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Supplemental Energy Influenced on *Leucaena leucocephala* Leaf Meal in Swamp Buffaloes

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Abstract: Four Thai-rumen fistulated swamp buffaloes male (*Bubalus bubalis*), about 3 years old with 360±18 kg liveweight were assigned according to a 2×2 factorial arrangement in a 4×4 Latin square design to receive dietary treatments. The treatments were as follows: T1) level of concentrate at 0.1% BW with *Leucaena leucocephala* Leaf Meal (LLLM) at 300 g/hd/day; T2) concentrate at 0.2% BW with LLLM at 300 g/hd/day; T3) concentrate at 0.1% BW with heated *Leucaena leucocephala* Leaf Meal (HLLLM) at 300 g/hd/day and T4) concentrate at 0.2% BW with HLLLM at 300 g/hd/day. The results revealed a significant increase in roughage and total DM intake ($p<0.05$) by concentrate level at 0.2% BW (T2 and T4) as compared with concentrate level at 0.1% BW (T1 and T3). Digestion coefficient (%) of DM, OM and CP were increased by level of concentrate at 0.2% BW while NDF and ADF were similar among treatments. However, there was no effect of neither energy level nor HLLLM on ruminal pH and temperature ($p>0.05$). Concentration of ruminal NH_3-N was decreased by HLLLM as compared with LLLM ($p<0.05$) while blood urea-nitrogen was not changed and was in normal range. Total bacterial direct counts were found significantly different ($p<0.05$) whereas fungi zoospores and protozoal populations were similar among treatments. Nevertheless, viable bacterial counts were found affected by both concentrate level and HLLLM. The treatments with HLLLM were lower than those in LLLM and concentrate level at 0.2% BW were higher than those supplemented at 0.1% ($p<0.05$). Based on this study, it could be concluded that HLLLM could be used as a protein source in terms of rumen undegradable protein while the combination of HLLLM and concentrate level at 0.2% of BW could enhance the voluntary feed intake, nutrient digestibility, rumen fermentation and ecology in swamp buffalo fed supplementation on 2+2% urea-lime treated rice straw.

Key words: *Leucaena leucocephala* leaf meal, heat treatment, digestibility, rumen ecology, rice straw, swamp buffalo, Thailand

INTRODUCTION

Ruminant production in tropical is usually closely integrated into overall food production. The majority of small-holder farmers in Asia have ruminants on their farms to provide power, transport and manure and to utilize crop residues and other forages to produce meat and milk (Pralomkarn *et al*., 1995). Feed, commonly is low in protein and high in fibre and also its quality and quantity vary considerably throughout the year (Evans, 1986). Moreover, many local roughages and crop residues, generally have limited factors such as low digestibility and Nitrogen (N) contents which further reduce voluntary intake (Leng, 1991; Dryhursta and Wood, 1998). Ruminant feeding systems based on poor quality roughage where protein is one of the 1st limiting factors may require additional protein to maintain an efficient rumen ecosystem that will stimulate nutrient intake and improve animal performance (Preston and Leng, 1987a). According to Wanapat (2008), energy and protein sources are of prime importance for ruminants as they stimulate microorganisms in the rumen and enhance the productive function of the animals. However, the supplementation of high protein and energy concentrates involves extra cost. On the contrary, Ruminants raise in the tropical largely depend on seasonal feed resources which are relatively low in quality hence, the manipulation of rumen efficiency through the uses of local feed resources would be as advantage (Wanapat, 2000). Foliages from locally grown shrubs and trees such as Leucaena (*Leucaena leucocephala*) have been successfully investigated as protein supplements for ruminants (Hove *et al*., 2001;

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Kahindi *et al*., 2007; Saha *et al*., 2008). *Leucaena leucocephala* is a tropical forage legume which is rich in protein and which has consequently become important in the world of research as a protein supplement for ruminants fed poor quality roughages such as maize stover (Jones, 1979; Devendra, 1983, 1984). It has a great potential in animal nutrition according to Jones (1979), Devendra (1983, 1984), Gutteridge and Shelton (1994) and Nhan (1998). Presently, the greatest use of this plant in animal nutrition is its incorporation in cattle feed. Leucaena leaf meal with its rich protein, minerals and vitamin content is also becoming a popular ingredient in poultry feeds in the tropics (D'Mello and Talpin, 1978). Its protein content is at high levels of 29.2% Crude Protein (CP) in leaf meal and 22.03% CP in forage (Garcia *et al*., 1996). Moreover, it contains condensed tannin content of 2-6% (Tropical forage) that can protect protein from rumen microbial degradation and reduce methane production (Suchitra and Wanapat, 2008).

The ruminant animals derive their amino acids supply jointly from dietary protein which escapes rumen degradation and microbial protein synthesized in the rumen. The amount of protein and amino acids that escapes rumen degradation varies greatly among different feeds, depending on their solubility and the rate of passage to the small intestine. Microbial protein synthesis however is regulated by the quantity of plant organic matter fermented in the rumen, provided that ammonia concentration and mineral elements are not limiting (Kaufman and Lupping, 1982). It is often the case in some situation that animal's requirements for amino acids not fully met from the normal sources of dietary protein. Rapid and extensive degradation of valuable proteins in the rumen lead research to develop the concept of protein protection from ruminal degradation with the principal objective of enhancing the supply of essential amino acids to the productive animal further reduce wasteful ammonia production in the rumen and reduction of nitrogen losses as urea in the urine (Annison, 1981). Heat treatment of feedstuffs can decrease degradation of dry matter and crude protein by blocking reactive sites for microbial proteolytic enzymes (Broderick and Craig, 1980) and increase the supply of dietary protein to the duodenum (Tagari *et al*., 1986). Several studies (Faldet and Satter, 1991) on various protein sources have shown a correlation between decreased ruminal degradation of protein and increased milk production.

Various heat treatments are available for decreasing degradability of oilseeds: oven-heating, roasting, extruding and autoclaving. Heat treatment has the advantage of being safe rather inexpensive and easily available (not requesting complex equipment). However, the knowledge on the optimal condition of heat treatments of *Leucaena leucocephala* leaf meal is scarce. Whereas data on the effect of the *Leucaena leucocephala* leaf meal heat treating, it was yet been found no data of the effect on feed intake and rumen ecology in swamp buffalo. Therefore, the objectives of this study was to determine the effect of energy level and heat treatment on *Leucaena leucocephala* leaf meal on feed intake and rumen ecology in swamp buffalo.

MATERIALS AND METHODS

Animals, diets and experimental design: The *Leucaena leucocephala* (*LL*) was collected around the area and sundried. Only the leaf of LL was collected and ground used meal for the experiment. After that the leaf meal was kept and half of the leaf meal was heated in the oven at temperature 100°C for 60 min. The 2+2% urea-calcium hydroxide treated rice straw was prepared by adding 2 kg urea and 2 kg $Ca(OH)_2$ (purchased as hydrated lime) in 100 L to 100 kg air-dry (91% DM) straw. The relevant volume of urea and lime solution was sprayed onto a stack of 5 whole straw bales (mechanically baled straw each bale weighing approximately 20 kg) and then covering the stack with a plastic sheet for a minimum of 10 days before feeding directly to animals (Wanapat and Pimpa, 1999).

Four, Thai-rumen fistulated swamp buffaloes male (*Bubalus bubalis*), about 3 years old with 360±18 kg liveweight were assigned according to a 2×2 factorial arrangement in a 4×4 Latin square design to receive dietary treatments. The treatments were as follows: T1) level of concentrate at 0.1% BW with *Leucaena leucocephala* Leaf Meal (LLLM) at 300 g/hd/day; T2) concentrate at 0.2% BW with LLLM at 300 g/hd/day; T3) concentrate at 0.1% BW with heated *Leucaena leucocephala* Leaf Meal (HLLLM) at 300 g/hd/day and T4) concentrate at 0.2% BW with HLLLM at 300 g/hd/day. Each period of the four periods last 21 days in length with the 1st 14 days as feed adaptation and intake measurement while the last 7 days as for sample collection. Ingredient compositions of concentrate mixture, LLLM and roughage (2+2% urea-lime treated rice straw) are shown in Table 1. All animals were individually penned and water and mineral block were available at all times. All animals were fed on urea-lime treated rice straw *ad-libitum*.

Data collection and sampling procedures: Feed offered and refusals were recorded daily through the experimental period for DM intake calculation and feed samples were randomly collected twice a week for DM analysis using

Table 1: Feed ingredients and chemical composition of dietary treatments used in experiment

Items	Percentage	LLLM	HLLLM	2+2% urea-lime rice straw
Ingredients				
Cassava chip	75.0	-	-	-
Rice bran	7.0	-	-	-
Coconut meal	7.0	-	-	-
Palm kernel meal	5.0	-	-	-
Molasses	1.5	-	-	-
Urea	1.5	-	-	-
Mineral mixed	1.0	-	-	-
Salt	1.0	-	-	-
Sulfur	1.0	-	-	-
Total	100.0	-	-	-
Chemical composition of DM (%)				
DM	92.3	86.2	94.6	54.2
OM	90.7	91.6	91.7	86.2
CP	10.8	27.3	27.1	5.8
NDF	18.2	35.4	36.4	76.5
ADF	12.5	16.3	17.2	56.2

DM = Dry Matter, OM = Organic Matter, CP = Crude Protein, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber, LLLM = *Leucaena leucocephala* Leaf Meal, HLLLM = Heat *Leucaena leucocephala* Leaf Meal

hot air oven (AOAC, 1990). Samples of concentrate mixture, LLLM and treated rice straw including refusals were collected daily during the collection period. Samples of rice straw were composited by period as well as sample of concentrate mixture, LLLM and refusals were composited by period and by animal and store at -20°C for later chemical analysis in laboratory. The samples were divided into two parts, 1st part analyzed for DM while second part kept and pooled at the end of each period for analyses of Ash, CP according to AOAC (1990), NDF, ADF, ADL according to Goering and van Soest (1970). Rumen pH and fermentation characteristics were measured at the last day of each period at 0, 2, 4 and 6 h post morning-feeding. Approximately, 200 mL of rumen fluid were taken from the middle part of the rumen by using a 60 mL hand syringe at each time. Rumen fluid was immediately measured for pH and temperature using a glass electrode pH meter and temperature meter, respectively. Fluid samples were then strained through four layers of cheesecloth and divided in three parts.

The 1st 50 mL of rumen fluid sample were collected and kept in a plastic bottle to which 5 mL of 1M H_2SO_4 were added to stop fermentation process of microbe activity and then centrifuged at 3,000×g for 10 min. About 20-30 mL of supernatant were collected and analysis of NH_3-N by the hypochlorite-phenol procedure (Beecher and Whitten, 1970). The second portion of 1 mL rumen fluid were collected and kept in a plastic bottle to which 9 mL of 10% formalin solution (1:9 v/v, rumen fluid: 10% formalin) are added and stored at 4°C for measuring microbial population. The total direction counts of bacteria, protozoa (Holotrich and Entodiniomorhp) and fungal zoospores content of rumen fluid were done according to the method of Galyean (1989) based on the use of a haemacytometer (Boeco). Differentiations of rumen fungal zoospores from small protozoa were based on characteristics having flagellae while protozoa had ciliates around. Rumen fluid were diluted using autoclave distilled water (121°C for 15 min) as a medium by 100, 10 and 10 time and counting using 10×40, 10×10 and 10×40 ocular x objective of microscope for bacteria, protozoa and fungal zoospores, respectively. The 3rd portion for the total direct count of bacteria groups (cellulolytic, proteolytic, amylolytic) and total viable count bacteria were used the roll-tube technique described by Hungate (1969).

A blood sample (about 10 mL) was drawn from the jugular vein at at 0 and 4 h post feeding. 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 3500×g for 20 min (Table Top Centrifuge PLC-02, USA). The plasma were removed, stored at -20°C and analyzed for Blood Urea Nitrogen (BUN) composition according to the method of Roseler *et al.* (1993).

Statistical analysis: All data obtained from the experiment were subjected to ANOVA for a 4×4 Latin square design with 2×2 factorial arrangements of treatments using the General Linear Models (GLM) procedures of the Statistical Analysis System Institute (SAS, 1996). The statistical model included terms for animal, period, concentrate level, LLLM and the concentrate level×LLLM interactions. Treatment means were compared by Ducan's New Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Chemical composition of feeds: The experimental feed ingredient of concentrate, LLLM, treated rice straw and their chemical compositions are shown in Table 1. Concentrate ingredients were based on local resources, consisting of cassava chip, rice bran, coconut meal and palm kernel meal had a higher quality in term of CP and low in fiber (10.8 and 18.2% of DM, respectively). This concentrate was well consumed by animals during experimental periods. The nutritive value of the rice straw has been improved by the treatment. Crude protein content of 2+2% urea-lime rice straw was 5.8%. Moreover, urea and lime could decrease the proportion of NDF and ADF in rice straw from 76.5-56.2%, respectively. This value was similar to those values reported by Wanapat *et al.* (2009) who used 2.2+2.2% urea-lime treated rice straw. As reported by Schiere and Ibrahim (1989), rice straw can be treated with urea which released ammonia after dissolving in water. For practical use by farmers, urea is safer than using anhydrous or aqueous ammonia, it also provides a source of nitrogen (crude protein) in which straw is economically deficient. However, there is no change in the N-content of the straw when treated with lime. On the other hand, lime is a weak alkali agent with a low solubility in water.

It has been reported that lime 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. Furthermore, using a mixture of urea and calcium hydroxide has the advantage of reducing strong odor of free ammonium or ammonium carbonate.

Under this study, there were no differences between chemical composition of HLLLM and LLLM. It was also reported by Nasri *et al.* (2008) and Mahala and Gomaa (2007) who used heated whole soybean and sesame cake that there was no effect on chemical composition by heating. The CP content of both leaf meals were 27.3 and 27.1% in unheated and heated treatment, respectively. The NDF and ADF of unheated and heated leaf meals were 35.4, 16.3 and 36.4, 17.2%, respectively. It was similar to the value of Yousuf *et al.* (2007) who reported the values; 30.2, 30.2, 17.3 and 24.7, 32.0, 21.1%, CP, NDF and ADF, respectively. The variable values could due to differences in collection method and distinctive storage methods.

Effect on feed intake, N utilization and digestibility: The effects of energy level and LLLM on voluntary feed intake in swamp buffalo are shown in Table 2 including rice straw intake, concentrate intake, LLLM intake and total intake in terms of kg day^{-1}, BW (%) and g kg^{-1} BW$^{0.75}$. The results revealed a significant increase in roughage and total DM intake ($p<0.05$) by concentrate level at 0.2% BW (T2 and T4) as compared with concentrate level at 0.1% BW (T1 and T3) but not by LLLM.

Roughage and total DM intakes ranged from 5.9-6.5 and 6.6-7.4 kg day^{-1}, respectively and the highest was in 0.2% concentrate treatment. As shown by Singh *et al.* (2009), Thang *et al.* (2010) and Sahoo and Walli (2008) who reported that when increased level of energy intake there was an increase in DM intake. Moreover under this study, it was shown that low intake was found in the heated treatment.

This could be explained by the effect of high rumen undegradable protein. According to Swartz *et al.* (1991) who found the same effect that there was a slightly decrease in DM intake when more undegradable protein was consumed. It was also found in heated soybean meal with a slight decrease of DM intake (Ahrar and Schingoethe, 1979). Digestion coefficients (%) of DM, OM and CP were increased by level of concentrate at

Table 2: Effect of energy level and LLLM on voluntary feed intake and nutrient digestibility

	LLLM		HLLLM			Interaction		
Items	0.1	0.2	0.1	0.2	SEM	LLLM	Conc.	LLLM*Conc.
DM intake								
Roughage intake								
kg day^{-1}	6.10^{a}	6.50^{b}	5.90^{a}	6.40^{b}	0.060	NS	**	NS
BW (%)	1.58^{a}	1.66^{b}	1.52^{ac}	1.64^{b}	0.020	NS	**	NS
g kg^{-1} BW$^{0.75}$	69.90^{a}	73.50^{b}	67.40^{a}	72.80^{b}	0.760	NS	**	NS
Concentrate intake								
kg day^{-1}	0.40^{a}	0.70^{b}	0.40^{a}	0.70^{b}	0.010	NS	***	NS
BW (%)	0.09^{a}	0.19^{b}	0.09^{a}	0.19^{b}	0.001	NS	***	NS
g kg^{-1} BW$^{0.75}$	4.10^{a}	8.20^{b}	4.10^{a}	8.20^{b}	0.010	NS	***	NS
Lllm intake								
kg day^{-1}	0.26^{a}	0.26^{a}	0.28^{b}	0.28^{b}	4.000	**	NS	NS
BW (%)	0.07	0.07	0.07	0.07	0.002	NS	NS	NS
g kg^{-1} BW$^{0.75}$	3.00^{a}	3.00^{a}	3.20^{b}	3.20^{b}	0.060	**	NS	NS
Total intake								
kg day^{-1}	6.70^{a}	7.40^{b}	6.60^{a}	7.30^{b}	0.060	NS	*	NS
BW (%)	1.74^{a}	1.91^{b}	1.68^{a}	1.90^{b}	0.020	NS	*	NS
g kg^{-1} BW$^{0.75}$	77.00^{a}	84.70^{b}	74.70^{a}	84.20^{b}	0.780	NS	*	NS
Apparent digestibility (%)								
DM	60.50^{a}	70.20^{b}	62.40^{ab}	66.00^{ab}	2.280	NS	*	NS
OM	63.70^{a}	73.10^{b}	65.70^{ab}	69.40^{ab}	2.190	NS	*	NS
CP	50.50^{a}	60.20^{b}	53.00^{c}	59.90^{b}	0.640	*	*	NS
NDF	58.40	66.00	60.20	63.00	2.420	NS	NS	NS
ADF	54.00	61.40	50.60	54.70	3.510	NS	NS	NS

^{abc}Means with differing superscripts differ ($p<0.05$), SEM = Standard Error of the Mean, BW = Body Weight, BW$^{0.75}$ = Metabolic Weight, LLLM = *Leucaena leucocephala* Leaf Meal, HLLLM = Heat *Leucaena Leucocephala* Leaf Mea, DM = Dry Matter, OM = Organic Matter, CP = Crude Protein, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber

0.2% BW while NDF and ADF were similar among treatments. The DM, OM and CP were high in 0.2% concentrate especially with LLLM treatment, 70.2, 73.1 and 60.2% while the lowest was in 0.1% concentrate with LLLM, 60.5, 63.7 and 50.5%, respectively. Digestibility coefficients remained indifferent for varying ratios of RDP/UDP diet fed to goats (Mishra and Rai, 1996). Similarly to the present findings nutrient digestibility was reported to be improved due to protein protection and high energy level (Kridi *et al*., 2001; Wankhede and Kalbande, 2001). Wing *et al*. (1988) reported an increase ($p<0.01$) in DM and OM digestibility in Holstein cows fed undegradable protein with citrus molasses distillers soluble (6% of concentrate).

In the present study, no improvement of apparent digestibility coefficients of NDF and ADF was observed in the cattle fed the high energy diet as compared to the low level. Similarly, Klevesahl *et al*. (2003) observed no increases in NDF and ADF digestibility when beef steers were fed a high level of energy with corn starch, arguing that the rapid fermentation of starch resulted in decreasing fiber digestion in the rumen, related to low ruminal pH. However, the rate and extent of ruminal fermentation vary widely and digestion depends on the type of grain and degree of processing (Chanjula *et al*., 2003). The low digestibility of fiber could also be due to the low quality of the fiber in the diet.

One strategy for using highly degradable carbohydrates is to use them in combination with readily available NPN sources such as urea (Wohlt *et al*., 1978; Khampa *et al*., 2009). Effect of energy level and LLLM in swamp buffalo on N utilization were shown significantly different ($p<0.05$) among treatments in terms of N-intake, N-faeces, N-absorption and retention while no difference were found on N-urine and total N-excretion (Table 3). Total N-intake and N-balance were found highest in 0.2% of concentrate supplementation ($p<0.05$).

Ruminal fermentation, microorganism population and blood metabolites: Ruminal pH, temperature, microorganisms, NH_3-N and BUN are shown in Table 4. There were no effect of energy level and LLLM on ruminal pH and temperature ($p>0.05$). However, ruminal pH and temperature were in normal range at 6.53-6.70 and 39.1-39.3°C, respectively. All of these values were in normal range as reported as an optimal range for microbial digestion of fiber and protein, pH (6.5-7.0), temperature (39.0-41.0°C) (Wanapat, 1990). Ahrar and Schingoethe (1979) who used heated soybean meal, found no effect on pH by heat treatment. Moreover, Robinson *et al*. (1991) and Dutta *et al*. (2009) found the same results when supplemented with different energy ratio and rumen undegradable protein. However, NH_3-N was affected by energy level and LLLM but not for BUN. The average values of NH_3-N in this study were 13.60-16.57 mg%. The optimal ruminal ammonia nitrogen concentration for microbial growth ranged 5.0-25.0 mg% (Preston and Leng, 1987b) or 15-30 mg% (Wanapat and Pimpa, 1999) or 8.5 to >30 mg% (McDonald *et al*., 1996). In this result, NH_3-N in heated LLLM was lower than unheated treatment; 16.03-16.57 and 13.60-14.51 mg%, respectively and in high concentrate level groups were higher than in lower level.

This could be due to heat treatment of feedstuffs in which can decrease crude protein degradation by blocking reactive sites for microbial proteolytic enzymes (Broderick and Craig, 1980) and/or increased the supply of dietary protein to the duodenum (Tagari *et al*., 1986). Robinson *et al*. (1991) reported that when increased intake of rumen undegradable protein resulted in low ammonia nitrogen concentration, similarly to the result reported by Dutta *et al*. (2009). Although, there is a highly significant difference on NH_3-N concentration by heating, however no effect was found on BUN concentration. All the treatments were not changed, 10.0-11 mg% and were

Table 3: Effect of energy level and LLLM on N utilization in swamp buffalo

	LLLM		HLLLM			Interaction		
Items	0.1	0.2	0.1	0.2	SEM	LLLM	Conc.	LLLM*Conc.
N utilization								
N intake (g day^{-1})								
Rice straw	83.0[a]	87.5[b]	80.6[a]	86.5[b]	0.88	NS	**	NS
Concentrate	6.3[a]	12.8[b]	6.3[a]	12.4[b]	0.43	NS	**	NS
LLH	11.3	11.4	12.3	12.4	0.35	NS	NS	NS
Total	100.7[a]	111.6[b]	90.2[a]	111.3[b]	1.07	NS	***	NS
N excretion (g day^{-1})								
Feace	49.4[a]	44.1[b]	45.0[b]	43.9[b]	0.89	*	*	*
Urine	8.4	8.7	10.6	9.0	1.12	NS	NS	NS
Total	57.8	52.8	55.6	52.9	1.41	NS	NS	NS
N balance (g day^{-1})								
Absorption	51.3[a]	67.5[b]	54.2[a]	67.4[b]	1.52	NS	***	NS
Retention	42.9[a]	58.8[b]	43.6[a]	58.4[b]	1.86	NS	***	NS

[ab]Means with differing superscripts differ ($p<0.05$), SEM = Standard Error of the Mean, LLLM = *Leucaena leucocephala* Leaf Meal, HLLLM = Heat *Leucaena leucocephala* Leaf Meal

Table 4: Effect of energy level and LLLM on rumen ecology, NH_3-N, BUN

	LLLM		HLLLM			Interaction		
Items	0.1	0.2	0.1	0.2	SEM	LLLM	Conc.	LLLM*Conc.
Ruminal pH	6.69	6.53	6.62	6.54	0.07	NS	NS	NS
Ruminal Temp (°C)	39.20	39.20	39.30	39.10	0.11	NS	NS	NS
Ruminal NH_3-N mg (%) h-post feeding								
0	14.30	14.90	11.70	13.90	1.54	NS	NS	NS
2	17.60^{a}	18.30^{a}	16.00^{b}	17.50^{a}	0.37	*	*	NS
4	16.20^{a}	16.70^{a}	14.60^{b}	14.80^{b}	0.23	***	NS	NS
6	16.00^{a}	16.50^{a}	12.00^{b}	11.80^{b}	0.33	***	NS	NS
Mean	16.00^{a}	16.60^{ab}	13.60^{c}	14.50^{ac}	0.45	***	NS	NS
BUN (mg %) h-post feeding								
0	11.30	11.00	9.30	8.50	1.73	NS	NS	NS
4	12.50	11.80	11.50	11.50	2.06	NS	NS	NS
Mean	11.90	11.40	10.40	10.00	1.71	NS	NS	NS
Direct count×cell mL^{-1}								
Bacteria (×10^9)	3.30^{a}	4.40^{b}	2.90^{c}	3.20^{ac}	0.10	**	**	**
Protozoa (×10^5)	8.10	7.90	8.30	7.90	0.38	NS	NS	NS
Fungi (×10^5)	2.60	3.90	2.80	2.60	0.35	NS	NS	NS
Roll-tube technique (cfu mL^{-1})								
Amylolytic (×10^8)	4.60	5.10	4.30	4.40	0.75	NS	NS	NS
Proteolytic (×10^8)	2.80^{a}	3.10^{ab}	2.30^{c}	2.70^{a}	0.15	*	*	NS
Cellulolytic (×10^8)	10.00^{a}	10.50^{ab}	8.60^{c}	9.50^{a}	0.49	*	*	NS
Total (×10^9)	4.90^{a}	5.60^{ab}	4.00^{c}	4.80^{a}	0.43	*	*	NS

^{abc}Means with differing superscripts differ ($p<0.05$), SEM = Standard Error of the Mean, LLLM = *Leucaena leucocephala* Leaf Meal, HLLLM = Heat *Leucaena leucocephala* Leaf Meal, BUN = Blood Urea Nitrogen

lower than the values reported by Wanapat and Pimpa (1999). BUN was determined to investigate their relationship with rumen NH_3-N and protein utilization. However, Ahrar and Schingoethe (1979) found that BUN was affected by heating soybean meal. This was consistently with Hudson *et al*. (1970) which indicated that concentrations of plasma urea from ruminant animals fed heated soybean remained below those fed the unheated soybean meal. This suggested that the protein in the HSBM was degraded at a slower rate by the ruminal microorganisms than protein from unheated meal or that ammonia liberated from HSBM was utilized more efficiently for microbial protein synthesis.

Total bacterial direct counts were found significantly different by concentrate level and LLLM ($p<0.05$) whereas fungi zoospores and protozoal populations were similar among treatments. The treatment with at 0.2% concentrate with LLLM was the highest while the others three were similar. Nevertheless, viable bacterial counts were found affected by both concentrate level and HLLLM. The treatments with HLLLM were lower than those in LLLM and concentrate level at 0.2% BW were higher than those supplemented at 0.1% ($p<0.05$). Verbic (2002) revealed that energy supply was usually the 1st limiting factor for microbial growth in the rumen. The problem of low microbial protein yield in diets containing low quality forages cannot simply be solved by supplementing diets with high amounts of concentrates. It has been shown that in diets containing high levels of concentrates the efficiency of microbial protein synthesis in the rumen is lower than in well-balanced forage based diets. Moreover, nitrogen compounds which are released during the protein degradation are crucial for microbial growth in the rumen. Russell (2001) has reported protein sources which are low in Degradable Intake Protein (DIP) may limit the microbial protein synthesis when calculated to meet animal requirements based on dietary CP. In order to obtain maximal microbial protein synthesis, the nitrogen requirement of the rumen bacteria has to be met first. Carbohydrates are the main energy source for bacteria and most importantly, they can also be used as carbon skeletons for protein synthesis in combination with ammonia.

CONCLUSION

Based on this study, it could be concluded that HLLLM could be used as a protein source in terms of rumen undegradable protein while the combination of HLLLM and concentrate level at 0.2% of BW could enhance the voluntary feed intake, nutrient digestibility, rumen fermentation and ecology in swamp buffalo fed supplementation on 2+2% urea-lime treated rice straw.

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