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Effect of Various Plant Protein Sources in High-quality Feed Block on Dry Matter Intake, Digestibility and Rumen Fermentation in Swamp Buffalo

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Abstract: This study was designed to determine the effect of various plant protein sources in High-Quality Feed Block (HQFB) on feed intake, digestibility and rumen fermentation characteristic in swamp buffalo. Four, rumen fistulated swamp buffaloes (*Bubalus bubalis*) were randomly assigned according to a 4×4 Latin square design. Four kinds of plant protein sources [Coarse Rice Bran (CRB), Cassava Hay (CH), *Phaseolus Calcaratus* Hay (PCH) and Mulberry Hay (MH)] were mixed in the HQFB. HQFBs were allowed to be licked at free choice in a wooden box and Urea-Lime Treated Rice Straw (ULRS) were fed *ad libitum*. It was found that HQFB intakes were similar among treatments while ULRS intake in CH fed group was higher than those in other groups ($p<0.05$). Moreover, nutrient digestibility in terms of CP, NDF and ADF in CH fed group were significantly higher than those in other groups ($p<0.05$). Ruminal temperature, NH_3-N concentration, pH, BUN, acetate (C2), propionate (C3) but yrate (C4) and C2:C3 ratio were similar among treatments ($p>0.05$) while total VFA were highest in CH fed group ($p<0.05$). In addition, N absorption was highest in CH fed group ($p<0.05$). Based on this study it could be concluded that cassava hay, *Phaseolus calcaratus* hay and mulberry hay are potential to be used as protein sources in the HQFBs especially cassava hay which resulted in improved rumen fermentation efficiency and digestibility.

Key words: High quality feed block, rumen fermentation, microbial population, swamp buffalo, local feed resources, rice straw

INTRODUCTION

In the tropics, buffaloes and cattle are raised as an integral part of the crop production system, especially where rice is the main commodity (Chantalakhana, 2001). Wanapat *et al*. (2003) reported some differences between buffaloes and cattle in terms of nutrition and feed utilization. It was found that buffaloes could utilize feed more efficiently, particularly where the feed supply is of low quantity and/or quality, with the digestibility of feed about 3% units higher than in cattle. Wanapat (2000) suggested that this may be explained by buffaloes having a different rumen ecology than in cattle with higher population of cellulolytic bacteria and fungal zoospores, lower protozoa population and a greater capacity to recycle nitrogen to the rumen.

It was recognized that when animals are offered a low-nitrogen, high fiber roughage such as rice straw, one of the critical nutrients is fermentable nitrogen available to rumen microbes (ARC, 1980). The use of urea/molasses blocks is a convenient way for avoiding excessive intake of urea and will ensure an almost continuous supply of ammonia nitrogen in the rumen (Preston *et al*., 1965). Urea/molasses block feeding yielded positive results in many parts of the world (De and Singh, 2003; Rafiq *et al*., 2007).

High-quality feed block (HQFB) is one of strategic alternative feed block (Wanapat *et al*., 1999). Wanapat and Khampa (2006) reported that supplementation of HQFB could increase feed intake, nutrient digestibility and rumen VFA. Moreover, supplementation of HQFB in lactating dairy cow indicate that rumen ecology, milk yield and milk composition were significantly improved (Koakhunthod *et al*., 2001). Furthermore, supplementation of leguminous feeds or tree fodder as well as leaf has been shown to improve rumen ecology and ruminant performance (Devendra, 1990).

Recently, cassava hay (*Manihot esculenta*, Crantz) has been grown as a protein foliage supplement in ruminant feeding especially for dairy cattle, beef and

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buffalo production (Wanapat, 2003; Khang and Wiktorsson, 2006). Cassava hay contains a high level of protein (18-25% of DM) and a strategic amount of condensed tannins (4% of DM; Wanapat *et al*., 1997; Dung *et al*., 2005; Hue *et al*., 2010). The use of cassava hay was successfully implemented in several ways by either direct feeding or as a protein source in concentrate mixtures (Hong *et al*., 2003; Kiyothong and Wanapat, 2004), as component with soybean meal and urea in feed supplements (Wanapat *et al*., 2007) and as an ingredient in high quality feed blocks (Wanapat and Khampa, 2006).

Other attractive crops are *Phaseolus calcaratus* (leguminous crop) and mulberry for ruminant feeding in the tropics. *Phaseolus Calcaratus* (PC) has quite a high protein content (15-20% in DM) and can grow well in poor soil and dry areas. The whole PC crop can be sun-dried as PC hay as an animal feed while the seeds can be a protein source for human consumption. *Phaseolus calcaratus* is therefore a promising legume for intercropping and hay making for animals and human food (food-feed system). There have been very few data on PC hay for use in ruminant feeding, research work with this plant is therefore warranted. Mulberry (*Morus alba*) has a high protein content (15-27.6% in DM) (Makkar *et al*., 1989), high palatability and high intake. Moreover, mulberry has 50% of rumen undegradable protein and net energy for milk production about 1.48 Mcal kg^{-1}. Bodthaisong *et al*. (2008) reported that replacement soybean meal by mulberry hay could increase by-pass protein and milk production in lactating dairy cows.

All these leguminous feeds or tree fodders are potential sources of protein for ruminants. However, using these plants protein sources in HQFB in swamp buffaloes have been quite limited. Therefore, this present study was conducted to investigate the effect of various plant protein sources in high-quality feed block on feed intake, digestibility and rumen fermentation characteristic in swamp buffaloes fed on urea-lime treated rice straw.

MATERIALS AND METHODS

Animals, diets and experimental design: Four, ruminally fistulated swamp buffalo male with initial BW of 276±26 kg were randomly assigned to receive four dietary treatments according to a 4×4 Latin square design. The feed ingredients and chemical composition of experimental diets and Urea-Lime (2% urea +2% lime) treated Rice Straw (ULRS) are shown in Table 1. All animals were kept in individual pens, mineral block and water were available for ad libitum consumption. Four dietary treatments were as follows: High-Quality Feed Block (HQFB) with Coarse rice Bran (CRB), Cassava Hay (CH), *Phaseolus calcaratus* Hay (PCH) and Mulberry Hay (MH), respectively.

Table 1: Ingredients and chemical compositions of diets in the experiment

	HQFB				
Ingredients	CRB (T1)	CH (T2)	PCH (T3)	MH (T4)	ULRS
Coarse Rice Bran (CRB)	30	-	-	-	-
Cassava Hay (CH)	-	30	-	-	-
Phaseolus Calcaratus Hay (PCH)	-	-	31	-	-
Mulberry Hay (MH)	-	-	-	32	-
Molasses	40	42	40	40	-
Urea	13	11	12	11	-
Cement	12	12	12	12	-
Sulfur	1	1	1	1	-
Mineral mix	1	1	1	1	-
Salt	1	1	1	1	-
Tallow	2	2	2	2	-
Total	100	100	100	100	-
Chemical composition (%)	DM (%) 75.3	78.5	78.9	78.3	49.6
OM	72.8	76.1	70.6	74.1	84.2
CP	35.7	37.6	39.8	37.2	5.7
NDF	26.1	30.7	42.5	21.7	75.2
ADF	22.7	18.0	29.1	19.0	59.8
Ash	27.2	23.9	29.4	25.9	15.8

CRB = Coarse Rice Bran, CH = Cassava Hay, PCH = *Phaseolus calcaratus* Hay, MH = Mulberry Hay; ULRS = Urea-Lime (2% urea +2% lime) Treated Rice Straw, DM = Dry Matter, OM = Organic Matter, CP = Crude Protein, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber

Urea-Lime treated Rice Straw (ULRS) was prepared by using 2% (w/w) urea and 2% calcium hydroxide mixed with 100 kg of water, 100 kg of Rice Straw (RS) (50:50, water to straw) and poured over a stack of straw and then covered with a plastic sheet for 10 day before feeding to animals.

The CH, PCH and MH were prepared from whole fresh crop, chopped and sun-dried for 2-3 day, ground to pass a 1 mm screen using a Cyclotech Mill (Tecator, Hoganas, Sweden) and then mixed with other ingredients in respective HQFBs mixture. The mixture was put in a hydraulic compressive machine and left to sun-dry for 2-3 day to reduce moisture. HQFB were allowed to be licked all days in wooden box while ULRS were fed *ad libitum*. The experiment was conducted for 4 periods and each period lasted 21 day. During the first 14 day, all animals were fed with respective diets whereas during the last 7 day, the animals were moved to metabolism crates for total collection of urine and feces.

Data collection and sampling procedures: Feeds were sampled and fecal samples were collected from the total collection of each individual swamp buffalo on each treatment during the last 7 day of each period at the morning and afternoon feeding. Composited samples were dried at 60°C, ground (1 mm screen using Cyclotech Mill, Tecator, Sweden) and then analyzed for DM, ash, CP content (AOAC, 1995) and NDF and ADF (Van Soest *et al.*, 1991). At the end of each period, rumen fluid and jugular blood samples were collected at 0, 2, 4 and 6 h post feeding. Approximately 200 mL of rumen fluid was taken at each time from the middle part of the rumen using a 60 mL hand syringe.

Temperature and pH of rumen fluid were measured using a portable pH and temperature meter (HANNA instruments HI 8424 microcomputer, Singapore). Rumen fluid samples were then filtered through 4 layers of cheesecloth. Samples were divided into 3 portions; 1 portion was used for NH_3-N analysis with 5 mL of 1 M H_2SO_4 added to 50 mL of rumen fluid. The mixture was centrifuged at 16,000×g for 15 min and the supernatant was stored at -20°C before NH_3-N analysis using the micro-Kjeldahl methods (AOAC, 1995) and VFA analysis using HPLC (Samuel *et al.*, 1997). A second portion was fixed with 10% formalin solution in sterilized 0.9% saline solution. The total direct count of bacteria, protozoa and fungal zoospores were made by the methods of Galyean (1989) based on the use of a haemacytometer (Boeco, Hamburg, Germany). Another portion was cultured for group of bacteria using a roll tube technique (Hungate, 1969) for identifying bacteria groups (cellulolytic, proteolytic, amylolytic and total viable count bacteria).

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 500×g for 10 min and stored at -20°C until analysis of plasma urea N according to the method of Crocker (1967). Urine samples were analyzed for Urinary N excretion.

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} = The observation from animal j, receiving diet i in period k
- μ = The overall of mean
- M_i = The mean effect of HQFB (i = 1-4)
- A_j = The effect of animal (j = 1-4)
- P_k = The effect of period (k = 1-4)
- ε_{ijk} = The residual error

Treatment means were statistically compared 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 AND DISCUSSION

Chemical composition of diets: Chemical compositions of HQFB and ULRS are shown in Table 1. HQFB with CRB, CH, PCH and MH contained 35.7, 37.6, 39.8 and 37.2% CP, respectively. Similar CP content for HQFB with CRB have been previously reported by Koakhunthod *et al.* (2001) and Khampa *et al.* (2009) with values of 36.0 and 36.6% CP, respectively while Wanapat and Khampa (2006) reported at 37.8% CP.

The CP content for HQFB with CH similarly with Khampa *et al.* (2009), (37.3% CP) but lower than Wanapat *et al.* (1999) (43.6% CP) and higher than Koakhunthod *et al.* (2001) (33.2% CP). This could be due to CH was harvested at a younger stage of growth (3-4 months) or regrowth (2-3 months) for having higher nutritive value and/or difference in cultivars, age of plant, plant density, soil fertility, harvesting frequency and climate (Gomez and Valdivieso, 1984). In whole cassava plant, the forage especially leaf is usually less than stem (Ravindran, 1993).

Therefore, this variation may have resulted from ratio of stem: leaf. As the leaf and stem fraction in CH differed greatly in their nutrient content. In addition, NDF content was greatest in HQFB with PCH treatment. The differences could be attributed to age of plant because the amount of NDF in whole plants increased linearly with age of plant, especially increased in NDF and ADF in stems and indicated the formation of tannin-fiber complexes that were not solubilized in the acid detergent solution (Getachew *et al.*, 2001). The ULRS contained 5.7% CP, 75.2% NDF and 59.8% ADF on a DM basis and was similar to that reported by Wanapat *et al.* (2009).

Effect on feed intake and nutrient intake: Table 2 shows data on feed intakes and nutrient intakes. HQFB intakes in terms of BW% and g kg^{-1} $BW^{0.75}$ were not significantly affected ($p>0.05$) by treatments while ULRS and total intakes was highest in HQFB with CH ($p<0.05$) but lowest in HQFB with CRB fed group.

These data were in accordance with the report by Wanapat *et al.* (1997) that CH had are markable high DM digestibility (71%) and high ruminal by pass protein since it contained tannin-protein complex. Moreover, medium CT concentrations (30-40 g kg^{-1} DM) had no effect upon voluntary feed intake (VFI) (Terrill *et al.*, 1992; Wang *et al.*, 1996) but could reduce protein solubility and degradation in the rumen increased the absorption essential amino acids (EAA) from the small intestine (Waghorn, 2008; Barry and McNabb, 1999).

Table 2: Effects of various plant protein sources in high-quality feed block on HQFB, urea-lime treated rice straw, total intakes and nutrient intakes in swamp buffaloes

Items	HQFB CRB (T1)	CH (T2)	PCH (T3)	MH (T4)	SEM
HQFB intake (kg day^{-1})	0.30	0.31	0.27	0.27	0.010
Percentage of BW	0.11	0.11	0.10	0.10	0.004
g kg^{-1} BW$^{0.75}$	4.20	4.50	3.90	3.90	0.170
ULTR intake (kg day^{-1})	5.56^{a}	5.93^{b}	5.61^{a}	5.78^{ab}	0.070
Percentage of BW	1.91^{a}	2.06^{c}	1.94^{ab}	2.02^{bc}	0.030
g kg^{-1} BW$^{0.75}$	78.90^{a}	85.00^{c}	79.80^{ab}	83.30^{ab}	1.040
Total intake (kg day^{-1})	5.86^{a}	6.25^{b}	5.88^{a}	6.05^{ab}	0.070
Percentage of BW	2.02^{a}	2.18^{b}	2.03^{b}	2.12^{ab}	0.030
Percentage g kg^{-1} BW$^{0.75}$	83.20^{a}	89.50^{b}	83.70^{a}	87.20^{ab}	1.090
Nutrient intake (kg day^{-1})					
Organic matter	4.90^{a}	5.14^{b}	4.91^{a}	5.01^{ab}	0.060
Crude protein	0.42	0.45	0.43	0.42	0.010
Neutral detergent fiber	4.26^{a}	4.47^{b}	4.33^{ab}	4.37^{ab}	0.050
Acid detergent fiber	3.39^{a}	3.54^{b}	3.43^{ab}	3.48^{ab}	0.040

^{abc}Value within the row with different superscripts are significantly different ($p<0.05$), CRB = Coarse Rice Bran, CH = Cassava Hay, PCH = *Phaseolus Calcaratus* Hay, MH = Mulberry Hay

Nutrient intakes in term of OM, NDF and ADF were highest in HQFB with CH fed group ($p<0.05$) while CP intake was not significantly different ($p>0.05$).

Effect on digestibility and nitrogen balance: Apparent digestibility of CP, NDF and ADF were highest in HQFB with CH fed group as compared with other treatment groups ($p<0.05$). These data were in accordance with the report by Phengvichith and Ledin (2007) that supplementing Wilted Cassava Foliage (WCF) in the diet could increase the apparent digestibility of DM and resulted in a higher N retention and weight gain of growing goats.

N intake, Urinary N and N retention were not affected ($p>0.05$) by treatments while Fecal N was lowest and N absorption was highest in HQFB with CH fed group ($p<0.05$). With regards to N utilization, Owens and Zinn (1988) stated that N excretion and N retention should reflect differences in N metabolism, because N retention was the most important index of the protein nutrition status of the ruminants.

Characteristics of ruminal fermentation and blood metabolites: Measured rumen variables included temperature, pH, NH_3-N and VFA. Plasma urea N was also determined to investigate the relationship with rumen NH_3-N and protein utilization.

The pattern of ruminal fermentation and overall means are shown in Table 3. Rumen fluid pH and temperature were not altered among treatments and the values were stable at pH 6.6-6.7 and temperature of 38.8-39°C and the pH was within the range considered optimal for microbial digestion of fiber and protein [6.0-7.0; Lyle *et al.* (1981); Hoover (1986); Firkins (1996); Wanapat (1990)]. Ruminal NH_3-N is a major source of N for microbial protein synthesis (Erdman *et al.*, 1986). Ruminal NH_3-N concentrations were 11.35-11.85 mg dL^{-1} and were close to those previously reported by Wanapat and Pimpa (1999) for improving rumen ecology, microbial protein synthesis, digestibility and voluntary feed intake. Decreasing rumen NH_3-N concentrations also resulted in decreasing concentrations of blood urea N. Concentrations of blood urea N are highly correlated to the concentration of NH_3 production in the rumen (Preston *et al.*, 1965). This study revealed that HQFB with CH has been higher in NH_3-N concentration. This would indicate that available rumen NH_3-N could be used and/or absorbed in the rumen for further synthesis. There were no significant differences ($p>0.05$) in acetic acid, propionic acid and butyric acid proportions and acetic:propionic

Table 3: Effects of various plant protein sources in high-quality feed block on nutrient digestibility and nitrogen balance in swamp buffaloes

Items	HQFB CRB (T1)	CH (T2)	PCH (T3)	MH (T4)	SEM
Nutrient digestibility (%)					
Organic matter	67.62	68.87	68.33	68.44	0.65
Crude protein	61.92^{a}	68.79^{b}	66.09^{ab}	66.67^{ab}	0.90
Neutral detergent fiber	60.62^{a}	64.98^{b}	61.32^{a}	60.52^{a}	0.67
Acid detergent fiber	54.70^{a}	59.92^{b}	52.40^{a}	55.69^{a}	1.05
N balance (g day^{-1})					
N intake	67.9	73.2	68.6	69.0	1.06
Fecal N	25.7^{a}	22.9^{b}	23.3^{ab}	23.1^{ab}	0.54
Urinary N	29.4	30.9	30.7	29.9	0.44
N absorption	42.2^{a}	50.3^{b}	45.3^{ab}	45.9^{ab}	1.10
N retention	12.9	19.4	14.6	15.0	1.13

^{ab}Value within the row with different superscripts are significantly different ($p<0.05$), CRB = Coarse Rice Bran, CH = Cassava Hay, PCH = *Phaseolus Calcaratus* Hay, MH = Mulberry Hay

Table 4: Effects of various plant protein sources in high-quality feed block on rumen fermentation characteristic in swamp buffaloes

Items	HQFB CRB (T1)	CH (T2)	PCH (T3)	MH (T4)	SEM
Ruminal pH	6.64	6.60	6.60	6.67	0.01
Temperature (°C)	38.90	39.00	38.80	39.00	0.06
NH_3-N (mg dL^{-1})	11.35	11.85	11.64	11.59	0.09
BUN (mg dL^{-1})	12.50	13.30	13.30	11.90	0.35
Total VFA (mmol L^{-1})	102.20^{a}	116.00^{b}	110.30^{ab}	108.60^{ab}	3.69
VFAs (mol/100mol)					
Acetate (C2)	63.00	63.20	62.20	62.20	0.34
Propionate (C3)	28.20	28.80	29.00	29.20	0.22
Butyrate (C4)	8.80	8.00	8.50	8.20	0.31
C2:C3 ratio	2.20	2.20	2.10	2.10	0.02

^{ab}Value within the row with different superscripts are significantly different ($p<0.05$), CRB = Coarse Rice Bran, CH = Cassava Hay, PCH = *Phaseolus Calcaratus* Hay, MH = Mulberry Hay ratio (Table 4). However, total VFA concentrations was highest in HQFB with CH fed group and ranged from 102.2-116.0 mmol L^{-1} These values were similar to those reported by Koakhunthod *et al.* (2001).

CONCLUSION

Based on this experiment it could be concluded that cassava hay, *Phaseolus calcaratus* hay and mulberry hay are potential to be used as protein sources in HQFB especially cassava hay which can improve rumen fermentation efficiency by increasing total VFA.

Moreover, CP, NDF and ADF digestibilities and ULRS and nutrient intakes were significantly improved by cassava hay as protein sources in HQFB. HQFB are therefore recommended as lick-blocks for ruminants fed on low-quality roughages such as rice straw.

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