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# Rumen fluid profiles and environmental cost of production in weaned thin-tailed lambs given different levels of feeding

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**ABSTRACT**

This study was conducted to examine the effect of different levels of feeding on rumen fluid profiles and environmental cost of weaned Thin-tailed lambs. As many as 21 weaned male lambs were allocated into 3 feeding levels in a completely randomized design. The treatments were low (4% BW; T1), medium (5.5% BW; T2) and high feeding level (*ad libitum*; T3). Each treatment consisted of seven replicates. This study showed that lambs fed T3 had highest dry matter intake (DMI; 7.9%BW; 1516g/d) than that of T1 and T2 which were significantly different ($p<0.05$). The digestibility was similar in all treatments (averaged 60.21%; $p>0.05$). Lambs fed T3 had higher acetate (112.86 mM), propionate (41.14 mM), and butyrate (31.30 mM) concentrations at 6h after feeding ($p<0.05$). There was no significant effect of feeding levels on ammonia concentrations of rumen fluid at ($p>0.05$). Microbial nitrogen production of lambs was not significantly different (averaged 2.69 g/d; $p>0.05$). Lambs given T3 had the highest ADG (202 g/d; $p<0.05$) and the lowest methane emission production per unit body weight gain (0.54 L/gADG; $p<0.05$). In conclusion, the rumen fluid ammonia and VFA in the lamb are stable at high feeding level. The environmental cost of lamb fattening is lower when the lamb is kept under a high feeding level (*ad libitum*).

## Introduction

Rearing weaned lambs is one of the keys to increasing productivity and this is a common practice in the modern small ruminant industry. In the feedlot system, high weight gain is an important indicator for good performance and economic efficiency where the main product produced is meat (Rianto *et al*., 2024; Luthfi *et al*., 2025). Increasing livestock productivity starts from the efficiency of feed utilization. The good or bad utilization of feed can be seen from nutrient fermentation in the in rumen (Wang *et al*., 2019; Luthfi *et al*., 2024). In the rumen, microbes break down the feed and produce energy volatile fatty acids (VFA) as energy sources for the ruminant needs, its microbes and ammonia (NH3) as nitrogen sources for the microbes'growth. On the other hand, during VFA synthesis, methanogenic bacteria utilize hydrogen (H2) and carbon dioxide (CO2) produced acetic acid's formation in the rumen to produce methane gas (Nguyen *et al*., 2020; Luthfi *et al*., 2024). Methane gas emission in the rumen is calculated as lost energy (around 8 – 12% of digestible energy) that could otherwise be metabolized by ruminants and being a major factor in the greenhouse gas (Purnomoadi *et al*., 2010; Jayanegara *et al*., 2013; Luthfi *et al*., 2024; Waters *et al*., 2025). Not only methane gas, but also nitrogen excretion through urine and faeces from ruminants have an impact on greenhouse gases (Mohajan 2012; Scoones, 2023). Reisinger *et al*. (2021) claimed that methane emissions produced by ruminants have been a focus of recent studies for climate mitigation. Worldwide, methane emission from livestock contributes approximately 28% of global emissions (Mirzaei-Aghsaghali and Maheri-Sis, 2011; Glasson *et al*., 2022). This becomes a problem, what are the next steps that need to be taken to increase productivity but are also environmentally friendly. Therefore, it is necessary to take a strategic step as an effort to reduce methane and nitrogen emissions. Luthfi *et al*. (2024) found that methane emissions and nitrogen excretion per body weight production of Kacang goat can be reduced by increasing feed intake (in young Kacang goat resulted in 0.30 g $CH_4$/BWG and 0.15g Nitrogen/BWG, respectively, in mature age resulted in 0.22 g $CH_4$/BWG and 0.20g Nitrogen/BWG, respectively). However, studies on the different levels feed intake on the rumen profile and environmental costs have not been widely conducted in lambs. Based on that explanation above, this study aimed to examine the effect of different feeding levels on rumen fluid profiles and environmental costs in increasing the productivity of Thin-tailed lambs.

## Materials and methods

*Experimental design*

The maintenance and rearing methods of the animals in this study were approved by ethical committee with administration number: No. 60-02/A-09/KEP-FPP. Twenty-one male lambs (aged 3 mo and weighed 11.93±1.14 kg) were randomly divided into three feeding groups: low feeding (4% body weight), medium feeding (5.5% body weight), and high feeding (*ad libitum*). The feed was pelleted and consisted of 20% sugarcane, 15% rice bran, 36% cassava flour, 11% pollard, 10% soybean meal (SBM), 6% molasses, and 2% mineral mixture. The nutritional content was 14% crude protein (CP); 65% total digestible nutrients (TDN); 21% crude fiber (Cfi); and 2.27% ether extract (EE). The feed for low and medium feeding levels was provided twice daily, while the high feeding level was provided throughout the day.

*Experimental procedures and parameters measured*

This study was conducted in three stages, namely adaptation, the preliminary, and the data collection stage. The adaptation stage (2 weeks) was conducted to provide animals adapted to the feed and environment of the study and to determine the ability of lamb to consume the diet. In preliminary stage (1 week), The animals were randomly assigned to pens


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and fed. The feed provided and remaining feed were weighed to calculate daily feed consumption during the data collection stage (10 weeks). Body weight was measured weekly to calculate weight gain and adjust feed amounts for the following week.

Rumen fluid sampling was carried out 3 times a day, namely 0, 3 and 6 h after feeding. Rumen fluid samples were taken using a tube and pump. The pump hose was inserted into the mouth and into the rumen cavity of the sheep. The hose from rumen cavity sucked 20 ml of rumen fluid and then connected to the hose that flowed into the tube (Luthfi *et al*., 2024). After the rumen fluid was taken, the collected rumen fluid samples were mixed with $H_2SO_4$ (20% concentration) to maintain a pH of 3 or less. The samples were then filtered to obtain the fluid and stored in a freezer prior to VFA and $NH_3$ analysis. Rumen fluid VFA was analyzed using chromatography (Isac *et al*., 1994) while the measurement of $NH_3$-N of rumen fluid concentration was using the phenol-hypochlorite colorimetric method as described by Broderick and Kang (1980).

Acetate, Propionate and Butyrate concentrations were used to estimate the potential for methane production in sheep using the Moss *et al*. (2000) formula. The calculation of methane emission estimates uses the following formula:

$CH4 = 0.045\ C2 - 0.275\ C3 + 0.40\ C4$

Where:

C2 = acetic acid

C3 = propionic acid

C4 = butyric acid

*Microbial nitrogen production*

Microbial nitrogen production (MNP) was determined using the purine derivatives excreted in the urine. The urine samples were analyzed for allantoin according to the method of Chen and Gomes (1992). The formulae were:

$Y = 0.84X + (0.150BW0^{75- e\ -0.25X})$

MNP (gN/d) = (Xx70) / (0.116x0.83x1000) = 0.727 x X

MNP efficiency (gN/kg OM) = (MNP)/(OM digested).

where:

X is the total amount of microbial purine absorption (mmol/day)

Y is the total amount of microbial purine derivatives excreted in the urine (mmol/day)

$BW^{0.75}$ is the metabolic weight of body

0.83 is assumed to be the microbial purine digestibility.

70 is the purine N content (mg/mmol)

0.116 is the purine-N: total N ratio in the measured mixed microbes.

## Results

*Intake and rumen fluid profiles*

Feed intake, digestibility and rumen fluid profile are presented in Table 1. In this study, lambs fed *ad libitum* had the highest feed intake ($p<0.05$). In this study, the feeding levels had no effect ($p>0.05$) on the VFA concentration of lamb rumen fluid at 0 and 3 hours (Table 1). However, the feeding levels had an effect ($p<0.05$) on the VFA concentration at 6 hours in sheep fed T3 for acetate, propionate and butyrate. Total VFA at 6 hours showed a significant difference ($p<0.05$). Lambs fed *ad libitum* had the highest VFA concentrations ($p<0.05$) at 6 hours compared to VFA in lambs fed T1 and T2. The VFA profile at 6 hours in lambs fed *ad libitum* did not decrease as in lambs fed 4% and 5%. The increase in feeding level in this study did not change the A/P value ($p<0.05$). In this study, the mean of A/P value was 2.8. The $NH_3$ concentration in rumen fluid of lambs was similar among treatment ($p>0.05$) either at 0, 3 or 6 hours. In this study, it also found that the increase of feeding level resulted in a higher digestible organic matter ($p<0.01$). This study also found that production of microbial protein in the rumen fluid of lambs did not change ($p>0.05$).

Table 1. Rumen Fluid Profile of Thin-tailed lambs raised under different level feeding.

| Parameters | T1 | T2 | T3 | SEM | *p* values[1)] |
|---|---|---|---|---|---|
| DMI (g/d) | 609.63$^{C}$ | 869.16$^{B}$ | 1516.00$^{A}$ | 86.14 | 0 |
| DMD (%) | 60.7 | 59.04 | 59.41 | 0.88 | 0.74 |
| Acetate | ------------------------- | ------------------------- (mmol) | ------------------------- | | |
| 0 h | 72.04 | 81.66 | 94.17 | 7.25 | 0.48 |
| 3 h | 99.43 | 102.36 | 117.85 | 7.91 | 0.16 |
| 6 h | 86.13$^{C}$ | 96.18$^{B}$ | 112.86$^{A}$ | 8.08 | 0.02 |
| Propionate | | | | | |
| 0 h | 25.85 | 28.56 | 33.37 | 2.45 | 0.36 |
| 3 h | 35.26 | 38.38 | 41.91 | 2.96 | 0.62 |
| 6 h | 30.88$^{C}$ | 33.16$^{B}$ | 41.14$^{A}$ | 2.6 | 0.02 |
| Butyrate | | | | | |
| 0 h | 16.78 | 19.65 | 22.76 | 2.48 | 0.29 |
| 3 h | 24.7 | 24.07 | 22.76 | 2.21 | 0.65 |
| 6 h | 18.69$^{B}$ | 19.12$^{B}$ | 31.30$^{A}$ | 2.68 | 0.05 |
| A/P ratio | | | | | |
| 0 h | 2.9 | 2.8 | 2.7 | 0.08 | 0.94 |
| 3 h | 2.8 | 2.7 | 2.8 | 0.13 | 0.82 |
| 6 h | 2.8 | 2.8 | 2.7 | 0.11 | 0.87 |
| $NH_3$ (mg/ 100 ml) | | | | | |
| 0 h | 27.95 | 28.35 | 25.25 | 1.69 | 0.74 |
| 3 h | 22.82 | 24.97 | 25.14 | 1.95 | 0.88 |
| 6 h | 22.78 | 24.35 | 23.82 | 1.61 | 0.93 |

DMI= Dry matter intake; DMD= Dry matter digestibility; [1)]The different superscripts in the same row indicate significant differences among treatments ($p< 0.05$).

Table 2. Microbial production and microbial efficiency of lamb raised under different feeding level.

| Parameters | T1 | T2 | T3 | SEM | *p* value[1)] |
|---|---|---|---|---|---|
| DOMI (g/d) | 521.62$^{C}$ | 723.09$^{B}$ | 1018.12$^{A}$ | 38.48 | 0 |
| N microbial production (g/d) | 2.67 | 2.7 | 2.71 | 0.1 | 0.98 |
| Microbial protein production (g/d) | 13.33 | 13.49 | 13.53 | 0.52 | 0.99 |
| N microbial efficiency (g/kg DOMI) | 25.59$^{A}$ | 18.68$^{B}$ | 13.31$^{C}$ | 1.32 | 0 |

DOMI= Digested organic matter intake; N= Nitrogen; [1)]The different superscripts in the same row indicate significant differences among treatments ($p< 0.05$).

*Productivity and environmental cost*

Lamb production, methane emission and nitrogen excretion are presented in Table 3. Lambs fed T3 had highest ADG than that of lambs fed T1 and T2 ($p<0.01$). This finding showed that lambs with *ad libitum* intake had highest methane production and nitrogen excretion than those of lambs fed low and medium feeding ($p<0.01$). The environmental cost of lamb production in this study is presented in Table 3. Lambs fed T3 had lowest methane production per ADG than that of T1 and T2 ($p<0.05$).

## Discussion

The higher amount of feed resulted in high DMI. It also indicated that the lambs fed *ad libitum* were able to have DMI (up to 7.9% of BW). This finding was higher than previous study by Prima *et al*. (2019) which found that Thin-tailed lambs fed *ad libitum* were able to consume feed as much as 5.05% of BW. This difference in results was due to digestibility in current study (60.21%). It was higher than previous study by Prima *et al*. (2019) which was 51.3%. Dry matter digestibility in this current study was not significantly different among treatments (averaged, 60.21%; $p>0.05$). It was speculated that the digesta passage rate of lambs was not different in all treatments. The difference in feed intake did not alter the feed digestibility when there is no change in the rate passage of feed in digestive tract (Rianto *et al*., 1998; McDonald *et al*., 2010).

Table 3. The productivity, methane production and nitrogen excretion of lambs.

| Parameters | T1 | T2 | T3 | SEM | *p* value[1)] |
|---|---|---|---|---|---|
| ADG (g/d) | $85.35^{C}$ | $113.06^{B}$ | $202.63^{A}$ | 0.01 | 0 |
| FCR | 7.44 | 7.94 | 7.52 | 0.28 | 0.77 |
| Methane Prod. (L/d) | $6.53^{C}$ | $8.90^{B}$ | $10.87^{A}$ | 0.53 | 0 |
| Nitrogen Excr. (g/d) | $4.58^{C}$ | $7.18^{B}$ | $12.00^{A}$ | 0.79 | 0 |
| Methane Prod/ADG (L/ADG) | $0.78^{B}$ | $0.80^{B}$ | $0.54^{A}$ | 4.51 | 0.02 |
| Nitro prod/ADG (g/ADG) | 0.55 | 0.68 | 0.6 | 4.96 | 0.58 |

ADG= Average daily gain; FCR= Feed conversion ratio; [1)]The different superscripts in the same row indicate significant differences among treatments ($p< 0.05$).

A high level of feeding caused lambs fed T3 still have a higher nutrient intake to be fermented longer, so that acetic, propionic and butyric acid at 6 hours remain high. Previous study by Luthfi *et al*. (2024) found that the higher the nutrient content of the feed consumed resulted in the more organic matter fermented in rumen. Khan *et al*. (2016) claimed that VFA concentration in the rumen improves as increase as feed consumed and nutrient availability and the absorption process and feed rate. McDonald *et al*. (2011) stated that the total VFA production required for livestock survival ranges from 70-150 mmol.

The similar A/P ratio in this study was due to the amount of feed intake with the same ingredient compositions (Table 2) in the diet was directly proportional to the composition of VFA concentration in rumen fluid in acetic, propionic, butyric acids. There were no differences in composition of the ingredients of the diet. It caused the concentration of acetic acid and propionate was also similar among treatments in this study. Cheng *et al*. (2021) and Luthfi *et al*. (2024) found that fermentation conditions in the rumen were influenced by the composition of the ingredients in the ration, microbes and feed properties such as degradation rate and feed molecular structure. Previous studies by Lin *et al*. (2020) and Luthfi *et al*. (2023) showed that rations dominated by fibrous feed (forage) stimulated the formation of acetic acid while rations dominated by concentrates encouraged the formation of propionate. Bannink *et al*. (2006) stated that feed with higher starch and protein content increased propionate production, hemicellulose in feed increased butyrate production, and cellulose increased acetate production. This A/P value was in line with the previous study by Mu *et al*. (2019) which showed that lambs of Dorper and Small Thin-tailed crossbreed fattened with a balance of buckwheat and corn straw had an A/P ratio of 2.23 - 3.09. Luthfi *et al*. (2018) and Luthfi *et al*. (2024) stated that the lower the ruminal A/P value, the more efficient the dietary energy utilization, so that the productivity of ruminant would be higher. At least, the optimum of A/P in feedlot system should be 3 or less.

The average NH3 in this study was 25.05 mmol. It was due to the higher amount of DMI caused higher a rapid flow rate of feed into the post-rumen. In other words, protein degradation in the rumen in lambs fed high DM decrease as an increase the passage rate in digestive tract. Luthfi *et al*. (2024) and Wang *et al*. (2023) stated that NH3 concentration in rumen fluid is greatly influenced by crude protein of feed, the digestibility, length of time of the feed to stay in the rumen and rumen pH. Li *et al*. (2019) and Luthfi *et al*. (2024) found that ammonia concentration was not in line with the increase of feed intake because high DMI reduced the rate of protein degradation in the rumen. On the other hand, Manoni *et al*. (2023) stated that stable and non-excessive ammonia concentrations in the rumen prevent livestock from bloating cases. Regarding the previous study by McDonalds *et al*. (2011) that the optimal rumen ammonia concentration range for microbial growth is 8.5 - 30 mg / 100 ml. Wang and Tan (2013) recommend that for optimum microbial growth, it is necessary to strive for ammonia produced in the rumen to range from 2.5 mM to 18.0 mM.

This study found that an average of microbial protein production was 13.45 g/d. It was due to the concentration of NH3 was similar among treatments (Table 2) and resulting in similar protein production as well. For microbial protein synthesis in the rumen, NH3 is the main source of nitrogen in the rumen (Ran *et al*. 2021). Rumen NH3 concentration is a rough predictor of the efficiency of conversion of feed nitrogen to microbial nitrogen (Luthfi *et al*. 2024). Previous studies by Roman-Garcia *et al*. (2016) and Sok *et al*. (2017) found that increasing DMI was able to provide more substrate for microbial protein production, increase rumen flow rate, thereby increasing microbial protein efficiency. On the other hand, NRC (2007) stated that microbial protein efficiency is low if livestock receive high feed intake due to a decrease in pH. Pazoki *et al*. (2017) Rumen pH is very important for the development, fermentation and health of sheep. In general, rumen fluid pH is influenced by the feed rate and the presence of buffering in the rumen. This allows fermentation to still occur in the rumen and causes a decrease of pH value, thus affecting the efficiency of microbial protein production. In this study, microbial protein production was lower than the findings by Dorri *et al*. (2021) which showed that lambs given different levels of rice straw and Rumen Undegradable Protein (RUP): Rumen Degradable Protein (RDP) ratios had microbial protein production of 15-24 g/d. The different results might be due to differences in the feed given.

Lambs given T3 had highest productivity. It was due to the DMI of lambs fed T3 was high as the treatment conducted. The higher feed intake of lambs, the higher nutrient would be utilized for inducing high ADG as well (Aluns and Luthfi, 2018; Luthfi *et al*., 2024). The higher the DMI is directly proportional to the ADG produced. Therefore, it also similar FCR among treatments.

Lambs fed T3 also had highest methane production. This was because the high feed intake produced fermentation products, especially VFA as explained previously. Therefore, methane production also increased along with the increasing of VFA concentration especially acetic acid (Luthfi *et al*. 2024; Quail *et al*. 2025). The higher DMI also caused the increase of nitrogen excretion through feces and urine. It indicated lambs in this study had the same ability to digest and metabolize feed. Therefore, the higher feed intake resulted in high nitrogen excretion. Prima *et al*. (2019) claimed that nitrogen excretion through feces and urine highly affected the feed intake. On the other hand, this study showed that high feed intake (*ad libitum*) decreased proportion of methane emission per unit body weight gain. This indicated that the energy produced due to high DMI is used more to increase body weight and more efficiently decrease the environmental cost. However, nitrogen excretion per ADG this study was similar among treatments ($p>0.05$). Rianto *et al*. (2024) and Luthfi *et al*. (2024) claimed that the capacity of body cells to metabolize protein has limitations. Therefore, the high DMI did not affect the proportion of nitrogen excretion per unit body weight gain.

## Conclusion

Rumen fluid ammonia and VFA in the lamb are stable at a high feeding level. The environmental cost of lamb fattening is lower when the lamb is kept under high feeding level (*ad libitum*).


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## Conflict of interest

The authors have no conflict of interest to declare.

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