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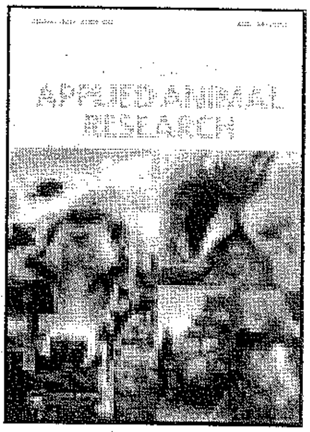

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SHORT COMMUNICATION

Effects of urea supplementation on rumen fermentation characteristics and protozoa population *in vitro*

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Cotton straw is used as a roughage source in conjunction with maize in draught prone regions in the world for animal production. However, this diet generally contains relatively low rumen degradable protein compared with its supply of fermentable metabolizable energy; therefore leading to a suboptimal animal performance and high methane production. Although urea supplementation is known to improve microbial crude protein (MCP) production and animal production, the recommended levels of urea supplementation range between 1% and 6.7% according to literatures. This *in vitro* study was conducted as a preliminary investigation to determine the impact of urea supplementation up to 3% (as dry matter in the diet) on rumen fermentation characteristics and protozoa population, on typical maize meal and cotton straw-based diet used for sheep production in XinJiang province, China. MCP production was improved by 64% when urea increased from 0% to 2% in the diet, with no additional benefit observed at 3% urea in the diet. On the other hand, methane production was reduced when urea increased from 0% to 3% in the diet. These results indicated that 2% urea supplemented under the current feeding condition may improve MCP production and reduce methane production. However, further *in vivo* study is needed to confirm that 2% urea in the diet would not cause adverse effects on animal health.

Keywords: microbial crude protein; methane; pH; cotton; maize; rumen

1. Introduction

Rumen microbes ferment feed carbohydrates to produce volatile fatty acids, and they also degrade feed nitrogen (N) to synthesis microbial crude protein (MCP; Cottle 1991). The MCP production contributes to protein supply to the small intestine, accounting for 40–90% of total absorbable protein (Koenig et al. 2000). The availability of rumen degradable protein (RDP) and fermentable metabolizable energy (FME) largely determines the MCP production in the rumen. Insufficient RDP relative to FME in the diet does not only limit animal performance, but also increase methane (CH_4) production as waste product due to surplus carbohydrate; which contributes to greenhouse gas emissions (Johnson & Johnson 1995). Ruminal methanogens are associated with the existence of protozoal species (Clark et al. 2011). This suggests CH_4 production may be changed through manipulating ruminal protozoa population (Johnson & Johnson 1995).

Cotton straw is a by-product of cotton production and it has been used as a roughage source together with maize in draught prone regions in the world; to form a basal diet for sheep production (Osuji et al. 1993). However, this diet generally contains relatively low RDP compared with its supply of FME; therefore leading to a suboptimal animal performance and high CH_4 production. Urea can be supplemented as an inexpensive RDP source to improve MCP production and animal performance. The common industry practice is to supplement less than 1% of urea as dry matter (DM) in the diet to prevent ammonia toxicity (Cottle 1991), but Currier et al. (2004) suggested up to 6.7% of feed can be consumed as urea if animal and dietary factors are ideal. To prevent ammonia toxicity on animal, this *in vitro* study was conducted as a preliminary investigation to determine the impact of urea supplementation up to 3% as DM in the diet on rumen fermentation characteristics and protozoa population, on typical maize meal and cotton straw-based diet for sheep production.

2. Materials and methods

A healthy non-lactating karakul sheep weighed 30 kg, fitted with permanent ruminal cannula was housed in a well-ventilated pen and maintained under strict animal ethical standards of Tarim University. Sheep was offered with 1 kg DM per day of chopped cotton straw and maize meal to fulfil their nutrient requirement for maintenance (Nicol & Brookes 2007). In addition fresh water was

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Table 1. Ingredients of the experimental diets.

Ingredients	0% urea	1% urea	2% urea	3% urea
Urea (g kg DM^{-1})	0	10	20	30
Maize meal (g kg DM^{-1})	225	222	221	217
Wheat bran (g kg DM^{-1})	30	39	29	29
Fish meal (g kg DM^{-1})	15	15	15	15
Cotton straw (g kg DM^{-1})	405	401	397	393
Maize straw (g kg DM^{-1})	135	134	132	131
Dry grass (g kg DM^{-1})	135	134	132	131
Salt (g kg DM^{-1})	5	5	5	5
Lime stone (g kg DM^{-1})	10	10	10	10
Cotton seed meal (g kg DM^{-1})	30	30	29	29
Premix mineral (g kg DM^{-1})	10	10	10	10

offered to the sheep *ad libtum*. Rumen content was sampled via ruminal cannula in the morning at 10.00 h prior to feeding. Rumen liquid was obtained through filtering rumen sample with four layers of cheesecloth. Rumen liquid was mixed with pre-prepared rumen buffer solution (by adding 10.0 mg $CaCl_2$, 10.0 mg $MnCl{\cdot}4H_2$, 1.0 mg $CoCl_2{\cdot}6H_2O$, 8.0 mg $FeCl_3{\cdot}6H_2O$, 1.2 g NH_4HCO_3, 10.8 g $NaHCO_3$, 1.8 g Na_2HPO_4, 1.9 g KH_2PO_4, 0.2 g $MgSO_4{\cdot}7H_2O$, 1.7 g NaOH and 0.2 g Na_2S into 650 ml of distilled water) in a ratio of 1:2 to make artificial rumen fermentation solution, and was kept under the continuous flushing with CO_2 for 10 min before transferring into a water bath at 39°C.

A basal diet (0% urea) was formulated using feed ingredients presented in Table 1. Dietary treatments were formulated into four levels of dietary urea concentration (0–3% as DM in the diet; Table 1). *In vitro* digestibility of organic matter on DM basis (DOMD) was measured as described by Clarke et al. (1982). The metabolizable energy (ME) level of the diet was calculated using the equation of ME (MJ kg DM^{-1}) = DOMD (g kg DM^{-1}) × 0.016. Kjeldahl method (Buchi, K-370, Switzerland) was used to analyze N concentration in the diet. Dietary crude protein (CP) was calculated by CP (g kg DM^{-1}) = N (g kg DM^{-1}) × 6.25.

Feed (200 mg) and artificial rumen fermentation solution (30 ml) were added into syringe (100 ml) with sealed tip for *in vitro* fermentation. The starting level of piston was recorded before placing the syringe into a shaking water bath at the speed of 10 shakes per min at 39°C. Follow the same process, each dietary treatment was repeated for six times, three of them were later used for pH, MCP production measurement, protozoa population quantification and other three were measured for CH_4 production.

At the end of 24 h fermentation, pH was measured and fermented rumen liquid was sampled through filtering 15 ml fermented rumen content with two layers of cheesecloth. Protozoa population was quantified by adding 35% of formalin into 1 ml of fermented rumen liquid and stained with methyl green (0.6 g l^{-1}) and NaCl (8.0 g l^{-1}) in a counting chamber according to Meng et al. (2000).

According to the method described by Makkar et al. (1982), the fermented rumen liquid was centrifuged at 1500 rpm at 4°C for 20 min. Supernatants were discarded, and cells obtained were washed with distilled water and this process was repeated for three times. The supernatants were discarded and the cells were transferred to flask for MCP determination by Kjeldahl method (Buchi, K-370, Switzerland). Gas chromatography (GC-14B, Shimadzu, Japan) was used for CH_4 measurement followed the procedures described by Lila et al. (2003).

Statistical analysis was performed using SPSS 13.0 (SPSS Science, Chicago, Illinois). General analysis of variance was conducted to determine the significant effect of dietary treatments. Multiple comparison was used to compare treatment means when $P < 0.05$.

3. Results and discussion

Maize meal and cotton straw represented 63% of the diet (Table 1). The results showed that when urea increased from 0% to 3% in the diet, the calculated CP concentration in the diet increased from 76 to 155 g kg DM^{-1}, while little ME change was detected across the treatments (Table 2). The MCP production was increased by 64% when urea increased from 0% to 2% in the diet. No MCP production difference was observed between 2% and 3% urea treatments (Table 3). On the other hand, CH_4 production was decreased by 8% when urea increased from 0% to 3% in the diet. Urea supplementation increased both pH and protozoa population (Table 3).

According to Nicol and Brookes (2007), the maintenance ME requirement of the sheep used in the current study was 5.8 MJ per day; calculated using formula

Table 2. Chemical compositions of the experimental diets.

Chemical composition	0% urea	1% urea	2% urea	3% urea
Crude protein (g kg DM^{-1})	76	102	128	155
Dry matter (g kg DM^{-1})	898	898	898	898
Ash (g kg DM^{-1})	111	110	109	108
Neutral detergent fiber (g kg DM^{-1})	291	289	286	284
Acid detergent fiber (g kg DM^{-1})	207	205	203	201
Ether extract (g kg DM^{-1})	29	29	29	29
Metabolizable energy (MJ kg DM^{-1})	8.8	8.7	8.6	8.5

Table 3. Urea supplementation effects on pH, concentrations of MCP (MCP; mg/ml), protozoa population (10^4/ml) and methane production (CH_4; ul/ml) after 24 hr *in vitro* rumen fermentation.

	0% urea	1% urea	2% urea	3% urea	SE	*P* value
MCP	1.1^b	1.3^b	1.8^a	1.8^a	0.101	0.013
Protozoa	57.1^b	58.8^b	61.0^a	60.3^a	0.463	0.017
CH_4	19.8^{ab}	20.0^a	19.2^b	18.3^c	0.254	0.031
pH	6.56^d	6.61^c	6.69^b	6.74^a	0.021	0.001

Note: Means with different superscripts are significantly different at the 5% confidence level.

0.45 MJ ME per kg of $BW^{0.75}$. This is 52% lower than the ME supply of 8.8 MJ per day from the basal diet (assuming feed consumption was 1 kg DM per day). The basal diet provided 76 g kg DM^{-1} CP, which is same as the estimated CP maintenance requirement of the sheep used in the current study (Brookes & Nicol 2007). Therefore, animal performance under the current feeding condition is likely to be limited by protein supply rather than energy supply.

Adding urea as a source of RDP should increase MCP production and contribute to metabolizable protein supply to the animal (Johnson & Johnson 1995). This is evident by the improved MCP production when urea increased from 0% to 2% in the diet. Zhou et al. (2009) reported that MCP production ranged between 0.6 to 2.0 mg ml^{-1}, which is comparable to the results from the current study. The average protozoa population from the current study is also comparable to previously reported, using faunated sheep (Koenig et al. 2000). Both MCP production and protozoa population increased as urea was supplemented in the diet up to 2%, with no further change at 3% urea in the diet (Table 3). This may reflect the 2% urea was the upper limit for microbial activity which also constrained the change of MCP production. Nevertheless, the overall pattern of urea supplementation led to an increase in pH is similar to Bernard et al. (2001) demonstrated, and ammonia accumulation from urea degradation may be responsible to the increased ruminal pH (Cottle 1991).

Methane is a waste product from carbohydrate fermentation; it contributes to greenhouse gas emissions and causes environmental pollution (Clark et al. 2011). The change of CH_4 production in the rumen is related to the sources of carbohydrate and protein, and also ruminal environment (e.g., methanogens population and pH; Johnson & Johnson 1995). The reduction in CH_4 production when urea was supplemented in this study may be related to increased ammonia accumulation from urea break down in the rumen and it inhibited the methanogenic activity (He et al. 2005).

4. Conclusions

The 3% urea supplementation in this *in vitro* study did not provide additional MCP production and protozoa population compared with 2% urea supplementation. Methane production decreased as urea supplementation increased. This study showed higher levels of urea supplementation (2% in the diet) than currently industry used (1% in the diet) on maize meal and cotton straw-based diet, can improve MCP production and reduce CH_4 production. However, *in vivo* study is needed to confirm 2% urea in the diet has no adverse effect on animal health under the current feeding condition.

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Charts Data

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Evolution of Citations per Document to a journal's published documents during the two, three and four previous years. The two years line is equivalent to journal impact factor ™ (Thomson Reuters) metric.

International Collaboration

International Collaboration accounts for the articles that have been produced by researchers from several countries. The chart shows the ratio of a journal's documents signed by researchers from more than one country.

Journal's Citable vs. Non Citable Documents

Not every article in a journal is considered primary research and therefore "citable", this chart shows the ratio of a journal's articles including substantial research (research articles, conference papers and reviews) in three year windows.

Journal's Cited vs. Uncited Documents

Ratio of a journal's items, grouped in three years windows, that have been cited at least once vs. those not cited during the following year.