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Original Research

The Effect of Cattle Breed and Forage-Concentrate Ratio on Fecal Methane and Nitrous Oxide Emissions

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Abstract

A field experiment was carried out to assess the methane (CH_4) and nitrous oxide (N_2O) emission from feces of different Indonesian cattle breeds and forage-concentrate ratio. The objectives of this study were to calculate the amount of CH_4 and N_2O emission and to analyze the effect of different cattle breeds and forage-concentrate ratio on CH_4 and N_2O emissions. The experiment used 3 x 2 factorial design. The first factor was three Indonesian local cattle breeds (Bali cattle, Madura cattle and Peranakan Ongole cattle). The second factor was two ratios of forage-concentrate (70:30 and 30:70). The gas was collected manually by using the 20 mL plastic syringe on 11 observation days in a month. On each observation day, the gas was collected five times with 10-minute interval (minute 10, 20, 30, 40 and 50 after chamber closure) between 2 and 3 pm. Analysis of variance (Anova) was conducted to analyze the effect of cattle breeds and forage-concentrate ratio. The result showed emission peak of CH_4 was on day 0 to 6, while the N_2O peak was on day 9 to 15. The highest amount of CH_4 emission occurred on feces of Bali cattle with 30:70 forage-concentrate ratio (895 mg CH_4/kg/day) on the first day of observation. The highest amount of N_2O emission was occurred on feces of Peranakan Ongole cattle with 30:70 forage-concentrate ratio (71,781.62 µg N_2O/kg/day) on day 15. The cattle breed and forage-concentrate ratio had no significant effect on both CH_4 and N_2O emission from feces.

Keywords: cattle breed, feces, emission, methane, nitrous oxide

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Introduction

Greenhouse gases (GHG) trap infrared heat from the earth's surface and increase the temperature of the earth [1]. These gasses are commonly carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). Livestock activities contribute to the emission of considerable amounts of GHG. Sources of GHG from livestock mostly come from enteric fermentation and manure management. Livestock manure emits CH_4, N_2O, and CO_2, depending on the way they are produced and managed [2].

Livestock also indirectly contribute to releasing large amounts of carbon in feed production, land clearing, and in the production, processing and marketing of livestock products. According to FAO [3], the livestock sector accounts for 9% of anthropogenic CO_2 emissions, 37% of anthropogenic CH_4 and 65 % of anthropogenic N_2O. Total emissions from global livestock are 7.1 gigatons of CO_2-eq per year, representing 14.5 percent of all anthropogenic GHG emissions [3].

Concerning N_2O, although its atmospheric concentration is lower than of CO_2, but its global warming potential is much higher. N_2O is very persistent in the atmosphere where it may last for up to 150 years [2]. UNEP [4] also stated that N_2O is the most significant ozone-depleting emission to the atmosphere. The presence of high levels of anthropogenic N_2O in the atmosphere will continue to cause ozone layer depletion. N_2O emissions and concentrations will continue to increase in future under most current projections [4].

N_2O is produced during the biological degradation of nutrients in animal feces and, to some extent, their formation is influenced by the same parameters, for example temperature and substrate availability [5]. In addition, Oenema et al. [6] also mentioned that manure management systems are conducive to nutrient, but the loss depends on the nutrient element, manure management system and the environmental conditions.

Total N_2O emissions are related to type and number of animals, N excretion per animal, and the management of animal wastes [6]. In detail of proportion on each type of N losses, Webb et al. [7] reported that the greatest of N losses (38%) was as NH_3, while other losses were via emissions of nitric oxide (NO), N_2O and N_2 (7%) and 4% via leaching and runoff. Increased N_2O emissions from soils are associated with animal manure and N fertilization, N derived from biological N_2 fixation and enhanced N mineralization [8].

Upon field application, factors such as manure composition, application method and soil conditions together determine the potential for N_2O emissions, but given the unpredictable effects of climate, effects of treatment and management can vary dramatically [9]. Losses from manure occur during storage, shortly after application to land and during crop growth [2]. Also, the storage conditions, the usage treatment technologies, and handling on the properties of field-applied manure are defining nitrogen transformations and emit N_2O emission [9]. Concerning manure deposition to land, Manure-induced soil emissions are the largest livestock source of N_2O worldwide [2]. Therefore, management during manure deposition become important [9].

Webb et al. [7] defined that nitrogen use efficiency (NUE) is the overall efficiency by which N is used in the entire farm system to produce outputs. Manure N efficiency is the proportion of manure-N that can be recovered by crop roots over more than one season [7]. UNEP [4] also mentioned the importance of boosting NUE to reduce N_2O emissions from agriculture, especially by efficiently use of fertilizer, manure and feed. Improved NUE also would bring added benefits of higher crop and livestock productivity, reduced air and water pollution due to decreased nitrogen losses [4].

In Indonesia, smallholder cattle farmers produce mostly kept local cattle, such as Bali, Madura and Peranakan Ongole (PO) Cattle. Some cattle farmers utilize forage and concentration for the feed. It is hypothesized that different cattle breed and forage-concentrate ratio leads to different CH_4 and N_2O emission However, it is not yet known how is the emission profile of different cattle breed, forage-concentrate ratio and their effect on CH_4 and N_2O emission. Therefore, the aims of this research were to determine actual quantities CH_4 and N_2O emissions from the most commonly used maintenance systems and to analyze the effect of different cattle breeds and forage-concentrate ratio on CH_4 and N_2O emissions.

Materials and Methods

The experiment was conducted at the Beef Cattle Research Station, Pasuruan, Indonesia from 1 September to 15 October 2021. The method was approved by the Institutional Animal Care and Use Committee of Indonesian Agency for Agricultural Research and Development, Ministry of Agriculture (reg. nr.: Rm/04/2021).

Experimental Design

The experiment used 3 x 2 factorial design. The first factor was three Indonesian local beef cattle breeds (Bali cattle, Madura cattle and PO cattle). The cattle were 1-2 years old heifer with average body weight of 240.28 kg. The body weight and body condition score of each cattle is shown in Table 1. The second factor was two ratios of forage and concentrate (70:30 and 30:70). The treatments combination is shown in Table 2. The experiment used five cattle as replication. Since this experiment used three cattle breeds, there were 30 cattle that took part in this experiment. Each cattle placed in an individual pen (Fig. 1). The housing used double slope roof shed. The floor made from concrete with cubicle rubber for each cattle. No specific

Table 1. The body weight and body condition score of cattle prior to faces sampling.

Nr.	ID	Breeds	Body weight (kg)	Body condition score*	
				Observer 1	Observer 2
1	20/17	PO	203	2.75	2.75
2	20/13	PO	318	3	3
3	B 20/09	Bali	108.5	3	2.75
4	B 20/10	Bali	144	3	3
5	M 20/05	Madura	159.5	2.5	2.75
6	M 19/20	Madura	236	2.5	2.75
7	M 19/34	Madura	250	2.75	2.75
8	20/10	PO	299	2.5	2.75
9	20/05	PO	296	3.25	2.75
10	B 19/24	Bali	277	3	3
11	M 20/23	Madura	238	3.25	2.75
12	M 19/31	Madura	237	3	3
13	B X3	PO	165	3	2.75
14	20/16	PO	207	2.5	2.75
15	B 20/38	Bali	274	3.25	3
16	M 19/37	Madura	256	3.25	3
17	B 20/4	Bali	202	2.5	2.75
18	B 20/2	Bali	227	2.5	2.75
19	20/23	PO	229	2.5	2.75
20	M 19/28	Madura	241	2.75	2.75
21	B 19/40	Bali	247	3.5	3
22	B 20/01	Bali	204	3.25	3
23	20/01	PO	267	2.5	2.75
24	M 20/18	Madura	245	2.5	2.75
25	20/02 A	PO	264	2.75	2.75
26	M 19/22	Madura	230	3	2.75
27	B 19/25	Bali	329	3.25	3.5
28	B 19/31	Bali	183.5	3	3
29	M 20/07	Madura	244	3	2.75
30	20/29	PO	228	2.5	2.75

* Minimum = 1, maximum = 5

litter used in this experiment to prevent the feces mixed with other substances from the litter. The cattle were head-to-head with the feed bunks in front of them.

Feed Treatments

The experiment used forage and concentrate as feed treatment. The forage was chopped fresh Elephant grass (*Pennisetum purpureum cv. Taiwan*), while the concentrate was mixed ingredients, mainly consisted of wheat pollard and copra meal. The ingredients composition of concentrate is shown in Table 3. The feed treatments were conducted 14 days before the feces collection of the cattle. The concentrate was fed at 7.30 am and forage was fed at 10 am on a daily basis. The cattle received ad libitum water. The total amount of daily concentrate and forage feeding was 3.5% of dry matter requirement based on body weight. The ratio of concentrate and forage in the total amount of feeding were 70:30 and 30:70.

Table 2. List of treatments with different cattle breeds and feed ratios.

Treatment numbers	Cattle breeds	Feed ratios
1	Bali (B)	Forage:Concentrate = 70:30 (A)
2	Madura (M)	Forage:Concentrate = 70:30(A)
3	PO (PO)	Forage:Concentrate = 70:30(A)
4	Bali (B)	Forage:Concentrate = 30:70 (B)
5	Madura (M)	Forage:Concentrate = 30:70(B)
6	PO (PO)	Forage:Concentrate = 30:70(B)

Fig. 1. The cattle were located in individual pen during feeding treatment and feces collection.

Gas Sampling

Gas sampling begins with collection of 1 kg of feces from each cattle at 8 pm. The feces were assumed as feces of the cattle in the last 24 hours. The feces from each cattle was mixed or homogenized manually using hoe, then 1 kg of the homogenized feces was taken. Sample from each cattle were placed into a cylinder chamber. Therefore, there were also 30 chambers in this experiment. The chamber was made from polyvinyl chloride pipe (Fig. 2a). The height and diameter of chamber were 40 cm and 20 cm, respectively. The chamber's lid has hole for digital thermometer and septum rubber hole for gas collection.

The gas collection was performed on 11 observation days in a month (day 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30). On each observation day, the gas was collected five times with 10 minutes intervals (minute 10, 20, 30, 40

Table 3. Ingredients composition of concentrate in feed treatments.

No.	Ingredients	Percentage (%)
1	Rice barn	10
2	Cassava powder	20
3	Corn gluten feed	18
4	Copra meal	22
5	Wheat pollard	28
6	Salt	1
7	$CaCO_3$	1
	Total	100

and 40 after lid closing) between 2 and 3 pm. It started with the installation of the chamber's lid, then water was poured at the connection between the lid and the chamber body to prevent gas leakage.

The gas was taken using a 20 mL syringe through a septum rubber, then transferred to a 10 mL vacuum vial. The same steps were performed at the 20th, 30th, 40th, and 50th minutes. Therefore, each feces sample in one chamber has 5 gas samples in 1 observation day. The gas collection for 30 chambers was done simultaneously at the same time (Fig. 2b). The temperature inside the chambers also recorded on each gas collection interval. Based on the Indonesia's Meteorological, Climatological, and Geophysical Agency (BMKG), the relative humidity of the environment in the research location during observation period was 80%.

The concentrations of CH_4 and N_2O were analyzed using a gas chromatograph (GC 2014, Shimadzu Corporation, Kyoto, Japan) with a flame ionization detector and an electron capture detector at the laboratory of the Indonesian Agricultural Environment Research Institute, Pati, Indonesia. The CH_4 and N_2O fluxes were calculated based on the linear regression in CH_4 and N_2O concentrations in the headspace of the chambers over time [10]. The amount of flux calculated using formula below. Analysis of variance (ANOVA) was conducted to analyze the effect of cattle breeds and forage-concentrate ratio.

$$E = \frac{dc}{dt} \times \frac{Vch}{Ach} \times \frac{mW}{mV} \times \frac{273{,}2}{273{,}2+T}$$

where, E is CH_4 flux (mg CH_4/kg/day) or N_2O flux (g N_2O/kg/day), dc/dt is the concentration change over time of CH_4 or N_2O (ppm/minute), Vch is chamber volume (m^3), Ach is chamber area (m^2), mW is molecular weight of CH_4 or N_2O (g), mV is molecular volume of CH_4 or N_2O (22.41 l) and T is the mean of temperature inside the chamber (ºC) [11].

a) b)

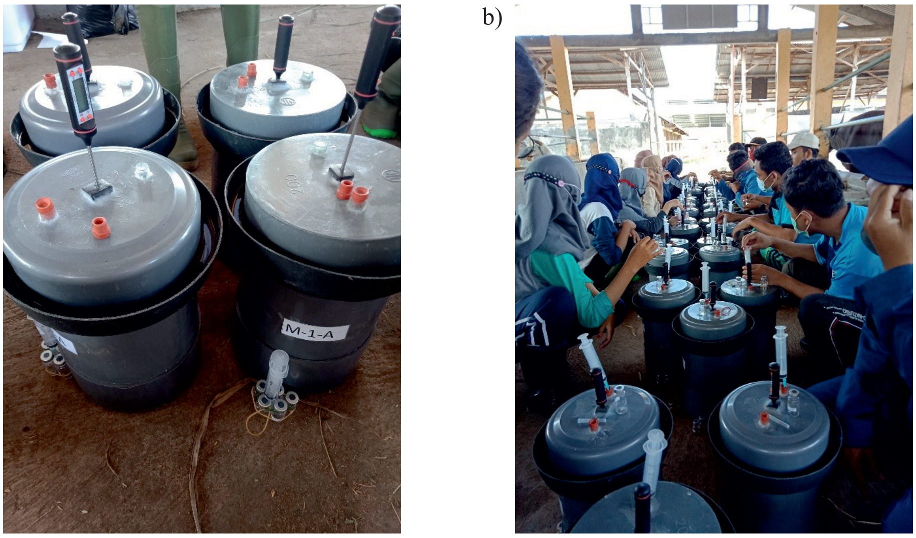

Fig. 2. The closed cylinder chambers contain feces samples a). The gas collection was done simultaneously at the same time b).

Results and Discussion

Methane Emission

On the initial day of gas collection (day 0), Bali Cattle fed with 70:30 Forage:Concentrate had the lowest CH_4 emission, while the Madura Cattle fed with 30:70 Forage:Concentrate had the highest, as shown in Table 4. CH_4 emission on day 3 was slightly different with day 0. The Madura Cattle fed with 30:70 Forage:Concentrate became the lowest emitters of CH_4, while the highest was PO Cattle fed with 70:30 Forage:Concentrate.

The pattern of CH_4 emission among treatments was shown in Fig. 3. It shows that the result showed emission peak of CH_4 was on day 0 to 6. The CH_4 emission continue to decrease until the last day of observation. The decrement of CH_4 in this research related to methanogenesis process. The process of methanogenesis is regulated by the concentration of O_2, the content of organic matter as a substrate, and the factors that determine its redox potential [12]. Organic matter is the main input for triggering methane production processes.

The increase of available organic matter, and its subsequent decomposition in soils under anaerobic conditions, stimulate methanogenesis by providing a substrate for the production of acetate and hydrogen and causing soil reducing conditions [13]. CH_4 emissions from feces deposited by animals on pastures range from 7 to 27% of total emissions by ruminants [14]. However, these emissions may become less significant depending on the environment and manure management [15].

Several factors affect the different pattern of CH_4, such as moisture content that plays an important role in methane production. When the moisture content of manure is reduced, the methane production trend falls, demonstrating that reducing the water content of manure could potentially cut CH_4 emissions from manure [16]. Therefore, it is important to investigate the possibilities of lowering water in manure as a methane mitigation technique [16].

Table 4. CH_4 emission (mg CH_4/kg/day) of treatments on each observation day.

Treatments*	Observation days										
	0	3	6	9	12	15	18	21	24	27	30
B-A	391.82	439.49	331.20	104.02	18.67	9.08	-3.48	-1.99	-1.04	-0.72	-17.47
M-A	486.95	636.66	336.69	20.32	121.69	9.45	-6.25	0.36	0.19	-0.25	-3.38
PO-A	482.56	692.18	74.37	189.00	18.58	11.51	-2.39	-4.01	-0.67	-0.24	-4.72
B-B	824.68	546.58	233.28	53.87	15.08	8.49	-0.23	-3.30	-0.63	0.68	-5.52
M-B	895.00	235.84	351.29	35.69	136.58	24.28	0.40	-3.92	0.12	0.15	-3.20
PO-B	606.93	553.40	123.31	103.66	25.21	23.50	2.77	-3.83	-0.69	0.78	-5.94

* B-A = Bali cattle with Forage:Concentrate = 70:30; M-A= Madura cattle with Forage:Concentrate = 70:30; PO-A = PO cattle with Forage:Concentrate = 70:30; B-B = Bali cattle with Forage:Concentrate = 30:70; M-B = Madura cattle with Forage:Concentrate = 30:70; PO-B = PO cattle with Forage:Concentrate = 30:70

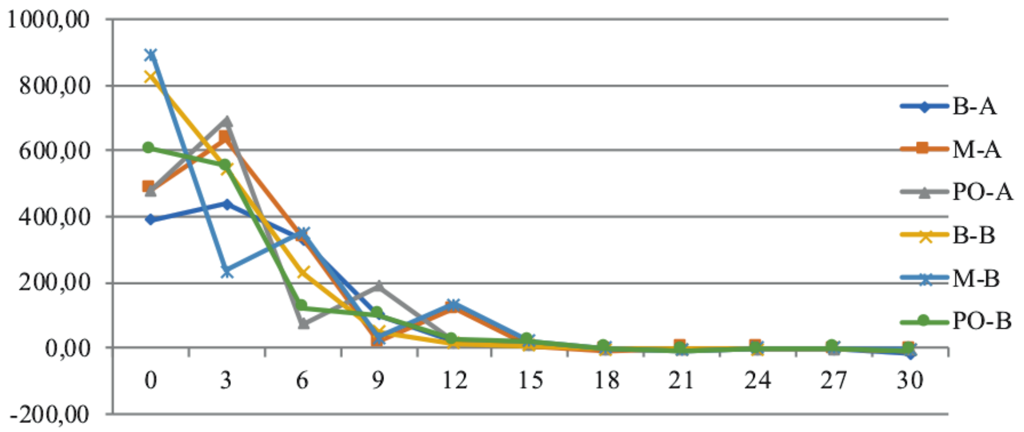

Fig. 3. CH_4 emission (mg CH_4/kg/day) of treatments on each observation day.
Note: B-A = Bali cattle with Forage: Concentrate = 70:30; M-A= Madura cattle with Forage:Concentrate = 70:30; PO-A = PO cattle with Forage: Concentrate = 70:30; B-B = Bali cattle with Forage:Concentrate = 30:70; M-B = Madura cattle with Forage:Concentrate = 30:70; PO-B = PO cattle with Forage: Concentrate = 30:70.

Nitrous Oxide Emission

With regards to N_2O emission (Table 5), PO Cattle fed with 30:70 Forage: Concentrate had the lowest N_2O emission, while the Bali Cattle fed with 70:30 Forage: Concentrate had the highest on the initial day of gas collection (day 0). Fig. 4 shows that the emission peak of N_2O was on day 9 to 15. Previous studies have shown that high N_2O emissions occurred in the first phase of manure application. Akiyama et al. [17] measured N_2O flux for six months with 14 times measurement. The result showed that N_2O flux was higher in the first month and reduced in the later months. A similar result was shown by Owens et al. [18] who measured N_2O fluxes of urine treated soil for 35 days with seven times measurement. The result showed that the peak of N_2O flux was in the first five days.

The production of N_2O is attributed mainly to microbial processes. Bacteria produce N_2O through nitrification (aerobic conditions) and denitrification (anoxic conditions) [1]. One of the main factors regulating nitrification, denitrification and release of N_2O is the availability of soil oxygen [18]. In the nitrification process, catabolic oxidation is transformed NH_4^+ to nitrite (NO_2^-) by Nitrosomonas sp. and then to nitrate (NO_3^-) by Nitrobacter bacteria [20].

During denitrification, N_2O is an intermediate in the dissimilatory nitrate and NO_2 reduction to N_2 under anaerobic conditions and consumed by denitrifying bacteria in soil, then N_2O arise during NH_4^+ oxidation to nitrite when O_2 supply is limited [8]. However, denitrification could also occur in aerobic environments because many denitrifying bacteria can produce N_2O over a wide range of oxygen pressures [18].

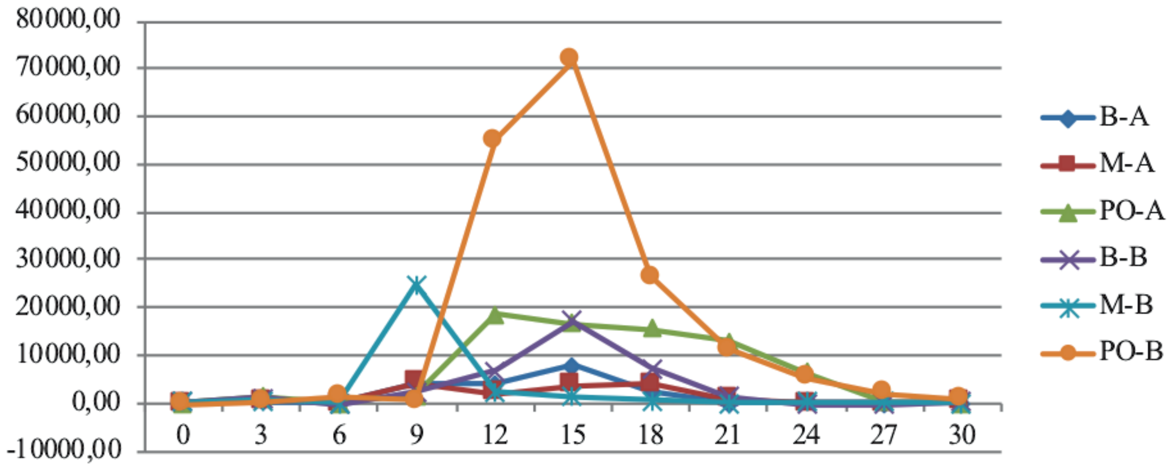

Fig. 4. N_2O emission (g N_2O/kg/day) of treatments on each observation day.
Note: B-A = Bali cattle with Forage:Concentrate = 70:30; M-A= Madura cattle with Forage:Concentrate = 70:30; PO-A = PO cattle with Forage:Concentrate = 70:30; B-B = Bali cattle with Forage:Concentrate = 30:70; M-B = Madura cattle with Forage:Concentrate = 30:70; PO-B = PO cattle with Forage:Concentrate = 30:70.

Table 5. N_2O emission (g N_2O/kg/day) of treatments on each observation day

Treatments*	Observation days										
	0	3	6	9	12	15	18	21	24	27	30
B-A	223.98	267.74	9.49	4,063.49	3,849.10	7,820.39	2,140.79	-80.70	398.63	84.01	73.64
M-A	-115.62	506.54	-53.43	4,282.05	1,997.91	3,768.70	3,910.04	711.06	-80.88	8.80	157.23
PO-A	55.57	1,310.93	-38.23	1,754.35	18,407.71	16,450.24	15,416.87	13,010.96	6,409.72	166.13	109.42
B-B	145.08	998.27	-179.09	2,200.18	6,785.16	17,151.44	7,420.83	1,327.00	-311.88	-315.48	223.40
M-B	212.03	525.32	97.15	24,594.27	2,096.18	1,343.63	552.46	63.97	163.58	288.71	68.40
PO-B	-200.37	279.65	1,522.61	417.24	55,118.35	71,781.62	26,424.71	11,146.51	5,294.80	1,877.09	944.68

* B-A = Bali cattle with Forage:Concentrate = 70:30; M-A= Madura cattle with Forage:Concentrate = 70:30; PO-A = PO cattle with Forage:Concentrate = 70:30; B-B = Bali cattle with Forage:Concentrate = 30:70; M-B = Madura cattle with Forage:Concentrate = 30:70; PO-B = PO cattle with Forage:Concentrate = 30:70

The magnitude of N_2O emissions depends on the interplay between prevailing soil microclimate, microbial activity, plant composition, biomass, and excreta composition, that in turn is defined by animal type and feed intake [21]. Micrometeorological N_2O flux measurements will have to be used more frequently in the future to provide researchers with answers in the face of a rapidly changing climate on earth [21].

The moisture content also plays important role in manure N_2O emission. Recent research on GHG emission from manure sun-drying showed that organic matter in the manure is not lost during the drying period and the methanogens seem to be inactivated due to the oxic conditions due to moisture loss, but methanogens detected after the 7-day drying period [22]. N contained in the manure also remains in the final dried manure and nitrification could occur during the drying process. This could be a potential source of N_2O after the application of the manure [22].

Effect of Cattle Breed and Forage-Concentrate Ratio

Cattle breed had no significant effect on both CH_4 and N_2O ($p>0.05$) as shown in Table 6 and 7. It is assumed that the three breeds on this study were tropical breed and well-adapted to the environment. Local cattle have a better ability to digest crude fiber, so they are more effective in digesting feed and reduce enteric fermentation that produces methane [23]. Local cattle breeds in Indonesia has a more effective ability to digest crude fiber. the amount of methane emission is also affected by differences in the effectiveness of feed digestion caused by differences in the composition and population of microbes in the rumen [24].

The previous study showed the effects of dairy cow breed (Holstein and Jersey) on greenhouse gas emissions from manure during storage and after field application was also not significant [25]. A meta-analysis showed that when reporting urine derived N_2O emissions, it was important to account for differences in animal diet, sex and breed, in addition to urine composition and nitrogen loads [26].

Previous study conducted gas measurement in nine cattle farms, seven were home to dairy cows and two to beef cattle [27]. The farms represented typical breeds, housing and management systems. Enteric methane emissions vary according to the animal feed intake and the energy used, therefore different breed with differences in weight will produce distinctive emissions [27]. As for manure emissions, the amount of manure stored in the house, the use of straw in the house or any other treatment the manure is subjected to can affect the strength of emissions [27].

Feed composition had no significant effect on both CH_4 and N_2O ($p>0.05$). However, feed composition had a significant effect in the previous study. The research on 21 dairy farms in Germany showed the total emissions had a high dependence on the diet

Table 6. The analysis of variance on cattle breed and feed ratio effect on CH_4 emission.

Source of Variation	SS	df	MS	F	P-value	F crit
Cattle Breed	1370.624	1	1370.624	0.63475	0.43343	4.259677
Feed Ratio	1826.37	2	913.1849	0.422905	0.659929	3.402826
Interaction	2107.394	2	1053.697	0.487978	0.619826	3.402826
Within	51823.54	24	2159.314			
Total	57127.93	29				

Note: SS = sum of squares; df = degree of freedom; MS = mean square

Table 7. The analysis of variance on cattle breed and feed ratio effect on N_2O emission.

Source of Variation	SS	df	MS	F	P-value	F crit
Cattle Breed	121936602.2	1	121936602.2	1.370936539	0.253145705	4.259677273
Feed Ratio	540676329	2	270338164.5	3.039419344	0.066588944	3.402826105
Interaction	101427793.9	2	50713896.93	0.570177724	0.572898831	3.402826105
Within	2134656397	24	88944016.56			
Total	2898697122	29				

Note: SS = sum of squares; df = degree of freedom; MS = mean square

composition; in particular, on the grass/maize ratio and the protein content of the animal diet, as well as from the manure management [28].

The effect of feed composition in the present study was not significant probably due to N content of Elephant grass and concentrate. The use of other forage and legume will give low N in urine. Plants with diuretic qualities, such as *Lolium perenne*, *Trifolium repens*, and *Plantago lanceolata*, have the ability to reduce urinary-N loading in individual urine patches by increasing grazing animals' urination frequency [29, [30]. Although increasing urination frequency results in more urine-affected soil coverage, overall paddock-scale N_2O emissions from urine patches are unlikely to be larger if total urinary-N excreted remains constant. In fact, if the N_2O emission factor decreases with N loading rate, they could be lower [30].

Conclusions

The result showed emission peak of CH_4 was on day 0 to 6, while the N_2O peak was on day 9 to 15. The highest amount of CH_4 emission occurred on feces of Bali cattle with 30:70 forage-concentrate ratio (895 mg CH_4/kg/day) on the first day of observation. The highest amount of N_2O emission occurred on feces of Peranakan Ongole cattle with 30:70 forage-concentrate ratio (71,781.62 g N_2O/kg/day) on day 15. The cattle breed and forage-concentrate ratio had no significant effect on both CH_4 and N_2O emission from feces.

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Conflict of Interest

We certify no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

Ethical Approval

The use of animals in this experiment approved by the Institutional Animal Care and Use Committee of Indonesian Agency for Agricultural Research and Development, Ministry of Agriculture (reg. nr.: Rm/04/2021).

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