Animal Feed Science and Technology 174 (2012) 131–139

Contents lists available at SciVerse ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Effects of energy level and *Leucaena leucocephala* leaf meal as a protein source on rumen fermentation efficiency and digestibility in swamp buffalo

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ARTICLE INFO

Article history:
Received 14 June 2011
Received in revised form 7 March 2012
Accepted 12 March 2012

Keywords:
Leucaena leucocephala leaf meal
Heat treatment
Energy level
Digestibility
Rumen ecology
Swamp buffalo

ABSTRACT

Four Thai – rumen fistulated male swamp buffaloes (*Bubalus bubalis*), about 3 years old with 360 ± 18 kg liveweight, were randomly assigned according to a 2 × 2 factorial arrangement in a 4 × 4 Latin square design to receive four dietary treatments. The treatments were as follows: a cassava based supplement (CS) at 1 g/kg BW and *Leucaena leucocephala* leaf meal (LLM) at 300 g/d (T1); CS at 2 g/kg BW with LLM at 300 g/d (T2); CS at 1 g/kg BW and heat treated LLM (HLLM) at 300 g/d (T3); and CS at 2 g/kg BW and HLLM at 300 g/d. During the experiment, urea–calcium hydroxide treated rice straw was given on *ad libitum* basis. The results revealed an increase in roughage and total dry matter (DM) intake (P<0.05) by CS at 2 g/kg BW (T2 and T4) as compared with CS at 1 g/kg BW (T1 and T3). Digestion coefficients of DM, organic matter (OM), and crude protein (CP) were increased by CS at 2 g/kg BW, while neutral detergent fiber (aNDF) and acid detergent fiber (ADF) were similar among treatments. However, there was no effect of neither energy level nor HLLM on ruminal pH and temperature (P>0.05). Concentration of ruminal ammonia nitrogen (NH_3-N) was decreased by HLLM as compared with LLM (P<0.05), while blood urea–nitrogen was not altered. There was an increase (P<0.05) in total volatile fatty acid (TVFA), acetic acid (C2), propionic acid (C3), and butyric acid (C4) concentrations and the highest were found in CS at 2 g/kg BW with HLLM (T4), while the lowest was in T1 and T3. However, no changes in C2–C3 ratio were found in this study. Total bacterial direct counts were found different (P<0.05), whereas fungal zoospores and protozoal populations were similar among treatments. Nevertheless, viable bacterial counts were found affected by both concentrate level and HLLM. The treatments with HLLM were lower than those in LLM and CS at 2 g/kg BW were higher than those supplemented at CS at 1 g/kg BW (P<0.05). In addition, efficiency of rumen microbial CP synthesis tended to be higher in treatment with higher level of energy and HLLM. Based on this study, it could be concluded that LLM could be used as a protein source, while the combination of HLLM and CS at 2 g/kg BW could enhance the voluntary feed intake, nutrient digestibility and rumen fermentation in swamp buffalo fed on treated rice straw (urea–calcium hydroxide treatment).

Abbreviations: BW, body weight; LLM, *Leucaena leucocephala* leaf meal; HLLM, heat treated *Leucaena leucocephala* leaf meal; DM, dry matter; OM, organic matter; CP, crude protein; ADF, acid detergent fiber; aNDF, neutral detergent fiber; TVFA, total volatile fatty acid; C2, acetic acid; C3, propionic acid; C4, butyric acid; NH_3-N, ammonia nitrogen; CS, a cassava based supplement.

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doi:10.1016/j.anifeedsci.2012.03.007

1. Introduction

Ruminants raised in the tropics largely depend on seasonal feed resources which are relatively low in quality in terms of low CP but high in crude fiber (CF); hence, the manipulation of rumen efficiency through the uses of local feed resources would be an advantage (Wanapat, 2000). Foliages from locally grown shrubs and trees such as Leucaena (*Leucaena leucocephala*) have been successfully investigated as protein a supplement for ruminants (Saha et al., 2008). 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 Taplin, 1978). Its protein content is at high levels of 292 g/kg CP in leaf meal and 220.3 g/kg CP in forage (Garcia et al., 1996). Moreover, it contains condensed tannin content of 10.1–10.5 g/kg that can protect protein from rumen microbial degradation and reduce methane production.

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. It is often the case in some situation that animal's requirements for amino acids are 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 DM and CP 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. 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 *L. leucocephala* leaf meal is scarce. Whereas data on the effect of the *L. 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 were to investigate the effect of energy level with heated and unheated treatment on *L. leucocephala* leaf meal on feed intake, nutrient digestibility, rumen fermentation and microbial population in swamp buffalo.

2. Materials and methods

2.1. Animals, diets and experimental design

L. leucocephala (LL) was harvested from the tree with average planting age of 4–5 years and sundried. Only the leaf of LL was sundried and ground, used 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 urea–calcium hydroxide treated rice straw was prepared by adding 2 kg urea and 2 kg $Ca(OH)_2$ (hydrated lime) in 100 l and poured over to 100 kg air dry (910 g/kg DM) straw. The relevant volume of urea and calcium hydroxide solution was sprayed onto a stack of 5 whole straw bales (approximately 20 kg) and then covered the stack with a plastic sheet for a minimum of 10 days before feeding directly to the animals (Wanapat et al., 2009).

Four Thai – rumen fistulated male swamp buffaloes (*Bubalus bubalis*), about 3 years old with 360 ± 18 kg liveweight, were randomly assigned according to a 2 × 2 factorial arrangement in a 4 × 4 Latin square design to receive dietary treatments. The treatments were as follows: a cassava based supplement (CS) at 1 g/kg BW and *L. leucocephala* leaf meal (LLM) at 300 g/d (T1); CS at 2 g/kg BW with LLM at 300 g/d (T2); CS at 1 g/kg BW and heat treated LLM (HLLM) at 300 g/d (T3); and CS at 2 g/kg BW and HLLM at 300 g/d. Each of the four periods lasted for 21 days, with the first 14 days as straw intake measurement, while the last 7 days for sample collection. Ingredient compositions of concentrate mixture, LLM and roughage (urea–calcium hydroxide 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–calcium hydroxide treated rice straw *ad libitum*.

2.2. Data collection and sampling procedures

Feed offered and refusals were recorded daily throughout the experimental period for DM intake calculation and feed samples were randomly collected twice a week for DM analysis. Samples of concentrate mixture, LLM 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. LLM and feed refusals were composited by period and by animal and stored at −20 °C for later chemical analyses.

Rumen pH, temperature and fermentation characteristics were measured at the last day of each period 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 measured for pH and temperature and the fluid samples were then strained through four layers of cheesecloth and divided into three parts. The first 45 ml of rumen fluid sample was collected and kept in a plastic bottle to which 5 ml of 1 M H_2SO_4 was added to stop fermentation process of microbe activity and then centrifuged at 3000 × *g* for 10 min. About 20–30 ml of supernatant was collected and analyzed for NH_3-N and VFA. 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

Table 1
Feed ingredients and nutritive values used in the experiment.

Item	Con[a]	LLM[b]	HLLM[c]	Urea–calcium hydroxide treated rice straw
Ingredients, g/kg dry matter				
Cassava chip	750			
Rice bran	70			
Coconut meal	70			
Palm kernel meal	50			
Molasses	15			
Urea	15			
Mineral mixture	10			
Salt	10			
Sulfur	10			
Chemical composition				
Dry matter, g/kg	923	862	946	542
Organic matter	907	916	917	862
Crude protein	108	273	271	58
Neutral detergent fiber	182	354	364	765
Acid detergent fiber	125	163	172	562

[a] Concentrate.
[b] *Leucaena leucocephala* leaf meal.
[c] Heat treated *Leucaena leucocephala* leaf meal.

formalin) was added and stored at 4 °C for measuring microbial population by using total direction counts. The third portion was for the total viable bacteria count (cellulolytic, proteolytic, and amylolytic) and total viable bacteria.

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 3500 × *g* for 20 min. The plasma were removed, stored at −20 °C and analyzed for blood urea nitrogen (BUN) composition. Urine samples were analyzed for total N and analyzed for allantoin concentration.

2.3. Analytical procedure

The samples were divided into two parts, first part for DM analyses, while the second part kept and pooled at the end of each period for analyses of Ash, CP, aNDF and ADF. Feeds, refusals and fecal samples were dried at 60 °C and ground (1 mm screen using the Cyclotech Mill, Tecator, Sweden) and analyzed using standard methods of AOAC (1995) for DM (ID 967.03) and ash (ID 942.05). ADF was determined according to an AOAC method (1995; ID 973.18) and was expressed inclusive of residual ash. aNDF in samples was estimated according to Van Soest et al. (1991) with addition of α-amylase but without sodium sulphite and results are expressed with residual ash. Total nitrogen (N) was determined according to AOAC (1995; ID 984.13).

Rumen fluid was immediately measured for pH and temperature using a portable pH temperature meter (HANNA Instruments HI 8424 microcomputer, Singapore) and NH_3-N by Kjeltech Auto 1030 Analyzer (AOAC, 1995; ID 973.18). VFA were analyzed using High Pressure Liquid Chromatography (HPLC, Instruments by Water and Novapak model 600E; water mode l484 UV detector; column novapak C18; column size 3.9 mm × 300 mm; mobile phase10 mM H_2PO_4 [pH 2.5]) according to Samuel et al. (1997). Rumen fluid was used for direct counts of bacterial, protozoa and fungal zoospores using methods of Galyean (1989) by haemocytometer (Boeco, Singapore). Groups of bacteria (*i.e.*, cellulolytic, proteolytic, amylolytic and total viable counts bacteria) were measured using the Hungate (1969) roll-tube technique. BUN was measured according to Crocker (1967).

Urine samples were analyzed for total N (AOAC, 1995; ID984.13) and allantoin in urine was determined by HPLC as described by Chen et al. (1993). The amount of microbial purines derivative absorption was calculated from purine derivative (PD) excretion based on the relationship derived by the equation of Liang et al. (1994): $Y = 0.12X + (0.20BW^{0.75})$. The supply of microbial N (MN) was estimated by urinary excretion of PD according to Chen and Gomes (1995): MN (g/d) = $70X/(0.116 \times 0.83 \times 1000) = 0.727X$; where X and Y are, respectively, absorption and excretion of PD in mmol/d. 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 650 g/kg OM of digestion in total tract, DOMR = DOMI × 0.65; DOMI = digestible organic matter intake).

2.4. 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, 1998). The statistical model included terms for animal, period, concentrate level, LLM and the concentrate level x LLM interactions. Treatment means were compared by Tukey's Multiple Comparison Test (Crichton, 1999).

Table 2
Effect of energy level and LLM on voluntary feed intake and nutrient digestibility.

Item	LLM[b]		HLLM[c]		SEM	Interaction		
	1[a]	2[a]	1[a]	2[a]		LLM[b]	Con[a]	LLM[b] × Con[a]
Dry matter intake								
Roughage intake								
kg/day	6.1	6.5	5.9	6.4	0.06	ns	**	ns
g/kg $BW^{0.75}$	69.9	73.5	67.4	72.8	0.76	ns	**	ns
Concentrate intake								
kg/day	0.4	0.7	0.4	0.7	0.01	ns	***	ns
g/kg $BW^{0.75}$	4.1	8.2	4.1	8.2	0.01	ns	***	ns
LLM intake								
kg/day	0.26	0.26	0.28	0.28	0.004	**	ns	ns
g/kg $BW^{0.75}$	3.0	3.0	3.2	3.2	0.06	**	ns	ns
Total intake								
kg/day	6.7	7.4	6.6	7.3	0.06	ns	*	ns
g/kg $BW^{0.75}$	77.0	84.7	74.7	84.2	0.78	ns	*	ns
Apparent digestibility								
Dry matter	0.61	0.70	0.62	0.66	0.02	ns	*	ns
Organic matter	0.64	0.73	0.66	0.69	0.02	ns	*	ns
Crude protein	0.51	0.60	0.53	0.60	0.006	*	*	ns
Neutral detergent fiber	0.58	0.66	0.60	0.63	0.02	ns	ns	ns
Acid detergent fiber	0.54	0.61	0.51	0.55	0.04	ns	ns	ns

[a] Concentrate (g/kg BW).
[b] *Leucaena leucocephala* leaf meal.
[c] Heat treated *Leucaena leucocephala* leaf meal.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

3. Results

3.1. Chemical composition of diet

Experimental feed and their chemical compositions are shown in Table 1. The mixture of concentrate, consisting of available local feed resources such as energy source (cassava chips), protein souces (rice bran, coconut meal, and palm kernel meal) and non-protein nitrogen (urea), had a higher quality in terms of CP and low in fiber (108, and 182 g/kg of DM, respectively). The mean level of CP of LLM and HLLM used in the experiment were 273 and 271 g/kg of DM, respectively. Moreover, fibrous fractions, aNDF and ADF were not diferent between LLM and HLLM. Rice straw quality was improved in CP by the treatment with urea–calcium hydroxide.

3.2. Feed intake and nutrient digestibility

Table 2 presents data of daily feed intakes and nutrient digestibility. Feed intakes were enhanced (P<0.05) by CS at 2 g/kg BW. Moreover, supplementation at CS at 2 g/kg BW increased rice straw intake, thus resulting in an increase in total intake. Apparent digestibility of DM, OM and CP were also found increased (P<0.05) in buffaloes consumed diet with CS at 2 g/kg BW and LLM, the highest was in T2 (0.70, 0.73, and 0. 60 kg/kg, respectively). The CP digestibility was increased by both factors CS at 2 g/kg BW and HLLM. In contrast, no effect on digestibility of aNDF and ADF by energy level and LLM supplementation was found (P>0.05).

3.3. Rumen fermentation and blood metabolites

Ruminal temperature, pH, and BUN were similar among treatments and the values were quite stable at 39.1-39.3 °C, pH (6.5–6.7), and BUN at 13.6–16.6 mg/dl, respectively (Table 3). However, BUN in the treatments with LLM (T1 = 11.9 and T2 = 11.4 mg/dl) tended to be higher than those with HLLM (T3 = 10.4 and T4 = 10.0 mg/dl). Treatments with HLLM were found lower in concentration of ruminal NH_3-N than with LLM. Both treatments with HLLM have lower concentration of NH_3-N (T3 = 13.6 and T4 = 14.5 mg/dl) than in the treatments with LLM (T1 = 16.0 and T2 = 16.6 mg/dl). In addition, the concentrate level has enhanced rumen NH_3-N concentration. The available rumen NH_3-N would be used in microbial protein synthesis by the rumen microbes. In this study, there were differences (P<0.05) in TVFA, C2, C3 and C4 concentrations when buffaloes were fed with different level of energy and LLM. As shown, the value of C2–C3 ratio were found no difference (P>0.05) among treatments. Overall, the VFA concentration was highly affected by level of energy, rather than LLM.

Table 3
Effect of energy level and LLM on ruminal fermentation and blood urea nitrogen.

Item	LLM[b]		HLLM[c]		SEM	Interaction		
	1[a]	2[a]	1[a]	2[a]		LLM[b]	Con[a]	LLM[b] × Con[a]
Ruminal pH	6.7	6.5	6.6	6.5	0.07	ns	ns	ns
Temperature, °C	39.2	39.2	39.3	39.1	0.11	ns	ns	ns
NH_3-N, mg/dl	16.0	16.6	13.6	14.5	0.45	***	ns	ns
Blood urea nitrogen, mg/dl	11.9	11.4	10.4	10.0	1.71	ns	ns	ns
Total VFA, mmol/l	82.9	95.6	82.2	99.5	1.22	ns	**	ns
VFA, mol/100 mol								
Acetic acid (C2)	59.3	67.4	58.7	68.8	0.90	ns	**	ns
Propionic acid (C3)	16.4	18.2	15.3	20.1	1.16	ns	*	ns
Butyric acid (C4)	7.2	10.0	8.2	10.6	0.67	ns	**	ns
C2:C3	3.6	3.7	3.8	3.4	0.62	ns	ns	ns

[a] Concentrate (g/kg BW).
[b] *Leucaena leucocephala* leaf meal.
[c] Heat treated *Leucaena leucocephala* leaf meal.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Table 4
Effect of energy level and LLM on microbial population in the rumen of swamp buffaloes.

Item	LLM[b]		HLLM[c]		SEM	Interaction		
	1[a]	2[a]	1[a]	2[a]		LLM[b]	Con[a]	LLM[b] × Con[a]
Ruminal microbes × cell/ml								
Bacteria, $\times 10^9$	3.3	4.4	2.9	3.2	0.10	**	**	**
Protozoa, $\times 10^5$	8.1	7.9	8.3	7.9	0.38	ns	ns	ns
Fungi, $\times 10^5$	2.6	3.9	2.8	2.6	0.35	ns	ns	ns
Viable bacteria, CFU/ml								
Amylolytic, $\times 10^8$	4.6	5.1	4.3	4.4	0.75	ns	ns	ns
Proteolytic, $\times 10^8$	2.8	3.1	2.3	2.7	0.15	*	*	ns
Cellulolytic, $\times 10^8$	10.0	10.5	8.6	9.5	0.49	*	*	ns
Total, $\times 10^9$	4.9	5.6	4.0	4.8	0.43	*	*	ns

[a] Concentrate (g/kg BW).
[b] *Leucaena leucocephala* leaf meal.
[c] Heat treated *Leucaena leucocephala* leaf meal.
* $P < 0.05$.
** $P < 0.01$.

3.4. Rumen microorganism population

As shown in Table 4, total bacteria counts were found different (P<0.05). Treatments with CS at 2 g/kg BW and LLM had the highest at 4.4×10^9 cell/ml and the lowest was in treatment with CS at 1 g/kg BW and HLLM, 2.9×10^9 cell/ml. Total counts of bacteria were affected by both of concentrate level and LLM, while protozoal and fungal zoospore population were not different. Total viable bacteria counts, cellulolytic bacteria, and proteolytic bacteria counts were found different (P<0.05), while amylolytic bacteria counts was not different among treatments. The treatment with CS at 2 g/kg BW and LLM was the highest in total viable bacteria counts, cellulolytic bacteria, and proteolytic bacteria counts (5.6×10^9, 10×10^8, and 3.1×10^8 CFU/ml, respectively). The treatments with LLM were higher than those with HLLM.

3.5. Nitrogen utilization and efficiency of microbial protein synthesis

Total N intake was found higher in treatment with CS at 2 g/kg BW (83.9 and 83.8 g/day; T2 and T4, respectively, Table 5). Moreover, feces N were found different by both concentrate and LLM, the highest was in T1 (49.4 g/day). Treatments with CS at 2 g/kg BW were found higher in terms of N balance (both absorption and retention). There was no difference of allantoin excretion among treatments, while allantoin absorption and Microbial crude protein (MCP) were affected by energy level and LLM, and tended to have the effect of the two factor (P=0.08); the higher were in treatments with CS at 2 g/kg BW, especially with HLLM. In contrast, EMNS were not affected neither by level of concentrate nor LLM, ranging from 28.0 to 32.0 gN/kg OMDR. However, HLLM treatments showed the higher value.

Table 5
Effect of energy level and LLM on N utilization, purine derivations (PD) and microbial crude protein supply (MCP).

Item	LLM[b]		HLLM[c]		SEM	Interaction		
	1[a]	2[a]	1[a]	2[a]		LLM[b]	Con[a]	LLM[b] × Con[a]
N utilization, g/day								
N intake	74.4	83.9	73.8	83.8	0.77	ns	***	ns
N excretion								
Feces	49.4	44.1	45.0	43.9	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								
Absorption	25.0	39.8	28.0	40.0	2.33	ns	***	ns
Retention	16.6	31.1	17.4	30.9	2.56	ns	***	ns
PD, mmol/d								
Allantoin excretion	27.8	31.6	29.0	33.2	3.14	ns	ns	ns
Allantoin absorption	89.7	116.2	94.6	120.4	4.94	ns	**	ns
Microbial nitrogen supply, gN/d	65.2	84.5	68.8	87.6	3.59	ns	**	ns
Microbial crude protein, g/d	407.7	528.2	429.9	547.3	7.82	*	***	0.08
EMNS[d], gN/kg OMDR[e]	28.0	28.6	30.5	32.0	2.16	ns	ns	ns

[a] Concentrate (g/kg BW).
[b] *Leucaena leucocephala* leaf meal.
[c] Heat treated *Leucaena leucocephala* leaf meal.
[d] Efficiency of microbial nitrogen synthesis.
[e] Digestible OM apparently fermented in the rumen.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

4. Discussion

4.1. Chemical composition of diet

Concentrate ingredients were based on local resources, consisting of cassava chip, rice bran, coconut meal and palm kernel meal, which had a higher quality in term of CP and low in fiber. This concentrate was well consumed by animals during the experimental periods. The nutritive value of rice straw has been improved by the treatment. CP content of urea–calcium hydroxide treated rice straw was 58 g/kg. Moreover, urea and calcium hydroxide could decrease the proportion of aNDF and ADF content in rice straw to 765 g/kg and 562 g/kg, respectively. This value was similar to those values reported by Wanapat et al. (2009) who used urea–calcium hydroxide treated rice straw. Under this study, there were no differences between chemical composition of HLLM and LLM. It was also reported by Fathi Nasir 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. It was similar to the value of Yousuf et al. (2007) who reported the values; 302, 302, 173 g/kg and 247, 320, 211 g/kg, CP, aNDF and ADF, respectively.

4.2. Feed intake and nutrient digestibility

The results revealed an increase in roughage and total DM intake (P<0.05) by CS at 2 g/kg BW (T2 and T4) as compared with CS at 1 g/kg BW (T1 and T3), but not by LLM. Roughage and total DM intakes ranged from 5.9–6.5 and 6.6–7.4 kg/d, respectively, and the highest was in CS at 2 g/kg BW treatment. However, it was suggested that supplementation of small amount by-pass protein to low quality diet often results in a higher intake than without. 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 slight 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 CS at 2 g/kg BW, while aNDF and ADF were similar among treatments (Table 2). 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. Similarly, Klevesahl et al. (2003) observed no increases in aNDF 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. The effects of energy and protein supply on intake and digestibility may be different, depending on the amount of nutrients delivered to the animal. In the present study of Thang et al. (2010), an improvement

of apparent digestibility coefficients of OM, gross energy (GE), aNDF and ADF were observed in the cattle fed the high energy diet (32 MJ/day) as compared to the low level (25 MJ/day).

4.3. Rumen fermentation and blood metabolites

There were no effect of energy level and LLM on ruminal pH and temperature (P>0.05). However, ruminal pH and temperature were in normal range at 6.5–6.7 and 39.1–39.3 °C, respectively. 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 LLM, but not for BUN. Ruminal NH_3-N concentration is a crude predictor of efficiency of dietary nitrogen conversion into microbial nitrogen (Firkins et al., 2007; Broderick and Muck, 2009). In this study, NH_3-N in HLLM was lower than LLM treatment; 16.0–16.6 and 13.6–14.5 mg/dl, 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 NH_3-N concentration, similarly to the result reported by Dutta et al. (2009). Although there is a highly difference on NH_3-N concentration by heating, however, no effect was found on BUN concentration. However, Ahrar and Schingoethe (1979) found that BUN was affected by heating soybean meal (HSBM). This was consistently with Hudson et al. (1970), which indicated that concentrations of plasma urea from ruminant animals fed HSBM remained below those fed the unheated soybean meal (SBM). 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. It may due to 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. That may be the reason in this study resulted in lower BUN in treatments with HLLM which was lower degrade than LLM as similar to the result of Mabjeesh et al. (1998) who conducted with heat whole cottonseed (HWCS) in dairy cows.

There were difference (P<0.05) in Total VFA (TVFA), C2, C3 and C4 when buffaloes were fed with different level of energy and LLM. This increase was strongly related with the number of ruminal cellulolytic bacterial species. *Fibrobacter succinogenes* mainly produces primarily succinate, the major precursor of propionate in the rumen while *Ruminococcus albus* is mainly a species which produces acetate. Therefore, with an increase in number of *F. succinogenes*, *R. albus*, both propionate and acetate concentration were increased. In contrast to the present result, according to Dutta et al. (2009), TVFA concentration in the rumen liquor was statistically similar among treatments groups with different ratio of energy and protein. Moreover, the TVFA concentration in ruminal fluid was not influenced by the level of energy in the diet of sheep (Merchen et al., 1986; Carro et al., 2000). Moreover, rumen VFA concentrations were similar for cows fed SBM and HSBM rations reported by Ahrar and Schingoethe (1979). However, the treatments with HSBM tended to be higher than those with SBM. This was expected since varying a protein source in the ration should not affect rumen VFA greatly unless such a change causes a great deficiency in nitrogen available to the rumen microorganisms. According to Mabjeesh et al. (1998), the proportion of propionate was the lowest for HWCS diet compared to unheated treatments and the acetate/propionate ratios were higher at all sampling times for this diet. This is in contrast with the present study which shown the mean values of TVFA, C2, C3, and C4 were the highest in CS at 2 g/kg BW with HLLM (99.5, 68.8, 20.1, and 10.6 mmol/l, respectively), while the lowest was in T1 and T3.

4.4. Rumen microorganism population

Total bacterial direct counts were found different by concentrate level and LLM, whereas fungal zoospores and protozoal populations were similar among treatments. The treatment with CS at 2 g/kg BW with LLM was the highest, while the others three were similar. According to Verbic (2002), energy supply is usually the first limiting factor for microbial growth in the rumen. More than that, this could be explained that NH_3-N is an essential source of nitrogen for microbial protein synthesis. The range of NH_3-N level for optimal rumen ecology has been reported to be 15.0–30.0 mg/dl (Leng, 1999). Treatments with CS at 2 g/kg BW and LLM had the highest ranged at 4.4×10^9 cell/ml and the lowest was in treatment with CS at 1 g/kg BW and HLLM, 2.9×10^9 cell/ml. This could be explained by decreasing protozoal population. Van Soest (1994) suggested that protozoa in the rumen have ability to engulf ruminal bacteria. Makkar et al. (1995), for instance, noted that at increased bacterial counts, quebracho tannins decreased the number of both *Entodiniomorph* and *Holotrich* protozoa, whereby the latter appeared to have an even greater sensitivity to tannins. For this reason, LLM contained moderate concentrations of tannins that had a positive effect on the nutrition of ruminants (Hervas et al., 2003) resulting in high digestibility and intake.

Viable bacterial counts were found affected by both concentrate level and HLLM. The treatments with HLLM were lower than those in LLM and CS at 2 g/kg BW were higher than those supplemented CS at 1 g/kg BW. Total viable bacteria counts, cellulolytic bacteria, and proteolytic bacteria counts were found different, while amylolytic bacteria counts was not different among the treatments. Dietary CP in ruminant diets serves as a source of metabolizable protein to the ruminant by providing both ruminal degraded protein for microbial protein synthesis and ruminal undegraded protein (Russell, 2001). It seems that proteins which have lower rates of ruminal degradation tend to improve the efficiency of microbial protein synthesis, probably because of the better capture of released N by rumen microbes. Forage heating decreased the rate of N disappearance

from Dacron bags incubated in the rumen of steers (Yang et al., 1993). Makkar et al. (1995) indicated that the efficiency of microbial protein synthesis was greater in forages containing saponin and tannins, which reduce ruminal N degradability. In this study, the treatment with CS at 2 g/kg BW and LLM was the highest in total viable bacteria counts, cellulolytic bacteria, and proteolytic bacteria counts and the lowest was in treatment with CS at 1 g/kg BW and HLLM (T3).

4.5. N utilization and efficiency of microbial protein synthesis

Effect of energy level and LLM in swamp buffaloes on N utilization is shown different among treatments in terms of N intake, N feces, N absorption and retention, while no difference were found on N urine and total N excretion (Table 5). Total N intake and N balance were found highest in CS at 2 g/kg BW supplementation. This indicates higher protein available for use by the buffaloes. However, N excretion through feces was higher in high energy–high protein fed group, but urinary N was no difference between energy and protein level. Consistency to the present result, Ahrar and Schingoethe (1979) reported that there were no differences in N balance however; N was utilized slightly more efficiently by cows fed HSBM. N losses in feces and urine were slightly less with HSBM. This agreed with Glimp et al. (1967) and Little et al. (1963) which indicated that heat treatment decreased urinary excretion in ruminant animals. Results of another study (Sherrod and Tillman, 1962) showed that as heating increased and solubility decreased, the percentage of N intake retained increased.

Urinary excretion of purine derivative is considered to be an indicator of microbial production in rumen. Protein degradation in the rumen is one of the main reasons for the inefficient utilization of protein in ruminants. It seems that proteins which have lower rates of ruminal degradation tend to improve the efficiency of microbial protein synthesis, probably because of the better capture of released N by rumen microbes (Russell, 2001). Similar to the present study, it was found that the treatment with HLLM have a higher microbial crude protein (MCP) than LLM. It may be due to heated treatment could protect rumen degradation in the rumen. More important than that, in this result, allantoin absorption and MCP were found higher in treatment with higher in energy. This was reported by Russell (2001) that energy supply is usually the first limiting factor for microbial growth in the rumen. The maximum potential of rumen microbes to produce microbial protein can be explored only by the provision of high quality forage. In addition, matching the release of ammonia-N from dietary protein with the release of usable energy may improve N utilization. In order to increase microbial yield, it seems that the manipulation of energy and N fermentation in the rumen should first be aimed at obtaining the most even ruminal energy supply pattern possible within a particular dietary regimen.

5. Conclusions

Based on this study, it could be concluded that LLM could be used as a protein source, while the combination of HLLM and concentrate level at 2 g/kg BW enhanced voluntary feed intake, nutrient digestibility, rumen fermentation and microorganisms in swamp buffalo supplementation with urea–calcium hydroxide treated rice straw.

Acknowledgments

The authors would like to express our most sincere gratitude to the Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, and the Norwegian Programme for development, Research and Education (NUFU Project) for their financial support for the first author's study at M.Sc. degree level and for the use of the research facilities, respectively.

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