The Use of Ammoniated Zeolite to Improve Rumen Metabolism in Ruminant

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Abstract. Objective of this research was to study the effects of graded level supplementation of ammoniated zeolite on rumen metabolism parameters. This in vivo experiment was conducted in a 4x4 latine square design (LSD) with 4 treatments in 4 periods, using 4 crossbreed etawa male goats. The treatments were T1 = basal diet + 0% ammoniated zeolite, T2 = basal diet + 2% ammoniated zeolite, T3 = basal diet + 4% ammoniated zeolite, and T4 = basal diet + 6% ammoniated zeolite. Level of ammoniated zeolite supplementation is based on dietary dry matter basis. The basal diet consists of 70% *Napir grass* and 30% concentrate. Each period of experiment consist of 2 weeks for adjustment, 1 week for preliminary, and 10 days for total collection. Result of the experiment showed that ammoniated zeolite supplementation significantly increased the total volatile fatty acids concentration and total organic acids concentration in the rumen. Improved total volatile fatty acids concentration in the rumen could be used as a good indicator for improving rumen metabolism. However, even the concentrations of ammonia in the rumen tend to increased by ammoniated zeolite supplementation the increasing is still not statistically significant. Ruminal pH measurement in this experiment revealed that the use of ammoniated zeolite not significantly affects the ruminal pH.

Key Words: ruminant, ammoniated zeolite, ruminal ammonia, volatile fatty acids, organic acids

Introduction

Feed digestibility and nutrients supply for the host animals in ruminants could be increased by improving rumen motility and metabolism processes (Erwanto and Sutardi, 1996). Considerable progress has been made in increasing the rate of protein synthesis in the rumen as well as rumen microbial growth rate. For optimal rumen microbial cell growth, all raw materials or cell preqursors supply should be available in their optimum amounts. The raw materials are included ammonia and sulfur. Otherwise, ruminal fermentation process will lead to low rumen microbial growth rate and uncouple or undesirable fermentation processes. Moreover, considering the nutrient requirements of rumen microbial also important to understanding nitrogen metabolism in the rumen (Batch et al., 2005).

It has long been demonstrated that adequate energy and nitrogen supply is necessity for optimal ruminal fermentation processes and biosynthesis of rumen microbial cells, especially ruminal bacteria. For example, when ruminants are fed with diets containing sufficient amount of energy but low in crude protein, ammonia may become a limiting factor for rumen microbial protein synthesis (Clark et al., 1987). Moreover, even though rate of rumen microbial growth depend on carbohydrate supply (Pathak, 2008), when ruminal bacteria have insufficient nitrogen and other nutrients, excess carbohydrate can be toxic (Russell, 1998).

Cellulolytic ruminal bacteria incorporate preformed amino acids in the rumen (Atasoglu et al., 2001). However, it was known that ammonium ion (NH_4^+) is one of a preferred source of nitrogen for rumen microbial growth (Bryant and Robinson, 1963; Erfle et al., 1977). Therefore, supplementation of non protein nitrogen (NPN) to a high energy diets with low crude protein content will in turn support the rumen microbial growth rate.

NPN supply in the rumen should be arranged in a suitable rate, so that the ammonia (NH_3) concentrations do not exceed the toxic level in the rumen. Regarding this condition, many efforts have been made to produce a slow release ammonia compounds in the rumen. However, a study by Males et al. (1979) revealed that most of the slow release ammonia products either released ammonia too pass or too slow in the rumen, so that the NPN products were of little benefit in the rumen for supporting microbial protein synthesis processes. The term ammonia is used to designate the sum of the protonated (NH_4^+) and unprotonated (NH_3) forms (Abdoun et al., 2003)

Natural zeolite is crystalline hydrated alluminosilicates of alkali and alkaline earth cation (Mumpton and Fishman, 1977). These materials could be used as a good sorbent, cation exchanger, catalyst, or molecular sieve. The cation exchange capacity property enabled the zeolite to have a buffer action in the rumen. Moreover, the cation exchange property of this crystal could be exploited properly, so that the zeolite could serve as a carrier for ammonium ion supply in the rumen. For this purpose, the exchangeable cations of zeolite could be displaced by ammonium ion (NH_4^+) and become ammoniated zeolite products.

In the rumen, the ammoniated zeolite fed toruminants will then slowly release its NH_4^+ through ion exchange mechanism process with other cations from continuous supply of regenerative saliva or cation from feed origin such as Na^+ and K^+. The objective of this research was to study the effects of graded level supplementation of ammoniated zeolite on rumen metabolism parameters.

Materials and Methods

This in vivo experiment was conducted at the Department of Animal Science, Faculty of Agriculture University of Lampung in April–August 2010. The experiment was conducted in a 4x4 latine square design (LSD) with 4 treatments in 4 periods using 4 crossbreed etawa male goats. The treatments were T1 = basal diet + 0% ammoniated zeolite, T2 = basal diet + 2% ammoniated zeolite, T3 = basal diet + 4% ammoniated zeolite, and T4 = basal diet + 6% ammoniated zeolite. The basal diet consists of 70% *Napir grass* and 30% concentrate, containing 68% total digestible nutrients (TDN) and 12% crude protein (CP) of dietary dry matter basis. Each period in LSD experiment consist of 2 weeks for dietary adjustment, 1 week for preliminary period, and 10 days for total collection. Dietary treatment formulations were presented in Table 1.

Parameters measured in this experiment were ruminal ammonia concentration, ruminal pH, ruminal volatile fatty acids concentration, and dry matter and organic matter digestibility.

Ruminal ammonia concentration was measured using micro diffusion Conway techniques, ruminal total volatile fatty acids concentration were measured using steam

Table 1. List of dietary treatments formulation

No	Dietary Treatment	Proportion of feed (% dry matter basis)		
		Napier Grass	Concentrate*)	Ammoniated Zeolite
1	Treatment 1 (T1)	70.0	30.0	0.0
2	Treatment 2 (T2)	68.0	29.4	2.0
3	Treatment 3 (T3)	67.2	28.8	4.0
4	Treatment 4 (T4)	65.8	28.2	6.0

T1 = basal diet + 0% ammoniated zeolite; T2 = basal diet + 2% ammoniated zeolite; T3 = basal diet + 4% ammoniated zeolite; and T4 = basal diet + 6% ammoniated zeolite. The basal diet consists of 70% napir grass and 30% concentrate, containing 68% total digestible nutrients (TDN) and 12% crude protein (CP) of dietary dry matter basis.

*) Concentrate: consists of 47% fermented cassava waste, 37% rice bran, and 16% coconut oil meal.

distillation, and total organic acids were measured using gas chromatograph. All data collected were subject to analysis of variance (anova) followed by least significant difference (LSD) test, using procedure of Morris (2006).

Metabolism experiment was conducted on individual metabolism barn. Animals were feed daily with experimental diets based on requirement of each animal. The feed was equally divided in to 7 AM and 15 PM feeding. At the end of each total collection periods, ruminal fluids samples were taken from the rumen using stomach tube, about 3 hours after morning feeding and then analyzed for ruminal pH. The samples were then centrifuged at 12,000 rpm for 20 minutes. The supernatant was collected for later ammonia, volatile fatty acids, and organic acids concentration measurement.

Results and Discussion

Dietary treatments effects on dry matter consumption, ruminal ammonia concentration, ruminal pH, and ruminal VFA concentration are presented in Table 2.

Dry matter consumption and ruminal pH

Dry matter consumption and ruminal pH were not significantly affected by ammoniated zeolite supplementation (P>0.05). It means that ammoniated zeolite could be used in ruminant diets without significant adversial effects in dry matter consumption. Data on ruminal pH also demonstrated that ammoniated zeolite could control ruminal pH, even in condition of increasing total volatile fatty acids and organic acids concentration in the rumen followed by zeolite supplementation. Moreover, the value of ruminal pH observed in this experiment still in the range of optimal ruminal pH (5.5–7.0) for rumen proteolytic enzymes (Kopecny and Wallace, 1982).

Ammonia concentration

It appears from Table 2 that ammoniated zeolite supplementation in the diet tend to increased the ruminal ammonia concentration. However, the increasing of ammonia concentration in the rumen by ammoniated zeolite supplementation in the diet was not statistically significant (P>0.05). Similar to this result, Bosi et al. (2002) reported that ammonia level in the rumen was not affected by feeding zeolite to lactating dairy cows. This phenomena indicates that ammoniated zeolite could release its ammonium ion into the rumen fluids and the process of releasing of ammonium ion is happened slowly or well controlled compared with using urea as current NPN sources.

The condition of slow release ammonium ion from ammoniated zeolite supplementation in the diet will be beneficial to the process of microbial protein synthesis in the rumen and also prevent the host animal from ammonia toxicity (Cherdthong and Wanapat, 2010). The ammonia released then used by rumen microbial for the novo synthesis of amino acids and growth (Pathak, 2008 and Serrato-corona et al., 2011).

In some cases, high producing dairy cows were fed large quantities of high quality proteins. Microbial protein degradation in the rumen usually not directly coupled to microbial protein synthesis, as a result ruminal ammonia production often excessive and ultimately lost by urinary N excretion (Sannes et al., 2002). In this case, ammoniated zeolite supplementation could be recommended to reduce the gap.

The process of releasing ammonium ion is possibly by ion exchange with generative cations from saliva and feed origin such us Na^+ and K^+. Strategy of using non-protein nitrogen (NPN) is to ensure a continuous supply of NPN to the rumen in order to maintain an optimal level of ammonia (Ortiz et al., 2002). Therefore, ammoniated zeolite could be used as a new

Table 2. Effects of ammoniated zeolite supplementation on ruminal ammonia (NH_3), ruminal pH, and ruminal VFA concentration

Rumen Parameters	Treatments (% ammoniated zeolite)			
	0	2	4	6
Feed consumption (g/head/day)	427.30^{a}	413.60^{a}	418.70^{a}	400.90^{a}
Ruminal pH	6.26^{a}	6.20^{a}	6.20^{a}	6.22^{a}
Ammonia concentration (mM)	6.37^{a}	6.62^{a}	6.80^{a}	6.81^{a}
Total volatile fatty acids concentration (mM)	80.00^{a}	75.00^{a}	85.00^{a}	110.00^{b}
Total organic acids concentration (ppm)	3892.5^{a}	4149.9^{ab}	4186.3^{ab}	4654.9^{c}

Values bearing different superscript at the same row differ significantly (P<0.05), tested by LSD test

alternative for developing a safe slow release ammonia product in the rumen, especially for small scale ruminant production in rural area.

Total volatile fatty acids and organic acids

It could be seen in Table 2 that ammoniated zeolite supplementation significantly increased the ruminal total volatile fatty acids and total organic acids concentration (P<0.05). However, substantially increased in VFA concentration is happen at high level of ammoniated zeolite supplementation, especially in treatment 4 (ammoniated zeolite 6% of dietary dry matter). This result confirmed that improving of ammonia supply in the rumen by ammoniated zeolite could further improved the rumen microbial growth and will in turn improve the bioprocess of ruminal fermentation. It has long been demonstrated that VFA is one of the main products of ruminal fermentation.

The positive effects of zeolite were also observed in sheep and dairy cow. Ghaemnia et al. (2010) reported that addition of natural zeolite in the ration could improve crude protein and neutral detergent fiber (NDF) digestibility in Arabic lamb. Milk yield in dairy cow was improved by zeolite supplementation, as resulted from possible enhancement of propionate production in the rumen (Katsoulos et al., 2006).

With regard to its capacity to provide ammonia safely in the rumen for microbial protein synthesis and to improve the ruminal fermentation for providing VFA for energy metabolism of the host animals, feeding ammoniated zeolite to ruminant that are fed with low protein diet would be beneficial. For example, dairy cow in early lactation usually fed with high concentrate diets containing large amounts of readily fermentable carbohydrate but usually fairly low in crude protein content.

Conclusions

Ammoniated zeolite could be used as a safe slow release ammonium ion in the rumen for supporting microbial protein synthesis and ruminal metabolism. However, the best level of ammoniated zeolite supplementation at graded level of dietary protein still needs to be further explored.

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References

Abdoun K, K Wolf, G Arndt and H Martens. 2003. Effect of ammonia on Na^+ transport across isolated rumen epithelium of sheep is diet dependent. Brit. J. Nutr. 90:751-758.

Atasoglu C, CJ Newbold and R.J. Wallace. 2001. Incorporation of [^{15}N] ammonia by the cellulolytic ruminal bacteria *Fibrobacter succinogenes* BL2, *Ruminococcus albus* SY3, and *Ruminococcus flavefaciens* 17. Appl. Environ. Biol. 67(6): 2819-2822.

Bach A, S Calsamiglia and MD Stern. 2005. Nitrogen metabolism in the rumen. J. Dairy Sci. 88:E9-E21.

Bosi P, D Creston and L Casini. 2002. Production performance of dairy cows after dietary addition of clinoptilolite. Ital. J. Anim. Sci. 1:187-190.

Bryant MP and IM Robinson. 1963. Apparent incorporation of ammonia and amino acid carbon during growth of selected species of ruminal bacteria. J. Dairy Sci. 46:150-156.

Cherdthong A and M Wanapat. 2010. Development of urea products as rumen slow release feed for ruminant production: A Review. Aust. J. Basic and Appl. Sci. 4(8):2232-2241.

Clark JH, MR Murphy and BA Crooker. 1987. Supplying the protein needs of dairy cattle from by product feeds. J. Dairy Sci. 70:1092-1097.

Erfle JD, FD Sauer and S Mahadevan. 1977. Effect of ammonia concentration on activity of enzymes of ammonia assimilation and on synthesis of amino acids by mixed rumen bacteria in continuous culture. J. Dairy Sci. 60:1064-1068.

Erwanto and T Sutardi. 1996. Nutritional manipulation of legumes tree and urea ammoniated *Napier grass* diet through partial defaunation and supplementation of $(NH_4)_2SO_4$, leucine and valine. Indon. J. Nutr. Feed Sci. Vol 1(1):39-44.

Ghaemnia L, M Bojarpour, Kh Mirzadeh, M Chaji and M Eslami. 2010. Effects of different levels of zeolite on digestibility and some blood parameters in Arabic Lambs. J. Anim. Vet. Sci. 9(4):779-781.

Katsoulos PD, N Panousis, N Roubies, E Christaki, G Arsenos and H Karatzias. 2006. Effects of long term feeding of a diet supplemented with clinoptilolite to dairy cows on the incidence of ketosis, milk yield and liver function. Vet. Res. 159:415-419

Kopecny J and RJ Wallace. 1982. Cellular location and some properties of proteolytic enzymes of rumen bacteria. Appl. Environ. Microbiol. 43:1026-1033.

Males JR, RA Munsinger and RR Johnson. 1979. In vitro and in vivo ammonia release from “slow release” urea supplements. J. Anim. Sci. 48:887-895.

Morris TR. 2006. Experimental Design and Analysis in Animal Science. CABI Publishing, UK.

Mumpton FA and PH Fishman. 1977. The application of natural zeolite in animal science and aquaculture. J. Anim. Sci. 45:1188-1203.

Ortiz RMA, MA Galina and MMA Carmona. 2002. Effect of a slow non-protein nitrogen ruminal supplementation on improvement of *Cynodon nlemfuensis* or *Brachiaria brizanta* utilization by Zebu steers. Livest. Prod. Sci. 78:125-131.

Pathak AK. 2008. Various factors affecting microbial protein synthesis in the rumen. Veterinary World 1(6):186-189.

Russell, JB. 1998. Strategies that ruminal bacteria use to handle excess carbohydrate. J. Anim. Sci. 76:1955-1963.

Sannes RA, MA Messman and DB Vagnoni. 2002. Form of rumen-degradable carbohydrate and nitrogen on microbial protein synthesis and protein efficiency of dairy cows. J. Dairy Sci. 85:900–908.

Serrato-Corona S, JIS Duarte, MM Ortiz and EC Perez. 2011. Oat hay apparent digestibility, rumen ammonia nitrogen and bun in goats supplemented with fermented molasses-poultry litter. J. Anim. Vet. Adv. 10(6):808-812.