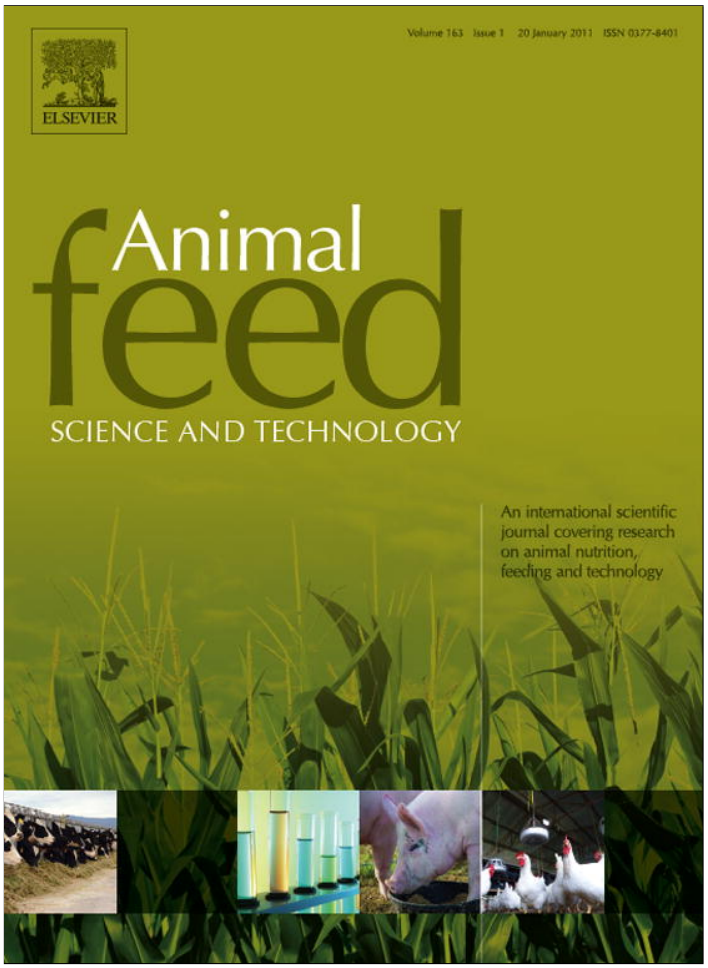

Animal Feed Science and Technology 163 (2011) 43–51

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Influence of urea calcium mixture supplementation on ruminal fermentation characteristics of beef cattle fed on concentrates containing high levels of cassava chips and rice straw

A. Cherdthong, M. Wanapat*, C. Wachirapakorn

Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand

ARTICLE INFO

Article history:
Received 19 May 2010
Received in revised form 7 October 2010
Accepted 12 October 2010

Keywords:
Slow release urea
Urea calcium mixture
Soybean meal
Cassava chips
Rumen fermentation
Microbial protein synthesis

ABSTRACT

The purpose of this study was to evaluate the effects of various N sources in concentrates containing high levels of cassava chips, with rice straw as the basal forage, on rumen ecology, rumen microbial counts, microbial crude (CP) protein synthesis, and digestibility of nutrients. Four ruminally fistulated crossbred (Brahman × native) beef steers with initial body weight (BW) of 400 ± 40.2 kg were randomly assigned according to a 4 × 4 Latin square design. The dietary treatments were different sources of N in the concentrates and were: T1 = urea (control; urea); T2 = soybean meal (SBM); T3 = urea $CaCl_2$ mixture (U-Cal); T4 = urea $CaSO_4$ mixture (U-Cas). All steers were kept in individual pens and supplemented with concentrate at 5 g/kg of BW daily. The experiment was 4 periods, and each lasted 21 d. During the first 14 d, all steers were fed their respective diets *ad libitum* and for during the last 7 d, they were moved to metabolism crates for total urine and fecal collection. Dry matter intake ranged from 9.8 to 10.5 kg daily and was not altered by diet, while digestibility of NDF differed among treatments and was highest with U-Cas supplementation ($P<0.05$). Ruminal NH_3 N and plasma urea N with U-Cal, U-Cas, and SBM diets were lower compared with the urea supplemented group ($P<0.05$). Ruminal volatile fatty acid concentrations were not altered by treatments. Total viable, and cellulolytic bacteria, differed among treatments and were highest with U-Cas (9.1×10^{11}, and 4.0×10^{9} cfu/mL, respectively). In addition, efficiency of rumen microbial CP synthesis based on organic matter (OM) truly digested in the rumen was increased by SBM or U-Cal supplementation, and was highest with U-Cas supplementation (18.2 g of N/kg of OM truly digested in the rumen). Supplementation of U-Cas to a concentrate containing a high level of cassava chips improved rumen ecology and microbial CP synthesis in beef cattle, suggesting that urea calcium mixtures can replace soybean meal or urea in beef cattle diets without adverse affects on rumen fermentation and other rumen parameters.

Abbreviations: ADF, acid detergent fiber; BW, body weight; DM, dry matter; NDF, neutral detergent fiber; NPN, non-protein N; SBM, soybean meal; VFA, volatile fatty acid; UCM, urea calcium mixtures; U-Cal, urea-$CaCl_2$ mixture product; U-Cas, urea-$CaSO_4$ mixture product.

* Corresponding author. Tel.: +66 43 202368; fax: +66 43 202368.
E-mail address: metha@kku.ac.th (M. Wanapat).

0377-8401/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.anifeedsci.2010.10.003

1. Introduction

Substitution of traditional feeds in diets of ruminants is common in response to changes in economic conditions (Devendra, 2007; Wanapat et al., 2009). Use of soybean meal (SBM) as a source of protein in animal feeding is well established. However, high feed prices in some parts of the world, and fluctuation in feedstuff production, have raised interest in alternative N sources for livestock feeding. Use of urea as a non-protein N (NPN) replacement is attractive in ruminant diets, because of its low cost compared with other protein feeds, such as SBM, with high rumen degradability (Wanapat, 2009; Cherdthong et al., 2010a,b; Xin et al., 2010). Urea is converted via ruminal ammonia into microbial protein, thereby supplying additional microbial protein to the host (Nocek and Tamminga, 1991; Calsamiglia et al., 2008; Cherdthong and Wanapat, 2010). However, the amount of urea that can be used in diets is limited, due to its rapid hydrolysis to NH_3 in the rumen by microbial enzymes (Golombeski et al., 2006; Highstreet et al., 2010). This rapid hydrolysis to NH_3 can occur at a much faster rate than NH_3 utilization by rumen bacteria, with as effects accumulation in the rumen and absorption through the rumen wall. The net result is that a potentially large part of the N from feed NPN sources is excreted in the urine, which can contribute to environmental pollution (Broderick et al., 2009).

The efficiency of protein use by ruminants has gained attention by environmentalists and government regulators in many parts of the world (Robinson, 2010). Many groups have suggested that ruminant production systems should be designed and managed in ways that minimize adverse effects on resource conservation and the environment. A partial solution could be to modify urea to control its rate of rumen release so that NH_3 production more closely parallels carbohydrate digestion (Pinos-Rodríguez et al., 2010). Ruminal slow release urea compounds, which have been fed to ruminants, include biuret, starea, urea phosphate, formaldehyde treated urea and polymer-coated urea (Taylor-Edwards et al., 2009). These compounds have not been as advantageous as urea because a part of their NPN may leave the rumen without being converted to NH_3, thereby reducing its incorporation into microbial protein (Galo et al., 2003; Firkins et al., 2007). More recently, slow urea release properties have been achieved by binding urea to substrates such as calcium chloride (Huntington et al., 2006; Golombeski et al., 2006). In an earlier *in vitro* experiment, urea calcium sulphate mixtures were shown to reduce ruminal NH_3 concentrations, as well as increase the cellulolytic bacterial population, when compared with urea (Cherdthong et al., 2010a). Since urea is inexpensive, it could be used for tropical ruminant production provided its ruminal release is controlled to slow ammonia production and/or synchronized with soluble carbohydrates in the rumen. This should be of value in improving the efficiency of rumen N utilization (Nocek and Tamminga, 1991; NRC, 2001).

Cassava (*Manihot esculenta*, *Crantz*) is grown widely in tropical areas and the price is generally relatively low. Cassava chips contain high levels of nonstructural carbohydrate and are highly degradable in the rumen compared with other energy sources, including corn meal (Chanjula et al., 2003). Recently, Wanapat and Khampa (2007) showed that a concentrate based on a high proportion of cassava chips with a high urea level could improve rumen fermentation efficiency and rumen microbial crude protein (CP) synthesis in dairy steers. However, no data has been reported on supplementation of slow release NH_3 products in concentrate cassava chips based diets of steers. Our study was conducted to evaluate effects of different urea N sources in concentrates containing high levels of cassava chips with rice straw as basal forage on rumen ecology, rumen microbial counts, microbial CP synthesis, and digestibility of nutrients in beef cattle.

2. Materials and methods

2.1. Animals, diets and experimental design

Four ruminally fistulated crossbred (Brahman × native) beef cattle steers with initial body weight (BW) of 400 ± 40.2 kg were randomly assigned to a 4 × 4 Latin square design. The dietary treatments were: T1 = urea (control); T2 = soybean meal (SBM); T3 = urea $CaCl_2$ mixture (U-Cal); T4 = urea $CaSO_4$ mixture (U-Cas), respectively. The urea calcium mixture (UCM) products were prepared according to Cherdthong et al. (2010a) by, in brief, providing an aqueous solution (23 g $CaCl_2$ or $CaSO_4$ + 17 mL H_2O) of $CaCl_2$ or $CaSO_4$ at 50 °C for 10 min and dissolving solid urea (60 g urea) in aqueous $CaCl_2$/$CaSO_4$ and then heating and agitating the mixture at 50 °C for 10 min prior to reducing the temperature of the solution to about 25 °C. Concentrates containing 161 to 162 g/kg of CP and 11 MJ/kg dry matter (DM) were fed at 5 g/kg of BW daily of concentrates, and rice straw was fed *ad libitum* allowing for 100 g/kg refusals. All steers were kept in individual pens, and clean fresh water and mineral blocks were available at all times. The experiment was 4 periods, and each lasted 21 d. During the first 14 d, all steers were fed their respective diets with *ad libitum* intake, whereas the last 7 d they were moved to metabolism crates for total urine and fecal collection during These days they were restricted to 900 g/kg of the previous voluntary feed intake of straw, but still supplemented with concentrate at 5 g/kg of BW daily. Table 1 shows the chemical composition of the concentrates and rice straw.

2.2. Data collection and sampling procedures

Feeds were sampled and fecal samples were collected by total collection of each individual steer during the last 7 d of each period at the morning and afternoon feeding. Feeds, refusals and fecal samples were dried at 60 °C and ground (1 mm screen using the Cyclotech Mill, Tecator, Sweden) and analyzed using standard methods of AOAC (1995) for DM (ID 967.03), N (ID 984.13), EE (ID 954.02) and ash (ID 942.05). Acid detergent fiber (ADF) was determined according to an AOAC method (1995;

Table 1
Ingredient and chemical composition of concentrates and rice straw used in the experiment (g/kg dry matter (DM)).

	Dietary treatments[a]				
	Control	SBM	U-Cal	U-Cas	Rice straw
Ingredients, g/kg DM					
Cassava chips	700	650	700	700	
Soybean meal (SBM), 440 g/kg CP solvent	–	193	–	–	
Rice bran	50	30	50	50	
Coconut meal, solvent	70	30	50	50	
Palm kernel meal, solvent	100	41	93	93	
Pure sulfur	10	10	10	10	
Mineral premix[b]	10	10	10	10	
Molasses, liquid	10	10	10	10	
Urea	40	16	–	–	
Urea $CaCl_2$ mixture (U-Cal)	–	–	67	–	
Urea $CaSO_4$ mixture (U-Cas)	–	–	–	67	
Salt	10	10	10	10	
Chemical composition					
Dry matter, g/kg	952	945	941	944	970
Organic matter, g/kg DM	911	922	914	920	889
Ash, g/kg DM	89	78	86	80	111
Ether extract, g/kg DM	25	27	26	26	20
Neutral detergent fiber, g/kg DM	128	134	125	131	771
Acid detergent fiber, g/kg DM	76	81	73	78	506
Crude protein, g/kg DM	161	162	162	162	25
Metabolizable energy (ME)[c], MJ/kg DM	11	11	11	11	8

[a] Control: urea; SBM = soybean meal; U-Cal (urea $CaCl_2$ mixture: 60 g urea, 23 g $CaCl_2$ and 17 mL H_2O; U-Cas (urea $CaSO_4$ mixture): 60 g urea, 23 g $CaSO_4$ and 17 mL H_2O.
[b] Minerals and vitamins (each kg contains): Vitamin A: 10,000,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU; Fe: 50 g; Zn: 40 g; Mn: 40 g; Co: 0.1 g; Cu: 10 g; Se: 0.1 g; I: 0.5 g.
[c] Metabolizable energy (ME) was calculated according to the equation of Robinson et al. (2004b).

ID 973.18) and is expressed inclusive of residual ash. Neutral detergent fiber (NDF) in samples was estimated according to Van Soest et al. (1991) with addition of α-amylase but without sodium sulphite and results are expressed with residual ash. Metabolizable energy (ME) was calculated according to the equation described by (Robinson et al., 2004b) as:

$$\text{ME (MJ/kg DM)} = 0.82 \times (((2.4 \times \text{crude protein (CP)}) + (3.9 \times \text{ether extract (EE)}) + (1.8 \times \text{organic matter (OM)})) \times \textit{in vitro} \text{ organic matter digestibility (ivOMD)})$$

where CP, EE and OM are in g/kg DM and ivOMD values obtained from our previous *in vitro* study with mean values of 530 g/kg DM. At the end of each period, rumen fluid and jugular blood samples were collected immediately after feeding and 2, 4 and 6 h after feeding as well. Approximately 200 mL of rumen fluid was collected at each time from the middle of the rumen using a 60 mL hand syringe. Rumen fluid was immediately measured for pH and temperature using a portable pH temperature meter (HANNA Instruments HI 8424 microcomputer, Singapore) and NH_3 N by Kjeltech Auto 1030 Analyzer (AOAC, 1995; ID 973.18). Volatile fatty acids (VFA) were analyzed using high pressure liquid chromatography (HPLC, Instruments by controller water model 600E; water model 484 UV detector; column novapak C18; column size 3.9 mm × 300 mm; mobile phase 10 mM H_2PO_4 [pH 2.5]) according to Samuel et al. (1997). Rumen fluid was used for direct counts of protozoa and fungal zoospores using methods of Galyean (1989) by haemacytometer (Boeco, Singapore). Groups of bacteria (*i.e.*, cellulolytic, proteolytic, amylolytic, total viable count bacteria) were measured using the Hungate (1969) roll-tube technique.

A blood sample (about 10 mL) was collected from the jugular vein at the same time as rumen fluid sampling into tubes containing 12 mg of EDTA, and plasma was separated by centrifugation at 500 × *g* for 10 min at 4 °C and stored at −20 °C until analysis of plasma urea N according to Crocker (1967). Urine samples were analyzed for total N (AOAC, 1995; ID 984.13) and allantoin in urine was determined by HPLC as described by Chen and Gomes (1995). The amount of microbial purines absorbed was calculated from purine derivative excretion based on the relationship derived by Chen and Gomes (1995).

2.3. *In vitro* urea release

An *in vitro* technique was used to measure the release characteristics of the UCM according to Galo et al. (2003). Urea was used as the positive control. Triplicate set of beakers at each sampling time within substrate treatments was prepared. Samples (600 mL of each) were placed in separate beakers containing 100 mL of 39 °C distilled water and incubated at 39 °C. The solutions were sampled immediately after incubate and after 0.5, 1.0, 1.5, 2.0, 4.0, 6.0 and 8.0 h. The 5 mL samples were analyzed for N concentration (AOAC, 1995; ID 984.13).

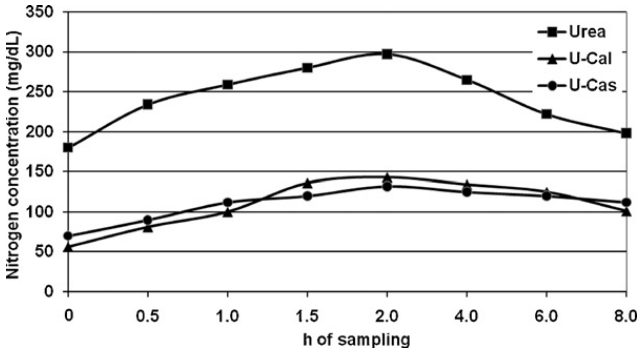

Fig. 1. Nitrogen release from UCM products in distilled water compared to urea. Urea (■), U-Cal (▲) and U-Cas (●) shown as mean values [SEM was 49.6 and was highest with urea than others (P<0.05) at every h of sampling].

2.4. Statistical analysis

All 4 × 4 Latin square design data from the experiment were analyzed using the SAS (1996) GLM procedure according to the model:

$$Y_{ijk} = \mu + M_i + A_j + P_k + \varepsilon_{ijk}$$

Y_{ijk}, observation from steer j, receiving diet i, in period k; μ, the overall mean, M_i, effect of the different N sources (i = 1, 2, 3, 4), A_j, the effect of steer (j = 1, 2, 3, 4), P_k, the effect of period (k = 1, 2, 3, 4), and ε_{ijk} residual effect. Results are presented as mean values with the standard error of the means. Differences between treatment means were determined by Duncan's New Multiple Range Test (Steel and Torrie, 1980) with P<0.05 level of significance.

3. Results

3.1. Product evaluation and chemical composition of feeds

In vitro incubations of urea, U-Cal and U-Cas show the slower release rates of urea in U-Cal and U-Cas *versus* uncoated urea (Fig. 1).

The composition of the diets and rice straw is shown in Table 1. Concentrate diets contained similar concentrations of DM, OM, CP, aNDF, ADF and ME. The nutritional values of the diets would be expected to support normal performance of these experimental beef cattle under tropical area conditions (Wanapat et al., 2008; Wanapat, 2009).

3.2. Feed intake and digestibility

Rice straw, concentrate and total DM intake (Table 2) were not influenced by feeding the different N sources. Apparent digestibility, and its components (Table 2), were also unaffected by N source, except aNDF digestibility was highest with U-Cas supplementation, intermediate with SBM and U-Cal, and lowest with control (P<0.05).

Table 2
Feed intakes and apparent digestibility of beef cattle fed different N sources.

	Dietary treatments				SEM
	Control	SBM	U-Cal	U-Cas	
DM intake					
Rice straw, kg/day	7.8	8.0	7.7	8.1	0.51
Concentrate, kg/day	2.0	2.4	2.2	2.4	0.94
Total, kg/day	9.8	10.4	9.9	10.5	1.54
Apparent digestibility					
Dry matter	0.58	0.63	0.60	0.62	0.046
Organic matter	0.61	0.65	0.64	0.66	0.048
Crude protein	0.61	0.63	0.62	0.62	0.025
Neutral detergent fiber[a]	0.53[a]	0.60[b]	0.59[b]	0.64[c]	0.011
Acid detergent fiber	0.40	0.44	0.43	0.45	0.035

[a] Means in the same row with different superscripts differ (P<0.05).
[b] Means in the same row with different superscripts differ (P<0.05).
[c] Means in the same row with different superscripts differ (P<0.05).

Table 3
Ruminal pH, rumen temperature and concentrations of rumen NH_3 N and VFA, and plasma urea N, as affected by feeding different N sources.

	Dietary treatments				SEM
	Control	SBM	U-Cal	U-Cas	
Ruminal pH	6.9	6.7	6.6	6.7	0.44
Ruminal temperature, °C	39.4	39.1	39.5	39.2	0.32
NH_3-N, mg/dL	19.2[a]	15.4[b]	14.6[b]	14.5[b]	1.01
Plasma urea N, mg/dL	16.8[a]	13.2[b]	12.2[b]	12.7[b]	1.06
Total VFA, mM	114.5	115.3	115.4	119.2	8.55
VFA, mol/100 mol					
Acetic acid (C2)	74.3	70.6	72.3	70.4	3.65
Propionic acid (C3)	18.9	21.5	19.5	22.3	4.11
Butyric acid (C4)	6.8	7.9	8.2	7.3	1.23
C2:C3	3.9	3.3	3.7	3.2	0.55
C2 + C4:C3	4.3	3.7	4.1	3.5	0.68

[a] Means in the same row with different superscripts differ (P<0.05).
[b] Means in the same row with different superscripts differ (P<0.05).

3.3. Rumen function and microbes

Rumen pH, temperature and VFA concentrations were not influenced by dietary N sources (Table 3). However, ruminal NH_3 N and plasma urea N concentrations were lower with the UCM and SBM diets than on the urea diet. Ruminal NH_3 N (at 2 and 4 h post feeding) and plasma urea N (at 4 h post feeding) were highest when urea was supplemented (Figs. 2 and 3, respectively). The U-Cas diet increased ruminal bacteria counts and cellulolytic bacteria counts (Table 4) compared with all other groups (P<0.05). Control values were lower than those with SBM and C-Cal (P<0.05). A similar pattern occurred for absorption and excretion of allantoin (P<0.01) whereas N retention was lower with control *versus* all treatments (Table 5).

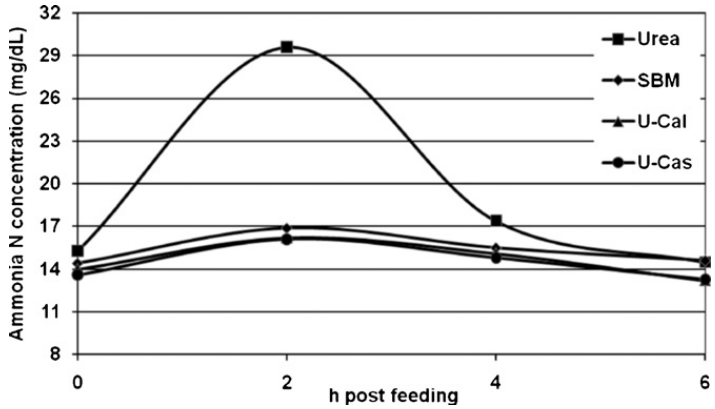

Fig. 2. Ruminal NH_3 N concentrations (mg/dL) in beef cattle at 0, 2, 4 and 6 h post feeding as a result of feeding urea (■), soybean meal (SBM) (♦), U-Cal (▲) and U-Cas (●) [SEM was 2.7 and was highest (P<0.05) with urea at 2 h post feeding].

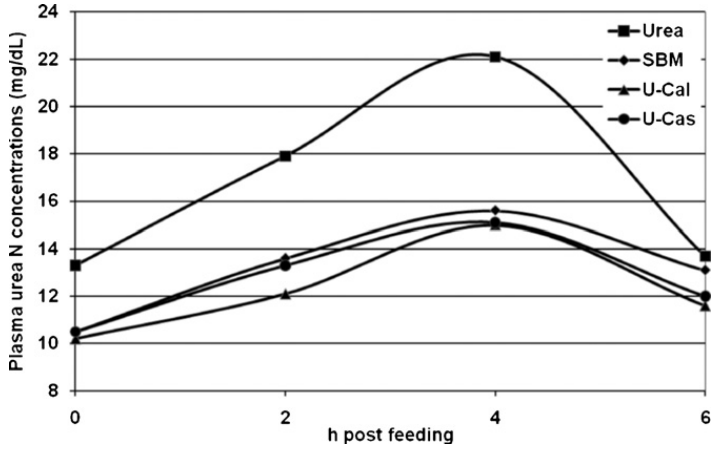

Fig. 3. Plasma urea N concentrations (mg/dL) in beef cattle at 0, 2, 4 and 6 h post feeding as a result of feeding urea (■), soybean meal (SBM) (♦), U-Cal (▲) and U-Cas (●) [SEM was 1.6 and was highest (P<0.05) with urea at 2 and 4 h post feeding].

Table 4
Effect of different N sources on ruminal microbes and viable bacteria.

	Dietary treatments				SEM
	Control	SBM	U-Cal	U-Cas	
Ruminal microbes, cell/mL					
Bacteria, $\times 10^{11}$	4.1[a]	6.7[b]	6.3[b]	9.1[c]	0.68
Protozoa, $\times 10^{6}$	3.5	4.0	4.2	3.9	1.65
Fungal zoospore, $\times 10^{4}$	1.2	1.5	1.5	1.8	1.50
Viable bacteria, cfu/mL					
Total, $\times 10^{9}$	3.5	3.9	3.8	4.1	0.98
Amylolytic, $\times 10^{8}$	1.2	1.5	1.7	1.6	2.11
Proteolytic, $\times 10^{8}$	0.9	1.1	1.4	1.3	0.98
Cellulolytic, $\times 10^{9}$	1.3[a]	2.9[b]	2.9[b]	4.0[c]	0.32

[a] Means in the same row with different superscripts differ (P<0.05).
[b] Means in the same row with different superscripts differ (P<0.05).
[c] Means in the same row with different superscripts differ (P<0.05).

Table 5
Effect of different N sources on N balance, excretion of urinary derivatives (PD), and microbial crude protein supply.

	Dietary treatments				SEM
	Control	SBM	U-Cal	U-Cas	
Nitrogen balance, g/d					
N intake	104.3	108.5	106.2	109.1	2.99
Faecal N	21.3	18.6	17.6	17.4	3.36
Urinary N	30.5	24.3	23.1	22.4	7.51
N absorption	83.0	89.9	88.6	91.7	5.85
N retention	52.5[a]	65.6[b]	65.5[b]	69.3[b]	4.11
PD, mmol/d					
Allantoin excretion	127.6[a]	165.8[b]	163.9[b]	216.0[c]	2.07
Allantoin absorption	115.7[a]	161.1[b]	158.7[b]	189.2[c]	6.65
Microbial crude protein[d], g/d	436.5[a]	567.7[b]	561.0[b]	736.3[c]	10.32
EMNS[e], gN/kg OMDR	12.9[a]	14.4[b]	15.2[b]	18.2[c]	0.39

[a] Means in the same row with different superscripts differ (P<0.05).
[b] Means in the same row with different superscripts differ (P<0.05).
[c] Means in the same row with different superscripts differ (P<0.05).
[d] Microbial crude protein (MCP) (g/d) = 3.99 × 0.856 × mmoles of purine derivatives excreted (Galo et al., 2003).
[e] Efficiency of microbial N synthesis (EMNS, g/kg of OM digested in the rumen (OMDR) = [(MCP (g/d) × 1000)/DOMR (g)], assuming that rumen digestion was 650 g/kg OM of digestion in total tract.

Microbial N synthesis in total and relative to OMDR was also lowest with control (EMNS = 12.9 g N/kg OMDR) and highest with U-Cas supplemented steers (18.2 g N/kg OMDR; P<0.05).

4. Discussion

4.1. Impacts of UCM on rumen fermentation

The release rate of the UCM products, which were much less than urea at 2 h of incubation, demonstrates that they may have the potential to be slowly released in the rumen. Similarly, Highstreet et al. (2010) showed that a slowly rumen released encapsulated urea, which was 0.9 urea and 0.1 fat according to the manufacturer, had a measured ruminal *in sacco* N release of 30, 37 and 41 mg/dL of at 0.5, 4 and 12 h of ruminal incubation respectively, where the 0.5 h value is an estimate of immediately released urea and 4 and 12 h bracket the most likely range of mean retention times of the material in the rumen.

4.2. Feed intake and digestibility

DM intake was not affected by adding the slow release N sources to the diet in substitution for urea. This is similar to Pinos-Rodríguez et al. (2010), who observed that DM intake was not affected by a slow release urea product compared to the urea treatment and Xin et al. (2010) who reported that DM intake of cows fed a polyurethane coated urea was similar to those fed SBM. In our study, it is likely that U-Cas was more slowly hydrolyzed to NH_3 than urea, which was used more efficiently by rumen microorganisms (Taylor-Edwards et al., 2009). Higher fibre digestibility in the SBM and UCM diets is consistent with higher counts of rumen bacteria, especially cellulolytic bacteria. Nevertheless, apparent digestibilities of DM, OM, CP and ADF were not affected by the UCM treatments. This finding is consistent with other experiments wherein substitution of a slow release urea product for urea did not affect DM, OM and ADF digestibilities (*e.g.*, Highstreet et al., 2010). In contrast

to our findings, a polymer-coated slow release urea was demonstrated to increase total tract DM and CP digestibilities when fed to lactating dairy cows (Galo et al., 2003). Moreover, Xin et al. (2010) found that urea and polyurethane coated urea diets had ($P<0.05$) lower digestibilities of DM, OM and ADF *versus* in a SBM fed diet.

4.3. Rumen fermentation parameters and blood metabolites

Ruminal NH_3 N concentration is a predictor of efficiency of dietary N conversion into microbial N (Firkins et al., 2007). In our study, ruminal NH_3 N concentrations were higher with the urea diet and NH_3 N concentrations of SBM, U-Cal and U-Cas were stable throughout the sampling periods through 6 h post feeding. This finding was likely due to UCM products controlling the rate of urea release in the rumen, leading to a slower rate of NH_3-N formation, compared to urea treatment. These results agree with Cherdthong et al. (2010a), who reported that, with an *in vitro* gas production technique, urea treatments rapidly increased concentrations of NH_3 N relative to UCM treatments with cassava chips or corn grain as energy sources. Slow NH_3 N formation in the rumen is likely due to hydrogen bonding in U-Cas between the sulphate from $CaSO_4^{2-}$ and amino group in the urea compound. Sulphate anions are linked between layers of sulphate and chelated by urea groups (Cherdthong et al., 2010b). The urea molecules take part in hydrogen bonding as both donors and acceptors, as described by Gale et al. (2010). Water molecules are also included, and form an additional hydrogen bond with sulphate. One water molecule further forms hydrogen bonds to the urea CO group (Custelcean et al., 2007).

Similar to our results, a urea–calcium product supplementation resulted in lower concentrations of ruminal NH_3 N in a treatment that represented consumption of a CP equivalent to 220–460 g/d of CP in steers (Huntington et al., 2006). Moreover, Taylor-Edwards et al. (2009) reported that a slow release urea product reduced the rapidity of NH_3 production in the rumen without affecting other ruminal fermentation metabolites. According to Taylor-Edwards et al. (2009), it could be inferred that slow release urea diets prolong microbial utilization of additional N sources during ruminal fermentation. Therefore, synchronization between ruminal NH_3 release and carbohydrate availability might be improved, thereby resulting in higher microbial protein synthesis. As concentrations of plasma urea N are highly correlated to concentrations of NH_3 in the rumen (Wanapat et al., 2008; Wanapat and Cherdthong, 2009; Wanapat et al., 2009) the finding in our study that beef cattle consuming the UCM and SBM diets had less urea in the blood than cattle fed the urea diet may indicate that, compared with the urea diet, more N was available for ruminal protein synthesis and relatively less NH_3 was absorbed from the rumen to be converted to urea in the liver.

Supplementation of UCM did not affect ruminal VFA patterns or concentrations when compared with the SBM or urea group, similar to previous experiments (Xin et al., 2010), suggesting that UCM does not negatively affect ruminal fermentation.

4.4. Rumen microorganism population

Ruminal bacteria count and cellulolytic bacteria were highest (9.1×10^{11}, and 4.0×10^9 cfu/mL, respectively) with U-Cas supplementation. If urea is more slowly released in the rumen it will be more consistently available to rumen cellulotytic bacteria (Russell and Rychlik, 2001; Udén, 2006).

4.5. N balance and efficiency of microbial protein synthesis

The more positive N retention in this study with the UCM *versus* urea diet demonstrates the positive practical influence of UCM with these cassava chip based diets in a rice straw based feeding system. One indicator of the efficiency of rumen N use is the amount of microbial CP (MCP) delivered to the small intestine, which is a consequence of microbial growth and its washout from the rumen (Sniffen and Robinson, 1987; Robinson et al., 2005; Cherdthong and Wanapat, 2010). In the current study, MCP (as calculated from purine derivative excretion using the equation (Chen and Gomes, 1995)) ranged from 437 to 736 g/d, which are intermediate values for beef cattle of this type (Wanapat et al., 2008).

Microbial CP synthesis in the rumen often provides the majority of the protein supplied to the small intestine of ruminants, accounting for 500 to 800 g/kg of total absorbable protein (Robinson et al., 2004a; Firkins et al., 2007). The higher EMNS with the SBM *versus* the urea diet might be due to use of peptide or amino acid N, which can enhance microbial growth (Galo et al., 2003; Xin et al., 2010). Supplementation of U-Cas resulted in the highest EMNS, possibly due to a slow urea release from U-Cas which prolonged microbial utilization of a more continuous supply of NH_3 N during ruminal fermentation (Xin et al., 2010). Because NH_3 produced in the rumen is used for microbial growth, which is dependent on energy availability, it is important that the rate of NH_3 production in the rumen be coordinated with the rate of carbohydrate digestion (Robinson et al., 2004a; Highstreet et al., 2010; Cherdthong et al., 2010b). Therefore, the potentially improved sychronization between ruminal NH_3 release and carbohydrate availability (*i.e.*, cassava chips) was improved, consequently resulting in higher microbial CP synthesis with U-Cas.

5. Conclusions

The urea calcium sulphate product (U-Cas) improved aNDF digestibility, rumen fermentation, and microbial CP synthesis in beef cattle, compared with inclusion of urea, a urea calcium chloride product (U-Cal) and soybean meal. Soybean meal

could be substituted by the U-Cas product without negatively affecting rumen fermentation and, hence, improve rumen fermentation efficiency.

Acknowledgments

The authors express sincere thanks to the Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Thailand and Thailand Research Fund (TRF) through the Royal Golden Jubilee PhD Program for providing financial support for the research and use of research facilities.

References

AOAC, 1995. Official Method of Analysis, 16th ed. Animal Feeds: Association of Official Analytical Chemists, VA, USA.
Broderick, G.A., Stevenson, M.J., Patton, R.A., 2009. Effect of dietary protein concentration and degradability on response to rumen-protected methionine in lactating dairy cows. J. Dairy Sci. 92, 2719–2728.
Calsamiglia, S., Cardozo, P.W., Ferret, A., Bach, A., 2008. Changes in rumen microbial fermentation are due to a combined effect of type of diet and pH. J. Anim. Sci. 86, 702–711.
Chanjula, P., Wanapat, M., Wachirapakorn, C., Uriyapongso, S., Rowlinson, P., 2003. Ruminal degradability of tropical feeds and their potential use in ruminant diets. Asian-Aust. J. Anim. Sci. 16, 211–216.
Chen, X.B., Gomes, M.J., 1995. Estimation of Microbial Protein Supply to Sheep and Cattle Based on Urinary Excretion of Purine Derivative—An Overview of the Technique Details. Occasional Publication 1992. International Feed Resources Unit, Rowett Research Institute, Aberdeen, UK.
Cherdthong, A., Wanapat, M., 2010. Development of urea products as rumen slow-release feed on ruminant production: A review. Aust. J. Basic Appl. Sci. 4, 2232–2241.
Cherdthong, A., Wanapat, M., Wachirapakorn, C., 2010a. Urea–calcium mixture as a rumen slow-release urea on in vitro rumen fermentation. In: Proceedings of the 14th Animal Science Congress of the Asian-Australasian Association of Animal Production Societies (AAAP), vol. 3, August 23–26, 2010. National Pingtung University of Science and Technology, Pingtung, Taiwan, Republic of China, pp. 1625–1628.
Cherdthong, A., Wanapat, M., Wachirapakorn, C., 2010b. Effects of urea–calcium mixture in concentrate containing high cassava chip on feed intake, rumen fermentation and performance of lactating dairy cows fed on rice straw. Livest. Sci., doi:10.1016/j.livsci.2010.08.002.
Crocker, C.L., 1967. Rapid determination of urea nitrogen in serum or plasma without deproteinization. Am. J. Med. Technol. 33, 361–365.
Custelcean, R., Sellin, V., Moyer, B.A., 2007. Sulfate separation by selective crystallization of a urea-functionalized metal–organic framework. Chem. Commun., 1541–1543.
Devendra, C., 2007. Perspectives on animal production systems in Asia. Livest. Sci. 106, 1–18.
Firkins, J.L., Yu, Z., Morrison, M., 2007. Ruminal nitrogen metabolism: perspectives for Integration of microbiology and nutrition for dairy. J. Dairy Sci. 90 (E. Suppl.), E1–E16.
Gale, P.A., Hiscock, J.R., Jie, C.Z., Hursthouse, M.B., Light, M.E., 2010. Acyclic indole and carbazole-based sulfate receptors. Chem. Sci. 1, 215–220.
Galo, E., Emanuele, S.M., Sniffen, C.J., White, J.H., Knapp, J.R., 2003. Effects of a polymer-coated urea product on nitrogen metabolism in lactating Holstein dairy cattle. J. Dairy Sci. 86, 2154–2162.
Galyean, M., 1989. Laboratory Procedure in Animal Nutrition Research. Department of Animal and Life Science, New Mexico State University, Las Cruces, NM, USA, pp. 107–122.
Golombeski, G.L., Kalscheur, K.F., Hippen, A.R., Schingoethe, D.J., 2006. Slow-release urea and highly fermentable sugars in diets fed to lactating dairy cows. J. Dairy Sci. 89, 4395–4403.
Highstreet, A., Robinson, P.H., Robison, J., Garrett, J.G., 2010. Response of Holstein cows to replacing urea with a slowly rumen released urea in a diet high in soluble crude protein. Livest. Sci. 129, 179–185.
Hungate, R.E., 1969. A roll tube method for cultivation of strict anaerobes. In: Norris, J.R., Ribbons, D.W. (Eds.), Methods in Microbiology. Academic Press, New York, NY, USA, pp. 117–313.
Huntington, G.B., Harmon, D.L., Kristensen, N.B., Hanson, K.C., Spears, J.W., 2006. Effects of a slow-release urea source on absorption of ammonia and endogenous production of urea by cattle. Anim. Feed Sci. Technol. 130, 225–241.
National Research Council, 2001. Nutrient Requirements of Dairy Cattle, 7th revised ed. National Academic Science, Washington, DC, USA.
Nocek, J.E., Tamminga, S., 1991. Site of digestion of starch in the gastro-intestinal tract of dairy cows and its effect on milk yield and composition. J. Dairy Sci. 74, 3598–3629.
Pinos-Rodríguez, J.M., Peña, L.Y., González-Muñoz, S.S., Bárcena, R., Salem, A., 2010. Effects of a slow-release coated urea product on growth performance and ruminal fermentation in beef steers. Ital. J. Anim. Sci. 9, 16–19.
Robinson, P.H., 2010. Impacts of manipulating ration metabolizable lysine and methionine levels on the performance of lactating dairy cows: A systematic review of the literature. Livest. Sci. 127, 115–126.
Robinson, P.H., Chalupa, W., Sniffen, C.J., Sato, H., Fujieda, T., Ueda, T., Suzuki, H., 2004a. Influence of ingredient reformulation to reduce diet crude protein level on productivity, and efficiency of dietary nitrogen use, in early lactation dairy cows. Anim. Feed Sci. Technol. 116, 67–81.
Robinson, P.H., Givens, D.I., Getachew, G., 2004b. Evaluation of NRC, UC Davis and ADAS approaches to estimate the metabolizable energy values of feeds at maintenance energy intake from equations utilizing chemical assays and *in vitro* determinations. Anim. Feed Sci. Technol. 114, 75–90.
Robinson, P.H., DePeters, E.J., Shinzato, I., Sato, H., 2005. Influence of feeding free lysine to early lactation dairy cows on ruminal lysine escape, rumen fermentation and productivity. Anim. Feed Sci. Technol. 118, 201–214.
Russell, J.B., Rychlik, J.L., 2001. Factors that alter rumen microbial ecology. Science 292, 1119–1122.
Samuel, M., Sagathewan, S., Thomas, J., Mathen, G., 1997. An HPLC method for estimation of volatile fatty acids of ruminal fluid. Indian J. Anim. Sci. 67, 805–807.
Statistical Analysis System, 1996. SAS/STAT User's Guide: Statistics, Version 6.12. Edition. SAS Inc., Cary, NC, USA.
Sniffen, C.J., Robinson, P.H., 1987. Protein and fiber digestion, passage, and utilization in lactating cows. Microbial growth and flow as influenced by dietary manipulations. J. Dairy Sci. 70, 425–441.
Steel, R.G.D., Torrie, J.H., 1980. Principles and Procedures of Statistics. McGraw Hill Book Co., New York, NY, USA.
Taylor-Edwards, C.C., Elam, N.A., Kitts, S.E., McLeod, K.R., Axe, D.E., Vanzant, E.S., Kristensen, N.B., Harmon, D.L., 2009. Influence of slow-release urea on nitrogen balance and portal-drained visceral nutrient flux in beef steers. J. Anim. Sci. 87, 209–221.
Udén, P., 2006. *In vitro* studies on microbial efficiency from two cuts of ryegrass (*Lolium perenne*, *cv.* Aberdart) with different proportions of sugars and protein. Anim. Feed Sci. Technol. 126, 145–156.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.
Wanapat, M., 2009. Potential uses of local feed resources for ruminants. Trop. Anim. Health. Prod. 41, 1035–1049.
Wanapat, M., Cherdthong, A., 2009. Use of real-time PCR technique in studying rumen cellulolytic bacteria population as affected by level of roughage in Swamp buffalo. Curr. Microbiol. 58, 294–299.

Wanapat, M., Khampa, S., 2007. Effect of levels of supplementation of concentrate containing high levels of cassava chip on rumen ecology, microbial N supply and digestibility of nutrients in beef cattle. Asian-Aust. J. Anim. Sci. 20, 75–81.
Wanapat, M., Cherdthong, A., Pakdee, P., Wanapat, S., 2008. Manipulation of rumen ecology by dietary lemongrass (*Cymbopogon citratus* Stapf.) powder supplementation. J. Anim. Sci. 86, 3497–3503.
Wanapat, M., Polyorach, S., Boonnop, K., Mapato, C., Cherdthong, A., 2009. Effects of treating rice straw with urea or urea and calcium hydroxide upon intake, digestibility, rumen fermentation and milk yield of dairy cows. Livest. Sci. 125, 238–243.
Xin, H.S., Schaefer, D.M., Liu, Q.P., Axe, D.E., Meng, Q.X., 2010. 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. Asian-Aust. J. Anim. Sci. 23, 491–500.