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Original Article

Effect of ammoniated rice straw with urine and urea on the amount of digested crude protein, methane gas production and VFA profile of fat tail sheep

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Abstract

Objective: This research aimed to find out the VFAs profile and methane gas production in fat-tailed sheep that fed rice straw ammoniated with commercial urea (T1), and rice straw ammoniated with urine (T2). Eight thin-tailed sheep aged 1.5 with an average body weight of 20 kg were used in this study. All the object studies were placed in individual metabolism cages. The feed concentrate used in this study was 2.3 % from the body weight of livestock, rice straw was ammoniated and water was given ad libitum, and feed was given twice a day at 7.00 a.m. and 4.00 p.m. Concentrate were given first, then 1 hour later the ammoniated rice straw was given. The drinking water was provided ad libitum.
Methods: This research was conducted in three stages, 1) total collection, 2) measure methane gas production, and 3) rumen fluid collection. A completely randomized design with 2 treatments and 4 replications was used in this study. After all data was collected, then it was analyzed using the Independent T-test using IBM SPSS Statistics 21 software.
Results: The results show no significance ($P > 0.05$) on rice straw ammoniated with commercial urea (T1), and rice straw ammoniated with urine (T2) for methane gas production (1,328.68±349.56 vs 1,463.95±215.41), VFA Total (40.74±13.09 vs 34.79±6.34), NH_3 production (9.17±3.67 vs 8.78±2.94). It indicates that ammoniated using commercial urea or urine does not interrupt rumen fermentation.
Conclusions: It could be concluded that crude protein digestible, methane gas production, and VFAs profile especially the fermentation conditions in the rumen are not affected by ammoniation from commercial urea or urine.

Keywords: Rice straw; Ammoniation; Methane; Crude protein; VFA

INTRODUCTION

The phenomenon of rising temperatures on Earth, currently experienced, is partly caused by global warming. Global warming occurs due to the trapping of heat produced by the sun in the atmosphere, which was previously absorbed by greenhouse gases (GHGs) [1]. Some GHGs responsible for global warming include carbon dioxide (CO_2), nitrogen oxide (NO_2), and methane (CH_4). Ruminant accounted for third as a contributor for GHGs [2].

Methane gas is one of the gases produced during the fermentation process in the rumen of ruminant animals. This phenomenon is known as methanogenesis, which is caused by archaea methanogens. The process of methanogenesis occurs rapidly in the rumen because archaea methanogens can utilize carbon and hydrogen skeletons to produce methane. The potential value of GHG is 25 times of CO_2 [2].

The formation of methane in the rumen leads to losses, one of which is the energy that should be utilized to form propionic acid [3]. Propionic acid serves as a precursor for muscle formation in ruminant animals. According to Mitsumori and Sun (2008), methane production in the rumen results in an energy loss of 2% to 12% [4].

Several factors contribute to the high production of methane gas in the rumen. These factors include the digestible protein content in the feed [5]. As the amount of digestible protein increases, more nitrogen (N) is produced. Additionally, another contributing factor is the crude fiber content in the feed. When the crude fiber content in the feed is higher, it leads to an increased production of methane gas.

Reducing methane production in the rumen can be achieved through feed management. Compounds such as phenolics, tannins, and saponins can inhibit methanogen bacteria development.

Rice straw is an abundant agricultural waste, but it has very low crude protein content and high crude fiber content. Therefore, efforts are needed to improve the quality of rice straw. One of the methods is ammoniation, which increases the nitrogen (N) content in rice straw.

Ammoniation of rice straw is expected to increase the nitrogen content in the straw. However, this excess nitrogen can alter fermentation patterns, impacting the production of hydrogen (H_2) and volatile fatty acids (VFAs). Furthermore, the elevated ammonia levels may encourage the growth of specific bacteria that produce less hydrogen, ultimately limiting the availability of substrates for hydrogenotrophic methanogens.

The objective of this study is to examine the profile of volatile fatty acids (acetate, propionate, and butyrate) which produced from variations in digestible protein levels and methane production.

MATERIALS AND METHODS

Materials

The research was conducted in experimental farm of the Faculty Animal and Agricultural Science, Diponegoro University, Semarang. The material used in this research consisted of eight fat-tailed sheep with an age of approximately 1.5 years and a weight of approximately 20 kg. Data collection was carried out in several stages, including: (1) Total collection stage to collect digestibility data, (2) Methane gas data collection stage to measure methane production resulting from rumen fermentation, and (3) rumen fluid collection stage to determine the levels of volatile fatty acids (VFAs) such as acetate, propionate, and butyrate.

Dietary feed was provided twice a day at 7.00 a.m. and 4.00 p.m., with 2.3 % of the body weight in concentrate and ad libitum access to ammoniated rice straw. Concentrate were given first, then 1 hour later the ammoniated rice straw was given. The nutritional content of the feed used can be found in Table 1.

Rice straw ammoniation

The ammoniation of rice straw with commercial urea is carried out according to Saputro *et al.* (2024) [5], while the ammoniation

Table 1. Nutritional Content of Feed

Feedstuffs	Dry Matter	Ash	Crude Protein	Crude Fat	Crude Fiber	NFE	TDN *)
	-------------	-------------	-------------	(%)	-------------	-------------	-------------
Concentrate	83.40	10.12	20.72	0.73	18.41	50.02	61.82
Rice straw amoniated with commercial urea	87.95	25.54	9.10	2.76	37.83	24.78	37.52
Rice straw amoniated with urin	86.59	21.95	8.75	4.00	34.60	30.70	39.50

NFE: Nitrogen-free extract, TDN: Total digestible nutrients, TDN was calculated based on the digestibility coefficients as determined by Haris *et al.* (1972) in Hartadi *et al.* (1990) [6].

of rice straw with urine is conducted according to Hidayat *et al.* (2009) [7]. The use of urine is expected to increase the nitrogen content in rice straw. Moreover, the phenolic compounds present in urine are predicted to decrease methane production by slowing down the metabolism of the specific archaea bacteria responsible for methane synthesis.

Feed digestibility

The digestibility measurement of feed was conducted using the total collection method over a period of seven days in a metabolic cage. Subsequently, the digestibility data were calculated according to the method described by Tillman *et al.* (1998) [8].

Measurement of methane gas

The measurement of methane gas resulting from enteric fermentation in the rumen is conducted using the CH_4 Analyzer from Horiba Ltd, Japan, along with an airflow meter (L/min) (Airflow meter from STEC, Horiba Ltd, Japan). The measurement method is based on Purnomoadi *et al.* (2003) [9].

VFAs production and NH_3

The measurement of Volatile Fatty Acid (VFA) production is conducted using the method described by Filipek and Dvorak (2009) [10]. Meanwhile, the measurement of NH_3 concentration is done according to Chaney and Marbach (1962) [11].

Data analysis

The data obtained were analyzed using the Independent T-test method using the IBM SPSS Statistics 21 software.

RESULTS

The research results presented in Table 2 indicate that there is no significant difference in the digestible dry matter (DDM), digestible organic matter (DOM), digestible crude protein (DCP), and methane gas production between the two treatments.

The digestible dry matter showed no significant between the treatment of rice straw ammoniated with commercial urea (T1) and ammoniated with urine (T2), but numerically, T2 was higher than T1. The digestible organic matter, digestible crude protein, and methane gas production also showed no significant between T1 and T2.

The VFAs profile for acetate, propionate, and butyrate showed no significant between T1 and T2, also for the A/P ratio and NH_3 production. Numerically, T1 showed a higher value than T2 for all parameters. The research results presented in Table 3.

Table 2. Digestible dry matter, digestible organic matter, digestible crude protein, and methane gas production.

Parameters	Treatments	
	T1	**T2**
Digestible dry matter (g/h)	576.25±92.71	680.50±36.08
Digestible organic matter (g/h)	531.00±78.59	633.25±25.71
Digestible crude protein (g/h)	110.50±15.93	124.50±4.80
Methane gas production (kJ/d)	1,328.68±349.56	1,463.95±215.41

T1 : rice straw ammoniated with commercial urea, T2 : rice straw ammoniated with urine

Table 3. VFAs profile, Acetate: Propionate ratio and NH_3

Parameters	Treatments	
	T1	**T2**
VFA total (mMol/l)	40.74±13.09	34.79±6.34
Acetate (mMol/l)	29.02±9.50	24.47±4.39
Propionate (mMol/l)	6.60±2.03	5.85±1.50
Butyrate (mMol/l)	5.13±1.66	4.46±0.87
A/P ration	4.39±0.32	4.24±0.29
NH_3 (mg N/100 ml)	9.17±3.67	8.78±2.94

T1 : rice straw ammoniated with commercial urea, T2 : rice straw ammoniated with urine,
A/P : acetate/propionate.

DISCUSSION

The digestible dry matter did not show significance for T1 and T2, this indicates that both rice straws which ammoniated with commercial urea or urine were acceptable. The digestible organic matter was also shown to not be significant on T1 and T2. The organic matter digestible indicates that the higher organic matter digestible, then higher methane gas production. Zaman *et al.* (2021), state that if feed intake and digestion efficiency are high, it would contribute to an increase in methane production, it is because the substrate to form methane gas is available [12].

Digestible crude protein does not show significance both for T1 and T2. The effect of crude protein on methane gas production was inconsistent between recent studies [13]. Some studies have shown that there is no effect on methane gas production that correlated with an increase in crude protein [14,15]. Arndt *et al.* (2015) reported that increasing CP can affect on increase of methane gas production [16]. A meta-analysis showed a negative correlation between methane production and crude protein [17]. The crude protein digestible also leads to enteric methane gas production indirectly by providing nitrogen for the formation of archaea methanogens. So, the higher the digestible crude protein, then higher the methane gas production. Methane gas production for T1 and T2 does not show significance, even though T2 showed higher numerically. This might be caused by higher digestible organic matter and digestible crude protein.

The VFAs profile, A/P ratio, and NH_3 production do not show significant differences in both treatments, although, in terms of numbers, T2 is lower than T1. The decline in VFA production might be caused by the utilization of nutrients for the formation of methane gas. Greening *et al.* (2019) report that the formation of methane gas by archaea methanogens needs hydrogen (H) [18]. The major source of hydrogen in ruminal fermentation is produced during carbohydrate fermentation, especially high fiber feed sources to produce acetate through the acetyl-CoA pathway [19]. Rice straw has a high crude fiber content, so the formation of acetate is higher than butyrate and propionate. Furthermore, ammoniation is not set up to decrease crude fiber, this is just simply adds nitrogen to the feed material.

The formation of propionate through the acrylate pathway or the succinate pathway needs hydrogen [20]. Similarly, the formation of methane gas also needs hydrogen, therefore an increased proportion of propionate within the VFA profile correlates with a reduction in methane emissions [21].

The butyrate acid has shown no significance for both treatments, but T2 is lower than T1 numerically. The butyryl-CoA pathway produces butyrate acid through fermented dietary fibers, which are predominantly composed of carbohydrates [22]. When producing butyrate, hydrogen is also generated in this process [23], although the amount is not as much as the hydrogen produced during acetate production. The decrease of butyrate acid might be caused by the phenolic that is found in the urine that is used for ammoniation. Phenols could inhibit rumen microbe activity through damage to the cell membrane and coagulating proteins in microbes [24].

The A/P ratio for both treatments does not show a significant difference, but T2 is lower numerically. The ratio A/P could influence methane gas production, this is because a higher ratio of acetate to propionate (A/P), which signifies a predominance of acetate production, generally results in heightened methane emissions owing to the increased availability of hydrogen for methanogenesis [25].

The NH_3 production for both treatments does not show a significant difference. It indicates that the addition of commercial urea or urine for ammoniation does not affect the ability of rumen microbe in the formation of N-microbe. The NH_3 acts as a crucial nitrogenous substrate for the proliferation and metabolic functions of ruminal microorganisms, including archaea methanogens [26].

CONCLUSIONS

The using of commercial urea or urine to ammoniate the rice straw did not affect the formation of methane gas production and VFA profile. In future studies, it might be the calculation of the best dosage for urine to use in ammoniation of rice straw which is more effective in reduction of methane gas production and increasing of VFA production.

CONFLICT OF INTEREST

I declare that there are no conflicts of interest related to funding sources or materials used in this research.

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