source in the ration for group two (T1), while for group three (T2), the protein source in the ration was soybean meal. Diet composition and the nutrient content for each group can be seen in Table 1. The goats were individually kept in metabolic cages. The animals were fed with total mixed ration (TMR) at about 4.5% in terms of dry matter (DM) of their BW three times a day at 8 am, noon, and 4 pm, while water was accessible at all times. Goats were adapted for 5 weeks followed by 14 weeks of data collection. Feces and urine were collected from the 10th week for 14 days, and feces were stored frozen until used for the anaerobic digestion test and for chemical composition analysis.

Respiratory methane emission

Respiratory methane emissions were evaluated using the facemask method [11]. The mask was connected to a methane analyser (VIA-510, Horiba Ltd., Japan) for measuring methane content, while air volume was measured by an air flow meter (STEC SEF-6470, Horiba Ltd., Japan). The data were recorded continuously using IBM PC/AT compatible computer running Test Point TM (Test Point TM Technique & Reference, 1999). Data were collected for 10 min at 3 h intervals over 2 days [12].

Table 1. The ration composition and nutrient content (Nutrient compositions are expressed as percentage of dry matter (DM) (%)).

Feedstuffs/Nutrients	T0	T1	T2
Feed ingredients:			
	(%)		
<i>P. purpureum</i>	60	30	30
<i>Gliricidia</i> leave	40	30	30
Cassava waste product	0	20.10	19.20
Wheat bran	0	13.75	13.80
Fish meal	0	6.15	0
Soybean meal	0	0	7.00
Nutrients content in the rations:			
Dry matter (% of DM)	92.04	91.26	91.53
Ash	11.80	10.41	10.11
Ether extract	2.36	2.48	2.56
Crude Fiber	36.35	29.68	29.18
Crude Protein	15.55	15.26	15.59
Nitrogen free extract	33.94	43.80	42.56
Total digestible nutrient	46.92	56.21	57.95
Gross energy (cal/gm)	4362.25	4318.64	4416.27

Feedstuffs were made Total Mixed Ration and 1% of mineral mix was added.

Anaerobic digestion test

Feces-derived methane production was analysed by anaerobic digestion batch test using 500 ml infusion bottle according to Møller et al. [13]. Each reactor contained substrate and inoculum except for the control that contained inoculum only. Inoculum to substrate ratio was 1:1 in terms of VS [13]. The inoculum was prepared by collecting fresh dairy cow feces at the Faculty of Animal and Agriculture Sciences, Diponegoro University farm. Feces were diluted with tap water at 1:1 ratio and kept under anaerobic conditions at 35°C for 3 weeks. Prior to use, the inoculum was filtered using a cloth with the aim to produce a more homogenous inoculum. Only the liquid fraction was subsequently used to inoculate the batch tests. pH, DM, and VS of filtered inoculum was 6.4z, 3.88%, and 2.79%, respectively. In order to gain anaerobic conditions, infusion bottles that had inoculum and substrate added were flushed with nitrogen for 2 min. Each bottle was sealed with a rubber stopper and connected to another 500 ml infusion bottle which contained NaOH solution to absorb CO₂ [14] using Teflon tubing. The digesters were incubated at 37°C for 90 days. Methane was collected using 1 L Tedlar gas bag and measured periodically using the liquid displacement method described by Møller et al. [7]. The net methane production from the substrate was calculated as the total gas production from each bottle that contained substrate and inoculum, with the gas from bottles that contained inoculum only (control) subtracted. The net methane yield was corrected to STP condition. The test was done in triplicate.

Analytical procedures

Dry matter contents of samples were analyzed by drying at 105°C for 7 h. Ash was determined by combusting the dried samples at 550°C for 6 h and VS was calculated by subtracting the ash weight from the DM [15]. Crude protein (CP) was analyzed using the Kjeldahl standard method, crude fat was determined using the Soxhlet extraction method, crude fiber was analyzed according to the Van Soest procedure [16], and pH was measured using a pH meter (Hanna® pH meter). Gross energy content of the ration was analyzed using a bomb calorimeter while VFA in the rumen fluid was analyzed using gas chromatography

(Shimadzu GC-8). Data were analyzed using ANOVA with 95% confidence level. Duncan's multiple range tests were used in post ANOVA analysis when differences were found to be significant [17].

Results and Discussion

Feces compositions from each treatment are presented in Table 2. Statistical analysis showed that only CP content was significantly different ($p < 0.05$) between treatments. The CP content of feces was 10.99%, 12.59%, and 12.53% of DM for treatment T0, T1, and T2, respectively.

The lower protein content of feces ($p < 0.05$) from treatment T0 compared to T1 and T2 may be caused by the different characteristics of the protein source in the feedstuff of each treatment. The protein source of treatment T0 was roughages. That protein source was probably not easy to decompose in the goat digestive tract yet microorganisms in the rumen can use it as a protein source. Therefore, the animal can utilize it effectively. On the other hand, the protein source in ration T1 (fish meal) is rumen undegraded protein [18]. Therefore, the greater part of the protein in T1 will go to the goat intestine. Nevertheless, since the absorption rate of nutrient in the goat intestine is limited, some of the protein in the ration will be lost through feces. The protein source in the ration T2 is expected to be easily degraded in the rumen; therefore, a large part of the protein content was excreted through feces. Ørskov and McDonald [19] reported that when soybean meal is supplemented to a dried-grass diet for sheep and the ration was given *ad libitum*, the final degradation of soybean meal protein after 24 h was estimated to be 66%. In addition, CP consumption in T1 and T2 was significantly higher than that in T0 ($p < 0.05$). Crude protein consumptions of this current study were 51.23; 93.07 and 110.13 gm/h/d for treatment T0, T1, and T2, respectively. However, since there is a limitation in the goat intestine regarding the absorption of CP in the ration, therefore some part of this CP ration is wasted through animal feces. These facts may probably explain the higher protein content in feces from treatment T1 and T2 than from treatment T0.

The average respiratory methane emission from each treatment is provided in Table 3. There was an effect

Table 2. Feces composition from each treatment (The values are expressed as percentage of DM except where noted).

Treatment	Ash	Crude fat	Crude protein	Fibre	Nitrogen free extract (NFE)	DM (%)	Volatile solid (VS) (%)
	(% of dry matter (DM))						
T0	15.14	1.91 ^a	10.99 ^a	48.81 ^a	23.15	58.55	39.20
T1	14.94	2.06 ^a	12.59 ^b	45.58 ^a	24.84	47.39	35.69
T2	15.39	1.95 ^a	12.53 ^b	45.08 ^a	25.05	53.15	39.95

^{a, b}Parameters in each column followed by the same superscript are not significantly different ($p > 0.05$)

Table 3. Respiratory methane emission and ultimate methane yield.

Treatments	Parameters				
	Respiratory methane emission			Ultimate methane yield	
	(L/h/d)	(L/kg DMI)	(L/kg DDM)	(L/kg BW)	(L/kg VS)
T0	5.81 ^a	34.94 ^a	42.01 ^a	0.40 ^a	152.63 ^a
T1	20.00 ^b	38.86 ^a	65.07 ^b	1.04 ^b	163.52 ^a
T2	32.06 ^b	46.25 ^a	90.21 ^c	1.38 ^b	209.68 ^a

^{a, b}Parameters in each column followed by the same superscript are not significantly different ($p > 0.05$).

($p < 0.05$) of different feed ration composition on goat respiratory methane emission per h/d; per kg digestible dry matter (DDM) and per kg BW. However, the treatments gave no effect ($p > 0.05$) on goat respiratory methane emission per dry matter intake (DMI). Cao et al. [20] reported that methane emission in sheep, which have a similar digestion tract to that of goats, fed non-fermented total mixed ration of whole crop rice was 39.84 l/h/d; 39.87 l/kg DMI; 60.87 l/kg DDM, and 2.12 l/kg BW^{0.75}. In addition, the study from Azlan et al. [21] found that methane production of goat fed 60% basal feed and 40% untreated rice straw was 20 l/h/d; 42.3 l/kg DMI and 60.5 l/kg DDM. Therefore, the data presented here are comparable with the results from the studies of Cao et al. [20] and Azlan et al. [21].

In general, goat respiratory methane emission in treatment T0 (except in terms of L/kg DMI) that was fed forage as a single feed was significantly lower ($p < 0.05$) than that from T1 and T2 that were fed forage and concentrate. However, there was no significant effect between T1 and T2. Methane production is associated with the concentration of propionic acid as the end of fermentation product; an increased propionic acid concentration will cause the decrease of methane production [22] since electrons are used during propionic acid formation [20].

In this present study, the propionic acid concentration of the rumen fluid was significantly higher ($p < 0.05$) in T0 at 3 and 6 h after feeding as compared with that in T1 (Table 4). Likewise, total VFA concentration was higher ($p < 0.05$) in T0 at 3 h after feeding than that in T1 (Table 4), but the effect of treatments was not observed on the total VFA concentration before feeding and at 6 h after feeding. In accordance with this study, Cao et al. [20] found that concentration of propionic acid and total VFA in the rumen fluid fed fermented total mixed ration (FTMR) at 2 h after feeding was significantly ($p < 0.05$) higher than the control that was fed non fermented TMR. Therefore, ruminal methane emission in sheep fed FTMR was significantly lower than that fed TMR.

The explanation for the lower respiratory methane emission and higher propionic acid and total VFA concentration in rumen fluid of treatment T0 than that in other

treatments in this current study may be due to: (1) a higher concentrate content in the ration of treatments T1 and T2 causing a high lactic acid production in the rumen. This phenomenon was confirmed by the fact that the pH value of rumen fluid in T1 and T2 before and 3 h after feeding tended to be lower than that in T0. This condition will depress cellulolytic bacteria, and hence depress their activity to degrade fiber in the rumen. It seemed that rumen microorganism in T0 can proliferate properly; therefore, the fermentation process may effectively run. Constable et al. [24] reported that when a ruminant ration contains a high rapidly fermented carbohydrate, the rates of acid especially the rates of lactic acid production by rumen bacteria will increase. Moreover, Dawson et al. [25] reported that this lactic acid is stronger than volatile fatty acids produced in the animal rumen. This phenomenon occurred in the feedlot industries that change their ration rapidly from forage-based ration to high concentrate ration. (2) A higher proportion of *Gliricidae* leaves in the ration of T0 than that in T1 and T2 seemed to be able to support the rumen microorganism activity better. Avilés-Nieto et al. [26] reported that the supplementation of *Gliricida sepium* (GS) hay to buffel grass-based ration can significantly increase CP digestibility in sheep. Moreover, CP concentration of GS was 183 gm/kg DM while the protein fractions in GS hay (% CP) were non-protein nitrogen: 4.42, rapidly degraded true protein: 0.21, true protein: 7.72, slowly degraded protein: 0.78, and unavailable protein: 5.16. This CP fraction of GS may able to improve the nitrogen intake thereby can increase nitrogen supply to rumen microorganism [26]. This fact, therefore, has a positive effect on rumen microorganism population and efficiency allowing them to increase the rate of nutrient decomposition [27].

Acetic acid, propionic acid, and total VFA concentrations in T0 were significantly ($p < 0.05$) increased at 3 and 6 h after feeding (Table 4). This circumstance also occurred in T1 and T2 (Table 4). The data can, therefore, explain that there was enhancement of microorganisms' activity in the rumen for all treatments at 3 and 6 h after feeding. Moreover, a large variation in methane emissions in terms of DDM intake in this study is in accordance with previous studies [20,28]. In addition, CP consumptions in

Table 4. Volatile fatty acid concentration and pH value of rumen fluid of goat fed different ration composition.

Parameters	Hours after feeding	Treatments		
		T0	T1	T2
Acetic acid (mMol)	0	76.03 ^{ax}	85.76 ^{ax}	99.39 ^{ax}
	3	150.85 ^{bx}	92.02 ^{ay}	105.42 ^{ay}
	6	125.47 ^{bx}	96.58 ^{ay}	113.42 ^{ayy}
Propionic acid (mMol)	0	17.40 ^{ax}	20.99 ^{ax}	23.77 ^{ax}
	3	36.07 ^{bx}	21.34 ^{ay}	25.95 ^{axy}
	6	33.57 ^{bx}	24.91 ^{ay}	28.96 ^{axy}
Butyric acid (mMol)	0	4.73 ^{ax}	7.28 ^{ax}	12.25 ^{ay}
	3	7.47 ^{ax}	5.28 ^{ax}	10.23 ^{ax}
	6	6.08 ^{ax}	8.18 ^{ax}	10.95 ^{ax}
Total VFA (mMol)	0	98.16 ^x	114.03 ^{x*}	135.40 ^{x*}
	3	194.39 ^x	118.64 ^{x*}	141.61 ^{xy*}
	6	165.11 ^x	129.67 ^{x*}	153.13 ^{x*}
pH	0	6.51 ^x	6.48 ^{x*}	6.41 ^{x*}
	3	6.39 ^x	6.31 ^{x*}	6.31 ^{x*}
	6	6.18 ^x	6.24 ^{x*}	6.36 ^{x*}
A/P ratio	0	4.47 ^x	4.09 ^x	4.15 ^x
	3	4.18 ^x	4.31 ^x	4.06 ^x
	6	3.76 ^x	3.89 ^x	3.94 ^x

^{a, b}Parameters in each column in the same treatment and in the same acid followed by the same superscript are not significantly different ($p > 0.05$).

^{x, y, z}Parameters in each row followed by the same superscript are not significantly different ($p > 0.05$).

*Adiwinarti et al. [23].

this experiment are above the threshold of protein concentrations that restrict microbial activity about 70 gm CP/kg DM [29]. Therefore, CP consumption in this experiment can support microbial activity and multiplication in the rumen, thus enhancing fermentation [30].

The ultimate methane yield (B_0) of feces from treatments T0, T1, and T2 in terms of VS is given in Table 3. There was no significant effect ($p > 0.05$) of different ration composition on the B_0 of goat manure. The B_0 of T0, T1, and T2 was 152.63 ± 18.41 ; 163.52 ± 12.19 , and 209.68 ± 51.07 ml $\text{CH}_4/\text{gm VS}$, respectively.

The lack of significant difference between feces-derived methane productions with different ration composition in this study could be due to the relatively similar feces composition from all treatments (Table 2). The compositional similarities and long anaerobic digestion time (90 d) could enable anaerobe microorganisms to decompose the greater part of organic material in the manure to produce methane.

This study found that B_0 of goat feces in all treatments was higher than the IPCC default value [31] for developing

countries. The IPCC values are 130 ml $\text{CH}_4/\text{gm VS}$ for developing countries and 180 ml $\text{CH}_4/\text{gm VS}$ for developed countries. For treatment T2 with soybean_addition as a protein source, this research found a B_0 that was higher than the IPCC default value for developed countries. Møller et al. [7] also found the same phenomenon; B_0 values (in developed countries) of dairy cattle manure fed early grass and maize were 8%–9% higher than the IPCC default value and for dairy cattle's added fat in the diet, the B_0 of the manure was 25%–31% higher than the IPCC value. Therefore, the result of this study indicates that manure methane emission (and manure methane potential when used as a substrate in biogas plants) of goat manure in developing countries can be higher than previously reported. Møller et al. [7] reported that in general, a higher ultimate methane yield (B_0) in a current study than that in earlier studies can be caused by improved feed composition, feeding practices, and higher DMI than those in the previous studies.

The aim of this present paper was to evaluate the effect of different feeding management on the methane yield of goat feces rather than goat manure, since manure quality is very much dependent on housing systems and management practices. In addition, urine is already hydrolysed to inorganic nitrogen in the animal pen, so there will be no methane production from the urine fraction [7].

This experiment cannot directly compare the ultimate methane yield (B_0) derived from goat feces with other studies since the information is rare. Arici and Koçar [4] have evaluated methane yield of goat manure as co-substrate with cattle manure and fermented cattle manure, but not as a single substrate. Hanafiah et al. [33] evaluated biogas production of goat manure; however, that study was performed for a shorter period (20 d) and the result was expressed only as biogas production rather than methane production.

If the total production of methane (B_0) from the feces can be generated completely by the biogas digester, the total methane production value (from respiration and from feces) were 95.19; 102.47; and 117.15 L $\text{CH}_4/\text{Kg DMI}$ for the T0, T1, and T2, respectively (Table 5). However, this would not occur in reality, since the biogas digester could only extract for about 70%–75% from the total methane production (B_0) of the sample [34].

This experiment was performed in Indonesia; therefore, the result of this study is expected to help improve the inventory of goat respiratory methane emission and goat feces methane yield in tropical developing countries. This is in accordance with IPCC [31] that recommends expanding the representativeness of the default value, particularly for livestock, in tropical regions and when varying diet is applied.

Table 5. Total methane production per Kg DMI.

Treatments	Parameters				
	Respiratory methane emission (A)	Feces production	Ultimate methane yield	Feces methane production per kg DMI (B)	Total methane production (A+B)
	(L/kg DMI)	(Kg DM feces/kg DMI)	(L/kg DM feces)	(L/kg DM)	L/Kg DMI
T0	34.94 ^a	0.59	102.12	60.25	95.19
T1	38.86 ^a	0.47	135.35	63.61	102.47
T2	46.25 ^a	0.48	147.71	70.90	117.15

Conclusion

Feeding grass and legume in goat can decrease methane respiration emission. However, since goats that are fed only grass and legumes have inferior performance compared to those fed grass, legume, and concentrate, the methane emission per kg of meat produced could be higher. Moreover, B_o of feces from goats in this experiment was higher than the international default value; therefore, the potential emission of goat manure in tropical developing countries could be higher than that in the present estimation.

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Conflict of interests

The authors declare no conflicts of interest.

Authors' contribution

SS performed the experiment and wrote the manuscript; RA, MK, and AP performed the work and revised the manuscript, and ALW revised the manuscript.

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