

Full Length Research Paper

## Effect of rice straw treatment on feed intake, rumen fermentation and milk production in lactating dairy cows

M. Wanapat\*, S. Kang, N. Hankla and K. Phesatcha

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

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This experiment was conducted to investigate the effect of urea and urea-calcium hydroxide treated rice straws on feed intake, rumen fermentation and milk production in lactating dairy cows. Three multiparous Holstein crossbred dairy cows, mid-lactation with  $365 \pm 30$  kg BW, were randomly assigned to receive three kinds of rice straw according to  $3 \times 3$  Latin square design: untreated (T1), 2 × 2% urea-calcium hydroxide treated (T2) and 3% urea treated (T3). Based on the present findings, urea-calcium hydroxide could enhance the nutritive value of rice straw. In addition, dry matter intake (DMI) and nutrient digestibility (DM, CP, OM, NDF and ADF) were significantly increased by straw treatments ( $P < 0.01$ ), however, no difference between urea and urea-calcium hydroxide treatments was found. In contrast, ruminal pH and temperature were similar among treatments ( $P > 0.05$ ). Ruminal  $\text{NH}_3\text{-N}$  concentration was increased in both treated straw groups while BUN was similar. While TVFA and C3 were improved in cows receiving urea and urea-calcium hydroxide treated rice straw, C2 and C4 remained stable. Furthermore, total bacterial and fungal zoospores were higher in the treated groups, while protozoa were not different. Ruminal viable bacterial groups were higher in the urea and urea-calcium hydroxide treated, except for proteolytic bacteria. Cows received treated rice straw consumed more N intake as well as N absorption ( $P < 0.05$ ). Remarkably, the PD, microbial protein and EMPS were enhanced by treated straw. Urea-calcium hydroxide treated rice straw significantly ( $P < 0.05$ ) increased milk yield and milk compositions. Based on this study, either 2 + 2% urea-calcium hydroxide or 3% urea treated rice straw could be fed to lactating dairy cows as for improvement of feed intake, nutrient digestibility, rumen fermentation and milk production, thus be recommended for use by dairy farmers.

**Key words:** Rice straw, urea, Calcium hydroxide, rumen fermentation, milk production, dairy cow.

### INTRODUCTION

Rice straw is the main crop residue which farmers usually store and use as ruminant feed in tropical areas especially during the long dry season whereby natural forages are being in constraint. Relative to low nutritive value of rice straw in terms of protein (2 to 5%), fiber and

lignin contents (NDF > 50%) and low digestibility (< 60%) (Wanapat et al., 1985), feeding rice straw only does not provide enough nutrients for optimum production requirement. Due to poor fermentation and low disappearance rate and passage through the rumen, feed

\*Corresponding author. E-mail: metha@kku.ac.th. Tel: +66 4320 2368. Fax: +66 4320 2368.

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

Items	Concentrate	Untreated rice straw	Urea-calcium hydroxide treated rice straw	Urea treated rice straw
<b>Ingredient (%)</b>				
Cassava chip	60.0			
Rice bran	12.2			
Coconut meal	5.8			
Palm kernel meal	13.6			
Molasses	2.0			
Sulfur	1.0			
Salt	1.0			
Mineral mixture	1.0			
Urea	3.4			
<b>Chemical compositions (%)</b>				
Dry matter	85.9	87.8	54.2	50.5
<b>%DM</b>				
Organic matter	93.0	88.8	86.2	88.1
Crude protein	16.0	2.7	5.8	6.0
Neural detergent fiber	18.2	84.8	76.5	74.5
Acid detergent fiber	8.2	63.7	56.2	54.1

intake was reduced (Conrad, 1966).

Various treatment methods have been used to improve nutritive value of rice straw including physical, biological and chemical treatment (Wanapat et al., 1996). Chemical and physical treatment of rice straw has been widely practiced as a method of improving intake and digestibility. Even though there are many researches on the use of chemical treatments on how to improve the nutrition value of rice straw such as urea, it is always an economical decision due to the increase of urea price. Recently, lime ( $\text{CaO}/\text{Ca}(\text{OH})_2$ ) has been thought as a potential alkali for straw treatment since it is cheap and readily available especially related to softening the lignin-cellulose bonds (Owen et al., 1984; Sundstøl, 1985; Doyle et al., 1986). Moreover, calcium (Ca) residues, which remains in the treated straw, causes no serious problems to the animal or the environment (Chaudhry, 1998) and can be a supplement to rice straw which is deficient in Ca when used as cattle feed.

Interestingly, combinations of lime (calcium hydroxide) and urea may be beneficial for straw treatment (Owen et al., 1984; Sundstøl, 1985). Such a mixture would be able to combine treatment effects on both chemicals together with the added calcium and nitrogen in the straw (Hadjipanayiotou, 1984; Sirohi and Rai, 1995). In such a combination either residual  $\text{NH}_3$  or Ca would be necessarily as high as if the chemicals were used alone. In addition, since the amount of urea can be reduced at the expense of a cheaper chemical (lime), the mixture would be more economic, as long as the overall treatment effect is maintained or enhanced. Therefore,

the objectives of this experiment were to compare untreated and urea-calcium hydroxide treated rice straw influenced on feed intake, nutrient digestibility, rumen fermentation and milk production in lactating dairy cows.

## MATERIALS AND METHODS

### Feed preparation, animals and experimental design

Three multifarious Holstein crossbred dairy cows (50% Holstein Friesian  $\times$  50% Thai native cows) in mid-lactation with  $365 \pm 30$  kg BW were randomly allocated to three kinds of rice straw: untreated (T1), 2+2% urea-calcium hydroxide treated (T2) and 3% urea treated (T3) according to a  $3 \times 3$  Latin square design. The urea treatment involved adding 3 kg of urea in 100 L water to 100 kg air-dry rice straw. The relevant volume of urea solution was then sprayed onto a stack of 5 whole straw bales (mechanically baled straw; each bale weighing approximately 20 kg) and then covered the stack with a plastic sheet for a minimum of 10 days before feeding directly to animals. Similarly, urea-calcium hydroxide treatment involved adding 2 kg urea and 2 kg calcium hydroxide ( $\text{Ca}(\text{OH})_2$ ) in 100 L to 100 kg air-dry rice straw. Concentrate ingredients and rice straw chemical compositions are shown in Table 1.

All cows were housed in individual pens and individually fed with concentrate diets at a ratio of concentrate to milk yield of 1:2 twice daily at 06.00 a.m. and 16.00 p.m. during milking time. Cows were fed *ad libitum* of rice straw as allowing for 100 g/kg refusals. The experiment was run in three periods; each lasted for 21 days which first 14 days for rice straw intake determination while the last 7 days for sample collection. Body weights were measured at the first and last days of samplings in each period. Milk yield was recorded during the 21 days of each period. Clean fresh water and mineral blocks were available at all times.

### Sampling procedure, data collection and analysis

Rice straw and concentrate were sampled daily during the collection period and were composited by period prior to chemical analyses. Feeds, fecal and urine samples were collected during the last seven days of each period. Fecal samples were collected at 09.00 or 12.00 h by rectal sampling, while urine samples were collected by spot sampling. Urination was induced by manual stimulation of the vulva, the sample was collected at 09.00 or 12.00 h. When fecal or urine samples were taken at 3 h intervals, two successive samples were combined and used as one sample. Composited samples were dried at 60°C, ground (1-mm screen using Cyclotech Mill and Tecator) and then analyzed for DM (ID 967.03), ash (ID 942.05) and CP (ID 984.13) (AOAC, 1995). Acid detergent fiber was determined according to AOAC (1995; ID 973.18) and was expressed inclusive of residual ash. Neutral detergent fiber in samples was estimated according to Van Soest et al. (1991) with addition of  $\alpha$ -amylase but without sodium sulphite and results are expressed with residual ash. Acid insoluble ash was used to estimate digestibility of nutrients (Van Keulen and Young, 1977).

Milk samples were composited daily according to the yield for both morning and afternoon milking, preserved with 2-bromo-2-nitropropane-1, 3 dial and stored at 4°C until analysis for fat, protein, lactose, totals solids and solids-not-fat content by infrared methods using Milko-Scan 33 (Foss Electric, Hillerod and Demark). Milk urea nitrogen was determined using Sigma kits #640 (Sigma Diagnostics, St. Louis, MO).

At the end of each period, rumen fluid samples were collected at 0 and 4 h post feeding. Approximate 200 ml of rumen fluid was taken from the rumen by a stomach tube connected with a vacuum pump at each time. Rumen fluid was immediately measured for pH and temperature using (Hanna Instruments HI 8424 microcomputer, Singapore) after withdrawal. Rumen fluid samples were then filtered through 4 layers of cheesecloth and divided into 3 portions. The first 45 ml of rumen fluid sample was collected and kept in a plastic bottle to which 5 ml of 1 M H<sub>2</sub>SO<sub>4</sub> was added to stop fermentation process of microbe activity and then centrifuged at 3,000 × g for 10 mins. About 20 to 30 ml of supernatant was collected and analyzed for NH<sub>3</sub>-N by Kjeltach Auto 1030 Analyzer (AOAC, 1995; ID 973.18) and VFA using High Pressure Liquid Chromatography (HPLC, Instruments by Water and Novapak model 600E; water mode I484 UV detector; column novapak C18; column size 3.9 mm × 300 mm; mobile phase 10 mM H<sub>2</sub>PO<sub>4</sub> [pH 2.5]) according to Samuel et al. (1997).

The second portion of 1 ml rumen fluid was collected and 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 by using total direction counts of bacterial, protozoa and fungal zoospores using methods of Galyean (1989) by haemocytometer (Boeco, Singapore). The third portion was for the total viable bacteria count (cellulolytic, proteolytic, and amylolytic) and total viable bacteria using the Hungate (1969) roll-tube technique. Total viable counts of bacteria were determined in roll tubes on a complete agar medium (Hobson, 1969) while the numbers of cellulolytic bacteria were estimated as the most probable number in agar (Hobson, 1969). In addition, while proteolytic bacteria were determined as casein medium, amylolytic bacteria were estimated as starch medium (Hobson, 1969). Culture methods were based on those described by Hungate (1969).

A blood sample (about 10 ml) was drawn from the jugular vein at the same time as rumen fluid. Blood samples were immediately placed on the ice and transported to the laboratory for separating plasma from the whole blood. Samples were refrigerated for 1 h and then centrifuged at 3,500 × g for 20 min. The plasma were removed, stored at -20°C and analyzed for blood urea nitrogen (BUN) composition according to method of Crocker (1967). Urine sample was analyzed for allantoin and creatinine 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).

### Statistical analysis

The data were analyzed in a 3 × 3 Latin square design by analysis of variance run in the PROC GLM of SAS (SAS Institute Inc., 1998). Data were analyzed using the model:  $Y_{ijk} = \mu + M_i + A_j + P_k + \epsilon_{ijk}$ ; where  $Y_{ijk}$ , observation from animal  $j$ , receiving diet  $i$ , in period  $k$ ;  $\mu$ , the overall mean;  $M_i$ , effect of roughage source ( $i = 1, 2, \text{ and } 3$ );  $A_j$ , the effect of animal ( $j = 1, 2, \text{ and } 3$ );  $P_k$ , the effect of period ( $k = 1, 2, \text{ and } 3$ );  $\epsilon_{ijk}$ , the residual effect. The results were presented as mean values and standard error of the means. Differences between treatment means were determined by Duncan's New Multiple Range Test (Steel and Torrie, 1980). Differences among means with  $P < 0.05$  were accepted as representing statistically significant differences.

## RESULTS

### Chemical compositions of feeds

Table 1 shows feed ingredient of concentrate, the three kinds of rice straw and their chemical compositions. The nutritive values of the rice straw were improved by treatments of urea-calcium hydroxide. The CP was increased up to 5.8 and 6.0% in urea-calcium hydroxide and urea treated rice straw, respectively, while untreated contained only 2.7%. Moreover, urea-calcium hydroxide and urea treatment could decrease the proportion of the aNDF and ADF down to 76.5, 74.5, 56.2 and 54.1%, respectively. On the other hand, concentrate ingredients were based on local resources, consisting of cassava chips, rice bran, coconut meal and palm kernel meal, which had a higher quality in terms of CP and low in fiber (16.0 and 18.2%, respectively). This concentrate was well consumed by the dairy cows during the experimental periods.

### Feed intake and nutrient digestibility

The effect of various treated rice straw on the voluntary feed intake and nutrients digestibility in lactating dairy cows are presented in Table 2. The findings under this investigation revealed that rice straw DMI was significantly different among all treatments ( $P < 0.05$ ) and the highest was in urea-calcium hydroxide treated group while the lowest was in untreated. However, there was no change in concentrate intake to all group while total DMI were found higher in either urea or urea-calcium hydroxide treated groups ( $P < 0.05$ ). Total DMI was increased to 11.4 and 10.8 kg/d in urea-calcium hydroxide and urea treated rice straw, respectively. Moreover, the present study had shown a significant increase in apparent digestibility as affected by urea-calcium hydroxide treatment on rice straw. The DM, OM, CP, aNDF and ADF digestibility were higher in treated

**Table 2.** Effect of various treated rice straw on voluntary feed intake and nutrients digestibility in dairy cows.

Item	Untreated rice straw	Urea-calcium hydroxide treated rice straw	Urea treated rice straw	SEM	Contrast <sup>1</sup>	
					L	Q
<b>Rice straw DM intake</b>						
kg/d	5.0 <sup>a</sup>	7.9 <sup>b</sup>	7.0 <sup>c</sup>	0.03	**	**
% BW	1.43 <sup>c</sup>	2.23 <sup>a</sup>	1.95 <sup>b</sup>	0.03	**	**
g/kg BW <sup>0.75</sup>	61.7 <sup>a</sup>	96.2 <sup>b</sup>	84.7 <sup>c</sup>	1.10	**	**
<b>Concentrate DM intake</b>						
kg/d	3.8	3.5	3.8	0.23	ns	ns
% BW	0.99	0.98	1.00	0.05	ns	ns
g/kg BW <sup>0.75</sup>	43.9	42.5	44.2	2.50	ns	ns
<b>Total DM intake</b>						
kg/d	8.8 <sup>a</sup>	11.4 <sup>b</sup>	10.8 <sup>b</sup>	0.20	*	*
% BW	2.43 <sup>c</sup>	3.21 <sup>a</sup>	2.96 <sup>b</sup>	0.03	*	**
g/kg BW <sup>0.75</sup>	105.4 <sup>a</sup>	138.8 <sup>b</sup>	128.8 <sup>c</sup>	1.70	*	*
<b>Apparent digestibility, kg/kg</b>						
Dry matter	0.49 <sup>a</sup>	0.58 <sup>b</sup>	0.60 <sup>b</sup>	0.05	**	*
Organic matter	0.55 <sup>a</sup>	0.64 <sup>b</sup>	0.64 <sup>b</sup>	0.04	*	*
Crude protein	0.50 <sup>a</sup>	0.61 <sup>b</sup>	0.60 <sup>b</sup>	0.03	*	*
Neutral detergent fiber	0.45 <sup>a</sup>	0.60 <sup>b</sup>	0.58 <sup>b</sup>	0.04	*	*
Acid detergent fiber	0.43 <sup>a</sup>	0.52 <sup>b</sup>	0.51 <sup>b</sup>	0.02	**	**

<sup>abc</sup> Values within the row with different superscripts are significantly different ( $P < 0.05$ ), \*  $P < 0.05$ , \*\*  $P < 0.01$ . <sup>1</sup> L = linear, Q = Quadratic.

than untreated rice straw. However, there was no difference between urea and urea-calcium hydroxide treatment on nutrients digestibility.

### Rumen fermentation and blood metabolites

The rumen fermentation and blood metabolites affected by various treated rice straw in dairy cows are presented in Table 3. The result revealed no significant difference on ruminal pH and temperature in all groups ( $P > 0.05$ ) and were in normal range 6.7 to 6.8°C and 39.1 to 39.4°C, respectively. In contrast, ruminal NH<sub>3</sub>-N was found higher in treated groups as compared to untreated group ( $P < 0.05$ ), while BUN concentration were stable in all groups ranging from 12.8 to 14.8 mg/dl. The average values of NH<sub>3</sub>-N in this study were 12.6 to 15.2 mg/dl. In addition, TVFA and C3 were significantly improved by the urea-calcium hydroxide treatment ( $P < 0.05$ ), while no difference were found in all groups on C2 and C4 ( $P > 0.05$ ). The shift increase in the molar proportion of C3 resulted in a lower C2:C3 ratio in ruminal fluid of animals receiving both kinds of treated rice straw.

### Ruminal microorganism population

Table 4 illustrates the result on ruminal microorganism

population affected by various treated rice straw in lactating cows. As shown, bacteria and fungi zoospores count were significantly different among all treatments and the highest was in either urea-calcium hydroxide treated rice straw ( $P < 0.05$ ). However, there was no effect of treated straw on protozoa population ( $P > 0.05$ ). Bacteria and fungi zoospores were found higher in treated rice straw than in untreated. In treated groups, the values ranged from 7.0 to 7.4 × 10<sup>9</sup> and 2.7 to 3.8 × 10<sup>5</sup> while in untreated group was 6.5 × 10<sup>9</sup> and 2.1 × 10<sup>5</sup> cell/ml; bacteria and fungi zoospores, respectively. Within the treated group, however, treatment with urea-calcium hydroxide treated rice straw tended to be higher than urea treated rice straw alone. Moreover, in this study, total viable, cellulolytic and amylolytic bacteria counts were significantly higher in treated rice straw groups as compared to untreated ( $P < 0.05$ ), while proteolytic bacteria was similar in all groups ( $P > 0.05$ ). However, no difference was found among treated groups on microorganism population.

### Nitrogen utilization and purine derivative

Nitrogen utilization and purine derivative in dairy cows affected by various treated rice straw are shown in Table 5. There was an increase in nitrogen intake and nitrogen absorption in treated rice straw, but no difference among

**Table 3.** Effect of various treated rice straw on ruminal fermentation and blood urea nitrogen in dairy cows.

Item	Untreated rice straw	Urea-calcium hydroxide treated rice straw	Urea treated rice straw	SEM	Contrast <sup>1</sup>	
					L	Q
<b>Ruminal (pH)</b>						
0-h post feeding	6.82	6.72	6.69	0.01	ns	ns
4-h post feeding	6.71	6.66	6.66	0.15	ns	ns
Mean	6.77	6.70	6.68	0.10	ns	ns
<b>Temperature (°C)</b>						
0-h post feeding	38.9	38.9	38.3	1.15	ns	ns
4-h post feeding	39.5	40.0	39.8	0.21	ns	ns
Mean	39.2	39.4	39.1	0.60	ns	ns
<b>NH<sub>3</sub>-N (mg/dl)</b>						
0-h post feeding	7.9	9.5	10.9	1.10	ns	ns
4-h post feeding	17.3 <sup>a</sup>	20.1 <sup>b</sup>	19.5 <sup>b</sup>	0.68	*	*
Mean	12.6 <sup>a</sup>	14.8 <sup>b</sup>	15.2 <sup>b</sup>	0.71	*	*
<b>BUN (mg/dl)</b>						
0h post feeding	9.0	11.5	10.5	1.18	ns	ns
4h post feeding	16.2	17.7	17.2	1.15	ns	ns
Mean	12.8	14.8	13.8	1.07	ns	ns
<b>Total VFA (mmol/L)</b>						
0-h post feeding	92.5 <sup>a</sup>	96.0 <sup>a</sup>	102.0 <sup>b</sup>	0.54	**	ns
4-h post feeding	110.4 <sup>a</sup>	119.1 <sup>b</sup>	110.1 <sup>a</sup>	0.74	ns	*
Mean	101.5 <sup>a</sup>	107.6 <sup>b</sup>	106.0 <sup>b</sup>	0.62	*	*
<b>VFA (mol/100 mol)</b>						
<b>Acetic acid (C2)</b>						
0-h post feeding	73.6	70.3	72.2	0.56	ns	ns
4-h post feeding	81.6	81.2	78.1	0.71	0.09	ns
Mean	77.6	75.8	75.2	0.61	ns	ns
<b>Propionic acid (C3)</b>						
0-h post feeding	16.4 <sup>a</sup>	19.1 <sup>b</sup>	20.3 <sup>b</sup>	0.65	**	ns
4-h post feeding	20.0 <sup>a</sup>	26.3 <sup>b</sup>	24.5 <sup>b</sup>	0.39	ns	*
Mean	18.2 <sup>a</sup>	22.7 <sup>b</sup>	22.4 <sup>b</sup>	0.13	**	**
<b>Butyric acid (C4)</b>						
0-h post feeding	7.5	6.7	7.4	0.27	ns	ns
4-h post feeding	7.7	11.6	10.6	3.75	ns	ns
Mean	7.6	9.1	9.0	1.82	ns	ns
<b>C2:C3 ratio</b>						
0-h post feeding	4.5 <sup>a</sup>	3.7 <sup>b</sup>	3.5 <sup>b</sup>	0.24	*	0.06
4-h post feeding	4.1 <sup>a</sup>	3.1 <sup>b</sup>	3.2 <sup>b</sup>	0.02	**	**
Mean	4.3 <sup>a</sup>	3.4 <sup>b</sup>	3.4 <sup>b</sup>	0.13	*	*

<sup>abc</sup> Values within the row with different superscripts are significantly different ( $P < 0.05$ ), \*  $P < 0.05$ , \*\*  $P < 0.01$ .<sup>1</sup> L = linear, Q = Quadratic.

treated groups was found under this study. However, nitrogen intake from rice straw was found the highest in

treatment with urea-calcium hydroxide and treated rice straw among the groups ( $P < 0.05$ ).

**Table 4.** Effect of various treated rice straw on microbial population in the rumen of dairy cows.

Item	Untreated rice straw	Urea-calcium hydroxide treated rice straw	Urea treated rice straw	SEM	Contrast <sup>1</sup>	
					L	Q
<b>Total direct count, cell/ml</b>						
<b>Bacteria × 10<sup>9</sup></b>						
0-h post feeding	6.2 <sup>a</sup>	6.8 <sup>b</sup>	6.0 <sup>a</sup>	0.65	ns	*
4-h post feeding	6.7 <sup>a</sup>	8.0 <sup>b</sup>	8.0 <sup>b</sup>	1.36	*	0.09
Mean	6.5 <sup>a</sup>	7.4 <sup>b</sup>	7.0 <sup>b</sup>	0.51	*	*
<b>Protozoa × 10<sup>5</sup></b>						
0-h post feeding	9.0	4.8	3.8	0.44	**	ns
4-h post feeding	12.3	12.0	14.2	2.02	ns	ns
Mean	10.7	8.4	9.0	1.20	ns	ns
<b>Fungi × 10<sup>5</sup></b>						
0-h post feeding	1.7	3.0	2.0	0.44	ns	ns
4-h post feeding	2.5	4.5	3.3	0.36	ns	ns
Mean	2.1 <sup>a</sup>	3.8 <sup>b</sup>	2.7 <sup>ab</sup>	0.22	ns	*
<b>Viable bacterial, CFU/ml</b>						
<b>Total × 10<sup>9</sup></b>						
0-h post feeding	4.0	4.7	4.4	0.30	ns	ns
4-h post feeding	7.5 <sup>a</sup>	12.7 <sup>b</sup>	11.9 <sup>b</sup>	0.23	**	*
Mean	5.7 <sup>a</sup>	8.7 <sup>b</sup>	8.1 <sup>b</sup>	0.26	*	*
<b>Cellulolytic × 10<sup>8</sup></b>						
0-h post feeding	0.8 <sup>a</sup>	1.5 <sup>ab</sup>	2.3 <sup>b</sup>	0.18	*	ns
4-h post feeding	1.8 <sup>a</sup>	3.4 <sup>b</sup>	1.6 <sup>a</sup>	0.07	ns	**
Mean	1.3 <sup>a</sup>	2.4 <sup>b</sup>	2.0 <sup>b</sup>	0.08	*	*
<b>Amylolytic × 10<sup>7</sup></b>						
0-h post feeding	2.7	3.15	2.53	0.16	ns	ns
4-h post feeding	5.7	7.8	6.8	0.41	0.06	ns
Mean	4.2 <sup>a</sup>	5.5 <sup>b</sup>	4.7 <sup>ab</sup>	0.16	ns	*
<b>Proteolytic × 10<sup>7</sup></b>						
0-h post feeding	0.3	0.7	0.8	0.26	ns	ns
4-h post feeding	2.8	4.2	3.8	0.31	ns	ns
Mean	1.6	2.5	2.3	0.28	ns	ns

<sup>ab</sup> Values within the row with different superscripts are significantly different ( $P < 0.05$ ), \*  $P < 0.05$ .<sup>1</sup> L = linear, Q = Quadratic.

Furthermore, purine derivatives (allantoin excretion and absorption) were found significantly different among the treatments ( $P < 0.05$ ), except for urine creatinine. In addition, the treatments with urea-calcium hydroxide were found higher than untreated rice straw in MCP and EMNS.

### Milk production and compositions

The effect of various treated rice straw on milk yield and

compositions in lactating dairy cows during the experiment are shown in Table 6. There was an increase in milk yield and production of 3.5% FCM in both urea-calcium hydroxide and treated rice straw as compared to untreated ( $P < 0.05$ ).

The milk yield and 3.5% FCM production ranged from 7.4 to 9.6 and 8.1 to 10.9 kg/d, respectively. However, under this investigation, milk composition were found no different among all treatments ( $P > 0.05$ ). Moreover, there was no difference in MUN in all groups and ranged from 10.7 to 13.7 mg/dl.

**Table 5.** Effect of various treated rice straw on nitrogen utilization, purine derivations and efficiency of microbial nitrogen synthesis in dairy cows.

Item	Untreated rice straw	Urea-calcium hydroxide treated rice straw	Urea treated rice straw	SEM	Contrast <sup>1</sup>	
					L	Q
<b>N utilization (g/d)</b>						
<b>N intake</b>						
Rice straw	21.6 <sup>a</sup>	73.0 <sup>b</sup>	67.3 <sup>c</sup>	0.42	**	**
Concentrate	97.7	90.8	99.7	6.00	ns	ns
Total	119.4 <sup>a</sup>	163.8 <sup>b</sup>	165.0 <sup>b</sup>	5.58	*	ns
N Faces	54.1	61.4	64.7	3.53	ns	ns
N Absorption	65.3 <sup>a</sup>	102.4 <sup>b</sup>	100.4 <sup>b</sup>	3.74	*	0.06
<b>Purine derivatives (mmol/d)</b>						
Allantoin excretion	125.4 <sup>a</sup>	171.1 <sup>b</sup>	181.6 <sup>b</sup>	7.71	*	*
Allantoin absorption	121.9 <sup>a</sup>	169.8 <sup>b</sup>	173.5 <sup>b</sup>	1.32	*	*
Urine creatinine	20.5	21.2	21.4	0.57	ns	ns
Microbial protein <sup>2</sup> , g/d	428.3 <sup>a</sup>	611.7 <sup>b</sup>	620.2 <sup>b</sup>	6.89	*	*
EMNS, gN/kg OMDR <sup>3</sup>	11.9 <sup>a</sup>	14.6 <sup>b</sup>	15.0 <sup>b</sup>	2.63	*	*

<sup>abc</sup> Values within the row with different superscripts are significantly different (P<0.05), \* P<0.05, \*\* P<0.01. <sup>1</sup> L = linear, Q = Quadratic. <sup>2</sup> Microbial protein (MCP) (g/d) = 3.99x0.856x mmoles of purine derivatives excreted (Galo et al., 2003). <sup>3</sup> Efficiency of microbial N synthesis (EMNS, g/kg of OM digested in the rumen (OMDR) = [(MCP (g/d) x 1000)/DOMR (g)], assuming that rumen digestion = 65% of digestion in total tract.

**Table 6.** Effect of various treated rice straw on milk yield and milk compositions in dairy cows.

Item	Untreated rice straw	Urea-calcium hydroxide treated rice straw	Urea treated rice straw	SEM	Contrast <sup>1</sup>	
					L	Q
<b>Production</b>						
Milk yield, kg/d	7.4 <sup>a</sup>	9.6 <sup>b</sup>	9.5 <sup>b</sup>	0.12	**	*
3.5% FCM <sup>2</sup> , kg/d	8.1 <sup>a</sup>	10.9 <sup>b</sup>	10.4 <sup>b</sup>	0.35	*	0.06
<b>Milk composition, %</b>						
Protein	3.2	3.5	3.3	0.08	ns	ns
Fat	4.0	4.5	4.2	0.03	ns	ns
Lactose	4.5	4.6	4.5	0.08	ns	ns
Solids-not-fat	8.4	8.4	8.5	0.13	ns	ns
Total solids	12.5	13.7	13.6	0.40	ns	ns
Milk urea N, mg/dl	10.7	13.7	12.0	1.01	ns	ns

<sup>a,b</sup> Means in the same row with different superscripts are different (P<0.05), \* P<0.05, \*\* P<0.01. <sup>1</sup> L = linear, Q = Quadratic. <sup>2</sup> 3.5% FCM (fat collected milk) = 0.432 (kg of milk/d) + 16.23 (kg of fat).

## DISCUSSION

### Chemical composition of feed

The nutritive value of the rice straw has been improved by urea-calcium hydroxide treatments. These values were similar to those values which were reported by Wanapat et al. (2009) who conducted by using 2.2 + 2.2% urea-calcium hydroxide treated rice straw. As having been reported by Schiere and Ibrahim (1989), rice

straw can be treated with urea, which releases ammonia after dissolving in water. However, there is no change in the N-content of the straw when treated with calcium hydroxide. On the other hand, calcium hydroxide is a weak alkali agent with a low solubility in water. It has been reported that calcium hydroxide can be used to improve the utilization of straw and also can be used to supplement the ration with calcium, which has been found to be in a negative balance in cattle fed only rice straw (Hadjipanayiotou, 1984; Pradhan et al., 1997;

Chaudhry, 1998). It was suggested that a combination of lime and urea would give better results than urea or lime alone. This combination has the advantage of an increased degradability and an increased content of both calcium and nitrogen. In addition, concentrate ingredients used in this study were based on local resources, consisting of cassava chips, rice bran, coconut meal and palm kernel meal, had a higher quality in terms of CP and low in fiber. This concentrate was well consumed by the dairy cows during the experimental periods.

### Feed intake and nutrients digestibility

The findings under this investigation revealed the highly significant difference in improvement on DMI when cows were fed with urea-calcium hydroxide and urea treated rice straw compared to the untreated treatment. These results were relatively higher than those found by Qingxiang (2002), who found that the treatment of urea and calcium hydroxide could slightly increase DMI in dairy cows when compared with untreated rice straw. This could be due to the differences in animals and their physiology. Moreover, Mould et al. (1982) found that when treated straw with 4 kg urea with 1 kg calcium hydroxide could increase intake from 2.9 to 4.2 kg/d, but if treated with urea only, the intake increased to 3.94 kg/d. This finding was similar to the present study which found urea-calcium hydroxide treated group had the highest result among all treatments. This means the combination between urea and calcium hydroxide could have more effectiveness on intake of rice straw. The increase in straw intake under the present experiment may thus be explained by virtue of its increased degradability in the rumen as reported by Trach et al. (2001). In addition, an increase in the outflow of straw cell walls into the abomasum as a result of alkali treatment has also been reported (Males, 1987). These possible effects of alkali treatment can aid in explaining the increases in straw intake in the present study.

Furthermore, the present study had shown a significant difference in apparent digestibility affected by various treated rice straw. The increases of DM, OM, CP, aNDF and ADF digestibility in urea-calcium hydroxide treated rice straw treatments were consistent with the earlier findings of other researchers (Fadel Elseed et al., 2003; Trach et al., 2001). In this study, with effect of alkali agents, linkage between lignin and hemicellulose was broken, which resulted in increasing the feed surface area for microbial attack to digest fiber; therefore an increased rice straw digestibility was obtained. Increased straw digestibility due to urea treatment has been well documented previously (Chenost and Kayouli, 1997; Madrid et al., 1997). Improvements in straw apparent digestibility as a result of treatment with calcium hydroxide and urea in combination have also been reported by Zaman and Owen (1990) and Sahoo et al.

(2000). Since the rumen is the primary site for fibre digestion, the increases in apparent digestibility of the treated straw were presumably due to increased rumen degradability resulted from increased susceptibility of structural carbohydrates of straw cell walls to rumen fermentation as well as more energy being made available for better growth of rumen microbes which degrade straw (Silva and Ørskov, 1988; Rai and Mudgal, 1988). The rumen retention time is actually not sufficient for the maximal fermentation of the substrate, thus an increase in degradation rate, as a result of increased straw degradability and better microbial activity, would cause a substantial improvement in digestibility and also in voluntary intake (Ørskov, 1994).

### Characteristics of ruminal fermentation and blood metabolites

There was no effect of lime and urea treatment on the rumen pH and temperature. However, ruminal  $\text{NH}_3\text{-N}$  were found higher with urea-calcium hydroxide treated rice straw fed group. The average values of  $\text{NH}_3\text{-N}$  in this study were 12.6 to 15.2 mg/dl and were in the optimal range in ruminal fluid for microbial growth which was reported from 5.0 to 25.0 mg/dl and (Preston and Leng, 1987). The present result was similar to the finding of Wanapat et al. (2009). However, there was no difference in BUN among all groups. This finding was not consistent with the finding of Wanapat et al. (2009), who found that there was an increase in BUN when cows were fed with urea-calcium hydroxide treated rice straw. However, this might have been due to the percentage of urea or calcium hydroxide used in the treatment and the lactation period of the experimental cows.

Moreover, total ruminal VFA and propionic acid were significantly different ( $P < 0.05$ ) and were higher in treated rice straw treatments. The shift in the molar proportion of C3 resulted in a lower C2:C3 ratio in ruminal fluid of animals receiving both of treated rice straw. Ørskov (1999) discussed that, when high fiber diets were offered, the VFAs in ruminal fermentation fluctuated from 65:25:10 to 70:20:10 (C2:C3:C4, in molar percentage) ratios. The results of VFAs in treated groups were similar and were higher ( $P < 0.05$ ) than those in the control group (untreated rice straw). Wanapat et al. (2009) also found the same result with the present study that when the animals were fed with urea or urea-lime treated rice straw would result in higher in total VFA and C3, which led to lower down the proportion of C2 to C3 ratio, if compared to untreated rice straw. The general increases in VFA due to the treatments were probably a reflection of the improvements in the ruminal fermentation rate as previously found (Trach et al., 2001), which resulted in increased OMD and NDF. Those groups with higher digestibilities showed higher values of VFA. Jayasuriya et al. (1987) have concluded that the output of VFA



increases with the increase in feed intake. This explains the increased VFA content under the present study.

### Ruminal microorganism population

Table 4 illustrates data on rumen microbes' population using direct count and roll-tube technique. There were significant differences in bacteria and fungi zoospores count among the treatments, while protozoa population was not affected by treated treatments. Bacteria and fungi zoospores were found higher in treated rice straw than in untreated. The treated group ranged from  $7.0$  to  $7.4 \times 10^9$  and  $2.7$  to  $3.8 \times 10^5$ , and untreated was  $6.5 \times 10^9$  and  $2.1 \times 10^5$  cell/ml bacteria and fungi zoospores, respectively. Within the treated group, however, treatment with urea-lime treated rice straw tended to be higher than urea treated rice straw alone. According to Chen et al. (2008) reported that chemical treatments enhanced the nutritive value of rice straw through increasing the number of accessible sites of microbial attachment on the surface of the particles, increasing fibrolytic microbe quantity and hence, fibrolytic enzyme activities, and improving rumen fermentation characteristics. Moreover, in this study total viable, cellulolytic and amylolytic bacteria counts were significantly different between treated and untreated groups, while proteolytic bacterial was not affected. The treated groups had more population than the untreated one. However, no difference within treated group was found. This is in contrast with the finding of Mapato et al. (2010) who compared between the untreated and urea treated rice straw with different level of vegetable oil and found no difference of microbial growth between treated and untreated rice straw group.

In the present study, it was found that in the treatment with urea-lime enhanced ruminal microbes than by urea treatment alone. This could be explained by Zaman (1994) who reported that a level of at least 1.5 kg urea is needed to prevent mold in straw ensiled with lime while Nguyen et al. (2001) observed that the combination of 3 kg lime and 2 kg urea can be biologically justified, at least as an alternative to 4 kg urea alone for straw treatment. Moreover, Goto et al. (1993) reported that alkaline treatment partially damages the lignin-polysaccharide bond that solubilizes hemicellulose and lignin in straw, and hence, exposes the cellulose to microbial attack. That is why under this study the combination between urea and lime in treated rice straw would be easily degraded on adhering rumen microorganisms by softening up the ligno-cellulosic bonding and thus improving the overall quality of the straw.

### Nitrogen utilization and purine derivative

There was an increase in nitrogen intake and nitrogen

absorption in treated rice straw, but no difference among treated groups was found under this study. This may be due to the nutrient composition of the rice straw as well as DMI and nutrient digestibility which had been improved by the urea and/or calcium hydroxide treatment (Tables 1 and 2). According to Chen and Gomes (1995), N excretion and retention could reflect on differences in N metabolism because nitrogen balance was the most important index of the protein nutrition status in ruminants (Ørskov, 1999). In this study, it was observed that N balance was high in treated rice straw. These values indicated that all treatments had more protein and therefore, higher protein available for use by cattle when animals were fed with treated straw as compared to untreated.

Urinary excretion of purine derivative is considered to be an indicator of microbial production in rumen. The maximum potential of rumen microbes to produce microbial protein can be explored only by the provision of high-quality forage (Russell, 2001). In the present study, purine derivatives (allantoin excretion and absorption) were found significant different among the treatments, except for urine creatinine were similar among treatments. In addition, the treatments with urea-calcium hydroxide were found higher than untreated rice straw in microbial crude protein and EMNS. According to Chen and Gomes (1995), urinary allantoin excretion was significantly increased by an increased proportion of increased level of intake. As reported by Galo et al. (2003), one indicator of the efficiency of rumen nitrogen use is the amount of microbial protein (MCP) flow from the rumen, which is a consequence of microbial growth and its washout from the rumen. It is clear that dietary DM intake affected the purine excretion. The allantoin excretion of this study significantly increased when animals were fed with urea-calcium hydroxide treated rice straw.

### Milk production and compositions

Table 6 presents the effect of various treated rice straw on milk yield and composition in lactating dairy cows during the experiment. Milk yield was not significantly different ( $P > 0.05$ ) among all treatments, but tended to increase in the two treated rice straw diet. Consistently, all cows receiving treated rice straw and untreated had a similar production of 3.5% FCM, ranged in 8.1 to 10.3 kg/d. Even though there was no significant difference among all treatments, both milk yield and 3.5% FCM in the treated groups tended to have a higher result than untreated. Moreover, this was consistent with the finding of Wanapat et al. (2009), Promma et al. (1984) and Djibrillou et al. (1998) who reported that treated rice straw with urea could improve feed intake, digestibility and rumen ecology. In this result, it was similar to who conducted the trial to compare between untreated, urea

treated, and urea-lime treated resulted in similar milk yield among treatments. According to Prasad et al. (1998), milk yield and 3.5% FCM production were not significantly different between the three rations indicating the rations containing urea treated rice straw which could maintain the milk production profiles similar to the ration containing untreated straw. Similar results were recorded by Lanting et al. (1989) and Talukder et al. (1990) with urea treated rice straw. However, Mapato et al. (2010) found the increase in milk yield and 3.5% FCM in the urea treated rice straw. There was an increase in fat concentration among the treatments and the highest was treatment with urea-lime treated rice straw, while milk protein, lactose, solids-not-fat, total solids, and milk urea nitrogen were similar in all treatments. It was reported by Mba et al. (1975) that urea treated of straw could increase in milk fat concentration. However, Wanapat et al. (2009) found both increase in fat and protein concentration in treated groups which in contrast with the result in this study that no difference in protein concentration. This could be due to the concentration of urea treated level of the treatment and was higher than in the present study. Prasad et al. (1998) have shown fat and solids-not-fat production tended to be higher on urea treated straw rations due to the tendency for a higher milk production.

## CONCLUSIONS AND RECOMMENDATIONS

Based on this study, it could be concluded that 2 + 2% urea-lime and 3% urea treated rice straw could improve nutritive value of rice straw through increasing the DM intake, nutrient digestibility, rumen ecology, and milk yield and composition in lactating dairy cows. The 2 + 2% urea-lime treated rice straw tended to have more effect on the rice straw efficiency than 3% urea treated rice straw. This study suggested that 2 + 2% urea-lime treated rice straw can be used as a good roughage source for dairy cows and the results under this experiment offer additional and practical data on the use of low quality roughage such as rice straw with effective chemical treatment and with lower cost as well as its applicability for use under practical farm conditions.

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