

Effect of Various Tropical Roughages on Rumen Ecology and Digestibility of Beef Cattle

Siwaporn Paengkoum^{1*}, Metha Wanapat²,
Chalong Wachirapakorn² and Ngarmnit Nontaso³

¹*Faculty of Animal Sciences and Agricultural Technology, Silpakorn University,
Phetchaburi, Thailand*

²*Tropical Feed Resources Research and Development Center (TROFREC), Faculty of Agriculture,
Khon Kaen University, Khon Kaen, Thailand*

³*Faculty of Science, Khon Kaen University, Khon Kaen, Thailand*

*Corresponding author. E-mail address: took_sw@yahoo.com, took_asat@hotmail.com

Accepted March 29, 2007

Abstract

Four-fistulated, castrated male crossbred cattle were randomly allocated to a 4 x 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). Faecal and feed samples were collected during the last seven days of each period and analyzed for DM, Ash, CP, NDF ADF and acid-insoluble ash (AIA). Rumen fluid and blood samples were collected on the last day of each period at 0, 2 and 4 h-post feeding and then analyzed for microorganisms (total viable and cellulolytic bacteria), NH₃-N, volatile fatty acid (VFA) and blood urea-nitrogen (BUN). The results revealed that total viable and cellulolytic bacterial population were enhanced ($P<0.05$) with UTS as the roughage source. Animals fed with FCF had a higher rumen propionate production ($P<0.05$) with a lower cellulolytic bacteria count. In addition, roughage intake and total DM intake were highest by using UTS (2.2 and 2.5 %BW, respectively) as the roughage source ($P<0.05$). Organic matter intakes were similar among UTS, CH and UTS: FCF treatments (8.0, 6.8 and 8.7 kg/d, respectively). Digestion coefficients of DM and OM were similar among treatments. However CP digestion coefficients were similar among CH, FCF and UTS: FCF treatments, but were higher ($P<0.05$) in CH than in UTS. 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 fresh as part of the feed or should 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; Rumen ecology

Introduction

“Feeding the bugs, feeding the cows” has been commonly referred to in ruminant production systems, the rumen is an essential fermentation vat to initiate anaerobic fermentation by prevailing microorganisms (bacteria, protozoa and fungi) to produce fermentation 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 *R. flavefaciens* are presently recognized as the major cellulolytic bacterial species found in the rumen (Koike and Kobayashi, 2001). Currently, many

researchers have been interested in studying rumen ecology and finding possible approaches to enhancing rumen functions. 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). 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 (Wanapat, 1999). Cassava (*Manihot esculenta*, Crantz) is an important cash crop widely grown in sandy loam soil, with low fertilizer input and under a hot long dry season. Whole cassava (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. However, the use of CH and fresh cassava foliage (FCF) in ruminant feeds have 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 voluntary feed intake and digestibility of beef cattle.

Materials and Methods

Animal and dietary treatments

Four, fistulated castrated male crossbred cattle were assigned randomly to a 4 x 4 Latin square design to investigate the effect of different tropical feeds on rumen ecology, voluntary feed intake and digestibility. There were four dietary treatments; T1) urea-treated (5%) rice straw (UTS); T2) cassava hay (CH); T3) fresh cassava foliage (FCF) and T4) UTS: FCF (1: 1 dry matter basis). Concentrate was offered at 0.3% of body weight. Animals were kept in individual pens and received of water freely. The experiment was conducted for four periods, each period lasted for 21 days. During the first 14 days,

animals were fed with respective diets *ad libitum*. Samples of UTS, CH, FCF and concentrate were collected to be analyzed for chemical composition. Cassava hay was prepared according to Wanapat et al. (1997). Whole cassava crop had grown for 3 months and then its regrowth every two months, chopped and sun-dried for about 2 days until the remaining dry matter was at least 85%. Fresh cassava foliage (FCF) was harvested every morning and afternoon, chopped by hand and fed to the animals immediately.

Data collection and sampling methods

All feeds and faeces were sampled for chemical composition analysis in terms of DM, Ash, CP (AOAC, 1985) and NDF, ADF (Goering and Van Soest, 1970) and acid-insoluble ash (AIA). Acid-insoluble ash (AIA) was used to estimate the digestibility coefficients of nutrients (Van Keulen and Young, 1977). Rumen fluid were collected at 0, 2, 4 h-post feeding on the last day of each period and measured for pH immediately, $\text{NH}_3\text{-N}$ concentration (Bromner and Keeney, 1965), VFA by using HPLC (Samuel et al., 1997) and analysis for total viable and cellulolytic bacteria by using the Roll Tube Technique (Hungate, 1969). Blood samples were taken from jugular vein at 0, 2, 4 h post-feeding after rumen fluid was sampled. The samples were kept on ice prior to plasma separation by centrifugation ($3,000 \times g$ for 15 min) and the supernatant was stored at -20°C for blood urea N (BUN) analysis (Crocker, 1967).

Statistical analysis

All data were subjected to analysis of variance using Proc. GLM (SAS, 1999). Data were analyzed using the model $Y_{ijk} = \mu + M_i + A_j + P_k + \Sigma_{ijk}$. Where Y_{ijk} = represents observation from animal (j), receiving diet (i), in period (k), μ , the overall of mean, M_i the mean effect of type of roughage sources ($i = 1, 2, 3, 4$), A_j , the effect of animal ($j = 1, 2, 3, 4$), P_k , the effects of period ($k = 1, 2, 3, 4$), and Σ_{ijk} , the residual effect. Treatment means were statistically compared by Duncan's New Multiple Range Test (Steel and Torrie, 1980).

Results

Chemical composition of feed

Feed ingredients and chemical composition of the feed 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 CH and FCF consisted of 24.7 and 25.3%CP, respectively.

Rumen ecology parameters

Rumen pH range from 6.6-6.9 and was higher in CH and FCF treatments than in UTS and UTS: FCF treatments. Rumen temperature, were similar among UTS, CH and FCF treatments but was slightly higher in UTS: FCF treatment. Ruminal $\text{NH}_3\text{-N}$ concentration was higher in FCF, CH and UTS: FCF treatments ($P<0.05$) (14.9, 14.7 and 14.0 mg%, respectively) than in UTS treatment (12.1 mg%). Blood-urea nitrogen (BUN) concentration were higher

in FCF treatment than those in UTS and UTS: FCF treatments ($P<0.05$) (Table 2). Ruminal VFAs after feeding in different treatments are presented in Table 3. Total volatile fatty acid (TVFA) were higher in UTS treatment than in CH treatments ($P<0.05$) but were similar FCF and UTS: FCF. Acetic acid (C_2) concentration were higher in UTS: FCF and UTS treatments ($P<0.05$), compared with to those in CH and FCF treatments. However, C_3 concentration was higher in FCF ($P<0.05$) compared with those in UTS: FCF treatment, but were similar to UTS and CH treatments.

Rumen bacteria species

Total viable bacteria and cellulolytic bacteria populations. The highest values were found in UTS treatment at 4.9×10^{10} CFU/ml and 6.8×10^9 CFU/ml ($P<0.05$), respectively (Table 4).

Table 1 Feed composition and chemical composition of urea-treated (5%) rice straw, cassava hay, and fresh cassava foliage.

Feedstuffs	Treatments			
	Concentrate	UTS	CH	FCF
Feed ingredient				
Cassava chip	58.0	-	-	-
Rice bran	37.0	-	-	-
Urea	3.0	-	-	-
Mineral mix	1.0	-	-	-
Sulphur	1.0	-	-	-
Total	100.0	-	-	-
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.

Table 2 Effect of various tropical roughages on ruminal pH, temperature, ammonia-nitrogen (NH₃-N) and blood urea-nitrogen (BUN) in beef cattle.

Items	Treatments				SEM
	UTS	CH	FCF	UTS : FCF	
Ruminal pH					
hr-post feeding					
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%					
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%					
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 (P<0.05)

SEM = standard error of the mean, UTS = urea-treated rice straw, CH = cassava hay, FCF = fresh cassava foliage, UTS : FCF at (1:1 DM basis)

Voluntary feed intake, digestibility and digestible nutrient intake

Table 5 shows data on roughage DM intake and total DM intake. The values were higher in UTS treatments (P<0.05) in terms of %BW and g/kgW^{0.75} (2.2, 96.0 and 2.5, 109.3, respectively) than those in other treatments. Crude protein intake (CPI) was highest in 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 anorexia and ataxia as well as frequent urination. Therefore, FCF should be fed only as part of the forage in the diet or should be wilted. In addition,

a combination use of FCF and UTS could be more suitable. Table 6 presents digestibility and digestible nutrient intake of feeds. There were no significant differences among treatments for DM or OM digestibility. Crude protein (CP) digestibility was highest in CH treatment (P<0.05) followed by FCF, UTS: FCF and UTS treatments, respectively. Neutral-detergent fiber (NDF) and acid-detergent fiber (ADF) digestibilities were higher in UTS treatment (P<0.05) as compared with other treatments (65.0 and 58.0%, respectively). These values could support for digestible crude protein (DCP) intake and digestible acid-detergent fiber (DADF) intake for

Table 3 Effect of various tropical roughages on ruminal total volatile fatty acid (TVFA), acetic acid (C₂), propionic acid (C₃) and butyric acid (C₄) in beef cattle.

Items	Treatments				SEM
	UTS	CH	FCF	UTS : FCF	
TVFA, mM					
hr-post feeding					
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 (C ₂), 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 (C ₃), 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 (C ₄), 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 (P<0.05)

SEM = standard error of the mean, UTS = urea-treated rice straw, CH = cassava hay, FCF = fresh cassava foliage, UTS : FCF at (1 : 1 DM basis)

animals. It was found that DCP intake was higher on CH treatment (P<0.05) than those in other treatments and DADF intake was highest on UTS treatment (P<0.05).

Discussion

The main objective of the present experiment was to investigate the use of different tropical feeds as a roughage source on rumen ecology, voluntary feed intake and nutrient digestibility in beef cattle. It had been shown that use of UTS, CH, FCF and UTS: FCF as a roughage source could provide effective fiber, maintain optimal range of ruminal pH (6.5-7.0)

and NH₃-N concentration. In addition, use of UTS resulted in higher VFA concentration and increased total viable and cellulolytic bacteria and could play an important role in changing the predominant rumen cellulolytic bacteria species. Table 2 demonstrated that rumen pH values were within the normal range (pH 6-7) (Weimer, 1996). An increase in ruminal pH in CH and FCF treatments could have been affected by condensed-tannin (CT) contained in CH, as CT stimulated saliva secretion and increases buffering to the rumen (Reed, 1995). Ammonia-nitrogen (NH₃-N) levels in FCF, CH and UTS: FCF (1: 1 DM basis) treatments were within the optimal range of

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

Items	Treatments				SEM
	UTS	CH	FCF	UTS : FCF	
Total viable bacteria, x 10 ¹⁰ CFU/g rumen content					
hr-post feeding					
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, x 10 ⁹ CFU/g rumen content					
0	6.5 ^a	4.5 ^b	3.3 ^c	5.3 ^a	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

^{a,b,c,d} values on the same row with different superscripts differ (P<0.05)

UTS = urea-treated rice straw, CH = cassava hay, FCF = fresh cassava foliage, UTS: FCF at (1:1 DM basis), Concentrate was offered at 0.3% of body weight/hd/d, CFU = colony forming unit

ruminal NH₃-N (15-30 mg %) for low quality roughages (Perdok and Leng, 1990). It also should be noted that these rumen NH₃-N levels were within a good range and suitable for rumen ecology particularly for further microbial protein synthesis (Kanjapruithipong and Leng, 1998; Wanapat and Pimpa, 1999). Blood-urea nitrogen concentration has been reported to be closely related to rumen NH₃-N. In addition, it was found that as dietary CP percentage increased, the concentration of BUN, solubility and degradability in the rumen of cattle increased (Cressman et al., 1980). Based on this experiment, type of roughage may play an important role in VFA production (Table 3). Wanapat et al. (2003) reported, that in beef cattle and swamp buffaloes, rumen fermentation pattern of VFAs was relatively consistent and were low in both cattle and buffaloes fed on rice straw. However, these values were increased by feeding UTS. It appeared that rumen function may be remarkably enhanced by dietary manipulation. With regards to total viable and cellulolytic bacteria (Table 4) the results were similar

to those reported by Hungate (1966) that indicates that the total culturable counts (TCC) in the rumen are usually 12-80 % of the direct count (DC). Krause et al. (1999) 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 x 10¹⁰, 9.6 x 10⁹ and 5 x 10⁸ 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. The data clearly shown that use of urea-treated rice straw as a roughage source could provide effective fiber, maintaining a higher pH, increase rumen NH₃-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 (Table 5 and 6) (Wanapat, 1985; Wanapat et al., 1986).

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/kgW ^{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/kgW ^{0.75}	109.3 ^a	93.4 ^b	87.5 ^{bc}	103.0 ^{ab}	4.86
Nutrient intake, kg/d					
OM	8.0 ^a	6.8 ^{ab}	5.5 ^a	8.7 ^b	0.69
CP	0.7 ^a	1.8 ^b	1.5 ^b	1.6 ^b	0.23
NDF	6.7	4.1	3.3	6.2	0.82
ADF	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 (P<0.05)

UTS = urea-treated rice straw, CH = cassava hay, FCF = fresh cassava foliage, UTS : FCF at (1 : 1 DM basis), OM = organic matter, CP = crude protein, NDF = neutral-detergent fiber, ADF = acid-detergent fiber, SEM = standard error of the mean

Table 6 Effect of various tropical roughages on digestion coefficients of nutrients and digestible nutrient intake.

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 ^b	63.1 ^{ab}	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
Digestible nutrient 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 (P<0.05)

UTS = urea-treated rice straw, CH = cassava hay, FCF = fresh cassava foliage, UTS : FCF at (1 : 1 DM basis), DM = dry matter, OM = organic matter, CP = crude protein, NDF = neutral-detergent fiber, ADF = acid detergent fiber, SEM = standard error of the mean

Conclusion

Based on this experiment, the results demonstrate that the use of UTS, CH, FCF and UTS:FCF as a roughage source could provide effective fiber, maintain optimal range of ruminal pH and $\text{NH}_3\text{-N}$ concentration. It was interesting that in CH as compared with FCF, there were higher in C_2 and cellulolytic bacteria while in FCF, C_3 was higher but with lower cellulolytic bacteria.

Further studies should be conducted using specific techniques such as Real Time PCR and Denaturing Gradient Gel Electrophoresis (DGGE) by using specific probes/primers to identify and quantify these bacterial populations.

Acknowledgements

The authors would like to express sincere thanks to the Tropical Feed Resources Research and Development Center (TROFREC), Khon Kaen University, Thailand. Faculty of Animal Sciences and Agricultural Technology, Silpakorn University and the National Research Council (NRC) of Thailand for financial support of the project. Special thanks to Dr. Peter Rowlinson, University of New Castle Upon Tyne, UK for his guidance.

References

- AOAC. (1985) *Official Method of Analysis*. Association of Official Analytical Chemists, Washington, D.C.
- Bromner, J.M., and Keeney, D.R. (1965) Steam distillation methods of determination of ammonium, nitrate and nitrite. *Analysis Chemistry Acta* 32: 485.
- Cressman, S.G., Grieve, D. G., McLoad, G.K., Wheeler, E.E., and Young, L.G. (1980) Influence of dietary protein concentration on milk production by dairy cattle in early lactation. *Journal of Dairy Science* 63: 1839-1847.
- Crocker, C.L. (1967) Rapid determination of urea nitrogen in serum or plasma without deprotenization. *American Journal Medical Technology* 33: 361.
- Goering, H.K., and Van Soest, P.J. (1970) Forage fiber analysis (Apparatus, Reagent, Procedures and some Application). In *Agricultural Handbook*. No. 397, ARS, USDA, Washington, D.C.
- Hungate, R.E. (1966) *The rumen and its microbes*. Academic Press, New York, NY.
- Hungate, R.E. (1969) A roll tube method for cultivation of strict anaerobes. In *Methods in Microbiology*, (Norris, J.R. and Ribbons, D.W., eds), New York: Academic 313: 117.
- Kanjanapruthipong, J., and Leng, R.A. (1998) The effects of dietary urea on microbial populations in the rumen of sheep. *Asian Australasian Journal of Animal Science* 11: 661-672.
- Koike, S., and Kobayashi, Y. (2001) Development and use of competitive PCR assays for the rumen cellulolytic bacteria: *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens*. *FEMS Microbiology Letter* 204: 361-366.
- Krause, D. O., Bunch, R. J., Smith, W. J. M., and McSweeney, C. S. (1999) Diversity of *Ruminococcus* strains: A survey of genetic polymorphisms and plant digestibility. *Journal of Applied Microbiology* 86: 487-495.
- Perdok, H.B., and Leng, L.A. (1990) Effect of supplementation with protein meal on the growth of cattle given a basal diet of untreated ammoniated rice straw. *Asian Australasian Journal of Animal Science* 3: 269-279.
- Reed, J.D. (1995) Nutritional toxicology of tannins and related polyphenols in forage legumes. *Journal of Animal Science* 73: 1516-1528.
- Samuel, M., Sagathewan, S., Thomas, J., and Mathen, G. (1997) An HPLC method for estimation of volatile fatty acids of ruminal fluid. *Indian Journal of Animal Science* 67: 805.
- SAS. (1999) *User's Guide: Statistics, Version 5 Edition*. SAS. Inst. Cary, N.C.
- Steel, R.G.D., and Torries, J.H. (1980) *Principles and Procedures of Statistic a Biomaterial*

- Approach*. (2nd ed), McGraw-Hill.
New York: U.S.A.
- Van Keulen, J., and Young, B.A. (1977) Evaluation of acid insoluble ash as a neutral marker in ruminants digestibility studies. *Journal of Animal Science* 44: 282-287.
- Wanapat, M. (1985) Improving rice straw quality as ruminant feed by urea-treated in Thailand. In *Proc. of Relevance of crop residues as animal feeds in developing countries*. (M. Wanapat and Devendra, C., eds) Funny Press, Bangkok, Thailand.
- Wanapat, M. (1999) *Feeding of ruminants in the tropics based on local feed resources*. Khon Kaen Publishing Company Ltd., Khon Kaen, Thailand. 236 p.
- Wanapat, M. (2000) Rumen manipulation to increase the efficient use of local feed resources and productivity of ruminants in the Tropics. *Asian Australasian Journal of Animal Science* 13 (Suppl.): 59-67.
- Wanapat, M., Sundstol, F., and Hall, J.M.R. (1986) A comparison of alkali treatment methods used to improve the nutritive value of straw. II. in sacco and in vitro degradation relative to in vivo digestibility. *Animal Feed Science and Technology* 14: 215-220.
- Wanapat, M., Pimpa, O., Petlum, A., and U. Boontao. (1997) Cassava hay: A new strategic feed for ruminants during the dry season. *Livestock Research for Rural Development* 9(2); LRRD Home Page. Accessed on March 10, 2005.
- Wanapat, M., and Pimpa, O. (1999) Effect of ruminal NH₃-N levels on ruminal fermentation, purine derivatives, digestibility and rice straw intake in swamp buffaloes. *Asian Australasian Journal of Animal Science* 12: 904-907.
- Wanapat, M., Notaso, N., Yuangklang, C., Wora-anu, S., Ngarmmaeng, A., Wachirapakorn, C., and Rowlinson, P. (2003) A comparative study between wamp buffalo and native cattle in feed digestibility and potential transfer of buffalo rumen into cattle. *Asian Australasian Journal of Animal Science* 16: 504-510.
- Weimer, P.J. (1996) Why don't ruminal bacteria digest cellulose faster. *Journal of Dairy Science* 79: 1496-1502.