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EFFECT OF *LEUCAENA LEUCOCEPHALA* PELLET (LLP) SUPPLEMENTATION ON RUMEN FERMENTATION EFFICIENCY AND DIGESTIBILITY OF NUTRIENTS IN SWAMP BUFFALO

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ABSTRACT

Four swamp buffaloes (*Bubalus Bubalis*) with average body weight of 238±27 kg were randomly assigned to receive four dietary treatments according to a 4 x 4 Latin square design. The treatments were supplemented with *Leucaena leucocephala* leaves (LLP) and were as follows: Treatment (T1) = LLP supplementation at 0 g/h/d (control); T2 = LLP supplementation at 150 g/ h/d; T3= LLP supplementation at 300 g/ h/d; T4= LLP supplementation at 450 g/ h /d. All animals were fed with concentrate (14.2 % CP, 76 % TDN) at 0.5% BW and offered with rice straw *ad libitum*. It was found that dry matter intake and ruminal NH_3-N (16.2 mg/dl) were increased at highest LLP supplementation, but no effect on rumen pH. Apparent digestibilities of DM, OM, CP, NDF and ADF were significantly increased ($P<0.05$) when increasing LLP supplementation and was highest at 450 g/h/d. Ruminal NH_3-N concentration was significantly different among treatments ($P<0.05$), and was highest when buffaloes were fed at 450 g/ h /d (16.2 mg/dl). Blood urea-nitrogen (BUN) concentrations were not significantly different ($P>0.05$) among treatments but tended to be increased with enhancing levels of LLP supplementation (9.0-11.6 mg/dl), respectively. Total volatile fatty acids (TVFA) and proportion of propionic acid (C_3) were differed among treatments and ranged from 88.6 to 100.8 mmol/l and 23.4 to 27.7 mmol/l, respectively. The concentrations of acetic acid (C_2) and butyric acid (C_4) were found decreased when increasing level of LLP from 300 to 450 g/ h /d while the ratio of acetic acid to propionic acid were decreased when supplementation of LLP from 300- 450 kg/ h /d. Based on this study, it could be concluded that supplementation of LLP at 450 g/ h /d with rice straw can improve voluntary intake, digestibility and rumen fermentation parameters in swamp buffaloes.

Key words *Leucaena leucocephala* pellet; supplementation; rumen fermentation; digestibility; swamp buffaloes; rice straw.

INTRODUCTION

Leucaena leucocephala, local tree legume resources has been successfully tested as a protein supplement for ruminants (Saha *et al.*, 2008). *Leucaena* can be used up to 30% of the diet as a protein source (Jetana *et al.*, 2011). It is high in nutritional quality and when managed as a browse supplement in grazing ruminant it may lead to improvement in growth and milk production (Cherdthong *et al.*, 2011b). Digestibility and intake values for *Leucaena leucocephala* range from 50 to 71% and from 58 to 85 $g/kg^{0.75}$ live weight, respectively (Saha *et al.*, 2008) because palatability or acceptability of *Leucaena leucocephala* is high, especially compared to other forage tree legumes such as *Calliandra calothyrsus* and *Gliricidia sepium* (Saha *et al.*, 2008; Jetana *et al.*, 2011).

Making a combination of cassava hay, soybean meal, urea and some binding agent as a pellet might be a suitable method to control urea release and provide a good source of RDP and RUP (Wanapat *et al.*, 2006). Pelleting of mulberry leaves meal based diets improved digestibility of most nutrients and rumen ecology in beef, which indicated that the nutrient digestibility could be improved by pelleting process (Tan *et al.*, 2011). However, using Leucaena leaves, protein sources with urea in a pellet have not yet been used as a protein source in ruminants especially when fed on rice straw. Therefore, this study was conducted to determine supplementation level of *Leucaena leucocephala* leaf meal pellet for better feed intake, nutrient digestibility and rumen fermentation in swamp buffaloes fed on rice straw.

MATERIALS AND METHODS

Animals, Diets and Experimental Design: Four, Thai male swamp buffaloes (*Bubalus bubalis*) with 238 ± 27 kg average live weight, were used in this experiment. They were randomly assigned according to a 4 × 4 Latin

square design to investigate the effect of *Leucaena* pellet. The dietary supplementations with LLP were: T1= Supplementation of LLP at 0 g/ h /d (Control); T2 = Supplementation of LLP at 150 g/ h /d; T3= Supplementation of LLP at 300 g/ h /d; T4= Supplementation of LLP at 450 g/ h /d. Concentrates containing 14.2% CP, 76% TDN were offered at 0.5% of BW, and rice straw provided *ad libitum.* The LLP was produced as follows: 1) Collecting Leucaena leaves, sun dried about 2-3 days; 2) *Leucaena* leaves were ground (1mm screen using Cyclotech Mill, Tecator, Sweden); 3) Mixing *Leucaena* leaves meal with urea, cassava starch, molasses, salt, mineral and sulfur in ratio shown in Table 1 ; 4) After mixing well all ingredients in step (3) were added with water with ratio 0.8:1 (water and mixture); 5). Pelletting by pellet-machine (under 105-110 ºC for 10 min.) and then sun dried until dried and stored in plastic bags for use in experiment periods (Wanapat *et al.*, 1996).

Animals were held in individual pens and received free choice of water. Animal were bathed with clean water every morning. The experiment was conducted for four periods and each period lasted for 21 days. During the first 14 days, all animals were fed with respective diets (LLP), LLP were offered to the animals in two equal portions, at 07:00 h and at 16:30 h and rice straw was fed *ad libitum*. Concentrate was offered at 0.5% BW to each animal into two equal portions.

The feed refusals were weighed daily prior to the morning feeding to determine daily dry matter intake (DMI). Body weights were measured twice, at the beginning and at the end of each period (21 days). During the last 7days, the animals were moved onto metabolism crates for total collection of feces and urine, while rumen fluid and blood were collected at the last day of each period.

Data Collection and Sampling Procedures: During the first 14 days of each period, feed offered and feed refusal were weighed daily for measuring voluntary feed intake and feed samples were randomly collected daily for DM analyses using hot air oven (AOAC, 1997).

Total feces were collected and weighed during the last 7 days of each period. The fecal samples were collected at 5% of total fresh weight and divided into two parts, first part analyzed for DM every day; second part kept in refrigerator and pooled at the end of each period. Composited samples were dried at 60 °C, ground (1 mm screen using Cyclotech Mill, Tecator, Sweden) for chemical analysis for DM, OM, CP according to AOAC (1997), NDF and ADF according to Van Soest (1991).

A blood sample (about 10 ml) was collected from a jugular vein into tubes containing 12 mg of EDTA, and plasma was separated by centrifugation at 8,000 × *g* for 10 min and stored at −20 °C until analysis of plasma urea N according to the method of Crocker (1967).

Rumen fluid sample were taken by using stomach tube with vacuum pump at each time. Temperature and pH of rumen fluid were measured by a portable pH and temperature meter immediately after collected rumen fluid (HANNA Instrument HI 8424 microcomputer, Singapore).

The 50 ml of rumen fluid sample were collected and added with 5 ml of 1M H_2SO_4 to stop fermentation process of microbial activity and the centrifuged at 16,000 x *g* for 15 minute. About 20-30 ml of supernatant were collected and breezed at –20 °C until analyzed in the laboratory for analysis of VFAs using High Performance Liquid Chromatography (HPLC; Model Water 600; UV detector, Millipore Crop) according to the method of Samuel *et al.* (1997) and rumen fluid samples were used for NH_3-N analysis using the micro-Kjeldahl methods (AOAC, 1997).

Statistical Analysis: Statistical analyses were performed using the GLM procedure of SAS Version 6.12 (SAS, 1998). Data were analyzed using the model $Y_{ijk} = \mu + M_i + A_j + P_k + \varepsilon_{ijk}$, where Y_{ijk} = observation from animal j, receiving diet i, in period k; μ = the overall of mean; M_i = the mean effect of LLP (i = 1, 2, 3, 4); A_j = the effect of animal (j = 1, 2, 3, 4); P_k = the effect of period (k = 1, 2, 3, 4); and ε_{ijk} = the residual error. Treatment means were statistically compared by Duncan's New Multiple Range Test (Steel *et al.,* 1997). Differences among means with $P<0.05$ were accepted as representing statistically significant differences.

RESULTS AND DISCUSSION

Effect of LLP supplementation on rumen pH, temperature, ammonia nitrogen (NH_3-N), and blood-urea nitrogen (BUN): Ruminal pH and temperature were similar among treatments with values at 6.8-6.9 and 38.4-38.6°C, respectively (Table 2). Ruminal NH_3-N utilization in swamp buffaloes were significantly increased ($P<0.05$) among treatments. This indicates that high protein in LLP is suitable by the buffaloes. A number of researchers including Devendra (2007) those resulted that swamp buffaloes were more efficient than cattle in many aspects i.e. N-recycling and fiber digestion, NH_3-N level in relation to efficient fermentation and intake. Wanapat and Rowlinson (2007) reported that increasing level of ruminal NH_3-N to 17.6 mg/dl result in increased DMI. Ruminal NH_3-N is a major source of N for microbial protein synthesis. The concentration of NH_3-N in this present study range from 9.4-16.2 mg/dl which agreed with previous results [Wanapat and Rowlinson (2007); 15-30 mg/dl].

The result of BUN concentration was not significantly different but it tended to be increased as a

result of an increase in ruminal NH_3-N concentration especially at supplementation of LLP at 300-450 g. BUN concentrations of treatment group ranged from 9.0-11.6 mg% (Table 2). BUN has been known as a factor, which highly related to dietary protein level (Cherdthong and Wanapat, 2010). Digestible protein in the diet of ruminants is either degraded in the rumen or escapes to the abomasum and small intestine where it is degraded to amino acids and small peptides then absorbed into the portal blood system. Nitrogen from protein that is degraded in the rumen is used for microbial protein synthesis (Cherdthong and Wanapat, 2010; Cherdthong *et al.*, 2011a,b,c). Yield of microbial protein produced in the rumen is maximized when the ratio of available energy (fermentable organic matter) to protein (nitrogen) is optimized. When there is an excess of nitrogen relative to energy in the rumen, ruminal ammonia concentration increases. Unused ruminal ammonia enters the portal blood through the rumen wall and is transported to the liver where it is detoxified by conversion to urea (BUN). Therefore, increasing dietary protein increases ruminal NH_3-N concentration and increases BUN concentrations. In addition, different protein diets have been shown to differently affect BUN. Huntington *et al.* (2001) reported that rumen ammonia concentration increases with increasing dietary CP intake, but decreases with increasing rumen upgradeable protein (RUP) as a percent of dietary CP. However, under this study, BUN concentrations were similar among treatments which responded with relatively, low levels of rumen NH_3-N. This NH_3-N could be well used in rumen microbial protein synthesis. The resultant concentration of NH_3-N and BUN indicated that supplementation of LLP could be well used as protein source based on rice straw fed to buffaloes.

Effect of LLP supplementation on ruminal total volatile fatty acids (VFAs) in swamp buffaloes: The production of total VFA, acetic acid (C_2), propionic acid (C_3), butyric acid proportions (C_4), and acetic:propionic (C_2:C_3) ratio are shown in Tables 3 and 4. There were significantly increases as level of LLP supplementation increases in total VFA and C_3 (100.8 mM/l and 27.7% for 450 g/h/d, respectively). Similarly, Nguyen (2005) who showed that total VFA were increased by Sesbania grandiflora leaves supplementation. As Wanapat and Rowlinson (2007) pointed out, the efficiency of microbial growth in the rumen depends on rumen biochemistry, mainly the factors that are involved in the production of VFA in fermentative processes. However, total VFA concentrations in all diets under this study were in good range according to Cherdthong *et al*. (2010a; 70 to 130 mM).

Effect LLP supplementation on DM intake and apparent digestibility in swamp buffalo: Intakes of roughages, concentrate, total DM, and apparent nutrient digestibility are shown in Table 5. Intake in terms of kg/d and % BW were higher when LLP supplementation at 450 g/ h /d and as a result untreated rice straw intake were significantly enhanced by LLP supplementation. Moreover, g/kg $BW^{0.75}$ of rice straw intake and total intake of DM, % BW were significantly different and improved ($P<0.05$) at 450 g/ h /d LLP supplementation.

Apparent digestibility (%) of DM, OM, CP, NDF and ADF were highest when supplementation of LLP at 450 g/ h /d ($P<0.05$) as compared with control. Similarly, Jetana *et al.* (2011) reported that supplementation of treated Leucaena leaves in cattle could increase fiber digestion. The finding of current study revealed that digestibility linearly increased when LLP supplementation was increased. The reason could be the effect of the crude protein on microbial activity and the digestion of N in the rumen. Traore *et al*. (2010) reported that the improvement in feed digestibility and microbial activity when degradable protein was added in the rumen. Faster and more complete digestion of the feed by microbes apparently reduces the fill of the feed in the rumen, and thus enables an increase in feed intake and digestion.

Table 1. Ingredients and chemical compositions (% DM) of concentrate, *Leucaena Leucocephala* pellet, and rice straw used in the experiment

Item	Experimental diets		
	Concentrate	LLP	Rice straw
Ingredients			
Cassava chip	76.0	-	
Leucaena leaves meal	-	81.5	
Cassava starch	-	0.5	
Rice bran	8.0	-	
Coconut meal	4.0	-	
Palm meal	4.0	-	
Urea	3.0	10.0	
Molasses	2.0	5.0	
Sulfur	1.0	1.0	
Mineral mixture	1.0	1.0	
Salt	1.0	1.0	
Chemical composition, %			
DM	91.1	92.9	90.0
	----------% DM---------		
OM	93.6	91.3	89.5
CP	14.2	42.2	2.4
NDF	16.0	44.0	83.9
ADF	8.4	20.0	60.4

LLP= Leucaena pellets with 10% Urea, DM=Dry matter, OM=Organic matter, CP=Crude protein, NDF=Neutral detergent fiber, ADF=Acid detergent fiber.

Table 2. Effect of LLP supplementation on ruminal pH, temperature, ammonia-nitrogen (NH_3-N) and blood-urea nitrogen (BUN) in swamp buffalo

Items	LLP (g/ h /d)				SEM	Contrast		
	0	150	300	450		L	Q	C
Ruminal Rumen pH								
0h-post feeding	6.7	6.8	6.8	6.9	0.07	ns	ns	ns
2h	6.9	6.9	6.9	7.0	0.03	ns	ns	ns
4h	7.0	6.9	7.0	7.0	0.13	ns	ns	ns
6h	6.9	6.9	6.9	7.0	0.09	ns	ns	ns
Mean	6.9	6.8	6.9	6.9	0.03	ns	ns	ns
Ruminal temperature, °C								
0 h-post feeding	38.9	38.1	38.0	38.2	0.28	ns	ns	ns
2	38.6	38.5	39.2	39.0	0.22	ns	ns	ns
4	38.6	39.4	38.3	38.3	0.44	ns	ns	ns
6	38.1	38.4	38.2	38.5	0.18	ns	ns	ns
Mean	38.5	38.6	38.4	38.5	0.12	ns	ns	ns
Ruminal NH_3-N , mg/dl								
0 h-post feeding	7.5^{a}	9.2^{ab}	9.2^{ab}	9.9^{b}	0.45	*	ns	ns
2	12.7^{a}	17.4^{ab}	21.5^{b}	22.8^{b}	2.13	*	ns	ns
4	9.7^{a}	14.2^{b}	15.1^{bc}	17.0^{c}	0.59	*	ns	ns
6	7.9^{a}	12.7^{b}	13.9^{bc}	15.3^{c}	0.59	*	*	ns
Mean	9.4^{a}	13.4^{b}	14.9^{bc}	16.2^{c}	0.58	*	ns	Ns
BUN, mg %								
0 h-post feeding	7.0^{a}	9.3^{ab}	10.0^{ab}	10.3^{b}	0.82	ns	ns	ns
4	11.0	9.8	13.0	12.3	1.86	ns	ns	ns
Mean	9.0	9.5	11.6	11.1	1.23	ns	ns	ns

LLP = Leucaena pellet with 10% Urea, SEM=Standard error of mean, $^{a, b, c,}$ Means in the same row with different superscripts differ (P<0.05), *P < 0.05, L= Linear, Q=quadratic C=Cubic, ns= non-significant

Table 3. Effect of LLP supplementation on ruminal volatile fatty acids (VFAs) in swamp buffalo

Items	LLP (g/ h /d)				SEM	Contrast		
	0	150	300	450		L	Q	C
Total VFA, mMol/l								
0 h-post feeding	85.0^{a}	89.8^{b}	92.0^{b}	95.8^{c}	0.66	*	ns	ns
2	93.6^{a}	103.2^{b}	105.1^{bc}	108.2^{c}	0.87	*	*	ns
4	88.9^{a}	95.1^{b}	98.8^{c}	100.0^{c}	0.80	*	*	ns
6	87.1^{a}	91.8^{b}	94.4^{c}	99.2^{d}	0.59	**	ns	ns
Mean	88.6^{a}	95.0^{b}	97.6^{c}	100.8^{d}	0.47	**	*	ns
C_2 , %								
0 h-post feeding	66.9	66.1	65.5	65.6	0.39	ns	ns	ns
2	64.8	66.6	66.1	65.9	0.49	ns	ns	ns
4	66.6	66.0	65.5	65.1	0.49	ns	ns	ns
6	67.9^{a}	66.4^{ab}	65.8^{b}	65.2^{b}	0.39	ns	ns	ns
Mean	66.6^{a}	66.3^{a}	65.7^{b}	65.5^{b}	0.11	*	ns	ns
C_3 , %								
0 h-post feeding	23.0^{a}	25.4^{b}	27.5^{c}	27.6^{c}	0.29	**	*	ns
2	24.0^{a}	24.8^{b}	26.8^{c}	27.2^{c}	0.19	**	ns	ns
4	23.6^{a}	26.2^{b}	27.2^{bc}	28.3^{c}	0.31	*	ns	ns
6	23.3^{a}	25.5^{b}	26.6^{c}	27.5^{c}	0.28	*	ns	ns
Mean	23.4^{a}	25.5^{b}	27.0^{c}	27.7^{d}	0.11	**	*	ns
C_4 , %								
0 h-post feeding	10.1^{a}	8.4^{ab}	7.0^{b}	6.9^{b}	0.51	*	ns	ns
2	11.2^{a}	8.6^{b}	7.1^{b}	6.9^{b}	0.48	*	*	ns
4	9.8^{a}	7.8^{b}	7.4^{bc}	6.6^{c}	0.25	*	ns	ns
6	8.8^{a}	8.1^{ab}	7.6^{b}	7.3^{b}	0.22	*	ns	ns
Mean	10.0^{a}	8.2^{b}	7.3^{c}	6.9^{c}	0.15	**	ns	ns

LLP = Leucaena pellet with 10% Urea, g/ h /d = Gram per head per day, SEM=Standard error of mean, $^{a, b, c,}$ Means in the same row with different superscripts differ (P<0.05), *P < 0.05, L= Linear, Q=quadratic C=Cubic, SEM= standard error of the means, NS= non-significant

Table 4. Effect of LLP supplementation on ruminal concentration of acids acetic to propionic acids ratio in swamp buffalo

Items	LLP (g/ h /d)				SEM	Contrast		
	0	150	300	450		L	Q	C
$C_2 : C_3$								
0 h-post feeding	2.9[a]	2.6[b]	2.4[c]	2.4[c]	0.03	**	ns	ns
2 h	2.7[a]	2.7[a]	2.5[b]	2.4[b]	0.03	*	ns	ns
4 h	2.8[a]	2.5[b]	2.4[bc]	2.3[c]	0.05	**	ns	ns
6 h	2.9[a]	2.6[b]	2.5[bc]	2.4[c]	0.04	*	ns	ns
Mean	2.8[a]	2.6[b]	2.4[c]	2.4[c]	0.01	**	*	ns

Table 5. Effect of LLP supplementation on voluntary dry matter feed intakes and nutrient digestibility in buffaloes

Items	LLP (g/ h /d)				SEM	Contrast		
	0	150	300	450		L	Q	C
Concentrate intake, kg/d								
Kg/d	1.2	1.3	1.3	1.3	0.22	ns	ns	ns
Rice straw intake, kg/d								
kg/d	3.3[a]	3.5[a]	3.8[b]	4.6[c]	0.06	**	ns	ns
%BW	1.2[a]	1.4[ab]	1.5[b]	1.8[c]	0.09	*	ns	ns
g/kg $BW^{0.75}$	54.7[a]	56.4[a]	60.3[ab]	73.0[b]	4.17	*	ns	ns
Total DM intake								
kg/d	4.5[a]	5.0[ab]	5.3[b]	6.4[c]	0.21	**	ns	ns
%BW	2.0[a]	2.0[ab]	2.1[ab]	2.4[b]	0.11	*	ns	ns
g/kg $BW^{0.75}$	76.5[a]	80.1[ab]	84.0[ab]	94.5[b]	4.76	*	ns	ns
Apparent digestibility, %								
DM	63.1[a]	65.1[ab]	66.6[ab]	70.2[b]	1.91	*	ns	ns
OM	66.8[a]	67.3[a]	68.4[a]	71.4[b]	0.76	*	ns	ns
CP	50.2[a]	54.5[ab]	59.8[bc]	68.1[c]	2.49	*	ns	ns
NDF	60.4[a]	62.7[ab]	64.4[b]	65.7[b]	0.93	*	ns	ns
ADF	57.8[a]	60.4[b]	62.0[bc]	63.2[c]	0.72	*	ns	ns

LLP = Leucaena pellet with 10% Urea, SEM=Standard error of mean, [a, b, c,] Means in the same row with different superscripts differ (P<0.05), *P < 0.05, L= Linear, Q=quadratic, C=Cubic, DM=Dry matter, OM=Organic matter, CP=Crude protein, NDF=Neutral detergent fiber, ADF=Acid detergent fiber, NS=non-significant.

Conclusion: Based on this study, it could be concluded supplementation of LLP at 450 g/ h /d significantly improved day matter intake, rumen fermentation efficiency and digestibility in swamp buffalo fed on rice straw as a roughage.

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