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Effect of Roughage Sources on Cellulolytic Bacteria and Rumen Ecology of Beef Cattle

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ABSTRACT : The effect of different tropical feed sources on rumen ecology, cellulolytic bacteria, feed intake and digestibility of beef cattle was investigated. Four fistulated, castrated male crossbred cattle were randomly allocated to a 4×4 Latin square design. The treatments were: T1) urea-treated (5%) rice straw (UTS); T2) cassava hay (CH); T3) fresh cassava foliage (FCF); T4) UTS:FCF (1:1 dry matter basis). Animals were fed concentrates at 0.3% of body weight on a DM basis and their respective diets on an *ad libitum* basis. The experimental period was 21 days. The results revealed that the use of UTS, CH, FCF and UTS:FCF as roughage sources could provide effective fiber and maintain an optimal range of ruminal pH and NH_3-N. Total viable and cellulolytic bacterial populations were enhanced ($p<0.05$) with UTS as the roughage source. Animals fed FCF had a higher rumen propionate production ($p<0.05$) with a lower cellulolytic bacteria count. Moreover, three predominant cellulolytic bacteria species, namely *Fibrobacter succinogenes, Ruminococcus albus* and *Ruminococcus flavefaciens,* were found in all treatment groups. Roughage intake and total DM intake were highest with UTS (2.2 and 2.5% BW, respectively) as the roughage source ($p<0.05$). Nutrient intake in terms of organic matter intake (OMI) was similar in UTS, CH and UTS:FCF treatments (8.0, 6.8 and 8.7 kg/d, respectively), while crude protein intake (CPI) was enhanced in CH, FCF and UTS:FCF as compared to the UTS treatment ($p<0.05$). Digestion coefficients of DM and organic matter (OM) were similar among treatments, while the CP digestion coefficients were similar in CH, FCF and UTS:FCF treatments, but were higher ($p<0.05$) in CH than in UTS. CP and ADF digestible intakes (kg/d) were highest ($p<0.05$) on the CH and UTS treatments, respectively. It was also observed that feeding FCF as a full-feed resulted in ataxia as well as frequent urination: therefore, FCF should only be fed fresh as part of the feed or be fed wilted. Hence, combined use of FCF and UTS as well as CH and FCF were recommended. (**Key Words** : Cassava Hay, Cassava Foliage, Urea-treated Rice Straw, Cellulolytic Bacteria Species, Roughage, Rumen)

INTRODUCTION

"Feeding the bugs, feeding the cows" has been commonly referred to in ruminant production systems since the rumen is an essential fermentation vat to initiate anaerobic fermentation by prevailing microorganisms (bacteria, protozoa and fungi) to produce end-products for animal uses. Bacteria are the most numerous of these microorganisms and play a major role in the biological degradation of dietary fiber. *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens* are presently recognized as the major cellulolytic bacterial species found in the rumen (Koike and Kobayashi, 2001; Deng et al., 2007).

Currently, many researchers have been interested in studying rumen ecology and finding possible approaches to enhancing rumen function. Local feed resources. particularly low-quality roughages and agricultural crop-residues are of prime importance for ruminants raised in the tropics. These feeds exhibit close relationships with rumen ecology, microbes and rumen fermentation patterns. A number of dietary factors could influence rumen fermentation. especially the basal roughage sources. their physical form and fermentation end-products (Wanapat. 2000). In Thailand. there is a lack of literature and knowledge on using molecular techniques to study rumen microorganisms. therefore the study of microbial population dynamics has been limited. Moreover. competitive and cooperative interactions between fibrolytic microorganisms may affect the degradation of fibrous feeds and hence the energy provided to the animal. Knowledge concerning these

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interactions is essential to further understanding of fiber degradation in the rumen. Classical methods for enumerating microorganisms, based primarily on pure-culture isolation, have numerous limitations. Thus, accurate methods for the identification and quantification of fibrolytic microorganisms are needed to study microbial interactions within the rumen or any other complex community.

During a long dry season, ruminants in the tropics are normally fed on low-quality roughages and agricultural crop-residues and by-products such as rice straw, sugarcane tops and corn stover (Wanapat, 1999). Cassava (*Manihot esculenta*, Crantz) is an important cash crop widely grown in sandy loam soil, with low fertilizer input and under hot and long dry seasonal conditions. Cassava root contains high level of energy which has been used as energy sources in ruminant diets (Chanjula et al., 2004; Khampa et al., 2006; Wanapat and Khampa, 2007). Whole cassava crop (cassava hay, CH) was introduced by Wanapat et al. (1997) into a dry season feeding system for ruminants by managing cassava crop growth in order to obtain optimal yield and good quality. Cassava hay is high in protein (20-30% CP) and contains condensed tannins (1.5-4%). The use of cassava hay was proved to be an excellent ruminant protein feed (Wanapat, 1999; 2003) and was successfully implemented in several ways by either direct feeding (Phengvichith and Ledin, 2007) or as a protein source in concentrate mixtures (Wanapat et al., 2000a, b, c; Hong et al., 2003; Kiyothong and Wanapat, 2004; Granum et al., 2007), as component in soybean meal and urea pellets (Wanapat et al., 2006) and as ingredient in high quality feed blocks (Wanapat and Khampa, 2006). However, the use of CH and fresh cassava foliage (FCF) in ruminant feeds has not yet been substantiated. It was therefore the objective of this experiment to investigate the use of different tropical feeds as a roughage source on rumen ecology and voluntary feed intake of beef cattle.

MATERIALS AND METHODS

Animal and dietary treatments

Four, fistulated castrated male crossbred cattle were randomly assigned to a 4×4 Latin square design to investigate the effect of different tropical feeds on rumen ecology and voluntary feed intake. There were four different roughage treatments as follows:

T1: urea-treated (5%) rice straw (UTS)
T2: cassava hay (CH)
T3: fresh cassava foliage (FCF)
T4: urea-treated rice straw+fresh cassava foliage in ratio of UTS to FCF at 1:1 dry matter basis (UTS:FCF)

Urea treatment of the straw was done by spraying with urea (46% N) dissolved in tap water (5% w/w) to a final moisture content of 50-60%. The treated rice straw was immediately closed and kept under plastic sheet for ten days before feeding (Wanapat et al., 1990).

Concentrate was offered in a similar amount for all treatments at 0.3% of body weight/hd/d. All animals were kept in individual pens and received water *ad libitum*. The experiment was conducted for four periods, each lasting 21 days, namely 14 days for adaptation and dry matter intake measurement and the remaining 7 days for sample collection. During the experimental period, all animals were fed their respective roughage diets *ad libitum*. CH was prepared according to Wanapat et al. (1997) using a whole cassava crop grown for 3 months and its regrowth every two months, which was chopped and then sun-dried for about 2 days until the dry matter content was at least 85%. FCF was harvested every morning and afternoon, chopped by hand (about 5-10 cm, in length) and fed to the animals immediately.

Data collection and sampling methods

Feed and fecal samples were collected during the last seven days of each experimental period and then analysed for dry matter (DM), ash and crude protein (CP) according to AOAC (1985) methods; neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the methods of Goering and Van Soest (1970) and acid-detergent lignin (AIA) analysed as described by Van Keulen and Young (1977). AIA was used to estimate the digestibility coefficients of nutrients. Rumen fluid and blood samples were collected at 0, 2 and 4 h-post feeding on the last day of each experimental period. Rumen fluid was measured for pH immediately, ammonia-N (NH_3-N) concentration as described by Bromner and Keeney (1965) and volatile fatty acids (VFA) by HPLC. Total viable counts of bacteria were determined in roll tubes on a complete agar medium (Hobson, 1969) and the numbers of cellulolytic bacteria were estimated as the most probable number in agar (Hobson, 1969). Culture methods were based on those described by Hungate (1969).

Populations of cellulolytic bacteria species were determined using specific primers (Qaigenr®) by the PCR technique.

Blood samples were taken from the jugular vein at 0, 2 and 4 h post-feeding after rumen fluid was sampled. The samples were kept on ice prior to plasma separation by centrifugation (3,000×g for 15 min) and the plasma was stored at -20°C for urea N (BUN) analysis (Crocker, 1967).

Statistical analysis

All data were subjected to analysis of variance using Proc. GLM (SAS, 1999). Data were analyzed according to the model $Y_{ijk} = \mu+Mi+Aj+Pk+\Sigma ijk$, where Yijk represents

Table 1. Chemical composition of urea-treated (5%) rice straw (UTS), cassava hay (CH) and fresh cassava foliage (FCF)

Feedstuffs	Treatments			
	Concentrate	UTS	CH	FCF
Cassava chip	58.0	-	-	-
Rice bran	37.0	-	-	-
Urea	3.0	-	-	-
Mineral mix	1.0	-	-	-
Sulphur	1.0	-	-	-
Total	100	-	-	-
Chemical composition				
DM (%)	95.2	55.0	87.0	30.6
	------------------- % of DM -------------------			
OM	92.2	84.5	85.9	87.2
Ash	5.8	15.5	14.1	12.8
CP	12.8	7.2	24.7	25.3
NDF	17.7	78.6	58.4	57.5
ADF	8.4	51.3	44.5	43.9

UTS = Urea-treated rice straw, CH = Cassava hay.
FCF = Fresh cassava foliage, DM = Dry matter.
OM = Organic matter, CP = Crude protein.
NDF = Neutral-detergent fiber, ADF = Acid detergent fiber.

observation from animal (j), receiving diet (i), in period (k); μ, the overall mean; Mi, the mean effect of type of roughage source (i = 1, 2, 3, 4); Aj, the effect of animal (j = 1, 2, 3, 4); Pk, the effects of period (k = 1, 2, 3, 4); and Σijk, the residual effect. Treatment means were statistically compared by Duncan's New Multiple Range Test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Chemical composition of feed

Feed ingredients and chemical composition are presented in Table 1. The concentrate was formulated using simple and locally available feed ingredients and contained 12.8% CP. Urea-treated rice straw contained 7.2% CP, while cassava hay (CH) and cassava foliage (FCF) consisted of 24.7 and 25.3% CP, respectively, which were slightly higher than the values reported by Wanapat et al. (2000b,c). The higher values may have been attributed to a higher proportion of leaf to stem in the CH. Moreover, the CH nutritive values could be influenced by cultivar, age of plant, soil fertility or harvesting frequency (Wanapat et al., 1997). UTS contained a lower crude protein than in an earlier report by Wanapat et al. (2000a).

Rumen ecology parameters

Rumen ecology parameters were measured at 0, 2 and 4 h-post feeding for pH, temperature, NH_3-N, volatile fatty acids (VFA) and microorganism population. The results are shown in Table 2. Rumen pH was in the range 6.6-6.9 and was higher in CH and FCF treatments than in UTS and UTS:FCF treatments. This result could be due to higher total volatile fatty acid concentration leading to lower pH in

Table 2. Effect of various tropical roughages on ruminal pH, temperature, ammonia-nitrogen (NH_3-N) and blood urea-nitrogen (BUN) in beef cattle

Items	Treatments				SEM
	UTS	CH	FCF	UTS:FCF	
h-post feeding					
Ruminal pH					
0	6.5	6.6	6.6	6.4	0.08
2	6.6^{a}	6.9^{b}	6.8^{b}	6.5^{a}	0.09
4	6.7^{ab}	6.9^{b}	6.9^{b}	6.6^{a}	0.07
Means	6.6^{a}	6.9^{b}	6.9^{b}	6.6^{a}	0.09
Ruminal temperature (°C)					
0	39.0	39.1	39.0	39.2	0.07
2	39.2^{a}	39.0^{a}	39.2^{a}	39.5^{b}	0.09
4	39.3	39.2	39.2	39.4	0.08
Means	39.3^{a}	39.3^{a}	39.3^{a}	39.6^{b}	0.07
NH_3-N (mg/dl)					
0	11.0^{a}	15.3^{b}	14.6^{ab}	13.6^{ab}	1.25
2	11.6	14.5	15.2	14.6	1.63
4	13.6	14.4	15.0	13.7	0.72
Means	12.1^{a}	14.7^{b}	14.9^{b}	14.0^{b}	0.39
BUN (mg/dl)					
0	6.6	8.6	8.0	6.5	0.52
2	6.9^{a}	8.6^{ab}	11.0^{b}	7.8^{ab}	0.88
4	6.8^{a}	8.9^{ab}	10.2^{b}	7.8^{ab}	0.73
Means	6.9^{a}	8.8^{ab}	10.3^{b}	7.5^{a}	0.88

$^{a, b}$ Values on the same row with different superscripts differ significantly ($p<0.05$).
SEM = Standard error of the mean. UTS = Urea-treated rice straw, CH = Cassava hay. FCF = Fresh cassava foliage. UTS:FCF (1:1 DM basis).
Concentrate was offered at 0.3% of body weight/hd/d.

UTS and UTS:FCF treatments compared to other treatments. However, these values were within the normal range (pH 6-7) (Weimer, 1996). The increased ruminal pH in CH and FCF treatments could have been affected by condensed-tannin (CT) contained in CH, as CT stimulates saliva secretion and increases buffering to the rumen (Reed, 1995). Rumen temperature was similar in UTS, CH and FCF treatments but was slightly higher in UTS:FCF treatment. However, these values do not necessarily reflect the values which could be generated by coarse feed in the experiment. The rumen temperature in this experiment was used as a monitor of rumen physiology and did not fall below the normal range (38.5-39.8).

Ruminal NH_3-N concentration was higher in FCF, CH and UTS:FCF treatments ($p<0.05$) at 14.9, 14.7 and 14.0 mg%, respectively, than in UTS treatment (12.1 mg%). This result could be due to the higher protein content of cassava foliage compared to urea-treated rice straw, thus leading to higher protein degradation in the rumen.

However, the higher NH_3-N levels in FCF, CH and UTS:FCF treatments were within the optimal range of ruminal NH_3-N (15-30 mg%) for low quality roughages (Perdok and Leng, 1990). It also should be noted that these rumen NH_3-N levels were within a suitable range for rumen ecology, particularly for further microbial protein synthesis

Table 3. Effect of various tropical roughages on ruminal total volatile fatty acids (TVFA), acetic acid (C_2), propionic acid (C_3) and butyric acid (C_4) in beef cattle

Items	Treatments				SEM
	UTS	CH	FCF	UTS:FCF	
h-post feeding					
TVFA (mM)					
0	102.5	80.1	101.9	87.8	9.67
2	105.9	93.2	116.1	114.5	13.14
4	116.4	86.2	92.5	103.3	11.60
Means	108.3^{a}	86.5^{b}	103.5^{a}	101.9^{ab}	5.89
Acetic acid (C2, mol%)					
0	71.1^{a}	65.8^{b}	68.0^{ab}	71.5^{a}	1.42
2	72.7	68.3	63.1	71.2	3.42
4	65.7^{ab}	66.0^{ab}	61.6^{a}	71.4^{b}	2.53
Means	69.7^{a}	66.7^{b}	64.2^{b}	71.4^{b}	1.45
Propionic acid (C3, mol%)					
0	18.7	20.8	21.5	16.4	1.92
2	17.2	18.2	27.5	19.3	3.71
4	25.4	21.6	25.0	17.9	3.19
Means	20.4^{ab}	20.2^{ab}	24.7^{a}	17.9^{b}	1.67
Butyric acid (C4, mol%)					
0	10.1	13.3	10.5	12.2	1.34
2	10.2	13.5	9.4	9.5	1.86
4	8.9	12.3	13.4	10.7	1.66
Means	9.7^{a}	13.0^{b}	11.1^{ab}	10.8^{ab}	0.77

$^{a, b}$ Values on the same row with different superscripts differ significantly ($p<0.05$).
SEM = Standard error of the mean, UTS = Urea-treated rice straw, CH = Cassava hay, FCF = Fresh cassava foliage, UTS:FCF (1:1 DM basis).
Concentrate was offered at 0.3% of body weight/hd/d.

(Kanjanapruthipong and Leng, 1998; Wanapat and Pimpa, 1999).

Blood-urea nitrogen (BUN) concentration has been reported to be closely related to rumen NH_3-N. In addition, it was found that when dietary CP percentage increased, the concentration of BUN and the solubility and degradability of protein in the rumen of cattle also increased (Cressman et al., 1980).

The average concentrations of ruminal VFAs after feeding the different treatments are presented in Table 3. The total volatile fatty acids (TVFA) were higher in the UTS treatment than in the CH treatment ($p<0.05$) and were similar to the FCF and UTS:FCF treatments. Acetate (C2) levels were highest in UTS:FCF and UTS treatments ($p<0.05$) compared to those in CH and FCF treatments.

This result was affected by numerically higher cellulolytic bacteria populations in UTS:FCF and UTS treatments. However, propionate (C3) concentration was highest in FCF ($p<0.05$) as compared to UTS:FCF treatment, but was similar with UTS and CH treatments. Based on this experiment, type of roughage could play an important role in VFA production. Wanapat et al. (2003) reported in a comparative trial with beef cattle and swamp buffaloes that rumen fermentation pattern of VFAs was relatively consistent and levels were low in both cattle and buffaloes fed on rice straw, but these values were increased by feeding UTS. It appeared that rumen function could be remarkably enhanced by dietary manipulation.

Rumen microorganism and cellulolytic bacteria species

Table 4 shows total viable bacteria and cellulolytic bacterial populations. Higher values were found in UTS and UTS:FCF treatments when compared with CH and FCF treatments. Higher cellulolytic bacteria populations in UTS and UTS:FCF treatments led to higher C2 concentration in the rumen as compared to other treatments. Hungate (1966) indicated that the total culturable counts (TCC) in the rumen are usually 12-80% of the direct count (DC). Krause et al. (1999b) found that total numbers of bacteria in the rumen of adult sheep when measured as the direct counts, total culturable count and total cellulolytic bacteria (CEL) were 1.6×10^{10}, 9.6×10^{9} and 5×10^{8}cells/g digesta, respectively. The TCC was 58.9% of the DC and the CEL count was 3.1% of the DC or 5.2% of the TCC. In addition, the molecular technique was used in this trial to obtain preliminary data, as well as to classify rumen cellulolytic species of bacteria in beef cattle fed on local feeds. The objectives of this research were to develop molecular methods for analysis of predominant cellulolytic species of bacteria by using specific primers. It was also found that there were three predominant cellulolytic bacteria species namely; *Fibrobacter succinogenes*, selected to allow amplification of 446-bp product, *Ruminococcus albus*, demonstrated species-specific amplification of 176-bp

Table 4. Effect of various tropical roughages on ruminal total viable bacteria count in beef cattle

Items	Treatments				SEM
	UTS	CH	FCF	UTS:FCF	
h-post feeding					
Total viable bacteria (×1,010 CFU/g rumen content)					
0	5.1^{a}	3.3^{b}	3.1^{b}	4.4^{b}	0.28
2	4.9^{a}	3.5^{b}	3.6^{b}	4.3^{b}	0.41
4	4.8^{a}	3.6^{b}	3.5^{b}	4.4^{b}	0.25
Means	4.9^{a}	3.5^{b}	3.4^{b}	4.3^{c}	0.10
Cellulolytic bacteria (×109 CFU/g rumen content)					
0	6.5^{a}	4.5^{b}	3.3^{c}	5.3^{d}	0.30
2	7.1^{a}	4.6^{b}	3.4^{c}	5.4^{d}	0.26
4	6.9^{a}	4.5^{b}	3.5^{c}	5.7^{d}	0.36
Means	6.8^{a}	4.5^{b}	3.4^{c}	5.5^{d}	0.11

Cellulolytic bacteria species	Treatments				Detection target DNA
	UTS	CH	FCF	UTS:FCF	
Fibrobacter succinogenes	X	X	X	X	446
Ruminococcus albus	X	X	X	X	176
Ruminococcus flavefaciens	X	X	X	X	295

a, b, c, d Values on the same row with different superscripts differ significantly ($p<0.05$).
UTS = Urea-treated rice straw, CH = Cassava hay, FCF = Fresh cassava foliage, UTS:FCF (1:1 DM basis).
X = Detected by PCR technique, SEM = Standard error of the mean. Concentrate was offered at 0.3% of body weight/hd/d.

Table 5. Effect of various tropical roughages on feed intake in beef cattle

Items	Treatments				SEM
	UTS	CH	FCF	UTS:FCF	
Roughage DM intake/day					
kg	8.3^{a}	6.7^{ab}	5.4^{b}	8.7^{a}	0.76
%BW	2.2^{a}	1.8^{b}	1.8^{b}	1.9^{b}	0.09
g/kg $W^{0.75}$	96.0^{a}	80.3^{b}	75.1^{c}	89.2^{ab}	4.64
Total DM intake/day					
kg	9.4^{ab}	7.8^{bc}	6.3^{c}	10.0^{a}	0.83
%BW	2.5^{a}	2.1^{b}	2.1^{b}	2.2^{b}	0.09
g/kg $W^{0.75}$	109.3^{a}	93.4^{b}	87.5^{bc}	103.0^{ab}	4.86
Nutrient intake (kg/d)					
OMI	8.0^{a}	6.8^{ab}	5.5^{a}	8.7^{b}	0.69
CPI	0.7^{a}	1.8^{b}	1.5^{b}	1.6^{b}	0.23
NDFI	6.7	4.1	3.3	6.2	0.82
ADFI	4.4^{a}	3.1^{ab}	2.4^{b}	4.3^{a}	0.46

a, b, c Values on the same row with different superscripts differ significantly ($p<0.05$).
UTS = Urea-treated rice straw, CH = Cassava hay, FCF = Fresh cassava foliage, UTS:FCF (1:1 DM basis). OMI = Organic matter intake.
CPI = Crude protein intake, NDFI = Neutral-detergent fiber intake, ADFI = Acid-detergent fiber intake, SEM = Standard error of the mean.
Concentrate was offered at 0.3% of body weight/hd/d.

product and *R. flavefaciens*, selected to allow species-specific amplification of 295 bp product (data not shown herein).

Voluntary feed intake, digestibility and digestible nutrient intake

Table 5 shows data on roughage DM intake and total DM intake; the values were highest in UTS treatments ($p<0.05$) in terms of %BW and g/kg $W^{0.75}$ (2.2, 96.0 and 2.5, 109.3, respectively) than in other treatments. As cassava used in this experiment was the bitter variety, residual flavor contained in the CH may result in lower intake since Ravindran et al. (1987) demonstrated that the bitter taste of cassava foliage could negatively influence its intake by animals. CPI was highest in the CH treatment ($p<0.05$), followed by UTS: FCF, FCF and UTS treatments, respectively. It was also observed that feeding FCF as a full-feed resulted in ataxia as well as frequent urination, therefore FCF should only be fed as part of the forage in the diet or should be wilted. In addition, combined use of FCF and UTS would be more suitable than offering FCF as a full feed. Advantageous strategies of using cassava for ruminant feed have been reported by Wanapat et al. (1997) showing that cassava hay (CH) harvested at a younger stage of growth (3 months) contained up to 30% CP and had a good profile of amino acids, particularly methionine and leucine. Fresh cassava leaves contain hydrocyanic acid (HCN) at approximately 8-400 mg%; 0-20 mg% in plant tissue (DM) is considered safe for grazing, 50-75 mg% as doubtful and greater than 100 mg% as highly dangerous. Sun-drying for

Table 6. Effect of various tropical roughages on digestion coefficients

Items	Treatments				SEM
	UTS	CH	FCF	UTS:FCF	
Digestion coefficient (%)					
DM	52.8	53.6	51.6	54.2	0.57
OM	53.0	53.9	53.8	55.3	0.50
CP	60.1[a]	70.1[b]	69.4[ab]	63.1[b]	2.42
NDF	65.0[a]	48.7[ab]	44.7[b]	63.5[a]	5.14
ADF	58.0[a]	40.0[ab]	37.4[b]	52.6[ab]	4.96
Nutrient digestible intake (kg/d)					
DM	4.9	4.2	3.3	5.4	0.46
OM	4.3	3.6	3.0	4.8	0.39
CP	0.4[a]	1.3[b]	1.0[ab]	1.0[ab]	0.17
NDF	4.4	2.0	1.5	3.9	0.70
ADF	2.5[a]	1.2[ab]	0.9[b]	2.2[ab]	0.39

[a, b] Values on the same row with different superscripts differ significantly ($p<0.05$).
UTS = Urea-treated rice straw, CH = Cassava hay, FCF = Fresh cassava foliage, UTS:FCF (1:1 DM basis), SEM = Standard error of the mean.

about 3 days could remarkably reduce HCN in leaves from 173 down to 93 mg/kgDM and in chopped leaves from 109 down to 53 mg/kgDM (Ravindran et al., 1987; Wanapat et al., 1997). In this trial, the use of urea-treated rice straw as a roughage source could provide effective fiber, maintaining a higher pH, improving rumen NH_3-N, VFA and increased total viable and cellulolytic bacteria, and could play an important role in changing predominant rumen cellulolytic bacteria species. This involves treating straw with a 5% urea solution and keeping the straw reasonably airtight under plastic for 10 days. The main purpose is to improve the digestibility and protein content of the straw (Wanapat, 1985; Wanapat et al., 1986).

Table 6 presents digestibility values and digestible nutrient intake of feeds. There were no significant differences among treatments for DM or OM digestibility. CP digestibility was highest on the CH treatment ($p<0.05$) followed by FCF, UTS:FCF and UTS treatments, respectively. These values are in agreement with those reported by Wanapat et al. (2000d) and Wanapat (2003) which showed that CH had a high DM digestibility (71.0%) and a high level of ruminal by- pass protein since it contained a tannin-protein complex. Also, digestibility of fibrous fractions, in terms of NDF and ADF, was highest in the UTS treatment ($p<0.05$) as compared with other treatments (65.0 and 58.0%, respectively). These values could support digestible crude protein intake (DCPI) and digestible acid-detergent fiber intake (DADFI) for animals, since it was found that DCPI was higher on CH treatment ($p<0.05$) than in other treatments and DADFI was highest on UTS treatment ($p<0.05$).

As shown above, feeding FCF fully did not significantly enhance rumen parameters and overall intakes; however, when used with UTS, it resulted in various improvements. The two sources of feeds are commonly prevailing during the dry season, and it is therefore most practical to use the combined feeds in order to enhance feed utilization and subsequent productivity.

Conclusions and recommendations

Based on the results of this experiment, the use of UTS, CH, FCF and UTS:FCF as a roughage source could provide effective fiber and maintain an optimal range of ruminal pH and NH_3-N. In addition, UTS resulted in higher VFA and increased total viable and cellulolytic bacteria and could play an important role in changing the predominant rumen cellulolytic species of bacteria. Moreover, it was found that there were three predominant cellulolytic species of bacteria, namely, *F. succinogenes*, *R. albus* and *R. flavefaciens*, by using specific primers; however, their concentrations could not be identified at this stage. It was interesting that in CH as compared with FCF there were higher C2 levels and cellulolytic bacteria, while in FCF C3 level was higher but with lower cellulolytic bacteria. In addition, it was also observed that feeding FCF as a full-feed resulted in anorexia and ataxia as well as frequent urination; therefore, FCF should only be fed as part of the dietary forage or should be wilted. Combined use of FCF with UTS and also FCF with CH could also be suitable as a strategy for feeding of ruminants, while CH can be fully fed.

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