Effects of Various Levels of Cassava Hay on Rumen Ecology and Digestibility in Swamp Buffaloes

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ABSTRACT : Four, mature rumen fistulated male swamp buffaloes with an average initial weight of 426±25 kg were randomly allocated to receive dietary treatments according to a 4×4 Latin square design. Four dietary treatments with varying proportions of urea-treated rice straw (UTRS) and cassava hay (CH) were offered (100:0, 75:25, 50:50, 0:100). Each feeding period lasted 21 days, the first 14 days for feed adjustment and intake measurement and the final 7 days for rumen fluid and faecal collections. The results demonstrated the potential use of both UTRS and CH as roughage sources. As levels of CH increased in the diets pH values were maintained (6.5-7.0). Ruminal NH_3-N concentrations were significantly ($p<0.05$) raised as higher levels of CH were incorporated into the diets. Moreover, cellulolytic and proteolytic bacterial populations were enhanced while total protozoal counts were decreased ($p<0.05$). In addition, DM, OM and CP digestibilities and their digestible intakes as well as estimated energy increased with increasing levels of CH in the diets. The results suggest a favorable effect of CH substituting for UTRS. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 5 : 663-669)***

Key Words : Cassava Hay, Urea-treated Rice Straw, Rumen Ecology, Condensed Tannins, Swamp Buffaloes

INTRODUCTION

To meet their production potential, ruminants have to consume the required amounts of energy, proteins, minerals and vitamins. However in the tropics, most ruminants have been fed on low quality roughages, crop-residues and agricultural by-products such as rice straw, which are common feed resources available during the dry season (Wanapat, 2000). The main nutritional constraints for crop-residues as animal feeds are their high fibre content and bulkiness, slow rate of digestion reducing rumen clearance rates and low nitrogen content (Lui et al., 1999) particularly of proteins which are essential for both the animal and rumen microbial growth. Treatment of rice straw with ammonia or urea (5%) has been reported to improve the nutritive value and overall utilization of straw in ruminants. (Sundst∅l and Coxworth, 1984; Hart and Wanapat, 1992; Wanapat, 1999). Furthermore, supplementation with leguminous feeds or tree fodder has been shown to alter rumen ecology and improve ruminant performance (Devendra, 1990; Wanapat et al., 1989, 1992)

Cassava (*Manihot esculenta*, Crantz) is widely grown in tropical regions (Calpe, 1992; Wanapat, 2003). Its root is a good source of carbohydrate whilst the leaf has potential as a protein source supplement in ruminants (Reed et al., 1982; Devendra, 1988; Johnson and Djajanegara, 1989; Ravindran, 1993; Preston, 2001). Dried cassava leaf resulting from the crop-residue of a root crop contained a high level of condensed tannins (>6% DM), which have an adverse effect on intake, palatability and digestibility in ruminants (Reed et al., 1982). However, cassava hay harvested at young stage of growth (3-4 month), contained 22 to 25% CP with condensed tannins down at <4%. Earlier reports by Wanapat et al. (2000a, b) have shown the beneficial effect of using cassava hay in lactating dairy cows by reducing the amount of concentrate supplement and increasing milk yield and composition whilst modifying rumen ecology. However, little information is available on combinations of cassava hay and urea-treated rice straw as sources of roughage.

The objective of this experiment was to study the interactive use of cassava hay and urea-treated rice straw as sources of roughage on rumen ecology and digestibility in swamp buffaloes.

MATERIALS AND METHODS

Animals, diets and experimental design

Four, mature rumen fistulated male swamp buffaloes with an average initial weight of 426±25 kg were randomly assigned to dietary treatments according to a 4×4 Latin square design. The dietary treatments were 100% urea-treated rice straw without cassava hay (0% CH), 75% urea-treated rice straw with 25% cassava hay (25% CH), 50% urea-treated rice straw with 50% cassava hay (50% CH) and 0% urea-treated rice straw with 100% cassava hay (100% CH).

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Table 1. Ingredients and estimated chemical composition of the diet consumed by rumen fistulated swamp buffaloes (DM %)

Ingredient	% DM basis
Cassava chip	72.7
Rice bran meal	22.8
Urea	2.5
Minerals and vitamins [a]	1.0
Salt	1.0
Estimated values (total diet)	
DM [b], %	88.7
CP, %	12.0
TDN, %	75.4

[a] Minerals and vitamins (each kg contains): Vitamin A: 10,000,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU; Fe: 50 g; Zn: 40 g; Mn: 40 g; Co: 0.1 g; Cu: 10 g; Se: 0.1 g; I: 0.5 g.
[b] DM: dry matter; CP: crude protein; TDN: total digestible nutrient.

All buffaloes were drenched for internal worms and injected with vitamins A, D_3 and E prior to commencing the experiment. Each buffalo was housed individually in a 4×7 m pen where water and mineral salt were available at all times. During each period, animals received 2% BW of respective roughages and 0.3% BW of concentrate. The composition of the concentrate is given in Table 1. The animals were fed twice daily in two equal portions at 07:00 and 15:00 hours. Each experimental period lasted for 21 days, the first 14 days as a period of adaptation and for feed intake measurements while the last 7 days were for sample collections of rumen fluid and faeces.

Feed management

Urea-treated rice straw (UTRS) was prepared by using 5% (w/w) urea 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 days before feeding to animals.

Cassava hay (CH) was made from a cassava crop managed as follows: it was planted at 30×40 cm between plants and rows in a well-prepared soil, which was fertilized with cow manure at 700 kg (fresh) per hectare. Cassava Hay was made from whole cassava crop (aerial above) that were harvested at 3 month maturity after planting and was chopped into small pieces (about 2-4 cm) and then sun-dried for 2-3 days to obtain CH. Sun drying consisted of spreading the CH on the ground and turning. Samples of the final dry product were taken for chemical analysis.

***In vivo* digestibility**

Feed intake was measured on a daily basis during an initial two week period. Feeds and faecal samples were collected during the following seven days of each period. Faecal samples were collected by rectal sampling. Feeds and faeces were chemically analyzed for DM, ash and CP (AOAC, 1990), NDF, ADF and ADL (Goering and Van Soest, 1970) and AIA. In addition, condensed tannins were analyzed in cassava hay (Burns, 1971). Acid insoluble ash was used to estimate digestibility of nutrients (Van Keulen and Young, 1977).

Ruminal studies

Rumen fluid samples were collected at 0, 2, 4 and 6 h post feeding. Approximately 200 ml of rumen fluid was taken from the middle part of the rumen using a 60 ml hand syringe at the end of each period. Rumen fluid was immediately measured for pH and temperature using a portable pH and temperature meter (Orion Research portable meter 200 series, USA) after withdrawal. Rumen fluid samples were then filtered through four layers of cheesecloth. Samples were divided into two portions. One portion was used for NH_3-N and VFA analyses where 5 ml of H_2SO_4 solution (1 M) were added to 50 ml of rumen fluid. The mixture was centrifuged at 16,000×g for 15 minutes and supernatant stored at -20°C prior to NH_3-N analyses according to Zinn and Owens (1986). Another portion was fixed with 10% formalin solution in normal saline (0.9% NaCl) (Galyean, 1989). The total direct count of bacteria, protozoa and fungal zoospores were made using the methods of Galyean (1989) based on the use of a haemacytometer (Boeco) and cultured groups of bacteria using roll-tube technique the method described by Hungate (1969), for identifying bacteria groups (cellulolytic, proteolytic, amylolytic and total viable count bacteria).

Samples of blood (about 10 ml) were drawn from the jugular vein at the same time as rumen fluid sampling (0, 2, 4 and 6 h post-feeding) and the serum was separated by centrifugation at 1,000×g for 10 minute and stored at -20°C until analysis of blood urea nitrogen (BUN) according to the method of Crocker (1967).

Statistical analysis

Statistical analyses were performed using the GLM procedure of SAS (1990). 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 level CH (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), ε_{ijk}, the residual effect. Means were statistically compared using Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980).

RESULTS

Chemical composition of feeds

Feed ingredients and chemical composition are presented in Table 1 and 2. Concentrates were formulated using locally available feed ingredients.

Effect on feed intake

Table 3 shows data on feed intake and digestibility of nutrients. Intakes in terms of % BW and g/kg $W^{.75}$/d, they

Table 2. Chemical composition (g/kg DM) of urea-treated rice straw (UTRS), cassava hay (CH) and concentrate

Items	DM,g/kg [a]	Ash	OM	CP	NDF	ADF	ADL	CT
Cassava hay (CH)	857	83	917	216	594	356	114	37
Urea-treated rice straw (UTRS)	557	141	859	85	793	525	72	ND[c]
Concentrate[c]	906	79	921	117	96	71	33	ND

[a] DM: dry matter; CP: drude protein; OM: organic matter; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; CT: condensed tannins. [b] ND: not determined. [c] The diet consumed by rumen fistulated swamp buffaloes.

Table 3. Effect of varying levels of cassava hay on feed intake, apparent digestibility and digestible nutrient intake (kg/d)

Items	Percentage inclusion of cassava hay				SEM
	0	25	50	100	
Total DM intake, kg/d	9.2[ab]	9.8[a]	9.5[a]	8.4[b]	0.34
% of BW	2.0[ab]	2.1[a]	2.0[ab]	1.8[b]	0.07
g/kg $BW^{0.75}$	92.5[ab]	98.2[a]	93.6[a]	82.6[b]	0.23
Weight gain at 21d, kg	15.5	16.8	15.8	14.8	1.30
Weight change, kg/d	0.7	0.8	0.8	0.7	-
Apparent digestibilities, %					
DM	56.2[a]	57.1[a]	57.4[a]	65.7[b]	2.04
OM	61.7	61.4	61.0	67.3	1.99
CP	46.4[a]	50.9[ab]	58.5[b]	71.4[c]	3.24
NDF	51.8[a]	55.1[b]	55.2[b]	56.3[b]	0.93
ADF	44.4[a]	46.2[b]	46.2[b]	45.7[ab]	0.49
Digestible nutrient intake, kg/d					
OM	5.0	5.2	5.1	5.3	0.15
CP	0.5	0.6	0.8	1.2	0.05
NDF	3.8[ab]	4.0[a]	3.5[ab]	3.1[b]	0.30
ADF	2.0[a]	1.8[a]	1.6[b]	1.3[b]	0.18
Estimated energy intake[a]					
ME, Mcal/d	19.9	19.9	19.6	20.0	0.56
ME, Mcal/kg/DM	2.0	2.1	2.1	2.3	0.08

[a-c] Means within rows not sharing a common superscripts are significantly different ($p<0.05$).
SEM: standard error of the mean. [a] 1 kg DOM=3.8 Mcal ME/kg (Kearl, 1982).

were no significant difference in total DMI between 0, 25 and 50% CH, but were significantly ($p<0.05$) higher than in treatment with 100% CH, although roughage offered was adjusted at 2% BW. Apparent digestibilities (%) of DM, OM, CP and NDF were significantly higher than the control treatment (0% CH), however, ADF showed a trend to be lower but was not significantly ($p>0.05$) different among treatment CH-based diets. As a result, digestible DM, OM and CP intakes as well as estimated energy intakes (ME Mcal/DM/d, ME Mcal/kg/DM) were not significantly different among treatment, but estimated energy intakes shown tended higher in treatment with 100% CH.

Effect on rumen ecology

As shown in Table 4, rumen temperatures were similar among treatments and the values were quite stable at 38-39°C. Ruminal pH values measured at 0, 2, 4 and 6 h post feeding, were found in the range of 6.6-7.0 and were significantly ($p<0.05$) different at 0, 2 and 4 h post feeding. As shown, incorporation of CH at 50 and 100% of diets resulted in higher and more stable pH values than in other treatments. Ruminal NH_3-N concentrations were also different ($p<0.05$) at each hour of sampling. However, higher NH_3-N levels were found ($p<0.05$) as level of CH increased in the diets. The increases in rumen NH_3-N levels also resulted in increasing levels of BUN and the values were linearly increased as levels of CH increased in the diets.

Effect on rumen microbes

Table 5 presents rumen microorganism populations. Total viable bacterial counts were increased from 0 to 4 h post feeding ($p<0.05$) in all treatments and were the highest in treatment with 50% CH +50% UTRS (9.8×10^8 CFU/g). Total bacterial counts were similar among treatments, meanwhile, protozoal populations were found to decrease as levels of CH increased 6.2 to 2.1×10^5 cell/g, in the control and 100% CH treatment, respectively. However, fungal zoospores were variable and were highest in the treatment with 50% CH.

DISCUSSION

Feed composition of diet

The chemical analysis of CH, UTRS 5% and Concentrate are presented in Table 2. Similar values for UTRS and CH have previously been reported by Ravindran (1993); Wanapat et al. (2000a); Hong et al. (2003), as CH

Table 4. Effect of varying levels of dietary cassava hay on blood urea nitrogen (BUN, mg/dl), temperature of rumen (°C), ruminal pH and ammonia nitrogen (NH_3-N, mg/dl)

	Percentage inclusion of cassava hay				SEM
	0	25	50	100	
Temperature of rumen, °C					
0 h post feeding	38.7	38.2	38.7	38.1	0.72
2	39.0	39.0	38.7	38.6	0.31
4	39.7	38.7	38.8	38.7	0.21
6	39.5	39.2	39.3	39.0	0.29
Mean	39.2	38.7	38.8	38.6	0.38
Ruminal pH					
0 h post feeding	6.6[a]	6.6[a]	6.9[b]	7.0[b]	0.06
2	6.9[a]	6.5[b]	6.8[ab]	6.9[a]	0.09
4	6.7[ab]	6.6[a]	6.9[bc]	6.9[c]	0.51
6	6.7	6.6	6.6	6.8	0.10
Mean	6.7	6.6	6.8	6.9	0.19
NH_3-N, mg/dl					
0 h post feeding	10.4[a]	12.9[ab]	14.6[b]	18.6[c]	1.10
2	14.6[a]	17.2[ab]	18.6[ab]	20.7[b]	1.51
4	13.9[a]	18.2[b]	18.2[b]	17.1[b]	0.76
6	12.5[a]	12.9[ab]	13.2[ab]	14.3[b]	0.46
Mean	12.9[a]	15.3[b]	16.2[b]	17.7[b]	0.96
BUN, mg/dl					
0 h post feeding	13.3[a]	14.1[ab]	16.3[b]	19.3[b]	1.07
2	15.3[a]	15.2[a]	17.4[a]	21.8[b]	0.94
4	15.4[a]	16.0[a]	18.6[ab]	21.9[b]	0.89
6	14.2[a]	16.1[a]	20.0[b]	22.4[b]	1.06
Mean	14.6[a]	15.3[a]	18.1[b]	21.3[b]	0.99

[a-c] Means within rows not sharing a common superscripts are significantly different ($p<0.05$). SEM: standard error of the mean.

was harvested at a younger stage of growth (3-4 months) or regrowth (2-3 months).

Effect on feed intake

Apparent digestibilities (%) of DM, OM, CP and NDF were highest in 100% CH fed group (Table 3). These data are in agreement with those reported by Wanapat et al. (2000a) that CH had a high DM digestibility (71%) and high ruminal by pass protein since it contained tannin-protein complex. In this study, CT in CH (*Manihot-esculenta*, Crantz) was present at 37 g/kg DM. It may have led to increased absorption of EAA from the small intestine and increased animal productivity without affecting VFI, thus improving the efficiency of food conversion. These data support earlier work (Terrill et al., 1992; Wang et al., 1996), medium CT concentrations (30-40 g/kg DM) had no effect upon voluntary feed intake (VFI) but have reduced protein solubility and degradation in the rumen (Min et al., 2001), increased the absorption essential amino acids (EAA) from the small intestine (Waghorn et al., 1987; Barry and McNabb, 1999).

Similarly, there were no significant effects on for estimated energy intake, however intake of ME Mcal/kg/DM for supplemented CH treatment was higher than the control treatment. This difference is probably related to the high apparent digestibility of DM and OM intake that were associated with the apparent digestibility of the diet. As a consequence, intake of ME Mcal/kg/DM increased.

Effect on rumen ecology

Rumen ecology was measured by temperature, rumen pH, ammonia nitrogen (NH_3-N) and blood urea nitrogen (BUN) concentration. Ruminal pH was significantly ($p<0.05$) different at 0, 2 and 4 h post feeding. As Shown, incorporation of CH at 50 and 100% resulted in higher and more stable pH values than in other treatments but all were within the normal range (6.7-6.9) which has been reported as optimal for microbial digestion of fibre (Lyle et al., 1981; Hoover, 1986; Firkins, 1996) and also digestion of protein in the rumen (range 6-7) (Wanapat, 1999). This data was in accordance with reports by Yuangklang et al. (2001). The increased ruminal pH with increasing CH levels seemed to be related to CT concentration because CT stimulates increased saliva production and as a consequence, increasing urea recycling in the rumen (Reed, 1995).

Increased ruminal NH_3-N levels were found ($p<0.05$) as level of CH increased in the diets and were closer to optimal ruminal NH_3-N (15-30 mg %, Boniface et al., 1986; Erdman et al., 1986; Perdok and Leng, 1990; Wanapat and Pimpa, 1999) for increasing microbial protein synthesis, feed digestibility and voluntary feed intake. The increases in rumen NH_3-N levels also resulted in increasing levels of BUN and the values were linearly increased as levels of CH increased in the diets. The differences in NH_3-N and BUN concentration among treatments may have been related directly to CP levels of CH. Preston et al. (1965) reported that concentrations of BUN are highly correlated to protein intake and reflect the level of ammonia production in the rumen (Lewis, 1975). This would indicate that available rumen NH_3-N could be used and/or absorbed in the rumen for further synthesis (Table 4).

This study revealed that incorporation of CH has increased NH_3-N concentration with ammonia being the main nitrogen source for growth and protein synthesis by ruminal bacteria to achieve maximum fermentation (Satter and Slyter, 1974; Hoover, 1986). Therefore, increasing ruminal NH_3-N concentration by dietary additions of CH should improve efficiency of digestibility by ruminants, resulting in maximum intake and digestibility of low quality forages.

Effect on rumen microbes

Incorporation of CH has shown a trend to increase cellulolytic and proteolytic bacterial populations whilst total protozoal counts were dramatically decreased ($p<0.05$)

Table 5. Effect of varying levels of cassava hay on ruminal protozoa, fungi, bacteria population, ruminal cellulolytic, proteolytic, amylolytic and total viable bacterial count

Items	Percentage inclusion of cassava hay				SEM
	0	25	50	100	
Total viable bacteria ($\times10^8$ CFU/g rumen content)					
0 h, post feeding	8.7	9.5	8.2	8.9	0.89
4	9.6^{ab}	9.6^{ab}	9.8^{a}	9.3^{b}	0.10
Mean	9.1	9.6	9.0	9.1	0.49
Bacteria ($\times10^{10}$ cell/g rumen content)					
0 h, post feeding	3.7	3.6	3.2	3.2	3.35
4	3.6	3.8	3.5	3.6	2.16
Mean	3.6	3.7	3.4	3.4	2.67
Protozoa ($\times10^5$ cell/g rumen content)					
0 h, post feeding	6.6^{a}	5.2^{a}	2.8^{b}	2.1^{b}	0.53
4	5.5^{a}	3.8^{ab}	2.7^{b}	2.1^{b}	0.52
Mean	6.2^{a}	4.4^{b}	2.7^{bc}	2.1^{c}	0.52
Fungal zoospores ($\times10^5$ cell/g rumen content)					
0 h, post feeding	7.3^{a}	10.7^{b}	10.6^{b}	8.0^{ab}	0.80
4	7.2^{bc}	7.5^{ab}	8.8^{a}	6.0^{c}	0.34
Mean	7.2^{a}	9.1^{b}	9.7^{b}	7.0^{a}	0.57
Cellulolytic bacteria ($\times10^7$ CFU/g rumen content)					
0 h, post feeding	3.7	6.8	6.8	7.0	1.07
4	6.8	6.2	8.7	7.4	0.78
Mean	5.3	6.5	7.8	7.2	0.92
Proteolytic bacteria ($\times10^6$ CFU/g rumen content)					
0 h, post feeding	7.5	6.2	8.1	6.7	0.69
4	4.9	6.7	7.0	6.8	0.70
Mean	6.2	6.5	7.6	6.8	0.69
Amylolytic bacteria ($\times10^6$ CFU/g rumen content)					
0 h, post feeding	8.3	8.2	7.0	6.9	0.58
4	6.0	4.1	5.7	7.4	1.14
Mean	7.1	6.2	6.4	7.1	0.86

[a-c] Means within rows not sharing a common superscripts are significantly different ($p<0.05$). SEM: standard error of the mean.

(Table 5). It is possible that condensed tannins (CT) present in the CH may play an important role in decreasing protozoal populations. This data is in agreement with previous reports Wang et al. (1994); Yuangklang et al. (2001); Koakhunthod et al. (2001). However, it should be noted that cellulolytic bacteria on all treatments were higher than those in the 0% CH (6.5-7.8^7 CFU/g), respectively. It could be speculated that the CT in the CH are having a similar effect on the rumen protozoa and the cellulolytic bacteria. As previously reported by Makkar et al. (1995); McSweeney et al. (1999) and McSweeney et al. (2000) condensed tannins altered rumen ecology and increased microbial protein synthesis, however, the mode of action of CT on rumen fermentation is yet to be elucidated. However, at high levels (>6% of dry matter), tannins can adversely affect the microbial activity (Makkar et al., 1989; Reed, 1995) and also these plants often contain CT (polyphenolics) which inhibit growth of dominant rumen bacteria (*Fibrobacter succinogens*, *Butyrivibrio fibrisolvens* and *Streptococcus bovis*).

CONCLUSIONS AND RECOMMENDATIONS

Based on this experiment it could be concluded that replacing diets based on UTRS with CH resulted in maintained ruminal pH, increased NH_3-N concentrations and cellulolytic bacteria and decreased protozoal populations. Moreover, DM, OM and CP digestibility and estimated energy intakes were significantly improved as levels of CH increased in the diets. However, the results of this study show that CH supplementation to UTRS based diets appeared to have an optimal effect up to 50% potentiality improved rumen ecology, digestibility and feed intake of roughage in swamp buffaloes. This result suggests that the combined use of UTRS and CH should be recommended during the dry reason for improving rumen ecology and subsequent performance in ruminants.

However, further studies of CH supplementation in UTRS based roughage on bacterial, protozoal and fungal population changes and their ruminal fermentation should be conducted, particularly in lactating dairy cows on straw based feeding.

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