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REGULAR ARTICLES

Rumen metabolism of swamp buffaloes fed rice straw supplemented with cassava hay and urea

Thiwakorn Ampapon[1] · Metha Wanapat[1] · Sungchhang Kang[2]

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Abstract The objectives of this experiment were to investigate effects of cassava hay (CH) and urea (U) supplementation on feed intake, digestibility, rumen fermentation, and microbial protein synthesis of swamp buffaloes fed on rice straw. Four rumen-fistulated swamp buffaloes, 365 ± 15.0 kg, were randomly assigned according to a 4 × 4 Latin square design to receive four dietary treatments: T1 = CH 400 g/head/day + U 0 g/head/day, T2 = CH + U 30 g/head/day, T3 = CH + U 60 g/head/day, and T4 = CH + U 90 g/head/day, respectively. Results revealed that feed intake was not affected while nutrient digestibilities were increased ($P < 0.05$) with increasing U level supplementation especially at 90 g/head/day. Ruminal pH and temperature were not altered by urea supplementation, whereas ammonia nitrogen (NH_3-N) and blood urea nitrogen were increased with urea supplement ($P < 0.05$). In addition, total volatile fatty acid and butyric acid were similar among treatments, while propionic acid (C_3) was increased by level of urea supplement ($P < 0.05$), but acetic acid (C_2) and C_2/C_3 ratio were significantly decreased ($P < 0.05$). On the other hand, protozoal population and methane production were decreased by CH and urea supplement, while bacterial population particularly those of proteolytic, cellulolytic, and amylolytic bacteria and efficiency of microbial nitrogen synthesis were linearly increased ($P < 0.05$). Based on this experiment, it suggested that supplementation of urea and cassava hay for buffaloes fed rice straw improved rumen ecology and increased fermentation end products and microbial protein synthesis while reducing protozoal populations and methane production. Urea supplements of 60–90 g/head/day when fed with cassava hay are recommended for swamp buffaloes consuming rice straw.

Keywords Cassava hay · Microbial protein synthesis · Rice straw · Rumen fermentation · Swamp buffaloes · Urea

✉ Metha Wanapat
metha@kku.ac.th

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

[2] Agricultural Unit, Department of Education, National Institute of Education, Phnom Penh, Cambodia

Introduction

The availability of local feed resources in various seasons can contribute as essential sources of carbohydrate and protein which significantly impact rumen fermentation and the subsequent productivity of the ruminants (Wanapat et al. 2013). Cassava or tapioca (*Manihot esculenta*, Crantz) is an annual tuber crop grown widely in the tropics and sub-tropics. It is a cash crop cultivated by small-holder farmers within the existing farming systems in many countries. Cassava tuber contains high level of energy and minimal level of crude protein (CP) and has been used well as readily fermentable energy in ruminant rations (Wanapat and Kang 2013; 2015). Cassava hay (CH) can be processed from whole crop of about 4 months, containing high level of CP (25 %) and condensed tannin (CT; 3–4 %). Patra and Saxena (2010), Calabrò et al. (2011), and Guglielmelli et al. (2011) reported that if CT in the feed exceeded 6 % of dry matter (DM), feed intake and digestibility would be dramatically reduced. If CT level was between 2 and 4 % DM, it would help to protect protein from rumen digestion, thereby increasing by-pass protein or rumen undegradable protein (RUP). Since CH contains 3–4 % CT, it could form tannin-protein complexes which could act as a source of RUP. As reported by Promkot and Wanapat

(2005), CH contained 25 % CP with rumen by-pass protein of 45.4 %.

As known, urea (U) is commonly hydrolyzed by bacterial enzyme in the ruminant to ammonia and carbon dioxide then ammonia as used further with available C-skeletal to synthesize into microbial protein (McGuire et al. 2013; Cappellozza et al. 2013; Khattab et al. 2013; Sweeny et al. 2014; Benedeti et al. 2014; Holder et al. 2015; Kang et al. 2015). Piao et al. (2012) also reported the synchronization of the hourly rate of carbohydrate and nitrogen released in the rumen would increase the amount of retained nitrogen for growth and thus improve the efficiency of microbial protein synthesis (EMPS) in Holstein steers. However, Kertz (2010) reported that fermentation of starch would favor the utilization of non-protein nitrogen (NPN) over fibrous carbohydrate and simple sugars, such as in molasses and cassava chip. Nevertheless, efficient utilization of CP and NPN in ruminants depends upon knowledge of the basic principles underlying ruminal microbial nitrogen metabolism.

However, the amount of U that can be used in the diets is limited, due to its rapid hydrolysis to NH_3 in the rumen by microbial enzymes and availability of non-structural carbohydrate (Golombeski et al. 2006; Highstreet et al. 2010) as well as little information is available on combination of CH and U as sources of CP in feeding buffaloes. Therefore, the objective was to study U level and CH supplementation on feed intake, digestibility, rumen fermentation, microbial population, and microbial protein synthesis in swamp buffaloes fed on rice straw-based diet.

Materials and methods

Animals, feeds, and management

Four rumen-fistulated swamp buffaloes (*Bubalus bubalis*), 365 ± 15.0 kg body weight (BW), were randomly allocated to four levels of U supplement (0, 30, 60, and 90 g/head/day) and CH at 400 g/head/day according to a 4 × 4 Latin square design. All buffaloes were kept in individual pens, where water and mineral blocks were provided freely. Rice straw was fed ad libitum while concentrate was offered at 0.2 % of BW. The experiment consisted of four periods, with each period lasting for 21 days. During the first 14 days, all buffaloes were fed their respective diets, while concentrate was fed to the buffaloes in two equal portions at 07:00 a.m. and at 16:30 p.m. Refusals of rice straw were weighed daily prior to the morning feeding to determine daily dry matter (DM) intake. The BW of each buffalo was measured at the beginning and end of each period. Feed ingredients and chemical compositions of concentrate mixture, CH, U, and rice straw are illustrated in Table 1.

Table 1 Feed ingredients and chemical composition of the experimental diets

Items	Concentrate	Cassava hay	Urea	Rice straw
Ingredients, g/kg dry matter				
Cassava chip	670			
Rice bran	80			
Palm kernel meal	90			
Coconut meal	100			
Minerals	10			
Salt	10			
Molasses	20			
Sulfur	10			
Chemical composition, g/kg dry matter				
Dry matter	897	901	–	905
Crude protein	110	235	2850	27
Neutral detergent fiber	265	463	–	811
Acid detergent fiber	131	406	–	436

Sampling method and sample analyses

After the first 14 days, all buffaloes were well adapted to respective feeds and then were on metabolism crates during the last 7 days of each period. Feces, urine, and dietary feed were collected and sampled by total collection and were analyzed for chemical compositions of DM, ash, and crude protein (CP) by the methods of AOAC (2012) and NDF and ADF according to Van Soest et al. (1991).

On day 21st of each period, rumen fluid samples were collected at 0, 2, 4, and 6 h post-morning feeding through rumen fistula and measured for pH and temperature immediately (HANNA Instrument HI 8424 microcomputer, Singapore). Rumen fluid was separated into three parts: (1) First part was fixed with 5 ml of sulfuric acid (1 M H_2SO_4) solution to determine the concentration of volatile fatty acids (VFAs) 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) and NH_3-N analysis using the micro Kjeldahl method (AOAC 2012). (2) The second part was fixed with 10 % formalin solution (1:9 *v*/*v*, rumen fluid, 10 % formalin) to measure microbial populations by total direct counts of bacteria, protozoa, and fungal zoospores (Galyean, 1989). (3) The third part was cultured for groups of bacteria (i.e., cellulolytic, proteolytic, amylolytic, and total viable bacterial counts) using the roll-tube technique (Hungate 1969).

Blood (about 10 ml) was sampled 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 2012), and urinary allantoin was

Table 2 Effects of supplementation of urea and cassava hay on feed intake and digestibility of nutrients in swamp buffaloes

Items	Urea, g/head/day				SEM	*P* value
	0	30	60	90		
Total dry matter intake						
kg/day	6.9	6.9	6.9	7.1	0.53	0.91
%BW/day	2.0	1.9	2.0	2.0	0.32	0.53
g/kg $BW^{0.75}$	95.8[a]	104.0[b]	108.0[bc]	111.0[c]	1.75	0.01
Apparent digestibility, %						
Dry matter	50.8	54.8	53.5	55.7	1.88	0.06
Organic matter	52.7	53.6	54.4	57.3	2.07	0.08
Crude protein	53.0[a]	62.6[ab]	67.6[b]	72.8[b]	3.15	0.02
Neutral detergent fiber	38.3[a]	42.3[ab]	45.8[ab]	50.5[b]	2.87	0.02
Acid detergent fiber	37.3[a]	40.4[ab]	43.6[ab]	48.4[b]	2.36	0.04

Means in the same row with different superscripts differ ($P < 0.05$). Supplementation of cassava hay at 400 g/head/day

SEM standard error of the mean

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) and Liang et al. (1994).

Statistical analysis

All data were statistically analyzed for a Latin square design using the GLM procedure (Statistical Analysis System (SAS) 2013) in which buffalo, period, and level of U supplementation were main factors. Treatment means were compared by polynomial comparison according to the model: $Y_{ijk} = \mu + T_i + C_j + R_k + e_{ijk}$, where Y_{ijk} = the criteria under study, in treatment i, column j, row k, and μ = over all sample mean; T_i = effect of treatment i; C_j = effect of treatment i at column j; R_k = effect of treatment i at row k; and e_{ijk} = error.

Results and discussion

Cassava hay was prepared from harvesting the top part of cassava crop after 4 months of growth and the re-growth after 2 months throughout the year (Wanapat and Kang 2013). Rice straw was purchased from the farmers after rice harvesting. Concentrate supplement was formulated using locally available feed resources. The nutritive value of these respective feeds is shown in Table 1.

Feed intake and digestibility

Feed intake was not affected while apparent digestibility was increased by dietary supplement ($P < 0.05$) especially at 90 g/head/day U (Table 2) which was in consistency to the studies of Khattab et al. (2013) and Kang et al. (2015). Kertz (2010) reported that U supplement at approximately 135 g/head/day for cows with <20 % total CP in concentrate should be employed. Digestibilities of NDF and CP were higher in cattle fed pellet containing CH and/or U and could increase ruminal fermentation and microbial protein synthesis (Wanapat et al. 2006).

Characteristics of ruminal fermentation

Rumen pH and temperature were not altered among treatments, while NH_3-N and blood urea nitrogen (BUN) were increased ($P < 0.05$) with a high level of urea supplementation

Table 3 Effects of supplementation of urea and cassava hay on rumen ecology and fermentation end product in swamp buffaloes

Items	Urea, g/head/day				SEM	*P* value
	0	30	60	90		
Temperature, °C	38.8	38.8	38.4	38.2	0.06	0.21
pH	6.8	6.7	6.7	6.6	0.06	0.48
NH_3-N (mg/dL)	5.5[a]	8.4[b]	12.3[c]	16.8[d]	0.31	0.01
BUN (mg/dL)	8.0[a]	11.1[b]	15.2[c]	18.4[d]	0.82	0.02
Total VFA, mmol/L	110.0	123.0	132.0	132.0	7.74	0.62
	VFA, mol/100 mol					
Acetic acid (C_2)	74.5[a]	73.4[a]	67.5[b]	66.1[b]	0.75	0.02
Propionic acid (C_3)	13.7[a]	17.4[b]	21.0[c]	22.5[c]	0.68	0.04
Butyric acid (C_4)	11.8	12.3	8.3	12.8	1.02	0.21
C_2/C_3	5.4[a]	5.3[a]	3.3[b]	2.9[b]	0.12	0.04
Methane, mmol/L	34.5[a]	33.2[b]	28.0[c]	28.7[c]	0.15	0.01

Means in the same row with different superscripts differ ($P < 0.05$). Supplementation of cassava hay at 400 g/head/day. Methane production (mmol/L) calculated by Moss et al. (2000) = 0.45 (C_2) − 0.275 (C_3) + 0.4 (C_4)

NH_3-N ammonia nitrogen, *BUN* blood urea nitrogen, *VFA* volatile fatty acid, *SEM* standard error of the mean

Table 4 Effects of supplementation of urea and cassava hay on rumen microorganisms in swamp buffaloes

Items	Urea, g/head/day				SEM	P value
	0	30	60	90		
Total direct counts, cell/ml						
Protozoa, ×10[5]	8.5	7.6	7.8	7.8	0.94	0.06
Fungal zoospore, ×10[5]	5.4	6.1	6.3	6.1	0.41	0.12
Roll-tube technique, CFU/ml						
Amylolytic bacteria, ×10[7]	2.5[a]	4.5[b]	4.5[b]	4.9[b]	1.43	0.03
Proteolytic bacteria, ×10[7]	3.6[a]	3.7[a]	4.6[b]	5.9[b]	1.01	0.02
Cellulolytic bacteria, ×10[8]	3.7[a]	3.8[a]	5.4[b]	5.4[b]	0.76	0.02
Total viable bacteria, ×10[8]	5.5	5.6	5.9	6.8	1.37	0.09

Means in the same row with different superscripts differ ($P < 0.05$). Supplementation of cassava hay at 400 g/head/day

SEM standard error of the mean

(Table 3) and were in optimal range for microbial growth (Boucher et al. 2007). Ruminal NH_3-N concentration was a predictor of efficiency of dietary N conversion into microbial N (Firkins et al., 2007). Furthermore, increased ruminal NH_3-N levels were obtained ($P < 0.05$) as level of CH increased in the diets and were closer to optimal ruminal NH_3-N (15–30 mg %; Perdok and Leng 1990; Kang et al. 2015) for increasing microbial protein synthesis, feed digestibility, and voluntary feed intake. On the other hand, BUN was increased when U was added to the diets and the increase in BUN could result in nitrogen losses from rumen fermentation (Xin et al. 2010).

In addition, propionic acid production was increased ($P < 0.05$) at 60 and 90 g/head/day of urea supplementation, while acetic acid was decreased and total VFA and butyric acid were similar among treatments ($P > 0.05$). Furthermore, methane production was significantly reduced as affected by level of urea supplementation (Table 3). Wanapat et al. (2014) report that when high fiber diets are offered, VFAs in ruminal fermentation fluctuate from 65:25:10 to 70:20:10 (acetate/propionate/butyrate, in molar percentage) ratios and reiterated the effect of rumen pH on changing rumen ecology. Cappellozza et al. (2013) reported that total VFA increased with CP supplementation in ruminants consuming low-quality forage which supports the present data. Nevertheless, Kang et al. (2015) also further reported that total VFA and propionic acid were increased in buffaloes that consumed concentrate at 160 g/kg containing using urea at 40 g/kg DM.

Rumen microbial population

Total direct count of fungi zoospores was similar while protozoal populations were decreased among treatments. Moreover, total viable bacteria and amylolytic, proteolytic, and cellulolytic bacteria were increased ($P < 0.05$) in buffaloes supplemented with U at 60 and 90 g/head/day (Table 4). McSweeney et al. (2000) reported that CT altered rumen ecology and increased microbial protein synthesis. However, the mode of action of CT on rumen fermentation was yet to be elucidated. At high levels (>6 % DM), CT can adversely affect the microbial activity (Patra and Saxena 2010; Calabrò et al. 2011). Chanjula et al. (2004) showed that level of CH and urea-treated rice straw increased cellulolytic and proteolytic bacterial populations, while total protozoal counts were dramatically decreased in swamp buffaloes.

Purine derivatives and microbial protein synthesis

As shown in Table 5, purine derivative (PD) both excretion and absorption, microbial nitrogen supply (MNS), and EMPS were increased with increasing level of U supplementation ($P < 0.05$) which agreed with other reports, showing that synthesis of microbial protein was affected by dietary protein (Cecava et al. 1991). On the contrary, an increase in the RUP/RDP ratio may

Table 5 Effects of supplementation of urea and cassava hay on microbial protein synthesis in swamp buffaloes

Items	Urea, g/head/day				SEM	P value
	0	30	60	90		
Purine derivatives, mmol/day						
Excretion	26.7[a]	27.1[a]	29.8[ab]	32.0[b]	1.36	0.01
Absorption	87.4[a]	97.2[ab]	116.8[b]	132.4[c]	3.93	0.04
MNS, gN/day	63.6[a]	70.6[ab]	84.9[c]	96.3[c]	2.31	0.02
MP, g/day	398.0[a]	441.0[ab]	531.0[b]	602.0[c]	10.2	0.01
EMNS (gN/kg OMDR)	28.8[a]	31.5[ab]	38.9[c]	43.9[c]	2.10	0.02

Means in the same row with different superscripts differ ($P < 0.05$). Supplementation of cassava hay at 400 g/head/day

MNS microbial nitrogen supply, *MP* microbial protein synthesis, *EMNS* efficiency of microbial nitrogen synthesis, *OMDR* digestible organic matter apparently fermented in the rumen, *SEM* standard error of the mean improve protein efficiency because at a high RDP, more N would be absorbed as ammonia or more amino acids would be deaminated which might increase N excretion in urine (Castillo et al. 2011). Microbial N synthesis increased linearly with increasing urea supplementation which reflected in increased PD in the urine (Khattab et al., 2013). Kang et al.(2015) reported that fecal and urinary N excretions were decreased in buffaloes consumed concentrate containing higher CP level especially with urea while PD increased which resulted in a higher N balance as compared to lower CP level treatments. Findings under this experiment of higher urinary purine derivative were in buffaloes that received U between 60 and 90 g/head/day. It is suggested that the lower the glomerular filtration rate (GFR) found in buffaloes may be the reason for the differences as PD stay longer in the blood to give more time for recycling to the rumen when the rumen is developed and are then metabolized by bacteria (Thanh et al. 2009).

Conclusions and recommendations

It can be concluded that supplementation of urea and cassava hay for buffaloes fed rice straw improved rumen ecology and increased fermentation end products and microbial protein synthesis while reducing protozoal populations and methane production. Urea supplements of 60–90 g/head/day when fed with cassava hay are recommended for swamp buffaloes consuming rice straw.

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Research involving human participants and/or animals All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Compliance with ethical standard

Competing interests The authors declare that they have no competing interests.

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