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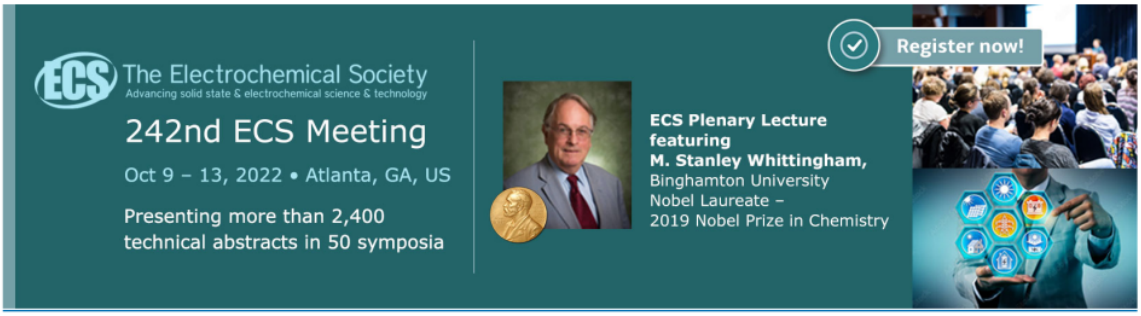

Increasing of rumen fermentation products and digestibility of diets through urea-zeolite supplementation *in-vitro*

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Abstract. The aim of this study was to find the optimal level of urea-zeolite supplementation in the substrate on rumen fermentation products and digestion in vitro. Testing the solubility level of urea-zeolite at a ratio of 1: 1 and 1: 2 in rumen fluid based on N-NH3 levels was carried out at the fermentation time of 0, 1, 2, 3, 4, and 5 hours. Completely randomized design was used in this research with urea-zeolite supplementation treatment (0.2, 4, 6, 8, and 10% of dry matter (DM) substrate). Substrate composed of corn sugarcane silage and concentrate with DM ratio was 70%: 30%. The variables measured were rumen fermentation products (pH, VFA, and N-NH3), DM and organic matter (OM) digestibility. The results showed that the solubility of urea-zeolite in rumen fluid at the same ratio of 1: 1 and 1: 2, the highest at 4 hours fermentation time of 40.81% and 39.13% and after 4 hours was stable. Urea-zeolite supplementation (ratio 1: 2) on the substrate has no effect (P> 0.01) on the pH of the liquid. N-NH3 fermentation products increased (P <0.01) linearly with increasing levels of urea-zeolite supplementation. The total VFA product and the degradation of dry matter were highly significant (P <0.01) influenced by urea-zeolite supplementation and were optimal at 6% and 4% DM substrate supplementation, respectively. DM and OM digestibility was highly significant (P <0.01) influenced by urea-zeolite supplementation and was optimal at the 6% BK substrate level. It was concluded that the use of urea-zeolite as a supplement in the substrate could increase fermentation products and digestibility in vitro

1. Introduction

Microorganisms that exist in ruminant reticulo-rumen use of non-protein nitrogen (NPN) and they produce amino acids used by host animals. In the rumen, NPN is degraded to ammonia and it will use as nitrogen sources to produce proteins that have high biological value when energy is available. Ruminants can digest microorganisms (bacteria, protozoa, and fungi) in intestines used as amino acids. Synthesis of protein microorganisms can supply 50% of the protein needed in ruminants [1] and the rest comes from undegradable protein in the rumen [2]

Generally, the source of NPN as a nitrogen source is urea (CO (NH2) 2 with nitrogen content between 42 to 46%. Supplementation of urea in ruminant diets can be done to supply ammonia-N. However, it is hydrolyzed easily by urease activity of rumen microorganisms so it is not fully used for protein synthesis of rumen microorganisms [3]. So, that the optimal use of urea in the rumen needs to be inhibited the rate of hydrolysis, inhibition can be done with natural materials that can be high-ion exchange capacity and absorb, so that it releases slowly in the rumen.

Zeolites can be mixed in animal feed to increase the use of ammonia for rumen microbs and buffer solutions [4]. Zeolites can also be a catalyst for the reaction of ammonia and carbon oxides to amino

acids or protein precursors in microorganisms. Research in 1970 showed that zeolites can absorb up to 15% of ammonia in the rumen and thus reduce ammonia toxicity to ruminants both in vivo and in vitro [5,6].

Binding of urea with zeolites in ruminant feed caused the release of ammonia slowly in the rumen, through the mechanism of ion exchange with cations. The purpose of this study was to study the effect of urea-zeolite supplementation based on rumen fermentation products and post-rumen digestibility in vitro.

2. Materials and Methods

Urea-zeolite were made by heating urea to liquid and adding zeolite with a ratio of 1: 1 DM (24.68% N) and 1: 2 DM (16.15% N). The substrate consisted of 70% corn sugarcane silage and 30% concentrate (Table 1). The inoculum source of three goats was taken in the morning before being fed. They were filtered and added with McDougall solutions as buffer at 39^0 C with ratio of 1: 4 (v / v).

The first experiment tested the hydrolysis level of urea-zeolite at a ratio of 1: 1 and 1: 2 according to manner [7] in goat's rumen fluid. Anaerobic incubation was performed at 0, 1, 2, 3, 4, and 5 h, with 4 replicates. In each incubation, the filtrate was analyzed for N-NH3 concentration by the phenol-hypoclorite reaction [8]. The obtained data were subject to analysis of t test and regression using the IBM SPSS Statistics program ver. 23.

In vitro experiment 2 used urea-zeolites supplement from Experiment 1 as much as 0, 2, 4, 6, 8, and 10% DM subtrat (Table 1). Chemical composition of subtrates dry mater (DM), ash, crude protein (CP), ether extract (EE), crude fiber (CF), and nitrogren free extract (NFE) of 86.62, 25.31, 11.13, 14.21, 31.04, and 18.32 %, respectively. *In vitro* experiment was conducted according to Tilley and Terry (1963). Incubation was performed for 96 h and each treatment was repeated 4 times. Glass tube consisted of two sets; first tube had 48 h anaerobic incubation then added with 1 ml mercuric chloride (50 mg/ml), second tube was added with 6 ml HCl 20% (v/v) then aerobic incubation continued for 48 h. The content of the first tube was filtered, then the sediment was analyzed for dry matter and the supernatant was analyzed for total VFA and NH_3-N using phenol-hypochlorite reaction [8]. The content of the second tube after 48 h aerobic incubation was filtered and the sediment was analyzed for dry and organic matter [9]. The obtained data were subject to analysis of variance in completely randomized design. Should different mean value occur, orthogonal polynomial and Duncan's multiple range test was conducted using IBM SPSS Statistics program ver. 23.

Table 1. Ingredient and chemical composition of the experimental subtrate on dry matter basis (% DM)

	% DM					
	S0	S1	S2	S3	S4	S5
Ingredient :						
Corn straw silage	70	70	70	70	70	70
Consentrate	30	30	30	30	30	30
Suplementation of urea-zeolit	0	2	4	6	8	10

S0 = basal subtrat, S1 = basal subtrat + 2 % DM UZS, S2 = basal subtrat + 4 % DM UZS, S3 = basal subtrat + 6 % DM UZS, S4 = basal subtrat + 8 % DM UZS, S5 = basal subtrat + 10 % DM UZS.

3. Results and Discussions

3.1. The Effect of Urea-zeolit on Percentage of Nitrogen Solubility

The concentration of N-NH3 in rumen fluid is used as an indicator of hydrolysis of urea-zeolite by urease of rumen microorganisms. The level of hydrolysis between the urea-zeolite ratio of 1: 1 and 1: 2 did not differ (P> 0.01). The level of urea-zeolite hydrolysis followed quadratic regression and its solubility was stable after 4 hours of incubation (Figure 1). The percentage of nitrogen hydrolyzed

from urea-zeolite at a ratio of 1: 1 follows the equation Y = 1.43 + 18.43 x - 2.20X² (R2 = 0.99) and at a ratio of 1: 2 with the equation Y = 1.85 + 17.88 x - 2.09 X² (R2 = 0.98). Based on these results, a 1: 2 ratio of urea-zeolite can be used as a substrate supplement for phase 2 research.

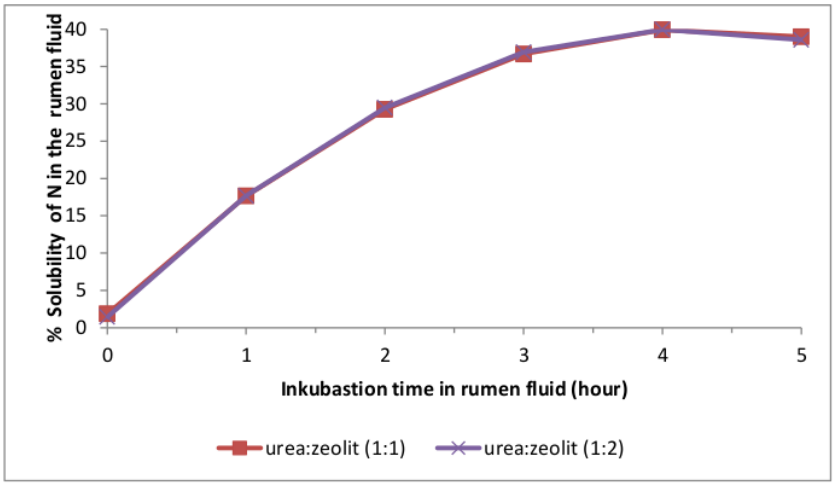

Figure 1. The relationship incubation time of urea-zeolit on % nitrogen solubility in the rumen fluid

3.2. pH, N-NH₃, VFA Total and Dry Matter (DM) Degradation in the Rumen In Vitro

The increased level of urea-zeolite supplementation did not affect the pH of the rumen fluid (pH 8.58 - 8.88) (Table 2). The exchange of cations from zeolites and ammonium (NH4 +) can act as a buffer in the rumen. Therefore, ammonium will be released slowly without affecting the pH.

Increasing of ammonia levels (Table 2) in the rumen fluid was affected (P <0.01) linearly by level of urea-zeolite supplementation (Y = 32.52 + 7.97X; R2 = 82.70%). Supplementation of urea-zeolites at dose of 4, 6, 8, and 10% DM subtrate, the average ammonia level was not different (P> 0.01). This phenomenon illustrates that the release of ammonia in the rumen fluid occurs slowly. These results differ from in vivo experiments that urea-zeolite supplementation results in stable ammonia levels, due to the slow release of ammonia and the absorption process in the rumen. In in vitro experiments, ammonia levels can be used to determine the solubility of urea-zeolites. In the absence of ammonia absorption in in vitro experiments, the increase in urea-zeolite supplementation will also be followed by an increase in ammonia levels in rumen fluid.

The total VFA fermentation product (Table 2) increased (P <0.01) with increasing levels of urea-zeolite supplementation and it was highest in the administration of 6% DM subtrate (116.5 mM / l rumen fluid). The effect of urea-zeolite supplementation on total VFA followed the quadratic regression equation (Y = 73 + 20.01 X -15.60X² + 6.11X³ - 0.90X⁴ + 0.042X⁵; R² = 94.89%). Increased rumen fluid ammonia with increased urea-zeolite supplementation up to 6% DM subtrate could increase the activity of microorganisms that degrade carbohydrates. The same result was reported by [10], that the addition of natural zeolite in the ration was able to increase the digestibility of protein and neutral detergent fiber (NDF) in Arabic sheep

DM degradation in the rumen (Table 2) followed the pattern of total VFA products. DM degradation increased (P <0.01) with increasing levels of urea-zeolite supplementation. The highest DM degradation was achieved in the administration of urea-zeolite 6% DM subtrate (74.48% DM) by following the quartic regression equation (Y = 66.25 - 3.97X + 4.21X² - 0.79X³ + 0.041X⁴; R² = 69.32 %). Based on the average value of DM in the administration of urea-zeolite supplements between 4

and 6% the DM subtrate showed no difference (P> 0.01). These results illustrate that supplementation with urea-zeolite in the subtrate could increase the rate of DM degradation, due to the availability of ammonia released slowly proportional to the availability of energy (VFAs)

Table 2. The effect urea-zeolit supplementation on pH, fermentation product (N-NH_3 and VFA total, DM degradation in the rumen and post rumen digestion

	Suplementation of zeolit-urea (% DM subtrat)						
	0	2	4	6	8	10	Significans
Rumen :							
pH	8.64	8.58	8.71	8.81	8.88	8.77	(P>0.01)
NH_3-N (mg/dl)	23.152^{a}	55.113^{b}	70.545^{bc}	82.631^{cd}	93.393^{cd}	109.288^{d}	(P<0.01)
Total VFA (mM/l)	73.0^{a}	86.5^{b}	108.0^{c}	116.5^{d}	70.5^{a}	70.0^{a}	(P<0.01)
DM degradation (%)	66.01^{ab}	70.67^{bc}	74.13^{cd}	78.48^{d}	65.19^{a}	65.85^{ab}	(P<0.01)
Post-rumen							
DM (%)	53.70^{a}	63.20^{b}	63.34^{b}	68.67^{b}	52.64^{a}	52.39^{a}	(P<0.01)
OM (%)	62.99^{a}	69.28^{bc}	70.40^{c}	77.81^{d}	62.46^{a}	62.46^{ab}	(P<0.01)

3.3. Dry Matter and Organic Matter Post Rumen Digestion In-Vitro

Dry matter digestibility (Table 2) in the post-rumen increased (P <0.01) with increasing levels of urea-zeolite supplementation. The highest digestibility of dry matter in the administration of 6% DM subtrate (68.67% DM) by following the quadratic equation ($Y = 53.70 + 25.11X - 18.59X^2 + 5.40X^3 - 0.65X^4 + 0.027X$ R; $R^2 = 68.62$ %). Based on the average digestibility value of dry matter between supplementation of urea-zeolite 4 and 6% DM subtrate did not differ (P> 0.01). Increased digestion of dry matter was in line with the increased degradation of dry matter in the rumen.

Digestibility of organic matter (Table 2) in the post-rumen as well as digestibility of dry matter increased (P <0.01) with increasing levels of urea-zeolite supplementation. The highest digestibility of organic matter in the administration of 6% DM subtrate (77.81% OM) by following the quadratic regression equation ($Y = 62.99 + 22.17X - 17.93X^2 + 5.43X^3 - 0.67X^4 + 0.028X^5$; $R^2 = 76.58\%$)

4. Conclusion

It was concluded that supplementation of urea-zeolite 6% DM substrate was the best in increasing the degradation of dry matter and fermentation products in the rumen and the digestibility of dry and organic material in the post-rumen

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