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Addition of cellulolytic bacteria in complete feed block based on agro-industrial by-products for Kacang goats

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Abstract

The research aimed at assessing the effects on nutrient digestion and ruminal fermentation by goats of a complete feed block (CFB) that incorporated agro-industrial by-products that were high in fibre and cellulolytic bacteria. Three Kacang goats, a native Indonesian breed, were used in a 3 × 3 Latin square experimental design with i) CFB without microbes (control), ii) CFB containing 1% *Pseudomonas aeruginosa* and 1% *Acinetobacter baumannii*, and iii) CFB containing 2% *P. aeruginosa* and 2% *A. baumannii*. Microbes in the CFBs consisted of lactic acid bacteria, yeast and cellulolytic bacteria that ranged from 10^6 to 10^8 cfu/g. The goats were fed each day at 08h00 and 16h00. The inclusion of *P. aeruginosa* and *A. baumannii* at 2% level reduced both neutral detergent fibre (NDF) and acid detergent fibre compared with other treatments. Goats fed on CFB with microbes had higher (P <0.01) digestibility of organic matter (OM) and NDF compared with control. The addition of *P. aeruginosa* and *A. baumannii* at 2% level increased (P <0.05) ruminal ammonia nitrogen (N-NH_3), acetate, and total VFA. However, goats fed on CFB with microbes had lower (P <0.05) urinary N excretion, which improved (P <0.05) N retention compared with the control. It was concluded that a combination of lactic acid bacteria, yeast and cellulolytic bacteria in the CFB could modify fermentation in the rumen and increase the use of nitrogen in goats.

Keywords: ammonia, cellulase, intake, rumen
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Introduction

The cost of feed in animal husbandry management is 60 - 70% of total production costs. Therefore it is necessary to attend to the quantity and quality of feed. Forages are often combined with concentrate and feed additives to increase livestock production and productivity. Rice straw and palm fronds are agricultural and plantation wastes that are abundantly available in Manokwari regency. Santoso and Hariadi (2009) and Santoso *et al*. (2017) reported that crude protein (CP) and neutral detergent fibre (NDF) contents in rice straw and oil palm fronds were 6.8% and 4.4%, and 71.1% and 79.2%, respectively, and thus require nutritional augmentation in order to be used effectively as feedstuffs for ruminant livestock.

Total feed block (CFB) is a product that is made up of forage, concentrate, and nutrients in chosen proportions that meet animals' nutritional requirements. Feeding CFB stabilizes the fermentation of rumen, lessens the loss of fermentation, and guarantees better utilization of ammonia (Prasad *et al*., 2001). The technology also boosts milk production, causes fewer environmental pollutants, raises farmers' income, has lower labour requirements, requires less time to feed, and lowers the cost of transporting straw (Karangiya *et al*., 2016).

Microbes digest feed in the rumen and the types and populations of these microbes affect nutrient digestibility. Lactic acid bacteria (LAB) are widely used as probiotics in cattle to enhance digestibility, microbial balance, and health of animals (Uyeno *et al*., 2015). Organisms such as *Lactobacillus*, *Streptococcus* and *Enterococcus* are among the commonly used probiotics for ruminants (Seo *et al*., 2010). Ridwan *et al.* (2018) reported that *L. plantarum* strain 32 isolated from cow rumen had higher in vitro dry matter and organic matter digestibilities and overall production of gas in comparison with the control. Oyeleke and Okusanmi (2008) stated that *P. aeruginosa* inhabited the rumen of cows, sheep, and goats and

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could hydrolyse cellulose. Chang *et al.* (2015) concluded that *Acinetobacter* sp. in the rumen of Korean native cattle belonged to cellulolytic bacteria. Moreover, Surabhi *et al*. (2018) noted that *A. baumannii* is a gut bacterium that has the ability to degrade cellulose. Santoso *et al.* (2016) added *Lactobacillus plantarum, Saccharomyces cerevisiae*, *A. baumannii* and *P. aeruginosa* to the concentrate and observed enhanced fermentation and in vitro nutrient digestibility. Moreover, Santoso *et al.* (2018) concluded that the additions of LAB, yeast, and cellulolytic bacteria to CFB altered in vitro patterns of ruminal fermentation by increasing the concentrations of ammonia nitrogen (NH_3-N) and total VFAs and reducing the production of methane. This study was conducted to evaluate the digestibility of nutrients, characteristics of rumen fermentation and the utilization of nitrogen in Kacang goats fed agro-industrial by-product-based CFBs treated with cellulolytic bacteria.

Materials and Methods

This study was performed according to the standard procedures of rearing and treating farm animals stated in the Law of the Republic of Indonesia, number 18, 2009.

The study collected rice straw and palm oil fronds from the paddy fields and palm oil plantation regions in Prafi district in Manokwari regency in Indonesia at longitude 133° 48’ E and a latitude of 00° 53’ S with an average altitude of 52 metres above sea level. To formulate the CFB, the rice straw and palm oil fronds were sun dried and chopped into lengths of one to two centimetres. Both types of waste were oven dried at 60 °C for 48 hrs. Then they were ground and passed through a 1-mm sieve using a Wiley mill. The *L. plantarum* was isolated from *Pennisetum purpureophoides* (Santoso *et al.,* 2017; Santoso *et al*. 2018). It was cultivated in MRS broth at 30 °C for 48 hours. In the meantime, *S. cerevisiae* was cultivated from malt extract broth at 30 °C for 48 hours (Santoso *et al.*, 2017; Santoso *et al*., 2018). Cellulolytic bacteria *P. aeruginosa* and *A. baumannii* were isolated from the rice straw and the palm oil seed wastes, and cultured with carboxymethyl cellulose. The CFB ingredients were mixed manually and then sprayed on top with *L. plantarum*, *Saccharomyces cerevisiae*, *P. aeruginosa*, and *A. baumannii* at >1.05 x 10^5, 9.45 x 10^8, 9.6 x 10^8, and >1.01 x 10^8 cfu/ml, respectively. All CFBs were formulated to be isonitrogenous (14% CP, DM basis). Around 300 g of blended material was placed in a hydraulic press to create a block measuring 15 × 10 × 8 cm. The three dietary treatments (Table 1) consisted of A) CFB without microbes, B) CFB with 1% *P. aeruginosa* and 1% *A. baumannii*, and C) CFB with 2% *P. aeruginosa* and 2% *A. baumannii*.

Table 1 Ingredients contained in complete feed blocks for Kacang goats made using agro-industrial by-products

Ingredients, %	Complete feed blocks		
	A	B	C
Rice straw	12	12	12
Palm oil frond	25	25	25
Cassava waste	23	23	23
Tofu waste	17	17	17
Molasses	20	20	20
Urea	1.5	1.5	1.5
Premix[1]	1.5	1.5	1.5
Microbes			
Lactobacillus plantarum	0	1.5	1.5
Saccharomyces cerevisiae	0	1.5	1.5
Pseudomonas aeruginosa	0	1	2
Acinetobacter baumannii	0	1	2

A: complete feed block without microbes; B: complete feed block containing 1% *P. aeruginosa* and 1% *A. baumannii*; C: complete feed block containing 2% *P. aeruginosa* and 2% *A. baumannii*

[1]Composition (per kg): 70 g calcium, 189 g phosphorus, 12 g magnesium, 300.000 IU vitamin A, 50.000 IU vitamin D3, 100 mg vitamin E, 100 mg vitamin K, 20 g trace elements (Zn, Mn, Fe, Cu, I, Co, Mo, Se)

Three male Kacang goats with a preliminary bodyweight (BW) of 19.8 ± 2.72 kg were used in a 3 × 3 Latin square design. Prior to the start of the experiment, the goats were dewormed with 10 mg/kg BW Kalbazen (PT Kalbe Farma, Jakarta, Indonesia). The goats were kept in three separate metabolism cages, which enabled the collection of urine and faeces separately. The goats were fed the CFBs twice daily at 08h00 and 16h00 at a dry matter (DM) intake of 66 g DM/kg $BW^{0.75}$/day (Kearl, 1982). Salt licks and fresh water were accessible ad libitum. Each phase lasted for two weeks, that is, eight days to acclimatize to the diet, five days to collect urine and faeces, and one day to sample rumen liquor. The goats were weighed at the start and the end of each phase.

At days 9 through 13 of each phase, each goat's overall faeces and urine excretions were collected and weighed. Individual feed refusals, if any, were collected and weighed every day, and samples were taken for analyses. Sub-samples of faeces were kept at -15 °C for further analysis. Urine was collected in buckets containing 50 ml of 100 mL/L (v/v) sulphuric acid to lower the pH and get it under 3.0 and to avert bacterial obliteration of N. Around 50 mL/L of the urine was sub-sampled and maintained at -15 °C until N was assessed.

On the final day of each period, around 20 ml of rumen fluid was collected via the oesophagus with a flexible stomach tube attached to a vacuum pump prior to the goats being fed and 1, 2 and 4 hours after they had been fed in the morning. Afterwards, this rumen fluid was strained through four layers of cheesecloth. Immediately after it was sampled, the pH was recorded with a digital pH meter (HANNA, Hi9321, Ronchidi Villafranca, Italy). To evaluate NH_3-N, 5 mL sub-samples of filtrate were added to 5 ml of 20 mL/L (w/v) NaCl. Samples to determine volatile fatty acids (VFA) were stabilized with a 46 mM mercuric chloride ($HgCl_2$) solution (200 μL/1.8 ml rumen fluid) and stored at -15 °C for analysis.

The faeces and feed samples were dried in an oven at 60 °C to 70 °C until constant weight, ground to pass a 1-mm sieve and then utilized in a chemical analysis. Feeds and faeces were dried in an oven at 105 °C for 24 hours to determine DM content. Organic material (OM) was obtained after ashing samples at 550 °C for four hours and N was determined with the Kjeldahl technique (AOAC, 2012). Neutral detergent fibre and acid detergent fibre (ADF) were analysed as prescribed by Van Soest *et al*. (1991). Neutral detergent fibre and ADF were expressed inclusive of the remaining ash. Nutrient digestibility was calculated as:

$$Digestibility = \frac{nutrient\ consumed - nutient\ in\ faeces}{nutrient\ consumed} x100$$

Data were analysed using a 3 × 3 Latin square design with three treatments and three replicates of treatments, following the GLM procedure of SAS version 9.1 (SAS Institute, Cary, North Carolina, USA). The mathematical model was:

$$Y_{ijk} = \mu + H_i + R_j + P_k + e_{ijk}$$

where: Y_{ijk} = a dependent variable for the ith animal, jth treatment, and kth trial period;
μ = general mean,
H_i = effect of the ith animal,
R_j = effect of jth treatment,
P_k = effect of the kth trial period, and
e_{ijk} = residual effect used as error.

Means were compared with Duncan's multiple range tests at probability levels of 5% and 1%.

Results and Discussion

All CFBs were light brown, compact in shape, and exuded a distinctive molasses aroma. After storage for three months, their colour had not changed, and no yeast had grown. These findings were consistent with observations by Santoso *et al*. (2017).

The chemical compositions of the CFBs in the experiment are summarized in Table 2. The average DM and OM contents in all CFBs were 77.7% and 91.1%, respectively, which were comparable with a palm oil frond-based CFB (Santoso *et al.,* 2017).

The mean CP in all the CFBs equalled 14%, which was above the threshold value of 7% required to support rumen microbial activity. Below this threshold, digestibility declines because microbial activity in the rumen has been found to be depressed due to the lack of N (Minson & Milford, 1966). The similar amount of CP in all the CFBs verifies the intent that the feeds were iso-nitrogenous.

Table 2 Chemical composition of complete feed blocks for Kacang goats made using agro-industrial by-products

Variables	Complete feed blocks		
	A	B	C
Dry matter (%)	78.6	77.7	76.9
Organic matter (%)	90.5	91.6	91.2
Crude protein (%)	14.7	13.3	14.0
NDF (%)	55.2	46.1	42.5
ADF (%)	36.9	33.1	27.9
Hemicellulose (%)	13.3	13.0	14.6
Total microbes (cfu/g)	-	$9.92{\times}10^8$	$>1.42{\times}10^9$

A: complete feed block without microbes; B: complete feed block containing 1% *P. aeruginosa* and 1% *A. baumannii*; C: complete feed block containing 2% *P. aeruginosa* and 2% *A. baumannii.* NDF: neutral detergent fibre; ADF: acid detergent fibre

The NDF and ADF contents of blocks C and B were lower than block A, which to some extent might have been because of cellulolytic bacteria in the CFB. Previously, Van Soest (1994) had commented that microbial enzymes could lower the levels of NDF.

The pH value, concentrations of NH_3-N, and individual and total VFAs are presented in Table 3. Propionate and butyrate concentrations and acetate to propionate ratio in the rumen were unchanged (P >0.05) by treatment.

Table 3 Rumen fermentation characteristics in Kacang goats fed a complete feed block

Variables	Complete feed blocks			SE	*P*-value
	A	B	C		
pH	6.75	7.00	6.94	0.13	0.49
N-NH_3 (mg/100 mL)	65.9^b	70.5^{ab}	85.5^a	1.06	0.01
Acetate (mM)	62.2^b	65.9^{ab}	71.3^a	0.49	0.01
Propionate (mM)	17.5	18.6	18.8	0.46	0.30
Butyrate (mM)	7.2	8.6	9.3	0.54	0.21
Total VFA (mM)	86.9^b	93.4^{ab}	99.4^a	0.53	0.01
Acetate : propionate	3.5	3.6	3.8	0.08	0.21

A: complete feed block without microbes; B: complete feed block containing 1% *P. aeruginosa* and 1% *A. baumannii*; C: complete feed block containing 2% *P. aeruginosa* and 2% *A. baumannii*; N-NH_3: ammonia nitrogen; VFA: volatile fatty acids

[a,b] Within a row means were a common superscript did not differ with probability (P <0.01)

The rumen pH values of goats fed on CFB were not different (P >0.05). Aschenbach et *al*. (2011) stated that rumen pH was dependent on saliva production, the absorption and generation of short-chain fatty acids, the type and amount of feed intake, and exchange of phosphates and bicarbonates via the ruminal epithelium. pH values in the rumen are relatively changeable, and the usual values range from 5.5 to 7.0 (Krause & Oetzel, 2006). Nonetheless, pH values in all treatments fluctuated from 6.75 to 7.00 and stayed within the range believed to be ideal for microbial digestion action. Zhang *et al.* (2017) determined that cellulose degradation by rumen microorganisms occurs at pH values between 5.5 and 7.5, with the highest degradation being attained at 6.5. Russell and Wilson (1996) demonstrated that rumen cellulolytic bacteria are exceptionally sensitive to acidity and a ruminal pH less than 6.0 to 6.2 was disadvantageous to bacterial growth. The diurnal pattern of ruminal pH in goats fed on CFB is presented in Figure 1.

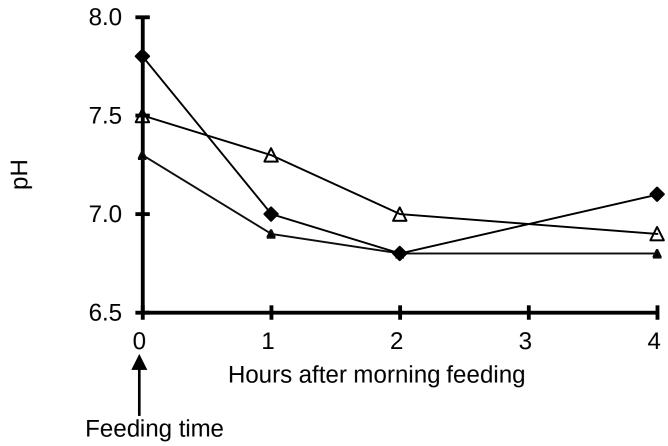

Figure 1 Diurnal pattern of ruminal pH in goats fed (♦) CFB without microbes; (∆) CFB containing 1% *P. aeruginosa* and 1% *A. baumannii*; (▲) CFB containing 2% *P. aeruginosa* and 2% *A. baumannii*

Protein and additional nitrogenous materials are broken down into peptides and ammonia in the rumen (Bach *et al.*, 1988). The microbes utilize the ammonia to synthesize protein. Goats that were fed block C had the highest (P <0.01) NH_3-N concentration compared with other diets. The trend in increase in NH_3-N concentration in goats fed on blocks B and C was supported by an increase in CP digestibility (Table 4).

Table 4 Apparent nutrient digestibility (%) in Kacang goats fed a complete feed block

Variables	Complete feed blocks			SE	*P*- value
	A	B	C		
Dry matter	55.7	57.7	58.7	1.77	0.59
Organic matter	58.2^b	60.2^a	62.0^a	0.13	0.01
Crude protein	52.6	54.3	57.3	0.75	0.09
NDF	42.2^c	45.9^b	48.6^a	0.17	0.01

A: complete feed block without microbes; B: complete feed block containing 1% *P. aeruginosa* and 1% *A. baumannii*; C: complete feed block containing 2% *P. aeruginosa* and 2% *A. baumannii.* NDF: neutral detergent fibre
^{a,b} Within a row means were a common superscript did not differ with probability P <0.01

The NH_3-N concentrations in the rumen varied from 65.0 to 85.5 mg/100 ml. Diurnal patterns of ruminal NH_3-N concentration in goats fed on CFB are illustrated in Figure 2. McDonald *et al.* (2010) stated that the required optimum concentrations of rumen NH_3-N to increase microbial protein synthesis was from 8.5 to 30 mg/dl and more. Abdulrazak *et al*. (1997) stated that between 5 mg/dl and 8 mg/100 mL of rumen liquor was enough to digest fibre. Consequently, in the current research, the ammonia N in every treatment of CFB was enough to guarantee optimal microbial development and digestion of fibre.

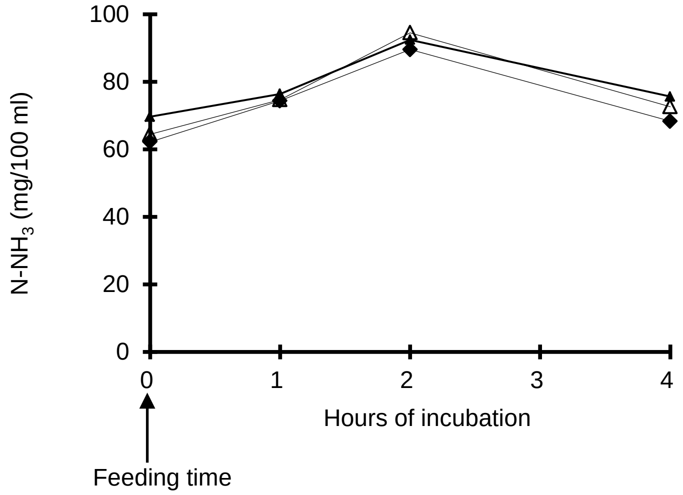

Figure 2 Diurnal patterns of ruminal N-NH_3 concentration in goats fed (♦) CFB without microbe; (∆) CFB containing 1% *P. aeruginosa* and 1% *A. baumannii*; (▲) CFB containing 2% *P. aeruginosa* and 2% *A. baumannii*

Carbohydrates are fermented by bacteria in the rumen and are transformed into volatile fatty acids (VFAs), which provide the main energy for ruminants. The ratios of butyrate, propionate, and acetate that are generated in the rumen are influenced by the type of forage and the composition and amount of rumen bacteria (Wang *et al*., 2020). Block C had higher (P <0.01) productions of acetate and total VFA than blocks B and A. The higher VFA concentration in goats fed on blocks B and C could be because of higher OM digestibility (Table 4). The capability to decompose cellulose is dependent on the kind of forage and crop maturity, along with the types of cellulolytic bacteria (Fondevila & Dehorty, 1996). Dijkstra (1994) determined that the fermentation of structural carbohydrates generated a large quantity of acetic acid and a small quantity of propionic acid compared with starch. Block C produced the highest (P <0.01) concentration of acetate, followed by blocks A and B. Wang *et al.* (2020) observed that fibre-degrading bacteria generated a large quantity of acetate in goat rumen. Acetate is associated with fermentation of the cell wall, and the increase in the level of acetate is typically related to the diminishing quality of the forage (Van Soest, 1994). In the present study, peak ruminal VFA concentration occurred at 1 hour after feeding in all treatments (Figure 3).

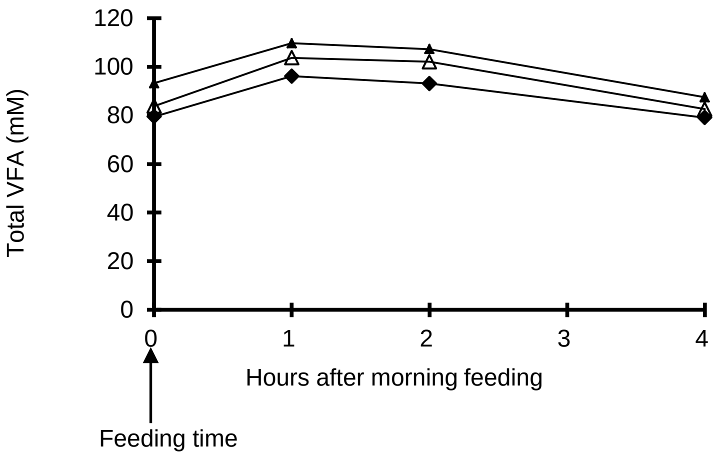

Figure 3 Diurnal pattern of ruminal total VFA concentration in goats fed (♦) CFB without microbes; (∆) CFB with 1% *Pseudomonas aeruginosa* and 1% *Acinetobacter baumannii*; (▲) CFB with 2% *P. aeruginosa* and 2% *A. baumannii*

Blocks B and C produced greater OM digestibility (*P* <0.05) and NDF digestibility (*P* <0.01) compared with block A (Table 4). The greater digestibility of NDF may be because of the addition of cellulolytic bacteria to the feed block. This result was consistent with the finding of Santoso *et al*. (2017) in which block B produced the greatest in vitro digestibility of DM, OM and NDF compared with the other feeds. Zain *et al*. (2015) reported that the addition of microbes to feed to act as probiotics stimulated rumen microbes and improved feed digestibility in ruminants.

There was no significant difference in N intake, digested N, faecal N and retained N among rations (Table 5). Urinary N excretion was at the lowest level (*P* <0.05) in block C, intermediate in block B, and highest in block A. Urinary N excretion as a percentage of N intake in blocks C and B was lower (*P* <0.05) than the control. Goats fed blocks B and C had higher (*P* <0.05) retained N expressed as a proportion of digested N compared with control. The lower N loss in urine was indicative of lower absorption of N and resulted in a higher retained N as a proportion of digested N. Santoso *et al*. (2007) concluded that lower N loss in urine reflected lower absorption of N and resulted in a higher ratio of retained N to N digested. In the present study, urine N excretion as a percentage of N intake fluctuated between 25% and 30.7%, which was lower than the findings reported by Zhu *et al.* (2020), which ranged from 37.8% to 43.2%. The amount of N excreted indicates protein consumption and the effectiveness of N deposition. Consequently, the lower total of N excretion (percentage of N intake) in goats given blocks C and B showed that these diets could reduce environmental N pollution. Urinary N is changed to ammonia quickly and is volatilized into the air, whereas faecal N is changed to ammonia at a slower rate (Bussink & Oenema, 1998).

Table 5 Nitrogen balance and nitrogen partition in Kacang goats fed complete feed blocks

Variables	Complete feed blocks			SE	*P*-value
	A	B	C		
Nitrogen (N) balance (g/day)					
N intake	12.7	11.9	12.9	0.15	0.09
Faecal N	6.3	5.7	5.6	0.21	0.21
Digested N	6.4	6.2	6.4	0.34	0.84
Urinary N	3.9[a]	3.2[b]	3.0[b]	0.11	0.05
Retained N	2.5	3.0	3.4	0.23	0.19
Nitrogen partition					
Faecal N/N intake	49.7	48.1	46.4	2.12	0.62
Urinary N/N intake	30.7[a]	26.6[b]	25.0[b]	0.68	0.05
Retained N/digested N	38.7[b]	48.7[a]	53.3[a]	1.39	0.03

A: complete feed block without microbe; B: complete feed block containing 1% *P. aeruginosa* and 1% *A. baumannii*; C: complete feed block containing 2% *P. aeruginosa* and 2% *A. baumannii*
[a,b] Within a row means were a common superscript did not differ with probability *P* <0.05

Conclusion

The addition of cellulolytic bacteria *P. aeruginosa* and *A. baumannii* to CFB reduced NDF and ADF contents. Block C was effective in modifying ruminal fermentation patterns by increasing concentrations of NH_3-N, acetate and total VFA. Digestibility of OM and NDF in blocks B and C was higher than that of control. The utilization of nitrogen expressed as ration of urinary N to N intake and retained N to digested N increased in goats fed on blocks B and C.

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Authors' Contributions

BS developed the concepts, designed the experiments, and drafted the manuscript. MNL, TWW and BTH conducted the experiments, collected samples and did statistical analysis. All of them approved the final version of the article.

Conflict of Interest Declaration

The authors declared that they had no competing interest.

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