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In vitro rumen fermentability of urea-limestone mixture combined with different sources of non-fiber carbohydrate

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Abstract

The study was conducted to determine the *in vitro* rumen fermentability of urea-limestone mixture (UL) combined with different sources of non fiber carbohydrate (NFC). Molasses (M) and banana plant root meal (BPRM) were used as different sources of NFC. The combinations were designed according to nitrogen (N) content of UL and NFC content of M or BPRM with ratios at 1:3, 1:6, and 1:12, respectively. The different NFC sources and dietary ratios of N to NFC were allocated according to a completely randomized design with factorial pattern of 2 x 3. First factor was source of NFC, and the dietary ratio of N:NFCwas the second factor. The interaction between NFC source and N:NFC ratio increased (p <0.01) DM degradability. The increasing N:NFC ratio enhanced (p <0.01) DM degradability and microbial protein, but lowered (p <0.01) nitrogen degradation and rumen NH_3 concentration. In conclusion, combination of UL and molasses or BPRM with N to NFC ratio at 1 : 12 effectively enhances some variables ruminal fermentation. Molasses is better than BPRM as NFC source.

Key words: *feed NFC, in vitro, rumen fermentability, urea-limestone mixture*

Introduction

Urea was commonly used as a N source to ruminant diet. Dietary urea normally leads to high rumen NH_3 concentrations immediately after feeding (Lee et al 2017). This rapid break down of urea causes excessive rumen NH_3, which result in more ammonia entering the blood (Holder et al 2015) and consequently lowering N utilization of urea (Taylor-Edwards et al 2009). Decreasing N release rate of urea prevents excessive ruminal NH_3 and improves the efficiency of urea utilization as a N source for ruminant.The mixture of urea, $CaSO_4$, and cassava chip is an alternative dietary supplement with a slow release ruminal N (Cherdthong et al 2011). In an previous study, limestone-urea mixture has a slower ruminal release of nitrogen compared with that of common urea (Harahap et al 2018). Combination of urea, limestone, and readily available carbohydrate source is postulated to be dietary supplement provided by a slower rumen N degradation.

Increasing amount of soluble carbohydrate is needed to synchronize the rapidly rumen degradation of urea. Likewise, urea N will be lost via urine if there is insufficient carbohydrate to match ruminal NH_3 release. Sugarcane molasses is usually used as a simple carbohydrate when urea is included in the diet (Salem et al 2013; Lizarazo et al 2014). Molasses provides an available energy for microbial protein synthesis (Soder et al 2011; Azizi-Shotorkhoft et al 2013). Banana plant root is traditionally used as dietary carbohydrate source, however there is a little information concerning with inclusion of banana plant root meal (BPRM) in the feed formulation.

Some studies clarified the use of dietary non fiber carbohydrate (NFC) source in the ruminants (Zhang et al 2012; Da-Cheng et al 2013; Li et al 2014; Spanghero et al 2017). A proper ratio of dietary N to NFC is needed to increase the efficiency utilization of dietary nitrogen and carbohydrate. Objective of the study was to determine the *in vitro* rumen fermentability of urea-limestone mixture combined with molasses or BPRM by applying some ratios of N to NFC.

Materials and methods

Materials and treatment

Rumen inoculum of this study was collected from two ruminal-cannulated Jawa Randhu crossbred goats. The goats were 30 months old with body weight average of 30 kg. The animals were kept in individual pens and clean fresh water was available continously. Animals were fed twice daily on diet containing 70% elephant grass, 10.7% rice bran, 17% coconut meal, 1.1% cassava waste, 1.0% sugarcane molasses, and 0.2% vitamin-mineral premix (based on dry matter).

Molasses and BPRM were used as the readily available carbohydrate, the chemical composition of two carbohydrate sources are shown in Table 1.

Table 1. Chemical composition of molasses and banana plant root meal (100% DM)

Component	Carbohydrate source	
	Banana Plant RootMeal	Molasses
Crude Protein	3.54	1.15
Extract Eter	0.21	0.71
Ash	12.30	7.09
Neutral Detergent Fiber	13.04	0.00
Nitrogen	0.57	0.18
Non-Fiber Carbohydrate[1]	67.40	91.04
Glucose	21.64	59.98
Starch	38.34	19.52
Total Digestible Nutrient	71.84	84.71

[1] Non fiber carbohydrates was calculated as = 100% - (% crude protein + %NDF + % extract eter + % ash) (Da-cheng et al., 2013).

The limestone-urea mixture (LU) was created similarly to previous study of ruminal nitrogen release by Harahap et al (2018). Molasses was purchased from a feedstuff supplier in Semarang city. The BRPM was made from part of ball shape like rhizome beneath stem of old plant. After peeling this ball shape like rizhome, the rhizome was sliced into 1 cm thin and then was sundried. The sundried matter was then crushed to create the BRPM. Combinations of urea-limestone mixture with soluble carbohydrate sources were made according to the ratios of N to

NFC, there were 3 combinations for each soluble carbohydrates (Table 2). The combinations using BRPM were ULBR3 (1 : 3 of N to NFC ratio), ULBR6 (1 : 6 of N to NFC ratio), and ULBR12 (1 : 12 of N to NFC ratio). The combinations using molasses were ULMR3 (1 : 3 of N to NFC ratio), ULMR6 (1 : 6 of N to NFC ratio) and ULMR12 (1 : 12 of N to NFC ratio).

Table 2. Ingredient and composition of urea-limestone mixtures combined with banana plant root meal and molasses

Treatment	**ULBR3**	**ULBR6**	**ULBR12**	**ULMR3**	**ULMR6**	**ULMR12**
Ingredient						
UL, %	67	50	32	74	58	41
BPRM, %	33	50	68	-	-	-
Molasses, %	-	-	-	26	42	59
	100	100	100	100	100	100
N and NFC composition						
N, %	7.33	5.62	3.79	7.93	6.25	4.47
NFC, %	22.24	33.70	45.83	23.67	38.24	53.71
Rasio N:NFC	1:3.03	1:6.00	1:12.06	1:2.98	1:6.11	1:11.99

UL = urea-limestone mixture; BPRM = Banana plant root meal.
ULBR3 = N:NFC ratio at 1:3 in urea-limetone mixture combined with BRPM.
ULBR6 = N:NFC ratio at 1:6 in urea-limetone mixture combined with BRPM.
ULBR12 = N:NFC ratio at 1:12 in urea-limetone mixture combined with BRPM.
ULM3 = N:NFC ratio at 1:3 in urea-limetone mixture combined with molasses.
ULM6 = N:NFC ratio at 1:6 in urea-limetone mixture combined with molasses.
ULM12 = N:NFC ratio at 1:12 in urea-limetone mixture combined with molasses.

The *in vitro* technique

Rumen fluid was collected in each goat before the morning feeding. Rumen fluid was strained through four layers of cheesecloth into pre-warmed thermo flasks. A strict anaerobic technique was applied during rumen fluid collection. The collected rumen fluid was then transported immediately to the laboratory.

Test of *in vitro* rumen fermentability for each combination was conducted using single stage of batch culture (Tilley and Terry 1963) with correction for blank (without substrate). McDougall buffer solution and rumen fluid were mixed at ratio of 4 : 1 for making a rumen inoculum. The test of *in vitro* rumen fermentability for combination of urea-limestone mixture with soluble carbohydrate was commenced for three hours. After 3 hours of incubation, the fermentation process was ceased by placing fermentor tube in an ice-cold bath, and then was centrifuged (3,000 x *g*, 15 minutes, 4 °C).

The filtrate was divided into 2 parts, one part was stored at -20 °C for volatile fatty acids and ammonia analysis. The other part was continued by centrifugation (10,000 x *g*, 15 minutes, 4 °C) and stored at -20 °C for determination of microbial protein content.

Chemical analysis

Analysis of DM and N content in residue were conducted according to AOAC (1990). Starch and glucose content were determined by Luff-Schoorl method (Koca et al 2014). Total volatile fatty acids concentration of rumen fluid was estimated by the method of steam distillation (Kromann et al 1967). Concentration of rumen partial volatile fatty acids were determined by using gas chromatography (GC-2010, Shimadzu, Tokyo, Japan) as described by Zhang et al (2008). Rumen NH_3 concentration was assayed using spectrophotometer as described by Chaney and Marbach (1962). The content of rumen microbial protein was analysed using Lowry method (Makkar et al 1982). Each parameter was assayed *in duplo*.

Statistical analysis

Two sources of NFC and three ratios of N to NFC were allocated according to a 2 x 3 of completely factorial design with three replicates of each. First factor was source of NFC, and the dietary ratio of N:NFCwas the second factor. The statistical model used was as follows :

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the observed value, μ is the overall mean, α_i is the effect of NFC sources, β_j is the effect of ratios of N to NFC, $(\alpha\beta)_{ij}$ is the interaction effect between NFC sources and ratios of N to NFC and ε_{ijk} is the random residual error. Data were tested using ANOVA and followed by Duncan's multiple range test.

Results

There were interactive effect between NFC sources and ratio of N to NFC on DM degradability (Table 3). Decreasing N to NFC ratio enhanced (p <0.01) DM degradability, and DM degradability of molasses was higher (p<0.01) than that banana plant root meal. The pattern of DM degradability was similar to the treatments effect on of rumen protein microbes (Table 3).

Table 3. Results of *in vitro* rumen fermentability test (nitrogen degradation)

Treatment	Parameters				
	Dry Matter Degradability	**Microbial Protein**	**Degraded Nitrogen**	**Undegraded Nitrogen**	**NH_3**
	--(%)--	-(mg/g)-	----------(%)---------		(mg/100ml)
ULBR3	14.72^{d}	4.38^{d}	96.30^{a}	3.70^{c}	109.71^{a}
ULBR6	16.37^{d}	5.16^{d}	90.78^{b}	9.22^{b}	80.50^{c}
ULBR12	16.77^{cd}	8.37^{c}	84.31^{c}	15.69^{a}	62.84^{d}
ULMR3	21.79^{c}	8.78^{c}	96.33^{a}	3.67^{c}	96.96^{b}
ULMR6	30.39^{b}	11.00^{b}	89.36^{b}	10.64^{b}	77.31^{c}
ULMR12	50.32^{a}	12.02^{a}	87.63^{b}	12.37^{b}	56.84^{d}
SEM	3.07	0.77	1.11	1.11	4.54
Comparison (*p value*)					
Carbohydrate Source	<0.001	<0.001	0.45	0.45	0.01
N : NFC ratio	<0.001	<0.001	<0.001	<0.001	<0.001
Interaction	<0.001	0.80	0.10	0.10	0.28

[a,b,c,d] Means in the same column with different letters show significant differences (p<0.05). ULBR6 = N:NFC ratio at 1:6 in urea-limetone mixture combined with BRPM. ULBR12 = N:NFC ratio at 1:12 in urea-limetone mixture combined with BRPM. ULM3 = N:NFC ratio at 1:3 in urea-limetone mixture combined with molasses. ULM6 = N:NFC ratio at 1:6 in urea-limetone mixture combined with molasses. ULM12 = N:NFC ratio at 1:12 in urea-limetone mixture combined with molasses.

Concentrations of rumen undegraded nitrogen were lowered (p <0.05) according to decreasing N:NFC ratio, and NFC sources did not effect on the concentrations of rumen undegraded nitrogen (Table 3). Rumen NH_3 concentrations were raised

(p<0.01) by NFC sources and ratio of N:NFC, though there was no interaction effect between NFC sources and N:NFC ratio on rumen NH_3 concentrations. Decreasing ratio of N:NFC enhanced (p<0.01) rumen VFA's concentration, and NFC sources did not effect on rumen VFA's concentration.

Rumen concentrations of C2 were rised (p<0.05) across NFC sources with increasing ratio of N:NFC, and BRPM had higher (p <0.05) rumen concentration of C2 than that of molasses, though NFC sources and N:NFC ration did not interact to effect on rumen concentration of C2 (Table 4). The ratio of N to NFC did not effect on rumen C3 concentration, though molasses resulted in more higher (p<0.05) rumen C3 concentration.

Table 4. Results of *in vitro* rumen fermentability test (carbohydrate degradation)

	Parameters							
Treatment	***VFA's***	**C2**	**C3**	**C4**	**Iso butyrate**	**Iso Valerate**	**Valerate**	**C2:C3**
	---(mM)---							
ULBR3	213.33^{a}	36.28^{a}	5.47^{ab}	1.91	1.00	0.86^{b}	0.18^{b}	6.64^{a}
ULBR6	190.00^{bc}	25.73^{b}	4.13^{b}	2.02	1.04	0.87^{b}	0.20^{ab}	6.30^{a}
ULBR12	186.67^{c}	25.63^{b}	4.15^{b}	2.35	1.18	1.07^{a}	0.21^{a}	6.20^{a}
ULMR3	210.00^{a}	25.13^{b}	4.70^{b}	1.69	0.79	0.51^{b}	0.20^{ab}	5.42^{ab}
ULMR6	203.33^{ab}	24.72^{b}	5.27^{ab}	1.70	0.80	0.59^{b}	0.22^{a}	4.69^{b}
ULMR12	193.33^{bc}	20.25^{b}	6.46^{a}	2.12	0.87	0.67^{b}	0.23^{a}	3.13^{c}
SEM	2.96	1.48	0.25	0.11	0.06	0.08	0.01	0.32
Comparison (*p value*)								
Carbohydrate Source	0.19	0.02	0.04	0.31	0.08	0.01	0.05	<0.001
N:NFC ratio	<0.001	0.03	0.47	0.32	0.68	0.09	0.01	0.02
Interaction	0.27	0.19	0.25	0.98	0.95	0.36	0.93	0.09

[a,b,c,d] Means in the same column with different letters show significant differences (p<0.05) ULBR3 = N:NFC ratio at 1:3 in urea-limetone mixture combined with BRPM. ULBR6 = N:NFC ratio at 1:6 in urea-limetone mixture combined with BRPM. ULBR12 = N:NFC ratio at 1:12 in urea-limetone mixture combined with BRPM. ULM3 = N:NFC ratio at 1:3 in urea-limetone mixture combined with molasses ULM6 = N:NFC ratio at 1:6 in urea-limetone mixture combined with molasses. ULM12 = N:NFC ratio at 1:12 in urea-limetone mixture combined with molasses.

The NFC sources and N:NFC ratio unchanged rumen concentrations of C4 and isobutyrate (Table 4). Decreasing N:NFC ratio lowered (p<0.05) rumen valerate concentrations. The use of molasses in the combination gave higher (p<0.01) rumen concentration of isovalerate compared with that of BRPM. Ratios of C2:C3 were increased (p<0.05) according to decreasing N:NFC ratio across the NFC sources.

Discussion

Molasses is widely used as a source of readily available carbohydrate for dietary urea supplement. Ball shape like rhizome beneath stem of the old banana plant is commonly utilized as a source of traditional food carbohydrate in Indonesia, but its use as feed supplement needs a clarification. Although the NFC content of banana plant root meal was lower than that of molasses (Table 1), the banana plant root meal could be used for diversification of readily available carbohydrate in balancing ruminant diet.

Rumen NH_3 concentration was higher when urea-limestone mixture was combined with BRPM compared with that of molasses (Table 3). Most rumen

NH_3 released from urea-limestone-molasses combination may be utilised for microbial protein synthesis, because there were more ATP and carbon skeleton. There are some factors affecting ammonia present in the rumen such as dietary protein or nitrogen content, rate of nitrogen hydrolysis and ammonia conversion to microbial protein synthesis (Jayanegara et al 2017; Kand et al 2018). For all the combinations, the average of ammonia concentration was more higher than 5 mg/dl (Table 3). Comparable results were reported by Cherdthong et al (2011), ammonia concentration of urea-calcium mixtures treatment ranged from 14 – 30 mg/dl. Although such a high ammonia concentration is not effective for nitrogen source in diet (Taylor-Edwards et al 2009; Holder et al 2015).

In this study, while ratio of N:NFC at 1:3 resulted in the highest rumen NH_3 concentration, the N:NFC ratio at 1:12 produced lowest rumen NH_3 concentration (Table 3). The rumen NH_3 concentration was the sum of degraded nitrogen and undegraded nitrogen in the rumen (Table 3). Beside this, the higher content of NFC in ULBR12 and ULMR12 resulted in lower rumen NH_3 than other combinations. This means that the higher NFC level may be provided with more ATP and carbon skeleton for protein microbial synthesis. Cherdthong et al (2011) reported that the decrease in rumen NH_3 concentration is attributed by an increase in utilization level of dietary nonstructural carbohydrates by rumen microbes. The declined after feeding ruminal NH_3 concentrations is an indication of increased N utilisation for microbial protein synthesis (Li et al 2014). The ULBR12 and ULMR12 could be considered as the better harmonization between rumen NH_3 release and carbohydrate availability to support microbial protein synthesis.

Dry matter degradation of urea-limestone mixture combined with molasses was higher than that of BPRM (Table 3). However, there was no different between DM degradation of ULBR12 and ULMR3 (Table 3). This reflected a higher microbial activity in the urea-limestone mixture combined with molasses than in urea-limestone mixture combined with BPRM. Ruminal degradation is well known supported by the population of rumen microbes. This phenomenon is supported by the higher NDF content of banana plant root meal than that of molasses. Ruminal fermentation of roughage feed may lower VFA concentration since high fiber is slowly degraded in the rumen (Suharlina et al 2016; Dal Pizzol et al 2017). Azizi-Shotorkhoft et al (2013) reported that molasses provides more available energy for microbial synthesis. The ratio of N to NFC at 1 : 12 gave the best efficiency of dry matter degradation. This fact was attributed with decreasing rumen ammonia concentration, increasing microbial synthesis and dry matter degradability (Table 3). Lopez-Soto et al (2014) stated that a synchronization between utilizations of soluble carbohydrate and ammonia in rumen may improve feed digestibility.

Volatile fatty acids are end product of carbon fermentation by rumen microbes. These products are absorbed across the rumen wall and utilized for biochemical synthesis of energy (Cherdthong et al 2014). Dietary non-fiber carbohydrate is rapidly fermented in rumen and used as carbon backbone source for rumen microbes protein. The ratio of N to NFC at 1 : 12 had lowest rumen VFA concentration, this was linearly with the decreasing rumen NH_3 concentration for protein microbes synthesis (Table 3).

Ruminal VFA production was affected by the content of feed fiber (Table 4). The NDF content of BRPM was higher than that of molasses (Table 1). Likewise, feed fiber is a precursor of acetate in rumen. This in agreement with the results of Sari et al (2015) and Maktabi et al (2016), greater feed NDF content may enhance rumen acetate concentration. On the other hand, feed NFC is a source of propionate (Cherdthong et al 2014). Fiber content of molasses is lower than that of BRPM, so that ruminal fermentation of molasses may result in a higher propionate concentration than that of BRPM. As expected, the rumen acetate concentration was increased by declining rasio of N to NFC (Table 4). Lowering NFC level is usually linear with the increasing NDF content (Da-Cheng et al 2013). Although glucose content was higher in molasses than in BPRM (Table 1), we did not find higher proportion of butyrate for molasses combinations. Previous study reported that high-sugar in diet was resulted higher butyrate production in the rumen (Gao and Oba 2016).

The ratio of rumen acetate to propionate combinations emphasized the effect of N to NFC ratio changes in the UL combinations. Increasing NFC content from the N:NFC ratio gradually at 1:3 and up to 1:12 caused decrease in acetate production and increase in propionate production (Table 4). Ruminal fermentation of subsrate producing acetate is attributed by high production of H_2 and CO_2 (Ungerfeld, 2013). Hydrogen is used as a precursor in the process of methane production by methanogenic bacteria. Accumulated hydrogen in large quantities during acetate production process causes elevated methane gas production in the rumen (Ahmed et al 2018). Li et al (2014) reported that increasing dietary fermentable carbohydrate (starch) content reduces the acetate to propionate ratio which is indicated by more propionate production in the rumen. Thus, the energy utilization of molasses is better compared to BRPM as a carbohydrate source in the feed.Concentration of valerate was greater in the ratio of N:NFC at 1:12 compared with 1:6 and 1:3. Higher portion of molasses and BPRM in ULMR12 and ULBR12, respectively, gave highest rumen true protein in all combinations of the UL (Table 3). This is in agreement with Zhang et al (2012) and Dal Pizzol et al (2017), iso-VFA production may be the result of dietary protein degradation by microbes in the rumen.

Conclusion

- The UL mixture combined with molasses or BPRM with N:NFC ratio at 1 : 12 resulted in higher DM degradability and amount of microbial protein compared with other N : NFC ratios.
- The NFC of molasses gave higher these parameter values compared to NFC of BPRM. Futher study is needed to determine the supplementation effect of slow release nitrogen of the UL on productive performance of ruminant.

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