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# Effect of polyurethane coated urea on ruminal ammonia release and fermentation characteristics *in vitro* of steam-flaked maize-based diet*

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ABSTRACT

Feed-grade urea (FGU), soyabean meal (SBM) and polyurethane coated urea (PCU) were used to investigate the effect of different nitrogen sources on ruminal ammonia release and other fermentation characteristics *in vitro*. The results showed $NH_3$-N concentrations of PCU and SBM diets were similar during first 8 h of fermentation and were significantly lower by 8.2-20.6% than that of FGU diet ($P<0.01$). *In vitro* ruminal pH, 24-h gas production, total VFA and individual VFA molar proportions were not affected by nitrogen sources, except for 24-h ruminal dry matter digestibility *in vitro* that was higher ($P<0.05$) for SBM diet than FGU and PCU diets. It was concluded that PCU supplement could slower ruminal ammonia release, which was similar with SBM and better than FGU.

KEY WORDS: nitrogen sources, ruminal ammonia release, fermentation characteristics, *in vitro*

## INTRODUCTION

Deficiency of protein feedstuff is a challenge to animal husbandry all over the world. So, it is necessary to exploit more protein sources and products used as an alternative substitute. As a common used and low cost non protein nitrogen (NPN) product, urea is popularly and widely used. However, farmers must be careful when feeding urea directly because of its some disadvantages such as poor feed intake, toxicity (Huber and Kung, 1981), and lower nitrogen utilization resulting from rapid release of ammonia in the rumen. Therefore, a slow-released

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urea has been developed and used in ruminant diets (Cass and Richardson, 1994; Galo et al., 2003). However, previous results were not consistent, what is partially due to different slow release techniques or different type of products used in the studies. In addition, when diets are high in ruminal available proteins, they have more microbial crude protein (MCP) production under the condition of highly nonstructural carbohydrate source. Therefore, steam-flaked maize as a high energy feed may provide lots of benefits over typical maize (Dhiman et al., 2002; Bargo et al., 2003). As a result, the purpose of the present study was to determine the effect of polyurethane coated urea on ruminal ammonia release and other fermentation characteristics *in vitro*, based on steam-flaked maize diet.

## MATERIAL AND METHODS

All diets were formulated to meet the nutrient requirements of Holstein cows according to NRC (2001). They were isonitrogenous (17.5% CP) and consisted of 43% mixed concentrate and 57% roughages (Table 1).

Table 1. Ingredients and chemical composition of the diets

| Item | Treatment | | |
|---|---|---|---|
| | SBM | FGU | PCU |
| *Ingredients, % DM* | | | |
| soyabean meal | 5.44 | 2.00 | 2.00 |
| feed-grade urea | - | 0.60 | - |
| coated-urea[1] | - | - | 0.60 |
| cottonseed meal | 9.13 | 9.13 | 9.13 |
| steam-flaked maize | 25.98 | 25.98 | 25.98 |
| yeast granule | 2.27 | 2.27 | 2.27 |
| Chinese wild rye grass | 17.63 | 20.47 | 20.47 |
| maize silage | 38.65 | 38.65 | 38.65 |
| dicalcium phosphate | 0.30 | 0.30 | 0.30 |
| salt | 0.30 | 0.30 | 0.30 |
| mineral and vitamin premix[2] | 0.30 | 0.30 | 0.30 |
| *Chemical composition, % of DM* | | | |
| CP | 17.52 | 17.38 | 17.38 |
| NDF | 36.03 | 37.40 | 37.40 |
| ADF | 22.26 | 23.05 | 23.05 |

[1] polyurethane coated urea (Granco Minerals, Inc. USA)

[2] contained g/kg: Mg 35; mg/kg: Cu 340, Mn 1500, Zn 2010, Se 15, Co 3.5, I 25; IU/kg: vit. A 80.000, vit. D 7000, vit. E 780

The *in vitro* gas production procedure described by Menke et al. (1979) was used with a slight modification. Namely, a water bath at 39°C was used for incubation instead of using an air-heated incubator. Gas production was recorded at the time intervals of 0, 1, 2, 4, 6, 8, 12, 18, 24, 36, 48 and 72 h. The same experiment was repeated on different days with ruminal fluid taken from three different Holstein cows.

Feed ingredients were analysed for crude protein (CP) based on the Dumas combustion method and dry matter according to AOAC (1990), NDF and ADF according to Van Soest et al., (1991). During fermentation *in vitro*, twelve syringes were taken out for sampling and analysis after 0, 1, 2, 4, 6, 8, 12, 18 and 24 h incubation, respectively. Ammonia nitrogen ($NH_3$-N) concentration was measured by the method of Broderick and Kang (1980). The pH value of culture solution was measured upon 24 h of incubation. And samples were analysed for VFA according to the description of Erwin et al. (1961).

Data were first subjected to statistical analysis using the GLM procedure of SAS (1996) for a randomized complete block design with treatment as the main effect. Runs served as blocks. Because the block effect was not significant ($P>0.05$), the data were pooled with eight replicates for each group and analysed as a one-way experimental design model.

## RESULTS AND DISCUSSION

The dynamic $NH_3$-N concentrations of the three diets during 24 h fermentation *in vitro* were shown in Table 2. The $NH_3$-N concentrations of all the diets were firstly enhanced within 1 h incubation, then declined gradually, and finally increased again to a stable level. However, PCU had the lowest concentrations of $NH_3$-N for all the time. During the first 8 h of fermentation, PCU decreased the $NH_3$-N concentration by 8.2-20.6% as compared with FGU, which was in agreement with the result of Prokop and Klopfenstein (1977), who reported that slow-release urea (combination of urea and formaldehyde) could decrease ruminal $NH_3$-N concentration by 25.3% compared to urea. No significant differences were found between SBM and PCU on ruminal ammonia release. Similar result was found in the report of Galo et al. (2003) in which urea release from the polymer-coated urea was 83% as extensive as uncoated urea after 1 h incubation in distilled water. Other products (such as urea-calcium combination) have the similar effect (Cass and Richardson, 1994). $NH_3$-N concentration began to step up at 8 h for FGU, which might indicate a self lysis of bacteria occurred. However, $NH_3$-N concentrations for SBM and PCU were increased at 12 h fermentation. So it could be inferred that the two slow releasing ammonia diets might prolong the effect time of microorganisms on nitrogen in ruminal fermentation. Consequently, they would

Table 2. The dynamic concentrations of $NH_3$-N for different diets in 24 h fermentation *in vitro*

| Time | Treatment | | | SEM | P-value |
|---|---|---|---|---|---|
| h | SBM | FGU | PCU | | |
| 0 | 17.13$^{b}$ | 18.72$^{a}$ | 16.85$^{b}$ | 0.26 | 0.0014 |
| 1 | 18.17$^{ab}$ | 18.83$^{a}$ | 17.29$^{b}$ | 0.29 | 0.0138 |
| 2 | 16.69$^{b}$ | 18.43$^{a}$ | 16.23$^{b}$ | 0.26 | 0.0005 |
| 4 | 13.93$^{b}$ | 16.38$^{a}$ | 13.74$^{b}$ | 0.27 | <.0001 |
| 6 | 12.85$^{b}$ | 14.98$^{a}$ | 12.20$^{b}$ | 0.46 | 0.0053 |
| 8 | 12.55$^{b}$ | 15.18$^{a}$ | 12.06$^{b}$ | 0.34 | 0.0002 |
| 12 | 17.63$^{ab}$ | 18.05$^{a}$ | 16.81$^{b}$ | 0.28 | 0.0356 |
| 18 | 20.39$^{ab}$ | 20.58$^{a}$ | 20.21$^{b}$ | 0.09 | 0.0514 |
| 24 | 20.69 | 20.70 | 20.54 | 0.04 | 0.0396 |

$^{a,b}$ means within the same row denoted by different letters differ each other (P<0.05)

relatively improve the balance between ammonia production and carbohydrate for the better microbial protein synthesis.

Supplementation of urea or coated urea did not affect other ruminal fermentation characteristics such as 72 h gas production, pH, total VFA concentration and the molar proportions of individual VFAs (P>0.05; Table 3). But SBM diets had a highest dry matter digestibility after 24 h fermentation (P=0.02), which might be due to the improved carbohydrate digestibility with true protein supplemented. This would be beneficial for the better synchronization of nitrogen and energy.

Table 3. The effect of different diets on other fermentation characteristics *in vitro*

| Item | Treatment | | | SEM | P |
|---|---|---|---|---|---|
| | SBM | FGU | PCU | | |
| 72 h gas production, ml | 55.80 | 54.06 | 54.13 | 0.71 | 0.23 |
| pH | 6.72 | 6.75 | 6.74 | 0.02 | 0.63 |
| Dry matter digestibility, % | 53.17$^{a}$ | 51.03$^{b}$ | 51.12$^{b}$ | 0.43 | 0.02 |
| Total VFA, mmol/l | 64.39 | 61.83 | 63.82 | 1.85 | 0.62 |
| *Molar proportion, mol/100 mol* | | | | | |
| acetic acid | 63.55 | 63.38 | 63.31 | 0.36 | 0.90 |
| propionic acid | 23.60 | 23.32 | 22.81 | 0.50 | 0.55 |
| isobutyric acid | 1.42 | 1.41 | 1.48 | 0.04 | 0.45 |
| butyric acid | 8.18 | 7.71 | 8.60 | 0.40 | 0.35 |
| isovaleric acid | 2.40 | 2.39 | 2.51 | 0.12 | 0.75 |
| valeric acid | 1.32 | 1.32 | 1.28 | 0.02 | 0.29 |
| Acetic : propionic acid ratio | 2.69 | 2.72 | 2.78 | 0.07 | 0.64 |

$^{a,b}$ means within the same row denoted by different letters differ each other (P<0.05)

## CONCLUSIONS

It was concluded that polyurethane coated urea delayed the ammonia release and did not affect other fermentation characteristics. The present results seemed to indicate that the raw soyabean may be partially substituted by coated urea. However, further study is needed to investigate ruminal microbial crude protein production and production performance in beef and dairy cows.

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