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Effects of Polyurethane Coated Urea Supplement on *In vitro* Ruminal Fermentation, Ammonia Release Dynamics and Lactating Performance of Holstein Dairy Cows Fed a Steam-flaked Corn-based Diet*

H. S. Xin[1], D. M. Schaefer[2], Q. P. Liu[3], D. E. Axe[4] and Q. X. Meng**

College of Animal Science and Technology, State Key Laboratory of Animal Nutrition
China Agricultural University, Beijing 100193, China

ABSTRACT : Three experiments were conducted to investigate the effects of polyurethane coated urea on *in vitro* ruminal fermentation, ammonia release dynamics and lactating performance of Holstein dairy cows fed a steam-flaked corn-based diet. In Exp. 1, a dual-flow continuous culture was run to investigate the effect of polyurethane coated urea on nutrient digestibility, rumen fermentation parameters and microbial efficiency. Three treatment diets with isonitrogenous contents (13.0% CP) were prepared: i) feed-grade urea (FGU) diet; ii) polyurethane coated urea (PCU) diet; and iii) isolated soy protein (ISP) diet. Each of the diets consisted of 40% steam-flaked corn meal, 58.5% forages and 1.5% different sources of nitrogen. PCU and FGU diets had significantly lower digestibility of NDF and ADF ($p<0.01$) than the ISP diet. Nitrogen source had no significant effect ($p = 0.62$) on CP digestibility. The microbial efficiency (expressed as grams of microbial N/kg organic matter truly digested (OMTD)) *in vitro* of the PCU diet (13.0 g N/kg OMTD) was significantly higher than the FGU diet (11.3 g N/kg OMTD), but comparable with the ISP diet (14.7 g N/kg OMTD). Exp. 2, an *in vitro* ruminal fermentation experiment, was conducted to determine the ammonia release dynamics during an 8 h ruminal fermentation. Three treatment diets were based on steam-flaked corn diets commonly fed to lactating cows in China, in which FGU, PCU or soybean meal (SBM) was added to provide 10% of total dietary N. *In vitro* NH_3-N concentrations were lower ($p<0.05$) for the PCU diet than the FGU diet, but similar to that for the SBM diet at all time points. In Exp. 3, a lactation trial was performed using 24 lactating Holstein cows to compare the lactating performance and blood urea nitrogen (BUN) concentrations when cows were fed PCU, FGU and SBM diets. Cows consuming the PCU diet had approximately 12.8% more ($p = 0.02$) dietary dry matter intake than those consuming the FGU diet. Cows fed the PCU diet had higher milk protein content (3.16% vs. 2.94%) and lower milk urea nitrogen (MUN) concentration (13.0 mg/dl vs. 14.4 mg/dl) than those fed the FGU diet. Blood urea nitrogen (BUN) concentration was significantly lower for cows fed the PCU (16.7 mg/dl) and SBM (16.4 mg/dl) diets than the FGU (18.7 mg/dl) diet. Cows fed the PCU diet had less surplus ruminal N than those fed the FGU diet and produced a comparable lactation performance to the SBM diet, suggesting that polyurethane coated urea can partially substitute soybean meal in the dairy cow diet without impairing lactation performance. (**Key Words :** Polyurethane Coated Urea, Rumen Fermentation, Ruminal Ammonia Release, Lactating Performance)

INTRODUCTION

Many species of rumen microorganisms can use ammonia as a nitrogen source for their growth and supply their microbial proteins to the host animal. The majority of ammonia captured by ruminal bacteria is released from deamination of amino acids and hydrolysis of non-protein nitrogen (NPN) compounds (NRC, 2001). Urea is widely used as a common NPN source in ruminant diets. However, urea is often degraded rapidly in the rumen by the action of urease and the resulting ammonia supply may exceed the capacity of rumen bacteria to assimilate it into amino acids (Huber and Kung, 1981). This rapid release of ammonia may result in inefficient N utilization in the rumen.

Although numerous studies have tested the effectiveness of slow-release urea product used in ruminant diets to

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** Corresponding Author: Q. X. Meng. Tel: +86-10-6273-3799, Fax: +86-10-6282-9099, E-mail: qxmeng@cau.edu.cn

[1] College of Animal Science and Technology, Northeast Agricultural University, Harbin 150030, China.

[2] Department of Animal Sciences, University of Wisconsin-Madison, WI 53706, USA.

[3] International Ingredient Corporation, St. Louis, Mossouri, 63026, USA.

[4] Agri-Nutrient Technology Group, Petersburg, Virginia, 23803, USA.

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improve ammonia assimilation in the rumen (Owens et al., 1980; Tedeschi et al., 2002; Galo et al., 2003), the results were considerably inconsistent. In an *in vitro* study, Cass and Richardson (1994) reported that a urea-calcium combination product reduced ruminal ammonia release compared with a typical urea product. In a later study, Cass et al. (1995) found that a supplement of slow-release urea/calcium compound in a feedlot steer diet yielded a 4.3% improvement in feed efficiency ($p>0.05$), a 2.8% decrease in ADG ($p>0.05$), and leaner carcasses. In another trial using lactating dairy cows (Golombeski et al., 2006), the addition of slow-release urea compound at the level of 0.61% dietary DM reduced feed intake, but increased milk production therefore resulting in improved feed efficiency. In contrast, Tedeschi et al. (2002) found that there was no improvement in growth performance when urea was substituted by a slow-release urea product at levels normally found in feedlot cattle diets. Similarly, Galo et al. (2003) reported that feeding lactating dairy cows with a diet including 0.77% polymer-coated urea had little impact on milk production.

A new slow-release urea product, polyurethane coated urea, has been commercially developed in which granular urea is coated with semi-permeable layers of organic polymer resins. Although semi-permeable membranes have been widely used to control the release of nitrogen fertilizer compounds in water or soil (El Sayed Mohamed Hassan and Loeb, 1964; Blaylock et al., 2005), the urea product coated with such semi-permeable membranes has not been tested for the rate of ammonia release in the rumen.

Because ammonia produced in the rumen is used for microbial growth, which is also dependent on energy availability, it is important that the rate of ammonia production in the rumen be coordinated with the rate of carbohydrate digestion. Steam-flaked corn grain has been shown to increase degradability of starch in the rumen, resulting in greater ruminal concentrations of VFA (particularly propionic acid) and more microbial CP synthesis (Chen et al., 1994; Dhiman et al., 2002). Therefore, we hypothesized that a supplement of coated urea in the steam-flaked corn-based diet of lactating dairy cows would result in a higher lactation performance than a traditional urea supplement and be comparable with a soybean meal supplement, because of its improvement of microbial nitrogen utilization through slow ammonia release and higher microbial synthetic efficiency in the rumen. To test this hypothesis, the objectives of this study were to evaluate the impact of supplementation of polyurethane coated urea on: i) rumen fermentation characteristics and microbial efficiency in continuous culture; ii) *in vitro* ruminal ammonia release dynamics; and iii) milk yield and blood urea nitrogen concentration in lactating cows fed a steam-flaked corn-based diet.

MATERIALS AND METHODS

Polyurethane coated urea source and steam-flaked corn

Polyurethane coated urea (PCU) was part of a concentrate mix provided by Granco Mineral Inc. (Virginia, USA). This product contained 99.5% urea and 0.5% coating materials with N content of 45.3% by weight. Steam-flaked corn (SFC) was provided by KAITE Feed Inc. in Hebei Province, China. The SFC was prepared with a steam-flaking machine (Model FX 1600-36, ROSCAMP CHAMPION, Waterloo, IA, USA). Briefly, a steam chamber was filled with corn grain and brought to a constant temperature (99°C) at atmospheric pressure using steam for 60 min and then the grains were passed through rollers with apertures adjusted to result in densities of 380 g/L. The flake thickness was 1.2 to 1.5 mm. The SFC was allowed to air-dry before use in the diet preparation.

Experimental design

Continuous culture study (Exp. 1) :

i) Continuous culture system and operating conditions

A dual-flow continuous culture system with 12 automated feeders as described by Meng (1999) was used in this study. The basic apparatus consisted of twelve independent 1,000 ml polycarbonate fermenters, with a working volume of 780 ml, which were immersed in a 39°C waterbath and stirred by a motor-driven stirrer at a speed of 37 rpm. Two peristaltic pumps (Model BT00-600M, Baoding Longer Pump Co., Ltd., Heei, China) were used to deliver buffer solution into each fermenter at a liquid dilution rate of 12%/h and to remove liquid effluent at 8%/h through a 100-mesh stainless steel filter (Model DFCCS-III, Hebei Wenanjingda Instrument Factory, Hebei, China) to allow the solid fraction to flow out with liquids at 4%/h. Solid and liquid effluents were individually received into jars immersed in a refrigerated waterbath (0 to 4°C) to terminate the microbial activity.

Mixed rumen contents as inoculum were collected from four ruminally-fistulated Holstein cows fed a basal diet similar to the diets provided to the fermenters (Table 1). Each of the pelleted diets (48 g/d) was delivered into the fermenter in 24 equal portions by the automated feeder installed on top of the fermenter. During fermentation, all fermenters were continuously stirred, purged with CO_2 to provide anaerobiosis, and maintained at a constant 39°C. A buffer solution, as described by Slyter (1990) without urea but plus L-cysteine HCl (250 mg/L), was infused continuously into each fermenter by the peristaltic pump to achieve a liquid dilution rate of 8%/h. Dilution rates of solid and liquid fractions were monitored twice daily by regulating the buffer input rate and liquid fraction removal rate.

Table 1. Ingredients and chemical composition of diets (Exp. 1) [1]

Item	Treatment diet		
	FGU	PCU	ISP
Ingredients (%)			
Steam-flaked corn	40.0	40.0	40.0
Chinese wild rye hay	50.5	50.4	46.6
Alfalfa hay	8.0	8.0	8.0
Feed-grade urea	1.6	-	-
Polyurethane coated-urea	-	1.7	-
Isolated soy protein	-	-	5.4
Chemical composition			
DM (%)	89.7	89.7	89.6
CP (% of DM)	13.1	13.0	13.1
NDF (% of DM)	51.0	50.9	49.5
ADF (% of DM)	34.6	34.6	32.4

[1] FGU = Feed-grade urea diet, PCU = Polyurethane coated urea diet, ISP = Isolated soybean protein diet.

ii) Treatment diets

Three isonitrogenous diets (13% CP) were formulated (Table 1) with steam-flaked corn, Chinese wild rye grass hay (*Leymus chinensis*), alfalfa hay and three N sources including polyurethane-coated urea (PCU), feed-grade urea (FGU) and isolated soy protein (ISP). In the treatment diets, additional N from different N sources provided about 35% of total dietary N. All the diets were pelleted (4 mm in diameter×10 mm in length) using a special pelletting machine (Model 9DKS-120, Beijing Wanqing Machinery Co. Ltd, Beijing, China) and were fed by automated feeders to each of the fermenters at 1-h intervals. Each diet was assigned to 4 fermenters as replicates and a total of 12 fermenters were used.

iii) Sampling procedure

According to Meng et al. (1999), continuous culture was run for a total of 7 d including the first 4 d for adaptation and then 3 consecutive days for sampling. During the sampling period, fermentation effluent (solid and fluid) and fermentation contents within fermenters were sampled once daily at 09:00 h. Prior to sampling, a 37% formaldehyde solution (Sigma-Aldrich) was added to each effluent jar at a rate of 2.5% of estimated effluent volume. The total solid or liquid effluents which were collected and recorded for volume over three sampling days (d 5 to 7) were pooled and stored at 4°C. Subsamples of formalinized effluent (approximately 1500 ml) were weighed and centrifuged at 30,000×g for 30 min. The pellet, containing undigested feed fractions, microorganisms and a small amount of buffer salt contamination was washed twice with distilled water, then frozen and lyophilized (10°C at shelf; Model FD-1A-50, Beijing Boyikang Experimental Instrument Co., Beijing, China). Samples then were placed at room temperature to balance moisture for 3 d, and then weighed and ground (1-mm screen). The pH of fermenter contents was determined by placing a glass-electrode pH probe (Model PHS-3C, Shanghai Leici Scientific Instrument Co., Ltd., China) into the fermenter vessel. Ten milliliters of ruminal fermentation fluid was taken from each fermenter and placed into a centrifuge tube containing 2 ml of 25% metaphosphoric acid. Samples were then frozen at -20°C until needed for ruminal VFA and ammonia analysis. To isolate pure bacterial samples, at the termination of each period 19.5 ml of 37% formaldehyde solution was added to the fermenter contents (780 ml) and the mixture was blended for 1 min to promote release of attached microorganisms from feed particles. After being strained through 4 layers of cheesecloth, the fluid fraction was centrifuged at 1,000×g for 5 min to remove feed particles and protozoa. The supernatant fluid was then re-centrifuged at 30,000×g for 30 min at 4°C. The pellet, containing bacteria and some protozoa, was washed three times with 0.9% (w/v) saline solution (first time) and distilled water (second and third time) by centrifugation (30,000×g, 30 min, 4°C). The resulting pellet was frozen and lyophilized as above.

In vitro fermentation study (Exp. 2) : An *in vitro* experiment was conducted to evaluate the rate of ammonia N release from 3 N sources. The 3 treatment diets (Table 2) were steam-flaked corn-based diets, commonly used in lactating cows in China, in which feed-grade urea (FGU), polyurethane coated urea (PCU), or soybean meal (SBM) were added to provide 10% of total dietary N. Each of the three diets was formulated to be isonitrogenous (17.5% CP) and added to a 100-ml glass syringe (HFT000025, Häberle Maschinenfabrik GmbH, Germany) for 8 h ruminal microbial fermentation *in vitro*. Ruminal fluid was obtained from three ruminally-fistulated Holstein cows fed a similar SBM basal diet. Then 30 ml of mixed culture medium (consisting of ruminal fluid and buffer; the ratio of ruminal fluid**:** buffer = 1:2; see Menke et al., 1979) were pipetted into each syringe containing 200 mg dietary DM followed by incubation in a water bath at 39°C. Three syringes for each treatment diet were incubated. At 0, 1, 2, 4, 6 and 8 h time points, the nine syringes were taken out and 10 ml culture medium was collected from each syringe. Subsamples were centrifuged for 15 min at 10,000×g for determination of ammonia N release.

Lactation trial (Exp. 3) : All procedures involving animals were approved by the Animal Care and Use Committee, Animal Science and Technology College, China Agricultural University (Beijing, China). Twenty-four lactating Holstein cows (12 primiparous and 12 multiparous cows in third lactation) were used in a 10 wk lactation trial (1 wk pre-treatment period followed by a 9 wk treatment period). Cows were blocked by parity and randomly

Table 2. Ingredient and chemical composition of the diets (Exp. 2 and 3)[1]

	Treatment diet		
	FGU	PCU	SBM
Ingredient	---------- % DM Basis ----------		
Wheat bran	2.95	2.95	2.95
Soybean meal	1.00	1.00	2.78
Feed-grade urea	0.60	-	-
Coated-urea[2]	-	0.60	-
Cottonseed meal	1.63	1.63	1.63
Whole cotton seed	4.55	4.55	4.55
Extruded soybean	1.00	1.00	2.66
Steam-flaked corn	13.99	13.99	13.99
Brewers grains	7.55	7.55	7.55
Fodder yeast	2.27	2.27	2.27
Cereal germs	4.44	4.44	4.44
Chinese wild rye grass hay	9.65	9.65	6.82
Alfalfa hay	10.23	10.23	10.23
Corn silage	38.65	38.65	38.65
Dicalcium phosphate	0.30	0.30	0.30
Salt	0.40	0.40	0.40
Sodium bicarbonate	0.59	0.59	0.59
Mineral and vitamin premix[3]	0.30	0.30	0.30
Chemical composition			
NE_L (MJ/kg of DM)[4]	6.19	6.19	6.40
DM (%)	57.35	57.26	58.70
CP (% of DM)	17.38	17.38	17.52
NDF (% of DM)	37.40	37.40	36.03
ADF (% of DM)	23.05	23.05	22.26
Ca (% of DM)	0.99	0.99	1.01
P (% of DM)	0.37	0.37	0.38

[1] FGU = Feed-grade urea diet, PCU = Polyurethane coated urea diet, SBM = Soybean meal diet.
[2] Polyurethane coated urea product is provided by Granco Minerals, Inc, USA.
[3] Contained 35 g/kg Mg, 340 mg/kg Cu, 1,500 mg/kg Mn, 2,010 mg/kg Zn, 15 mg/kg Se, 3.5 mg/kg Co, 25 mg/ kg I, 80,000 IU/kg vitamin A, 7,000 IU/kg vitamin D, 780 IU/kg vitamin E (Beijing Sanyuan Feed Co., China).
[4] Calculated from the tabular value (NRC, 2001).

assigned to each of three different N source diets (8 cows per treatment). At trial initiation, cows averaged 623±36 kg body weight, 89±12 DIM and 34.8±5.1 kg/d daily milk yield. During the trial (May to August, 2008), ambient temperature ranged from 19.5 to 43.5°C and humidity averaged 68.7%.

The treatment diets were identical to those used in Exp. 2 and dietary ingredient composition is presented in Table 2. In the three diets, FGU, PCU and SBM each provided 10% of total dietary N. Diets contained forage and concentrate mix in a 55:45 ratio (DM basis) and were fed as TMR three times daily (08:00, 15:00 and 22:00 h). Cows were housed in tie stalls and were fed individually for at least 5 to 10% refusal (as-fed basis). Daily amounts of feed offered and orts for individual cows were weighed and recorded. Orts were mixed for each dietary treatment and a representative composite sample was frozen. Samples of individual ingredients in the treatment diets were taken once weekly.

Cows were milked three times during feeding at 08:00, 15:00 and 22:00 h daily, and milk yield was recorded. Milk samples were collected from 3 daily milkings at the end of each week throughout the 9 wk trial. A portion (300 ml) of milk from each cow was thoroughly mixed and divided into two portions: one portion was analyzed immediately for fat, protein and lactose by a mid-infrared spectrophotometer (Model MilkoScan 4000, FOSS NIRSystems, USA) following the manufacturer's instructions; the other portion was frozen for later analysis of milk urea nitrogen (MUN). On the last day of the trial 2 h after morning feeding, blood

Table 3. Effect of treatment on true digestibilities in continuous culture fermenters

Digestibility (%)	Treatment diet[1]			SEM	p =
	FGU	PCU	ISP		
DM	46.3[b]	51.0[ab]	58.9[a]	2.43	0.02
OM	46.7[b]	51.2[ab]	58.9[a]	2.47	0.02
NDF	13.9[b]	18.5[b]	38.3[a]	1.70	<0.01
ADF	12.6[b]	16.5[b]	30.3[a]	1.83	<0.01
CP	43.5	44.6	45.5	1.46	0.62

[1] FGU = Feed-grade urea diet, PCU = Polyurethane coated urea diet, ISP = Isolated soybean protein diet.
[a, b] Means within the same row denoted by different letters differ each other ($p<0.05$).

was taken from the jugular vein of each cow into a 7 ml vacutainer tube containing lithium heparin (Sinopharm Chemical Reagent Beijing Co., Ltd. Beijing, China), and centrifuged at 2,500×g for 20 min. The plasma was harvested, divided into two aliquots and frozen at -20°C until analyzed.

Laboratory analyses

Feed samples from all three experiments, dietary orts from Exp. 3, isolated microorganisms and effluent residues from Exp. 1 were analyzed for DM and ash (AOAC, 1997), N content (Model Rapid N III, Elementar, Analyzer System GmbH, Germany), NDF and ADF (Van Soest et al., 1991). Isolated microorganisms and effluent residues from Exp. 1 were analyzed for RNA content according to the procedure of Zinn and Owens (1986). Microbial N of effluent residues was calculated from the RNA to N ratio of isolated microorganisms in conjunction with RNA content of the effluent residues. Microbial efficiency was expressed as grams of microbial N per kilogram of OM truly digested. True digestibilities of DM, OM, NDF, ADF and CP for all diets were calculated as the difference of DM, OM, NDF, ADF and CP between diets fed to fermenters and total effluent residues corrected for microbial contribution. Samples for VFA analysis were prepared as described by Li and Meng (2006), and VFA concentration was determined using gas chromatography (6890 N, Agilent technologies) with a 30 m HP-INNOWax 19091N-213 (Agilent) capillary column (0.32 mm i.d. and 0.50 μm film thickness. Ammonia concentration of fermenter contents was determined by the method of Broderick and Kang (1980) using a spectrophotometer (UV-VIS 8500, Shanghai Tianmei Scientific Instrument Co., Ltd., China). Frozen milk samples were thawed to room temperature (18°C) treated with cold trichloroacetic acid (TCA) for about 5 min for deproteinization, and centrifuged at 3,500×g for 30 min at 4°C. The clear supernatant fluid was used to analyze for MUN concentration (Roseler et al., 1993) using a spectrophotometer (UV-VIS 8500, Shanghai Techcomp Scientific Instrument Co., Ltd., China) at an absorbance of 535 nm. The plasma samples collected from each animal were assayed for blood urea nitrogen (BUN) according to the method described by Oltner and Berglund (1982).

Statistical analysis

Data of Exp. 1 and 2 were subjected to statistical analysis using the GLM procedure of SAS (1999) for a completely randomized design with treatment as the main effect. The data for the lactation performance in Exp. 3 were analyzed with repeated measures, using the PROC MIXED procedure of SAS (1999) according to the model: $Y_{ijkl} = \mu + T_i + W_j + C_k + TW_{ij} + e_{ijk,}$ where Y_{ijkl} is the observation, μ is the overall mean, T_i is the dietary treatment, W_j is the week effect, C_k is the cow effect (treated as a random factor), and e_{ijk} is the residual error term. Least squares means are reported throughout, and significance was declared at $p \leq 0.05$ unless otherwise noted. The PROC REG function of SAS was used to compute the regression equation and correlation coefficient between MUN and BUN.

RESULTS AND DISCUSSION

Exp. 1

In vitro nutrient digestibilities : The data on true digestibilities of DM, OM, NDF, ADF and CP are presented in Table 3. The PCU and FGU diets had similar digestibilities of DM, OM, NDF and ADF, which were significantly ($p<0.05$) lower than for the ISP diet. The present result of DM digestibility for the ISP diet (58.9%) was similar to another report (60.4%) in continuous culture (Griswold et al., 2003). Although the ISP diet showed higher NDF digestibility than the two urea diets, the absolute value was still lower (38.3%) than previous results (Meng, 1999; Griswold et al., 2003). This might be due to shortage of rapidly degradable nitrogen from the diet and buffer solution (no urea addition) for rumen microbial growth and cellulolytic activities. As shown in Table 4, ruminal ammonia concentrations for all diets were lower than 5 mg/dl. Bryant (1973) reported that ammonia nitrogen concentration higher than 5 mg/dl is essential for the growth of cellulolytic bacteria. On the other hand, more peptide or

Table 4. Effect of different nitrogen treatment on fermentation traits in continuous culture fermenters

Item	Treatment diet[1]			SEM	p =
	FGU	PCU	ISP		
pH	6.14	6.07	6.06	0.03	0.14
NH_3-N (mg/dl)	1.99[a]	1.39[b]	1.04[c]	0.07	<0.0001
Total VFA (mM/L)	64.08	66.08	63.27	2.50	0.72
Individual VFA (mol %)					
Acetic acid	56.81[a]	56.31[a]	47.18[b]	0.65	<0.0001
Propionic acid	33.33[b]	34.35[b]	41.07[a]	0.86	0.0002
Isobutyric acid	0.14[b]	0.08[b]	0.54[a]	0.02	<0.0001
Butyric acid	5.30[b]	5.25[b]	8.00[a]	0.27	<0.0001
Isovaleric acid	1.79	1.63	1.18	0.17	0.08
Valeric acid	2.64[a]	2.40[a]	2.04[b]	0.10	0.01
Acetic acid/propionic acid	1.71[a]	1.64[a]	1.16[b]	0.04	<0.0001

[1] FGU = Feed-grade urea diet, PCU = Polyurethane coated urea diet, ISP = Isolated soybean protein diet; additional N from different N sources provides about 35% of total dietary N.
[a, b] Means within the same row denoted by different letters differ from each other ($p<0.05$).

amino acid nitrogen from the ISP diet compared to the urea diets might explain the digestion difference between ISP and urea diets, because peptide or amino acids could improve microbial growth more than urea (Argyle and Baldwin, 1989; Griswold et al., 1996; Jones et al., 1998). Ruminal pH in fermenter contents was not altered ($p = 0.14$) by the dietary treatments. It has been recognized that ruminal pH is a key factor limiting fiber digestion in the rumen, especially at a pH below 6.2 (Orskov, 1992). Therefore, the relatively low digestibilities of NDF and ADF in all three diets appeared to be related not only to the low ammonia concentration, but also to the low ruminal pH. There was no significant effect ($p = 0.62$) of dietary nitrogen source on CP digestibility.

Microbial fermentation and growth efficiency : Ruminal microbial fermentation traits are presented in Table 4. The concentration of ammonia was highest for the FGU diet, followed by PCU and ISP diets. The PCU diet reduced ammonia concentration by 30.2% compared with the FGU diet, but it still had a significantly higher ammonia concentration than the ISP diet ($p<0.01$). However, the absolute values of ammonia concentration on all diets were much lower than the 5.0 mg/dl recommended for optimal microbial growth *in vitro* (Satter and Slyter, 1974), which might be considered as a crucial reason for the weak microbial efficiency. The SBM diet, however, could produce ammonia nitrogen above 5 mg/dl without urea addition in the buffer solution in continuous culture (Windschitl and Stern, 1988). The discrepancy between the current study and others (Windschitl and Stern, 1988) might be explained by the difference in CP content of the diets (13.0% vs. 16.0%).

There were no significant differences ($p = 0.72$) in total VFA concentration among the three dietary treatments. Because ruminal VFAs are derived mainly from dietary carbohydrate fermentation (Firkins, 1996), the similar total ruminal VFA concentrations reflected no adverse fermentation by addition of FGU or PCU to the diet. Molar percentages of individual VFAs were significantly altered ($p<0.05$) by the dietary treatment. Urea-based diets resulted in a higher proportion of acetate and less propionate than the ISP diet, which caused a significantly higher ratio of acetate to propionate ($p<0.01$). The isobutyrate molar percentage on the ISP diet was several fold higher than the other two urea treatment diets. This observation is in agreement with the report that isobutyrate concentration increased linearly with increasing level of peptides in continuous culture (Jones et al., 1998). Isobutyrate is considered to be a product of valine catabolism during ruminal fermentation (Blackburn, 1965), so the lower concentration of isobutyrate with FGU or PCU diets is presumably a result of lower dietary valine content. The lower molar percentage of butyrate on PCU and FGU diets might be attributed to interconversion between acetate and butyrate in the rumen (Sharp et al., 1982; Sutton et al., 2003). Less acetate was used to produce butyrate with urea-based supplementation in this study. The significance of valerate accumulation with the ISP diet in the present study was not clear, but the absolute values on all three diets were slightly higher than those noted by other researchers (Meng et al., 1999; Griswold et al., 2003) when urea was included in buffer solution in continuous culture.

Microbial efficiencies of the dietary treatments are illustrated in Figure 1. The FGU diet had the lowest microbial efficiency (11.3 g N/kg OMTD) and the ISP diet (14.7 g N/kg OMTD) had the greatest ($p = 0.05$), with the PCU diet (13.0 g N/kg OMTD) being intermediate. The higher microbial efficiency with the ISP diet might be explained by use of peptide or amino acid nitrogen to form true proteins to enhance microbial growth (Griswold et al.,

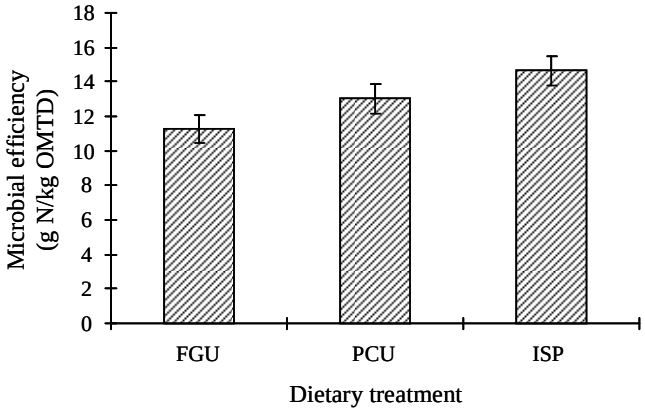

Figure 1. The microbial efficiency of different treatment diets in continuous culture fermentation. FGU = Feed-grade urea diet, PCU = Polyurethane coated urea diet, ISP = Isolated soybean protein diet.

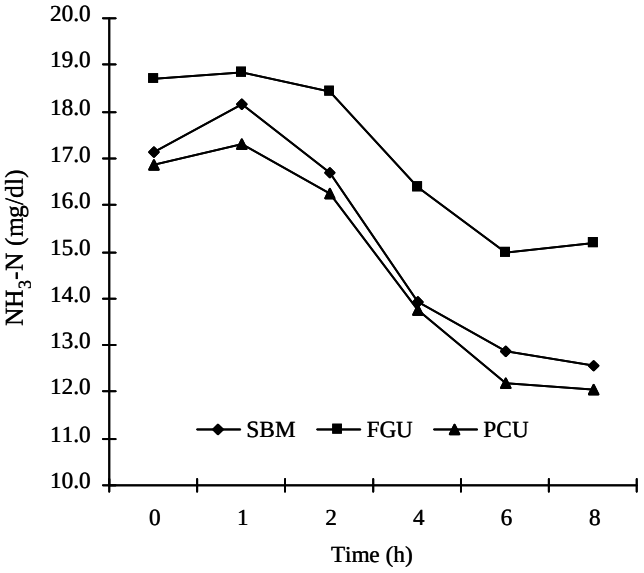

Figure 2. The dynamic concentrations of NH_3-N of different diets fermented *in vitro* during 8 h. FGU = Feed-grade urea diet, PCU = Polyurethane coated urea diet, SBM = Soybean meal diet.

1996; Koenig et al., 2004). However, according to NRC (2001), the microbial efficiency should be in the range of 12 to 54 g N/kg OMTD. The absolute values of microbial efficiency of all the diets in our study were slightly lower. This might reflect a limited nitrogen supply or lack of available nitrogen sources (peptide or amino acid) for ruminal microbial growth in the fermenters during incubation. Although all dietary treatments were under the same condition of limited nitrogen source which may constrain rumen microbial protein synthesis, the PCU diet had 15.6% greater microbial efficiency as compared to the FGU diet, which matched results of daily microbial nitrogen production (data are not shown).

Exp. 2

Ammonia nitrogen release dynamics : The ammonia-N concentrations of the three dietary treatments during 8 h fermentation *in vitro* are shown in Figure 2. Nitrogen utilization by rumen microorganisms can be reflected by ruminal ammonia-N concentration (Hungate, 1966). In our study, the ammonia-N concentrations of all the diets increased within 1 h, and then declined gradually. However, the PCU diet resulted in the lowest concentrations of ammonia-N at all time points. During 8 h *in vitro* fermentation, the PCU diet decreased ammonia-N concentration by 8.2-20.6% as compared with the FGU diet. This agrees with the result of Prokop and Klopfenstein (1977), who found that slow-release urea (combination of urea and formaldehyde) could decrease ruminal ammonia-N concentration by 25.3% compared to urea. No significant differences were found between PCU and SBM diets on ruminal ammonia release. A similar result was found in the report of Galo (2003), in which urea release from a polymer-coated urea was 83% as extensive as uncoated urea after 1 h incubation with distilled water. Other products, such as a urea-calcium combination, have had similar effects. Cass and Richardson (1994) made a comparison in an *in vitro* study and observed that a urea-calcium combination produced slower ammonia nitrogen release rate than regular urea. Ammonia-N concentrations began to increase at 8 h for the FGU diet, which indicates that bacterial autolysis may occur. However, ammonia-N concentrations with PCU and SBM diets still declined. Based on this result, it could be inferred that slow-release urea diets prolong microbial utilization of additional nitrogen sources during ruminal fermentation. Therefore, the sychronization between ruminal ammonia release and carbohydrate availability might be improved, consequently resulting in greater microbial protein synthesis.

Exp. 3

Lactation performance : Effects of PCU on dry matter intake (DMI) and lactation performance are presented in Table 5. DMI of cows fed the PCU diet was approximately 12.8% greater ($p<0.02$) than that of the FGU diet, and was similar to that of the SBM diet. This result is in agreement with the report of Galo et al. (2003), in which depressed DMI was not observed with a polymer-coated urea supplement. The decreased DMI for the FGU diet in the present study is consistent with the finding of Casper and Schingoethe (1986), where urea supplement reduced DMI of lactating cows. This might be attributed to its poor palatability resulting from the bitter taste of uncoated urea in the diets (Huber and Kung, 1981). In contrast, Knaus et al. (2001) reported that supplemental urea did not decrease the DMI of rations fed to steers, even when urea made up 1.8% of DM in the diet.

There was no effect of dietary treatment on milk fat,

Table 5. The effect of different treatments on dry matter intake and milk production in lactating cows

Item	Treatment diet[1]			SEM	p
	FGU	PCU	SBM		
DMI (kg/d)	20.19[b]	22.78[a]	22.74[a]	0.69	0.02
Yield/day					
Milk (kg)	32.48	34.53	34.48	1.51	0.22
ECM[2] (kg)	31.10	33.49	34.78	2.45	0.57
Protein (kg)	0.95	1.09	1.10	0.05	0.06
Fat (kg)	1.16	1.33	1.27	0.13	0.68
Lactose (kg)	1.55	1.68	1.70	0.12	0.64
ECM/DMI	1.55	1.49	1.53	0.12	0.93
Milk composition					
Fat (%)	3.71	4.01	3.72	0.23	0.51
Protein (%)	2.94[b]	3.16[a]	3.18[a]	0.07	0.04
Lactose (%)	5.09	4.99	5.07	0.10	0.70
Milk urea nitrogen (mg/dl)	14.44[a]	13.01[b]	12.77[b]	0.44	0.03
Blood urea nitrogen (mg/dl)	18.70[a]	16.68[b]	16.43[b]	0.67	0.05

[1] FGU = Feed-grade urea diet, PCU = Polyurethane coated urea diet, SBM = Soybean meal diet.

[2] Calculated using NRC (2001): Energy-corrected milk (ECM, kg/d) = milk yield (kg/d)×((0.0929×percent fat)+(0.0563×percent true protein) +(0.0395×percent lactose))/0.749 (NE_L, Mcal/kg).

[a, b] Means within the same row denoted by different letters differ each other ($p<0.05$).

lactose content, milk yield and energy-corrected milk yield (ECM, $p>0.05$). With respect to the content of milk protein, significant difference among the dietary treatments was detected. Cows consuming PCU and SBM diets had similar milk protein concentrations, both being greater than those fed the FGU diet ($p = 0.04$). This could be explained from two aspects: one is due to the difference in DMI and the other might be related to MCP production in the rumen. Cows fed with PCU or SBM diets had a higher milk protein yield ($p = 0.06$) and lower MUN concentrations ($p = 0.03$) compared with cows fed the FGU diet. MUN concentration represents the balance between energy and nitrogen in the rumen as well as the metabolism in liver of absorbed amino acids. The majority of MCP and ruminal degradable protein is broken down into amino acids and small peptides in the small intestine, and then used for synthesis of tissue or milk proteins. The remaining amino acids are deaminated to generate energy and ammonia, then the ammonia is converted to urea in the liver, the source of BUN and MUN. Furthermore, MUN concentration could reflect nitrogen losses from rumen fermentation (Hof et al., 1997). In the present study, we deduced that cows consuming PCU and SBM diets have less urea accumulation in the liver than cows fed the FGU diet. This indicates that, compared with the FGU diet, more nitrogen is available for ruminal protein synthesis and relatively less ammonia for urea formation due to feeding cows with PCU and SBM diets. Also, this tendency was in agreement with the report of Hojman (2004), who found that the relationship between MUN and milk protein percentage was negative.

BUN concentration was altered by the diets. Compared with the milk composition above, there was a similar trend in BUN concentrations for cows fed PCU and SBM diets to MUN concentration. Cows consuming the FGU diet had the highest concentration of BUN ($p = 0.05$). In a similar study, Casper and Schingoethe (1986) reported that urea supplement led to higher serum urea concentration than soybean meal because of its rapid hydrolysis to ammonia in the rumen. Based on the BUN and MUN concentrations in the present study, we could deduce that more urea should be formed in the liver and then excreted in urine for the cows consuming the FGU diet than other diets. This explanation may partly account for higher milk protein content for cows fed PCU or SBM diets than the FGU diet. Furthermore, we also found a strong positive correlation between BUN and MUN ($r^2 = 0.85$, data not shown). This is in accordance with other reports in which MUN concentration was highly correlated with BUN concentration (Broderick and Clayton, 1997; Kauffman, 2001).

CONCLUSIONS

A polyurethane coated urea resulted in significantly lower ruminal ammonia release during 8 h *in vitro* fermentation. To some extent, polyurethane coated urea can improve ruminal microbial efficiency in continuous culture, as compared with inclusion of feed grade urea in the diet. Milk protein percentage and yield were higher for cows receiving the PCU diet than the FGU diet but were similar to the SBM diet. The polyurethane coated urea supplement produced less surplus N (lower ruminal ammonia concentration, less MUN and BUN) than the FGU

supplement. It was concluded that lactation performance of dairy cows would be enhanced by polyurethane coated urea supplement, and soybean meal in the diet could be partially substituted by polyurethane coated urea without impairing lactation performance. Further study is needed in order to fully understand utilization of this new slow-released urea product in dairy practice.

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