EFFECTS OF UREA-LIME TREATED RICE STRAW ON RUMEN FERMENTATION EFFICIENCY, NUTRIENTS DIGESTIBILITY AND MICROBIAL NITROGEN SYNTHESIS IN SWAMP BUFFALOES

[1]N T T Giang, [2]M Wanapat, [1]V M Tuan, [1]B V Phong, [1]H T Hien and [1]P B Duy

[1]National Institute of Animal Sciences; [2]Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand

Corresponding author: Nguyen Thien Truong Giang, Email: nttgiangnias@gmail.com

ABSTRACT

This objective of this experiment was to determine effects of urea lime treatment of rice straw on rumen fermentation efficiency and nutrients digestibility in swamp buffaloes. Four rumen fistulated swamp buffaloes with initial weight were 353± 11 kg andwere randomly assigned to 2 treatments (T1 = untreated rice straw, T2 = 2% urea + 2% lime) according to T-test design. All treatments were fed ad libitum intake. Experimental time lasted for 21 days in which feed, feces, urine were collected during last 7 days and rumen fluid, blood were collected at last days for chemical analyzes. The dry matter intake and digestibility were improved significantly by using urea-lime treated rice straw (ULTRS) ($P < 0.05$). Rumen NH_3-N concentration were higher ($P < 0.05$) as compared with untreated rice straw (RS), while blood urea nitrogen was in normal ranges. The acetic acid concentration was decreased ($P < 0.05$) while propionic acid concentration and CH_4 prodution was increased ($P < 0.05$), thus acetic acid:propionic acid was subsequently lowered in buffaloes fed with 2% urea + 2% lime treated rice straw. Total viable bacteria, amylolytic, and cellulolytic bacteria were significantly enhanced by urea-lime rice straw treatment. The microbial protein synthesis and efficiency microbial N synthesis also were higher in urea-lime treated rice straw. Based on this study, it could be concluded that 2% urea + 2% lime treated rice straw improved digestibility of nutrients, rumen microbial population, rumen fermentation efficiency and enhanced microbial protein synthesis and efficiency of microbial N synthesis while the CH_4 production was lower than untreated rice straw.

Keywords: *Urea-lime, swamp buffaloes, rice straw, rumen fermentation, microbial protein*

INTRODUCTION

Agricultural by-products for ruminant feed source have been widely used in the world, especially in the tropical countries where feed resources are limited in the dry season. Rice straw is by-product of rice crop and used as a feed roughage source for ruminant animals. However, rice straw has low crude protein value and high fiber content, especially high level of lignification which drastically affected the dry matter intake and digestion leading to low efficient ruminant animals performance (Wanapat et al., 1985; Chemjon, 1991; Safari et al., 2011). The two well-known factors of rice straw that limit bacterial digestion in the rumen are its high level of lignification and low contents of nitrogen. Therefore, in principle, there are two approaches, which should be taken in combination, being straw delignification treatment and nutrient supplementation.Numerous methods of physical, chemical, biological treatment have been worldwide researched and developed in order to improve the utilisation of straw and other fibrous by-products as feed for ruminants.Chemical treatment involves the use of alkaline, acidic or oxidative agents. Urea is cheap crude protein source for microbial synthesis. Supplement of urea make rice straw increase its nutritive value (Sundstøl et al., 1978; Abate and Melaku, 2009). Treatment of rice straw with lime ($CaO/Ca(OH)_2$) should be given priority for research at the moment since it is cheap and readily available. Lime is not only an alkaline agent, it can also be used to supplement calcium which has been found in negative balance in cattle fed on rice straw (Nath et al., 1969). According to Sirohi and Rai (1995) and Pradhan et al. (1997) have found that combination of the lime and urea highly increased its intake, and *in-vitro* and *in-vivo* digestibility in rice straw. However, the data of

urea-lime treated rice straw was used for swamp buffaloes is limited. Therefore, the objectives of this experiment were to compare untreated and urea-lime treated rice straw on rumen bacterial diversity, feed fermentation and digestibility in swamp buffaloes.

MATERIALS AND METHODS

Animals, treatments and experimental design

Eight fistulated swamp buffaloes (353± 11kg BW) were used in this experiment. They were randomly allotted to study untreated rice straw and urea-lime treatede straw as roughage source, according to T-test design. Treatment 1 concentrate with untreated rice straw as a roughage (control), treatment 2 concentrate with urea-lime treated rice straw. All animals will be supplemented concentrate at 0.2% of BW/d (CP 12%; TDN 75%) and roughage will be given *ad libitum*. Urea-lime TRS was prepared by using 2 kg of urea plus 2 kg of lime, mixed with 100 kg of water then poured mixture water to 100 kg of RS (5 bales of RS, each bale weighting approximate 20 kg) and then covered with a plastic sheet for a 14 days before feeding to animals (Wanapat et al., 1985). Chemical composition of concentrate, rice straw and urea-lime treated rice straw were presented in Table 1. Swamp buffaloes were housed individually and fed the experiment diets twice at 8h and 16 h. Clean fresh water was provided *ad libitum*. The experiment lasted 28 days, first 7 days for adaptation,next 14 days for feed intake measurements and the last 7 days, buffaloes were move to metabolism crates for total urine and fecal collection.

Data collection and sampling procedures

The feed was sampled and analyzed. While fecal and urine samples were totally collected from each individual swamp buffaloes during the last 7 days of the experiment. Feeds, refusals and fecal samples were divided into two parts, the first part being analyzed for DM and the second part for chemical composition. DM, N and ash were analyzed by the standard method of AOAC (1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to Van Soest et al. (1991).

Rumen fluid will be collected at 0, 2, 4, 6 h post-feeding. Approximately 200 ml of rumen fluid was taken from the middle part of the rumen at the end of each period. Rumen fluid was immediately measured for pH and temperature using a portable pH and temperature meter (HANNA instrument HI 8424 micro-computer, Singapore).

Rumen fluid samples were then filtered through four layers of the cheesecloth. The samples weredivided into two portions. The first portion to analyze ammonia nitrogen (NH_3-N) where 5 ml of H_2SO_4 solution (1M) was added to 50 ml of rumen fluid. The mixture will be centrifuged at 16,000 x *g* for 15 minutes (Table Top Centrifuge PLC-02, U.S.A.) and supernatant will be stored at –20 °C prior to volatile fatty acid (VFAs) analyses using a HPLC (Instruments by controller water model 600E; water model 484 UV detector; column novapak C18; column size 4 mm x 150 mm; mobile phase 10 mM H_2PO_4 (pH2.5) according to Zinn and Owens (1986). The second portion quantified ruminal microorganism using the viable count of bacteria, based on the use of a roll-tube technique (Hungate, 1969).

At the same time as rumen fluid sampling, a blood sample (about 10ml) was collected from the jugular vein into tubes containing 12 mg of EDTA and the plasma was separated by centrifugation at 500 x *g* for 10 minutes (Table Top Centrifuge PLC-02, U.S.A.) at 4°C and stored at –20°C until analysis of blood urea nitrogen (BUN) according to the method of Crocker (1967). Urinary samples were an analyzed for total N determined according to AOAC (1995) and allantoin determined by HPLC as described by Chen and Gomes (1995). The amount of microbial purines derivative (PD) absorption was calculated from PD

excretion based on the relationship derived by the equation of Chen and Gomes (1995), Y = 0.85 X + 0.385 $BW^{0.75}$, where: X and Y are absorption and excretion of PD in mmol/d, respectively . The supply of microbial N (MN) was estimated by urinary absorption of purine derivatives: MN (g/d) = 0.727 X. The efficiency of microbial N synthesis (EMNS) was calculated using the following formula: EMNS = microbial N (g/d)/DOMR; where DOMR = digestible OM apparently fermented in the rumen (assuming that rumen digestion was 65% of digestion in total tract, DORM = DOMI x 0.65; DOMI = digestible organic matter intake).

Calculation of ruminal CH_4 production was estimated using VFA proportions according to Moss et al. (2000) as follows: CH_4 production = 0.45 x (acetate, C_2) – 0.275 x (propionate, C_3) + 0.4 x (butyrate, C_4).

Statistical analysis

All data were analyzed according to t-test design using the general linear procedure in PROC GLM of SAS (1998).Be careful Ttest design should use two sample test or paired T test for comparision between 2 samples.

Table 1. Feed ingredients and chemical composition of dietary treatment used in the experiment

Items	Concentrate	Untreated rice straw	Urea-lime treated rice straw
Ingredients			
Cassava chip	76.0		
Rice bran	8.0		
Coconut meal	4		
Palm meal	4		
Urea	3.0		
Molasses	2.0		
Sulphur	1.0		
Mineral mixed	1.0		
Salt	1.0		
Chemical composition (%)			
Dry matter	88.7	87.4	53.7
% DM			
Organic matter	92.7	88.4	86.1
Crude protein	15.8	2.6	5.72
Neural detergent fiber	18.5	83.9	75.9
Acid detergent fiber	8.3	63.2	57.1

RESULTS AND DISCUSSION

Chemical composition of feeds

Table 1 shows that the feed ingredient of concentrate, the 2 types of rice straw and their chemical composition. The nutritive values of rice straw were improved by urea-lime

treatment. The content of crude protein of urea-lime treated rice straw was significantly increased from 2.6 to 5.72%. However, NDF and ADF content was reduced being 75.9 and 57.1%, respectively by urea-lime treated rice straw. The improvement of urea-lime treated rice straw nutrient values was similar to those values were reported by Wanapat et al. (2009; 2013) who conducted by using 2.2 + 2.2% urea-lime and 2 + 2% urea-lime treated rice straw, respectively. The crude protein content in urea-lime treated rice straw was increased due to supplement of urea.

Feed and nutrients intake and apparent digestibility

The effect of rice straw and urea-lime treated rice straw on feed intake and apparent nutrient digestibility of swamp buffaloes was presented in Table 2. It indicated that DM intake was significantly increased with urea-lime treated rice straw ($P<0.01$) being from 5.5 – 6.0 kg/day. The apparent DM, OM, CP, NDF and ADF digestibilities were also markedly improved for the urea-lime treated rice straw compared to untreated rice straw treatment.

The feed intake of rice straw was improved due to treating with 2% urea + 2% lime. This result was lower than that found by Wanapat et al. (2013), who applied 2% urea + 2% lime treated rice straw improving rice straw DMI compared to untreated rice straw from 61.7 to 96.2 g/kg$BW^{0.75}$ in lactating dairy cow. The urea-lime supplementation to rice straw enhanced DMI was also found in many previous studies. According to Trach et al. (2001), improvement of DMI was due to increasing rice straw degradability.

Table 2. Effect of rice straw and urea-lime treated rice straw on feed intake and apparent nutrient digestibility of swamp buffaloes

Items	Treatment		Significancelevel[1]
	RS	*URL*	
DM intake			
Rice straw			
kg/d	5.3[a]	6.0[b]	**
g/kg $BW^{0.75}$	65.4[a]	73.4[b]	**
Concentrate			
kg/d	0.7	0.7	
g/kg $BW^{0.75}$	8.6	8.6	
Total			
kg/d	6.0[a]	6.7[b]	**
g/kg $BW^{0.75}$	74[a]	82[b]	**
Apparent digestibility (%)			
Dry matter	54.4[a]	63.3[b]	**
Organic matter	61.1[a]	66.2[b]	*
Crude protein	52.4[a]	67.8[b]	**
Neutral detergent fiber	56.2[a]	65.2[b]	**
Acid detergent fiber	54.7[a]	61.1[b]	**

*[1]RS = untreated rice straw, URL = urea-lime treated rice straw, * p<0.05, ** p<0.01, ns = non-significant (p>0.05).*

Ruminal fermentation and blood metabolites

Table 3. Effect of rice straw and urea-lime treated rice straw on rumen fermentation and BUN in swamp buffaloes

Items	Treatment		Significance level[1]
	RS	***URL***	
pH	6.59	6.52	ns
Ruminal temperature, °C	38.4	38.5	ns
NH_3-N (mg/dL)	6.9[a]	8.4[b]	*
BUN (mg/dL)	13.2	14.1	ns
Total VFA (mM)	113.3[a]	134.0[b]	*
Acetic acid,mM (C2)	72.9	71.8	ns
Propionic acid,mM (C3)	21.3[a]	23.2[b]	*
Butyric acid, mM (C4)	5.7	5.9	ns
C2:C3	3.4[a]	3.1[b]	*
Methane, mol/100mol[2]	29.2[a]	28.3[b]	*

*[1]RS = untreated rice straw, URL = urea-lime treated rice straw, * p<0.05,ns = non-significant (p>0.05).[2] Methane = 0.45(C_2) – 0.275(C_3) + 0.4(C_4) (Moss et al., 2000).*

The Table 3 indicates the effect of two types of rice straw on rumen fermentation and BUN in swamp buffaloes. The result revealed that no significant difference on pH, ruminal temperature and BUN between rice straw and urea-lime treated rice straw (P>0.05). The ruminal NH_3-N content, however, was significantly increased (P<0.05) from 6.9 to 8.4 (mg/dL) in untreated rice straw and urea-lime treated straw, respectively. Rice straw with urea-lime has improved total VFA compared to only rice straw (134 vs 113mM, respectively. The acetic acid (C2) and butyric acid (C4) had no change between two treatments while propionic acid content has been markedly improved (P<0.01) up to 23.2 mM in urea-lime treated rice straw compared to those untreated rice straw. As a result of that, the C2:C3 ration was significantlydecreased (P<0.01) and felt down to 3.1 mM.There was a decrease of methane production for urea-lime treated rice straw being 28.3 mM.

The ruminal NH3-N was higher for the urea-lime treated rice straw fed the animals. According to (Khejornsart et al., 2011; Wanapat et al., 2013), the combination of 2% urea and 2% lime in rice straw increased ruminal NH3-N (mg/dL) as compared to untreated rice straw on both buffalo and lactating dairy cattle. This result was consistent with that of Yuangklang et al. (2010). However, NH3-N values were in optimal range which was reported (from 5.0 to 25.0 mg/dl) by Preston and Leng (1987). The BUN content was similar in both treatments. The finding was consistent with that of Wanapat et al. (2013) without any changes. However Wanapat et al. (2009) presented that there was an increase of BUN, when cows fed urea-lime treated rice straw. This could be explained that the treatment levels of urea or lime were different.

The combination of 2% urea + 2% lime treating rice straw improved total VFA. The ratio of C2 and C3 was lower forthe urea-lime treated rice straw treatment which was similar to that of Wanapat et al. (2009 and 2013). This could be due to the improvement of rumen fermentation rate, which resulted in higher total VFA and propionic acid. Moreover, CH_4

production was lower for URL, treatment. Johnson and Johnson (1995) indicated that the methane production decreased as a result of higher soluble carbohydrate fermented in the rumen.

Ruminal microorganism population

The effect of untreated rice straw and urea-lime treated rice straw on microbial population in swamp buffaloes was shown in Table 4.

Table 4. Effect of rice straw and urea-lime treated rice straw on rumen microbial population (CFU/ml) by the roll tube technique

Items	Treatment		Significance[1]
	RS	***URL***	
Total viable bacteria, x 10^{10}	6.6^{a}	9.4^{b}	*
Cellulolytic bacteria, x 10^{10}	3.5^{a}	6.4^{b}	*
Proteolytic bacteria, x 10^{7}	2.1	2.7	ns
Amylolytic bacteria, x 10^{7}	4.4^{a}	5.9^{b}	*

*[1]RS = untreated rice straw, URL = urea-lime treated rice straw, * p<0.05, ns = non-significant (p>0.05).*

The improvement of total bacteria, cellulolytic and amylolytic bacteria population was significantly found in urea-lime treated rice straw treatment (P<0.05). The population of the total cellulolytic and amylolytic bacteria significantly increasedand were 9.4 x 10^{10}, 6.4 x 10^{10} and 5.9 x 10^{7} (CFU/ml) for the treated rice straw treatment, respectively. However, there was not signifcantly different of proteolytic population (P>0.05) between two treatments.

According to Goto et al. (1993), urea-lime treated rice straw softened up the ligno-cellulosic bond and partially damaged the lignin-polysaccharide bond, and hence increased the number of accessible sites of microbial attachment on the surface of the particles.Thus improvement of feed fermentation could be occurred. As a result of those, the bacterial population in the rumen could increase. Moreover, the findings showed that viable, cellulolytic and amylolytic bacterial populations were significantly higher for the urea-lime treated rice straw. This agreed with the finding of Wanapat et al. (2013), Similarly Bryant (1973) reported that in principle, cellulolytic bacteria species utilize ammonia as the main source of nitrogen for microbial protein synthesis. Thus a combination of urea-lime in the treatment could increase rumen microorganisms in this study.

Purine derivatives and microbial nitrogen synthesis

The Table 5 shows the effect of rice straw and urea-lime treated rice straw on urinary purine derivatives and MN synthesis in swam buffaloes.

The results revealed that the purine derivative excretion and absorption of urea-lime treated rice straw diet was higher (p<0.05) as compared to those untreated rice straw diet. In addition, microbial N synthesis and EMNS were higher (p<0.05) for the urea-lime treated treatment.

The purine excretion which is considered as an indicator of microbial production in rumen is originated from microbial purines and animal tissues absorption (Chen et al., 1992). In this study, the purine excretion, absorption and MNS increased in urea-lime rice straw diet, which was agreed with those in the findings of Wanapat et al. (2013). The microbial nitrogen synthesis was higher for the URL treatment, as a result of increase in purine excretion.

According to Hoover and Stokes (1991), the rate of digestion of carbohydrate as a major factor provides available energy for microbial synthesis. Microbial synthesis depends on NH3-N and C-skeletons synchronization, which fermentein the rumen, suitable for microbial protein synthesis. Under this study, the digestibility and NH_3-N content increased in URLtreatment leading to enhance the microbial protein synthesis.

Table 5. Effect of rice straw and urea-lime treated rice straw on urinary purine derivatives and microbial nitrogen synthesis in swamp buffaloes

Items	Treatment		Significance[1]
	RS	*URL*	
Urinary purine derivatives (mmol/d)			
Purine excretion	82.8	98.8	*
Purine absorption	60.6	79.3	*
MN supply[2] (g N/d)	44.0	57.6	*
EMNS[3] (gN/kg OMDR[4])	20.0	22.1	*

*[1]RS = untreated rice straw, URL = urea-lime treated rice straw, * p<0.05, ns = non-significant (p>0.05), [2]Microbial N supply, [3]Eficiency of microbial nitrogen synthesis, [4]Digestible OM apparently fermented in the rumen (assuming that rumen digestion was 650g/kg OM of digestion in the total tract).*

CONCLUSION

Based on the results of this study, it could be concluded that rice straw treated with 2% urea and 2% lime improved nutrient digestibility, rumen microbial populationand rumen fermentation efficiency, and enhanced microbial protein synthesis and microbial N synthesis efficiency. While CH_4 production was lower than untreated rice straw.

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