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REGULAR ARTICLES

Effect of protein level and urea in concentrate mixture on feed intake and rumen fermentation in swamp buffaloes fed rice straw-based diet

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Abstract Four rumen-fistulated Thai native swamp buffaloes were randomly assigned according to a 2×2 factorial arrangement in a 4×4 Latin square design to assess the effect of protein (CP) level and urea (U) source in concentrate diet on feed utilization and rumen ecology. The treatments were as follows: concentrate containing CP at 120 g/kg (soybean meal, SBM) (T1), 160 g/kg (SBM) (T2), 120 g/kg (U) (T3), and 160 g/kg (U) (T4), respectively. All buffaloes were fed concentrate at 10 g/kg of body weight, and rice straw was offered ad libitum. Feed intake and digestibilities of CP, neutral detergent fiber, and acid detergent fiber increased ($P<0.05$) in treatments with higher level of CP especially with U source ($P<0.05$). In contrast, CP level and source in concentrate did not affect on ruminal pH and temperature ($P>0.05$), while concentration of ruminal ammonia (N), blood urea (U), volatile fatty acids profile, microorganism populations, and variable bacterial growth increased in buffaloes consumed concentrate containing CP at 160 g/kg (T2 and T4; $P<0.05$). Fecal and urinary N excretions decreased in buffaloes consumed concentrate containing higher CP level especially with U source while purine derivatives increased which resulted in a higher N balance as compared to lower CP level and SBM source treatments ($P<0.05$). In summary, higher CP level in concentrate improved feed intake, nutrient digestibility, purine derivatives, and rumen ecology, and U had shown better result than SBM. Concentrate mixtures containing 16 g/kg CP with U 40 g/kg could improved nutrients utilization with no adverse effects for swamp buffaloes fed on rice straw.

Keywords Protein level · Urea · Soybean meal · Rumen ecology · Swamp buffalo · Rice straw

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Introduction

Poor quality roughages are characterized by their high contents of lignocelluloses and low level of nitrogen (N). Consequently, these roughages are poorly digested and often unable to support the maintenance requirements of ruminants. Supplementation of natural protein to ruminants consuming low-quality forage improved intake, digestibility, performance, and reproductive efficiency (McGuire et al. 2013). Most of these feeding systems use high-grain diets (Alvarez Almora et al. 2012); however, due to lower cost per unit of N compared with most sources of natural protein, urea (U) as non-protein N (NPN) is a popular source in ruminant feeding (McGuire et al. 2013; Cappellozza et al. 2013; Khattab et al. 2013; Wanapat and Kang 2013; Sweeny et al. 2014; Benedeti et al. 2014; Holder et al. 2015).

Researchers have suggested that NPN can effectively be used as a source of supplemental N to ruminants consuming low-quality forage in improvement of feed utilization and rumen ecology (McGuire et al. 2013; Cappellozza et al. 2013). Part of the inefficiency of NPN utilization in ruminant has been attributed to excess ammonia production in the rumen that is absorbed, converted to U, and excreted in the urine (Wanapat and Kang 2013). It has been theorized that synchronization of ruminal ammonia and energy availability will result in improving efficiency of NPN utilization and animal performance. Cassava root contains high levels of readily fermentable energy and could be used in combination with

readily available NPN sources such as U in ruminant rations (Wanapat et al. 2013; Wanapat and Kang 2013). However, the consequences of replacing true protein by NPN in diets with different levels of CP remain unclear in swamp buffaloes. Moreover, the use of NPN can result in management concerns mainly on palatability and refusal, urea toxicity, and reduction of N utilization efficiency compared with natural protein. Therefore, this study was conducted to investigate the effect of CP level and U source as NPN compared to SBM in concentrate mixture on feed utilization and rumen ecology in swamp buffaloes fed on rice straw.

Materials and methods

Animals, diets, and experimental design

Four male rumen-fistulated Thai swamp buffaloes (*Bubalus bubalis*), about 4 years old with 360±18 kg liveweight, were randomly assigned according to a 2×2 factorial arrangement in a 4×4 Latin square design to the following treatments; concentrate containing CP at 120 g/kg (T1) and 160 g/kg (T2) using SBM source; and 120 g/kg (T3) and 160 g/kg (T4) using U source, respectively. Each period lasted for 21 days with 14 days feed adaptation and 7 days samples collection on metabolism crates. All buffaloes were individually penned with availability of water and mineral block and were fed rice straw ad libitum with 10 g/kg of BW concentrate supplement. Table 1 shows feed ingredients and chemical compositions of each concentrate mixture and rice straw.

Data collection, sampling procedures, and analysis

Feed offered and refusals were recorded daily for dry matter (DM) intake calculation, and feed samples were randomly collected twice a week for DM analysis. Samples of each concentrate mixture, rice straw including refusals, feces, and urine, were collected daily during the last 7 days of each period as animals were moved to metabolism crate for total collection method. Feed and fecal samples were divided into two parts; first part was for DM analyses while second part was kept and pooled at the end of each period. 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) and ash (ID 942.05). Acid detergent fiber (ADF) was determined according to an AOAC method (1995; ID 973.18) and was expressed inclusive of residual ash while neutral detergent fiber (NDF) in samples was estimated according to Van Soest et al. (1991) with addition of α-amylase but without sodium sulfite, and results are expressed with residual ash.

Table 1 Ingredients and chemical compositions of the experimental diets

Items	Different concentrates[b]				Rice straw
	Soybean meal		Urea		
	120	160	120	160	
Ingredients, dry matter (g/kg)					
Cassava chip	600	600	630	650	
Rice bran	160	50	290	250	
Soybean meal	200	310	0	0	
Urea	0	0	20	40	
Molasses	20	20	40	40	
Sulfur	10	10	10	10	
Mineral mixture[a]	5	5	5	5	
Salt	5	5	5	5	
Chemical composition, dry matter (g/kg)					
Dry matter	880	881	858	840	925
Organic matter	931	935	908	892	872
Crude protein	125	160	120	167	21
Neutral detergent fiber	143	123	159	148	770
Acid detergent fiber	79	70	84	78	560
Ash	69	65	92	108	128

[a] Minerals and vitamins (each kilogram contain): 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; Se, 0.1 g; I, 0.5 g

[b] Crude protein levels containing in concentrates (g/kg dry matter)

On the last day of each period, approximate 200 ml of rumen fluid was taken from the middle part of the rumen using a 60-ml hand syringe at 0, 2, 4, and 6 h post feeding. Fluid was immediately measured for pH and temperature using a portable pH temperature meter (HANNA Instruments HI 8424 microcomputer, Singapore). The samples were then strained through four layers of cheesecloth and divided into three parts. The first 45 ml was kept in a plastic bottle to which 5 ml of 1 M H_2SO_4 was added to stop fermentation process of microbe activity and then centrifuged at 3000×*g* for 10 min. About 20–30 ml of supernatant was collected and analyzed for ammonia nitrogen (NH_3-N) using Kjeltec Auto 1030 Analyzer (AOAC 1995; ID 973.18) and volatile fatty acid (VFA) using high-pressure liquid chromatography (HPLC, Instruments by Water and Novapak model 600E; water mode l484 UV detector; column Novapak C18; column size 3.9 mm× 300 mm; mobile phase10 mM H_2PO_4 [pH 2.5]) according to Samuel et al. (1997). The second portion of 1 ml was kept in a plastic bottle to which 9 ml of 10 ml/l formalin solution (1:9 *v*/*v*, rumen fluid 10 ml/l formalin) was added and stored at 4 °C for measuring microbial population using total direction counts of bacterial, protozoa, and fungal zoospores according to Galyean (1989) by hemocytometer (Boeco, Singapore). The third portion (10 ml) was transported

immediately to laboratory for studying of total viable bacteria count using the Hungate (1969) roll-tube technique by determining in roll tubes complete, cellulose, casein, and starch medium for total viable, cellulolytic, proteolytic, and amylolytic bacteria, respectively (Hobson 1969).

Blood samples (10 ml) were drawn from the jugular vein at the same time as rumen fluid. Samples were then centrifuged at 3500×*g* for 20 min and the plasma were removed, stored at –20 °C, and analyzed for blood urea N (BUN) according to Crocker (1967).

Urine samples were analyzed for total N (AOAC 1995; ID984.13), and allantoin in urine was determined by HPLC as described by Chen et al. (1993). The amount of microbial purines derivative absorption was calculated from purine derivative (PD) excretion based on the relationship derived by the equation of Liang et al. (1994): $Y=0.12X+(0.20\ BW^{0.75})$. The supply of microbial N (MN) was estimated by urinary excretion of PD according to Chen and Gomes (1995): MN (g/day)$=70X/(0.116\times0.83\times1000)=0.727X$, where X and Y are, respectively, absorption and excretion of PD in mmol/d. Efficiency of microbial N synthesis (EMNS) was calculated using the following formula: EMNS=microbial N (g/day)/DOMR, where DOMR = digestible OM apparently fermented in the rumen (assuming that rumen digestion was 650 g/kg OM of digestion in total tract, DOMR=DOMI×0.65; DOMI = digestible organic matter intake).

Statistical analysis

All data obtained from the experiment were subjected to ANOVA for a 4×4 Latin square design with 2×2 factorial arrangements of treatments using the general linear models procedures of the Statistical Analysis System Institute (SAS 1998). Treatment means were compared by Tukey's multiple comparison test (Crichton 1999).

Results and discussion

Feed intake and nutrients digestibility

The results on feed intake and nutrients digestibility are shown in Table 2. Total intakes increased in buffaloes consumed concentrate containing higher level of CP especially with U source ($P<0.05$). These results agreed with Chen et al. (2010) who indicated that DMI of sheep was improved with the increasing CP level intake and additional U (Sweeny et al. 2014; McGuire et al. 2013). It is probable that a proportion of additional U contributed to improve rumen fermentation and digestibility. Compared with sources of natural protein, providing supplemental U to ruminants consuming low-quality forage increased intake, digestibility, and performance; primarily because of its lack of amino acid N and metabolizable protein

Table 2 Effect of protein level and urea on dry mater feed intake and nutrient digestibility

Items	Treatments				SEM	Interaction		
	Soybean meal		Urea			PL	PS	PL/PS
	120[a]	160[a]	120[a]	160[a]				
Dry matter intake								
Rice straw intake								
kg/day	5.0	5.1	5.2	5.5	0.09	0.08	0.04	NS
g/kg $BW^{0.75}$	66.3	67.8	68.4	72.2	1.45	NS	0.06	NS
Concentrate intake								
kg/day	3.1	3.1	3.1	3.1	0.05	NS	NS	NS
g/kg $BW^{0.75}$	41.3	41.3	40.9	41.6	0.55	NS	NS	NS
Total intake								
kg/day	8.1	8.2	8.3	8.6	0.11	NS	0.07	NS
g/kg $BW^{0.75}$	108	109	109	114	1.55	NS	0.08	NS
Apparent digestibility (kg/kg)								
Dry matter	0.62	0.65	0.65	0.70	0.24	NS	NS	NS
Organic matter	0.59	0.62	0.62	0.67	0.30	NS	NS	NS
Crude protein	0.49	0.62	0.57	0.71	0.30	0.004	0.04	NS
Neutral detergent fiber	0.43	0.46	0.51	0.57	0.22	NS	0.006	NS
Acid detergent fiber	0.47	0.56	0.56	0.64	0.24	0.01	0.01	NS

PL protein level, *PS* protein sources

[a] Crude protein levels containing in concentrates (g/kg dry matter)

(Cappellozza et al. 2013; McGuire et al. 2013). However, inconsistency to the present result, Khattab et al. (2013) and Chanjula et al. (2007) reported that U levels had no effect on feed intake in growing goats, probably due to high-urea-based diets being unfavored for goats.

Digestibilities of CP, NDF, and ADF (except for DM and OM) of the present results increased in buffaloes consumed concentrate containing higher CP levels and U source ($P<0.05$). Chen et al. (2010) and McGuire et al. (2013) also reported that DM and OM digestibilities were not affected by urea supplementation. Protein supplementation increases total tract digestibility in ruminants consuming low-quality forage. Khattab et al. (2013) reported that there was a linear increase in digestion of DM, OM, CP, and non-fiber carbohydrates with increasing U levels in the diets which could be due to the increased rate of rumen microorganisms growth as more available N in form of ammonia from the hydrolysis of U (Boucher et al. 2007). In the present study, using U as a CP source resulted in higher nutrient digestibility than SBM in swamp buffaloes.

Rumen ecology and blood metabolites

The data on rumen ecology, blood metabolites, and VFA production affected by CP level and U source are presented in Tables 3 and 4. There were no differences on ruminal pH among treatments. Similarly, mean of pH was not affected by U level according to Lizarazo et al. (2014) and Cappellozza

Table 3 Effect of protein level and urea on ruminal pH, temperature, ammonia nitrogen (NH_3-N), and blood urea nitrogen (BUN)

Items	Treatments				SEM	Interaction		
	Soybean meal		Urea			PL	PS	PL/PS
	120[a]	160[a]	120[a]	160[a]				
Ruminal (pH)								
Post feeding (h)								
0	6.57	6.46	6.84	6.59	0.06	0.03	0.02	NS
2	6.62	6.63	6.72	6.65	0.04	NS	NS	NS
4	6.58	6.68	6.77	6.64	0.09	NS	NS	NS
6	6.67	6.57	6.59	6.42	0.14	NS	NS	NS
Mean	6.61	6.59	6.73	6.58	0.05	NS	NS	NS
Ruminal temperature (°C)								
Post feeding (h)								
0	39.1	39.3	39.3	39.4	0.22	NS	NS	NS
2	38.8	39.2	39.1	39.0	0.14	NS	NS	NS
4	39.2	39.2	39.3	39.4	0.14	NS	NS	NS
6	39.3	39.6	39.3	40.0	0.12	0.007	0.08	NS
Mean	39.1	39.3	39.2	39.5	0.08	0.03	NS	NS
Ruminal NH_3-N (mg/dl)								
Post feeding (h)								
0	9.3	11.8	11.1	11.7	1.17	NS	NS	NS
2	9.9	18.0	14.5	23.2	1.96	0.005	0.04	NS
4	10.7	15.3	14.4	17.4	1.16	0.003	NS	NS
6	9.4	10.0	9.8	12.4	1.50	NS	NS	NS
Mean	9.8	13.8	14.7	16.2	0.78	0.001	0.03	NS
BUN (mg/dl)								
Post feeding (h)								
0	8.9	12.9	8.3	12.7	0.82	0.002	NS	NS
2	14.6	18.2	13.1	18.0	1.47	0.02	NS	NS
4	12.9	18.3	12.8	19.1	0.79	0.0003	NS	NS
6	13.6	16.5	12.7	17.0	0.96	0.006	NS	NS
Mean	12.3	16.5	11.7	16.7	0.90	0.002	NS	NS

PL protein level, *PS* protein sources

[a] Crude protein levels containing in concentrates (g/kg dry matter)

Table 4 Effect of protein level and urea on volatile fatty acids

Items	Treatments				SEM	Interaction		
	Soybean meal		Urea			PL	PS	PL/PS
	120[a]	160[a]	120[a]	160[a]				
Total volatile fatty acid (mmol/l)								
Post feeding (h)								
0	98.5	105	101	110	2.68	0.02	NS	NS
2	119	133	128	131	3.53	0.05	NS	NS
4	121	143	123	141	2.88	0.0005	NS	NS
6	98.5	118	109	127	3.16	0.001	0.02	NS
Mean	109	125	115	127	1.67	0.0002	0.04	NS
Acetic acid (mol/100 mol)								
Post feeding (h)								
0	70.3	66.0	68.7	66.2	1.13	0.02	NS	NS
2	68.7	59.3	66.5	61.9	2.74	0.04	NS	NS
4	65.9	58.0	61.9	56.8	2.72	0.05	NS	NS
6	69.0	62.6	66.5	62.6	2.38	0.07	NS	NS
Mean	68.4	61.5	65.9	61.9	1.92	0.02	NS	NS
Propionic acid (mol/100 mol)								
Post feeding (h)								
0	18.0	21.5	20.2	20.7	1.12	0.03	NS	NS
2	20.1	27.2	23.2	26.8	1.38	0.008	NS	NS
4	24.4	31.8	26.0	32.0	2.21	0.02	NS	NS
6	20.8	24.3	22.2	27.0	1.37	0.02	NS	NS
Mean	20.1	26.2	22.9	27.1	1.27	0.009	NS	NS
Butyric acid (mol/100 mol)								
Post feeding (h)								
0	11.7	12.5	11.1	11.2	0.79	NS	NS	NS
2	11.3	13.5	10.3	11.4	1.60	NS	NS	NS
4	9.7	10.2	12.1	11.2	1.83	NS	NS	NS
6	10.2	13.1	11.3	10.4	1.76	NS	NS	NS
Mean	10.7	12.3	11.2	11.1	1.14	NS	NS	NS
Acetic/propionic acid ratio								
Post feeding (h)								
0	3.8	3.3	3.6	3.0	0.27	0.05	NS	NS
2	3.4	2.3	2.9	2.3	0.20	0.05	NS	NS
4	2.8	1.9	2.4	1.8	0.28	0.03	NS	NS
6	3.5	2.6	3.0	2.3	0.29	0.03	NS	NS
Mean	3.4	2.5	3.0	2.3	0.21	0.01	NS	NS

PL protein level, *PS* protein sources

[a] Crude protein levels containing in concentrates (g/kg dry matter)

et al. (2013). However, U supplementation increased ruminal pH post feeding according to Van Soest (1994), since the ruminal pH is partly regulated by NH_3-N concentration and the variation in pH may be explained by U entering the rumen and being hydrolyzed by microbial ureases into CO_2 and ammonia. Changes in pH are a result of changes in ruminal fermentation and wide range CP level may affect ruminal fermentation. It seemed to be in agreement to the present study that the mean values of pH tended to be lower in treatments containing higher level of CP.

Ruminal NH_3-N and BUN increased in the treatments with higher level of CP especially with U source ($P<0.05$). This was similar to the result of Chen et al. (2010) who suggested that ruminal NH_3-N increased with the increasing level of

Table 5 Effect of protein level and urea on ruminal microorganism

Items	Treatments				SEM	Interaction		
	Soybean meal		Urea			PL	PS	PL/PS
	120[a]	160[a]	120[a]	160[a]				
Direct counts (cell/ml)								
Bacteria ($\times 10^9$)								
Post feeding (h)								
0	8.3	8.8	8.9	9.2	0.52	NS	NS	NS
2	9.5	14.0	10.6	16.7	0.72	0.0003	0.03	NS
4	11.1	16.2	11.8	17.4	0.75	0.0004	NS	NS
6	9.0	13.0	8.3	13.9	1.60	0.02	NS	NS
Mean	9.5	13.0	9.9	14.3	0.50	0.0002	NS	NS
Protozoa ($\times 10^5$)								
Post feeding (h)								
0	8.1	9.9	9.0	10.4	1.56	NS	NS	NS
2	9.0	11.4	11.8	13.0	1.53	NS	NS	NS
4	11.5	14.0	13.1	18.0	1.73	0.07	NS	NS
6	8.9	10.8	10.9	12.1	2.10	NS	NS	NS
Mean	9.4	11.5	11.2	13.4	0.64	0.01	0.02	NS
Fungi ($\times 10^5$)								
Post feeding (h)								
0	16.6	18.1	16.9	17.3	2.87	NS	NS	NS
2	20.9	26.1	22.4	29.6	2.32	0.01	NS	NS
4	29.3	34.6	28.9	36.3	1.89	0.01	NS	NS
6	15.6	20.0	19.3	23.1	1.69	0.05	0.09	NS
Mean	20.4	25.5	21.9	26.6	1.38	0.01	NS	NS
Roll-tube technique (CFU/ml)								
Amylolytic ($\times 10^8$)								
Post feeding (h)								
0	1.1	1.2	1.3	1.7	0.23	NS	NS	NS
4	1.3	1.6	1.9	2.4	0.43	NS	NS	NS
Mean	1.2	1.4	1.6	2.0	0.28	NS	NS	NS
Proteolytic ($\times 10^8$)								
Post feeding (h)								
0	2.0	2.3	2.9	3.0	0.54	NS	NS	NS
4	6.2	10.7	6.7	10.6	0.94	0.004	NS	NS
Mean	4.1	6.5	4.8	6.8	0.49	0.004	NS	NS
Cellulolytic ($\times 10^8$)								
Post feeding (h)								
0	14.6	17.3	17.3	21.1	2.85	NS	NS	NS
4	17.9	21.6	24.1	32.7	2.58	0.05	0.01	NS
Mean	16.2	19.5	20.7	26.9	1.63	0.02	0.01	NS
Total viable bacteria ($\times 10^9$)								
Post feeding (h)								
0	7.0	7.2	12.3	15.7	2.45	NS	0.02	NS
4	17.6	19.8	21.9	25.2	2.13	NS	0.06	NS
Mean	12.3	13.5	17.1	20.4	1.95	NS	0.02	NS

PL protein level, *PS* protein sources

[a] Crude protein levels containing in concentrates (g/kg dry matter)

dietary CP and U supplement compared with SBM (Cappellozza et al. 2013; Khattab et al. 2013). It was reported that U is more rapidly hydrolyzed into NH_3-N than other true protein sources. In addition, BUN was determined to investigate their relationship with rumen NH_3-N and CP utilization. The increases in rumen NH_3-N levels also resulted in increasing levels of BUN which is in agreement to the present study. It was reported that concentrations of BUN are highly positively correlated to the level of NH_3-N production in the rumen. The U content in the blood has been found to reach a maximum of 3 h after feeding and is commonly considered to reflect the protein degradability, level of forage intake, and N to energy ratio in ruminant diets. This would indicate that available rumen NH_3-N could be used and/or absorbed in the rumen for further synthesis. Chen et al. (2010) reported that protein levels did not show any effects on VFA profile which was consistent to the present study. However, in the present study, total VFA at 6 h post feeding and mean value increased in buffaloes consumed concentrate containing U source. According to Cappellozza et al. (2013), total VFA increased with CP supplementation in ruminants consuming low-quality forage which supports the present data.

Rumen microorganism population

Microorganisms were increased in buffaloes consumed concentrate containing higher level of CP (Table 5). This was consistent to the finding of Khampa et al. (2006) who revealed that fungal zoospores, protozoa, and total bacteria direct count increased in animals fed with higher CP level diet. The present number of protozoa in the rumen was decreased by CP level and this may attribute the increase in fungal zoospores per milliliter rumen fluid. Total viable bacterial counts, proteolytic, cellulolytic, and amylolytic bacteria did not changed with an increasing U level (Chanjula et al. 2007); however, overall populations tended to be slightly greater from 0 to 4 h post feeding for goats fed at the highest U level. These were in contrast to the present findings that amylolytic bacteria were not changed, while proteolytic, cellulolytic, and total viable bacteria were increased by CP level and U source.

Nitrogen utilization and purine derivatives

N intakes and excretion increased in buffaloes consumed concentrate containing higher CP level (Table 6); however, U

Table 6 Effect of protein level and urea on nitrogen utilization and purine derivative

Items	Treatments				SEM	Interaction		
	Soybean meal		Urea			PL	PS	PL/PS
	120[a]	160[a]	120[a]	160[a]				
N utilization								
N intake (g/day)								
Rice straw	16.9	17.2	17.3	18.3	0.31	0.08	0.04	NS
Concentrate	62.4	79.5	61.9	80.0	0.98	0.001	NS	NS
Total	79.3	96.7	79.2	98.3	1.03	0.001	NS	NS
N excretion (g/day)								
Feces	56.1	51.8	46.8	41.2	3.06	NS	0.02	NS
Urine	10.6	13.5	7.9	16.4	1.90	0.02	NS	NS
Total	66.7	65.3	54.7	57.7	3.44	NS	0.03	NS
N balance (g/day)								
Absorption	23.2	44.9	32.4	57.1	2.22	0.001	0.003	NS
Retention	13.1	31.3	24.5	40.7	2.88	0.001	0.01	NS
Purine derivative (mmol/day)								
Allantoin excretion	30.2	33.3	30.4	37.6	5.69	NS	NS	NS
Allantoin absorption	125	153	127	188	9.73	0.04	NS	NS
Microbial N supply (g N/day)	91.1	111	92.5	137	7.39	0.04	NS	NS
Microbial crude protein (g/day)	569	693	578	855	6.76	0.01	NS	NS
EMNS[b], (g N/kg) OMDR[c]	34.8	37.1	31.9	40.1	1.51	0.02	NS	NS

PL protein level, *PS* protein sources

[a] Crude protein levels containing in concentrates (g/kg dry matter)

[b] Efficiency of microbial nitrogen synthesis

[c] Digestible organic matter apparently fermented in the rumen

source in concentrate reduced N excretion. Similarly, N intake, urinary N excretion, and N retained increased linearly with increasing dietary CP (Chen et al. 2010; McGuire et al. 2013). Likewise, total excretion of fecal N was not significantly different, while the decline in urinary N, total N excretion, and N absorption were evident after U inclusion. According to Benedeti et al. (2014), the urinary excretion of N was linearly increased by the inclusion of U in the diets. The increase of urinary N loss with the increase of U levels in the diets might have occurred due to the rapid hydrolysis of ruminal NH_3-N resulting in escape of NH_3-N from the rumen (Benedeti et al. 2014). The effects of N balance on animal performance require further investigations to ensure the level of inclusion of U as a replacement of SBM in order to improve tissue deposition and animal performance. It is now well established that nitrogen retention depends on the intake of N, amount of fermentable carbohydrate of the diet.

The differences in the quality and routes of N excretion with consequent influences on N retention could reflect treatments feed difference in N metabolism, in which N retention is considered as the most common index of the protein nutrition status of ruminants. In addition, the present study showed that N absorption and retention increased in buffaloes supplemented with concentrate containing higher CP level and U source. Using urinary purine derivatives as microbial protein synthesis indicator has confirmed the improvement with higher level of protein while U supplementation resulted in similar results as those in SBM treatments. The maximum microbial growth efficiency can be reached by maximizing the synthesis of microbial N per unit of carbohydrates fermented in the ruminal environment. Microbial N synthesis increased linearly with increasing urea supplementation, which is reflected in increased PD in the urine (Khattab et al. 2013). The results are similar to Boucher et al. (2007) who reported the optimum ruminal ammonia N concentration required to support maximum synthesis of microbial and maximum efficiency of microbial protein synthesis.

Conclusions and recommendations

Supplementation of CP level using urea in concentrate can improve feed intake and rumen ecology. For swamp buffaloes fed on rice straw, concentrate mixtures containing 16 g/kg CP with U 40 g/kg improved nutrients utilization with no adverse effects.

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Conflict of interest The authors declare that they have no conflict of interest.

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