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Supplementation Levels of Concentrate Containing High Levels of Cassava Chip on Rumen Ecology and Microbial Protein Synthesis in Cattle

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Abstract: The object of this study was to determine the influence of supplementation of level concentrate containing high levels of cassava chip on rumen ecology, microbial protein synthesis in cattle. Four, rumen fistulated cattle with initial body weight of 400 ± 10kg were randomly assigned according to a 4 x 4 Latin square design. The dietary treatments were concentrate cassava chip based offering at 0, 1, 2 and 3 % BW with urea-treated rice straw fed *ad libitum*. It was found that ruminal pH was significantly decreased with increase of concentrate. Volatile fatty acids (VFA) concentration in the rumen was significantly different among treatments. In addition, a molar proportion of propionate was higher in supplemented groups at 2 and 3 % BW ($P<0.05$), leading to significantly decreased acetate:propionate ratio. Furthermore, microbial N supply was significantly improved and was highest at 2 %BW supplementation. The efficiency of rumen microbial-N synthesis based on organic matter (OM) truly digested in the rumen was highest in level of concentrate supplementation at 2 %BW (80 % of cassava chip in diets). Moreover, the total protozoal counts were significantly increased, while fungal zoospores were dramatically decreased in cattle receiving increased levels of concentrate. In conclusion, cassava chip can be use as energy source at 80% in concentrate and supplementation of concentrate at 2 %BW with urea-treated rice straw as roughage could improve rumen fermentation efficiency in cattle.

Key words: Concentrate, cassava chip, urea, rumen ecology, microbial protein synthesis, cattle

Introduction

Cassava (*Manihot esculenta*, Crantz) is an annual tuber crop grown widely in the tropical regions of Africa, Asia and Latin America. It thrives in sandy-loam soils with low organic matter, and in climate with low rainfall and high temperature (Hong *et al*., 2003). Cassava tubers contain high levels of energy and minimal levels of crude protein and have been used as readily fermentable energy in ruminant rations and has been used extensively as a feed for livestock (Wanapat, 2003; Kiyothong and Wanapat, 2004; Promkot and Wanapat, 2005).

Cassava chip (CC) or pellet contained high level of non-structural carbohydrate and were highly degradable in the rumen as compared with other energy sources including corn meal (Sommart *et al*., 2000; Chanjula *et al*., 2003). In addition, higher level of non-protein nitrogen (NPN) particularly urea could be incorporated in concentrate due to cassava chip's high rate of ruminal degradation. Current research work using high CC and urea levels in dairy steers (80% CC with 4% urea; Khampa *et al*., 2005), in lactating dairy cows resulted in good milk yield and quality (75% CC with 4.5 % urea). Most importantly CC could completely replace corn meal in concentrate and resulted in more lucrative productivity (Chanjula *et al*., 2004). Therefore, this present study was conducted to determine the influence of levels of supplementation of concentrate containing high levels of cassava chip on rumen ecology and microbial protein synthesis in cattle.

Materials and Methods

Animals, treatments and experimental design: Four-fistulated cattle with initial body weight of 400 ± 10kg were randomly assigned according to a 4 x 4 Latin square design to investigate the effects of levels of supplementation of concentrate containing high level of cassava chip with urea-treated rice straw (UTS) as a roughage source on feed intake, rumen ecology and microbial protein synthesis. The dietary treatments contained concentrate at 0, 1, 2 and 3 % BW, respectively.

Urea-treated rice straw (UTS) was prepared by using 5% (W/W) urea mixed with 100 kg of water in 100 kg of rice straw (RS) batches (50:50, water to straw) and poured over a stack of straw and then covered with a plastic sheet for a minimum of 10 days before feeding to animals (Wanapat, 1990).

All animals were kept in individual pens and water was available free choice. The experiment was conducted for four periods, and each period lasted for 21 days. During the first 14 days, all animals were fed on respective diets at *ad libitum* basis, while the last 7 days, the animals were kept in metabolism crates for total collection during which they were restricted to 90% of the previous voluntary feed intake of straw. Chemical and composition of concentrate and UTS used are shown in Table 1.

Table 1: Composition of concentrate diet and urea-treated rice straw (UTS) used in the experiment (%DM basis)

Item	Concentrate	UTS
Ingredient (%DM)		
Cassava chip	80	
Fine rice bran	6	
Whole cotton seed	5	
Urea	4	
Molasses	3	
Sulfur	0.5	
Salt	0.5	
Mineral mix	1	
Analyzed composition (%)		
DM	92.8	52.4
OM	91.2	84.7
Ash	8.9	15.3
CP	15.7	8.9
TDN	79.1	54.1
NDF	14.5	89.8
ADF	10.1	57.0
NDF protein	1.5	-
Fat	2.5	1.2
NSC[1]	59.9	-

(UTS) Urea –treated rice straw. [1]NSC = 100 – ((NDF – NDF protein) + protein + fat +ash), DM = dry matter, CP = crude protein, OM = organic matter, NDF = neutral detergent fiber, ADF = acid detergent fiber, TDN = Total digestible nutrient, NSC = non-structural carbohydrate.

Data collection and sampling procedures: Composites samples were dried at 60°C and ground (1 mm screen using Cyclotech Mill, Tecator, Sweden) and then analyzed for DM, ether extract, ash and CP content (AOAC, 1985), NDF, ADF and ADL (Goering and Van Soest, 1970).

Rumen fluid and jugular blood samples were collected at 0, 1, 2, 4, 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 each time at the end of each period. Rumen fluid was immediately measured for pH and temperature using a portable pH and temperature meter (HANNA instrument HI 8424 microcomputer, Singapore). 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 analysis where 5 ml of H_2SO_4 solution (1M) was added to 50 ml of rumen fluid. The mixture was centrifuged at 16,000 x *g* for 15 minutes and supernatant was stored at –20°C prior to NH_3-N and VFA analyses using a HPLC (Instruments by controller water model 600E; water model 484 UV detector; column novapak C_{18}; column size 4 mm x 150 mm; mobile phase 10 mM H_2PO_4 (pH2.5), ETL Testing Laboratory, Inc., Cortland, New York, 13045, U.S.A.) (Samuel *et al.,* 1997). Second portion was fixed with 10% formalin solution in sterilized 0.9% saline solution (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).

A blood sample (about 10 ml) was drawn from the jugular vein at the same time as rumen fluid sampling, separated by centrifugation at 500 x *g* for 10 minutes (Table Top Centrifuge PLC-02, U.S.A.) and stored at –20°C until analysis of blood urea nitrogen (BUN) according to the method of Crocker (1967). Urine samples were analyzed for urinary nitrogen (IAEA, 1997) and allantoin in urine was determined by high-performance liquid chromatography (HPLC) (Instruments by controller water model 600E; water model 484 UV detector; column novapak C_{18}; column size 4 mm x 150 mm; mobile phase 10 mM H_2PO_4 (pH2.5), ETL Testing Laboratory, Inc., Cortland, New York, 13045, U.S.A.) (Chen *et al.,* 1993).

The amount of microbial purines absorbed (X mmol/day) corresponding to the purine derivatives excreted (PD) (Y mmol/day) was calculated based on the relationship derived by Chen and Gomes (1995).

$Y = 0.85X+(0.385W^{0.75})$

where Y is the excretion of purine derivatives (mmol/day); X the microbial purines absorbed (mmol/day).

The supply of microbial N in gram per day was estimated as follows:

$$\text{Microbial N (g/day)} = \frac{X \times 70}{0.116 \times 0.83 \times 1000} = 0.727 \times X,$$

with X being the absorption of purine derivatives in mmol per day, following the assumptions made by Chen and Gomes (1995).

Digestibility of microbial purine is 0.83:

The N content of purines is 70 mg N/mmol.

The ratio of purine-N: total N in mixed rumen microbes is 11.6:100

The efficiency of microbial protein supply (EMNS) to denote the microbial N supplied to the animal per unit of DOMR was calculated using the following formula:

$$\text{EMNS} = \frac{\text{MN (g/day)}}{\text{DOMR (g)}} \times 1000 \text{ (g)}$$

Where DOMR = DOMI x 0.65 (ARC, 1990), DOMR = digestible organic matter apparently fermented in the rumen and DOMI = digestible organic matter intake.

Statistical analysis: Statistical analyses were performed using the GLM procedure of SAS (1998). Data were analyzed using the model $Y_{ijk} = \mu + M_i + A_j + P_k + e_{ijk}$. 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 concentrate (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), e_{ijk} the residual effect. Mean separations with a significant F ($P<0.05$) for treatment were statistically compared using the Duncan's New Multiple Rang Test (DMRT) (Steel and Torrie, 1980).

Table 2: Effect of levels of supplementation of concentrate containing high level of cassava chip on feed intake in cattle

Item	Level of concentrate (%BW)					Contrast	
	0	1	2	3	SEM	L	Q
DM intake (%BW)							
UTS	1.9^{a}	1.7^{ab}	1.2^{bc}	1.0^{c}	0.20	*	NS
Conc.	0^{a}	0.9^{b}	1.8^{c}	1.9^{c}	0.17	**	NS
Total	1.9^{a}	2.6^{b}	3.0^{b}	2.9^{b}	0.20	**	NS

^{abc} Values on the same row with different superscripts differ (p<0.05). UTS = Urea-treated rice straw, Conc. = concentrate. L = linear, Q = quadratic. * = P<0.05, ** = P<0.01, NS = P>0.05.

Results and Discussion

Feed intake and rumen fermentation parameters: Intake of urea-treated rice straw (UTS) had linearly decreased (P<0.05) when cattle received concentrates supplementation (Table 2). In addition, the concentration of NH_3-N, VFA, BUN and pH in the rumen fluid were used to monitor rumen fermentation pattern (Table 3). The rumen pH was significantly affected by level of concentrate supplementation. The cattle fed UTS with concentrate at 0 and 1 %BW supplementation had higher rumen pH (6.7 and 6.6) than those cattle fed UTS with concentrate at 2 and 3 %BW (6.3 and 5.7) supplementation. In this experiment, supplementation of concentrate at 3 %BW resulted in lowest ruminal pH (5.3) than those received concentrate supplementation at 2, 1 and 0 %BW, respectively. It has been suggested that concentrates containing high levels of cassava chip with high levels of nonstructural carbohydrate and readily degradable in rumen could decrease ruminal pH and be lower than optimal values (6.5-7.0) when cattle received high level of concentrate at 3 %BW supplementation (Wanapat, 2003).

Other studies Melaku *et al.* (2004) demonstrated inhibitory effects of rumen pH on cellulolysis only at values below 6.1 while Mould and Ørskov (1984) reported that lower pH have a major impact on fiber digestion. In addition, Cheng *et al.* (1984) reported that low ruminal pH appeared to prevent a strong attachment of bacteria to plant cell walls, resulting in lower fiber digestion. Based on this study, the rumen pH measured in cattle supplemented with any of the treatment feeds, supplementation of concentrate at 3 %BW resulted in inhibiting the fermentation of fiber in the rumen as well as microbial protein synthesis.

Ruminal NH_3-N concentrations were significantly different (P<0.05) among treatments at each hour of sampling and were in optimal ruminal NH_3-N range (15-30 mg/dl, Boniface *et al.*, 1986; Perdok and Leng, 1990; Wanapat and Pimpa, 1999) for improving rumen ecology, microbial protein synthesis, digestibility and voluntary feed intake. Furthermore, blood urea-nitrogen concentrations were not significantly different among treatments. The differences in NH_3-N and BUN concentrations among treatments may have been related directly to CP levels of concentrate. In addition, Preston *et al.* (1965) reported that concentrations of BUN were highly correlated with protein intake and reflected the level of ammonia production in the rumen. This study revealed that incorporation of concentrate 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, 1991; Wanapat, 2000). Similarly, Krebs and Leng (1984) suggested requirements for rumen NH_3-N of 20 mg/dl or more for sufficient voluntary intake of low quality roughages.

The influence of levels of supplementation of concentrate containing high levels of cassava chip with UTS as roughage on total VFA concentrate, production of total VFA, acetic acid proportion, propionic acid proportion, butyric acid proportion and acetic to propionic ratio are shown in Table 3. Mean total VFA concentration increased from 107.2 to 119.2 mM/L and proportion of propionic acid ranged from 17.5 to 26.1 mM/L (P<0.05) as linearly as increasing level of concentrate supplementation. The observed reduction in pH associated with increased concentrate feeding was due to increased VFA concentrations and were similar to the results of Robinson *et al.* (1986) and Sutton *et al.* (1993) who reported that increasing the starch content of the concentrate resulted in higher rumen propionate concentrations which may cause a depression in rumen pH. However, the results of total VFA concentration in all diets were found in normal concentrations (70 to 130 m*M*/L) and agreed with result of France and Siddons (1993).

Rumen microorganism populations: The effects of supplementation of concentrate with UTS as roughage in cattle on the ruminal microorganisms are summarized in Table 4. The supplementation of concentrate containing high level of cassava chip was significantly different among treatments (P<0.05). The higher concentrate supplementation decreased population of bacteria and fungi while protozoal population was decreased (P<0.05). The populations of protozoa were higher when receiving high levels of concentrate and it could be due to engulfment of starch by protozoa as substrate to produce end-product. Furthermore, Jouaney and Ushida (1999) reported that the number of protozoa per ml rumen fluid depended on the rate of soluble sugars and starches in the ration and also pH.

Table 3: Effect of levels of supplementation of concentrate containing high level of cassava chip on ruminal pH, ammonia-nitrogen (NH_3-N), blood urea nitrogen (BUN) and volatile fatty acid (VFAs) in cattle

Item	Level of concentrate (%BW)					Contrast	
	0	1	2	3	SEM	L	Q
Ruminal pH	6.7[a]	6.6[a]	6.3[b]	5.7[c]	0.08	*	NS
NH_3-N (mg/dl)	12.8[a]	15.4[b]	16.1[b]	17.1[b]	2.04	*	NS
BUN (mg/dl)	12.3	12.5	14.1	14.5	0.81	NS	NS
Total VFA (mmol/L)	107.2[a]	118.2[b]	119.2[b]	118.8[b]	3.76	*	NS
VFA (mol/100mol)							
Acetate (C2)	71.1[a]	67.9[ab]	64.8[ab]	63.4[b]	2.09	*	NS
Propionate (C3)	17.5[a]	21.5[b]	24.9[c]	26.1[c]	0.81	**	NS
Butyrate(C4)	10.9	10.6	10.3	10.5	0.82	NS	NS
C2 : C3 ratio	4.0[a]	3.1[ab]	2.6[b]	2.4[b]	0.04	*	NS
C2+C4 : C3 ratio	4.6[a]	3.6[b]	3.0[bc]	2.8[c]	0.20	**	NS

[abc] Values on the same row with different superscripts differ (p<0.05). L = linear, Q = quadratic * = P<0.05, ** = P<0.01, NS = P>0.05.

Table 4: Effect of levels of supplementation of concentrate containing high level of cassava chip on ruminal bacteria, protozoa, fungi population in cattle

Item	Level of concentrate (%BW)					Contrast	
	0	1	2	3	SEM	L	Q
Rumen microbes (cells/g)							
Bacteria (x 10^{11})	1.1[ab]	1.2[b]	1.0[ac]	0.8[c]	0.13	*	*
Protozoa (x 10^5)	4.1[a]	5.3[ab]	7.6[bc]	9.6[c]	0.87	*	NS
Fungal zoospores (x 10^5)	15.1[a]	9.6[ab]	7.0[c]	5.3[c]	2.04	*	NS

[abc] Values on the same row with different superscripts differ (p<0.05). L = linear, Q = quadratic, *= P<0.05, ** = P<0.01, NS = P>0.05.

Table 5: Effect of levels of supplementation of concentrate containing high level of cassava chip on excretion of purine derivatives (PD) (mmol/d) and microbial nitrogen supply in cattle

Item	Level of concentrate (%BW)					Contrast	
	0	1	2	3	SEM	L	Q
PD, mmol/d							
Allantoin excretion[1]	143.6[a]	211.6[b]	244.6[c]	130.7[d]	1.22	**	**
Allantoin absorption	137.8[a]	215.2[b]	256.6[c]	122.7[d]	2.06	**	**
Microbial N supply, gN/d[2]	98.9[a]	156.4[b]	186.6[c]	89.2[d]	0.85	**	**
EMNS, gN/kg OMDR[3]	18.3[a]	20.1[b]	23.3[c]	12.1[d]	0.12	**	**

[ab] Values on the same row with different superscripts differ (p<0.05).

[1]Allantoin in urine cattle was 80-85 % of total purine (IAEA, 1997).

[2]Microbial N (g N/day) = (X x 70)/ (0.116 x 0.83 x 1000) = 0.727 x X (where, X = total absorption of purine derivatives).

[3]EMNS = efficiency of microbial nitrogen supply (g N/kg OMDR), OMDR (kg) = 65 % of organic matter digestible in total tract.

Urinary excretion of purine derivatives and microbial nitrogen supply: In ruminants, allantoin is a main product of purine catabolism and the principal purine derivative in urine. The supplementation of level of concentrate on the purine derivative excretion, efficiency of microbial protein supply (EMNS) and microbial N supply are summarized in Table 5. Excretion of allantoin in urine was linearly and quadratically increased (P<0.01) with effects of different level of concentrate supplementation. The microbial nitrogen supplies as calculated from purine derivative excretion were from 89.2 to 186.6 g N/day. Moreover, EMNS ranged from 9.3 to 19.3 g N/kg OMDR. The higher microbial nitrogen supply and EMNS in beef steers fed concentrate at 2 %BW may be due to synchronization of the available fermentable energy and degradable nitrogen in the rumen. Hoover and Stokes (1991) reported that the rate of digestion of carbohydrates was a major factor controlling the energy available for microbial growth. In this experiment concentrates with high level of cassava chip at 80% and urea at 4% could be synchronized to produce ruminal NH_3-N and C-skeleton suitable for ruminal microbial protein synthesis.

Conclusion: Based on this study it was shown that locally available carbohydrate source of cassava chip could be effectively at high level of 80% of concentrate and high level of urea at 4%. Supplementation of concentrate with containing high level of cassava chip at 2 %BW was most suitable for rumen ecology and increasing of microbial protein synthesis efficiency in rumen. In addition, this concentrate was inexpensive, easily made by the farmers and could be used for beef cattle as well as dairy cows. Further use of cassava chip in ruminant diets should be widely recommended.

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References

ARC., 1990. The Nutrient Requirements of Ruminant Livestock. Suppl.1.Commonwealth Agricultural Bureaux, Slough, Farnham Royal, UK.

Boniface, A.N., R.M. Murrah and J.P. Hogan, 1986. Optimum level of ammonia in the rumen liquid of cattle fed tropical pasture hay. Proc. Aust. Soc. Anim. Proc., 1: 151-154.

Chanjula, P., M. Wanapat, C. Wachirapakorn, S. Uriyapongson and P. Rowlinson, 2003. Ruminal degradability of tropical feeds and their potential use in ruminant diets. Asian-Aust. J. Anim. Sci., 16: 211-216.

Chanjula, P., M. Wanapat, C. Wachirapakorn and P. Rowlinson, 2004. Effect of synchronizing starch sources and protein (NPN) in the rumen on feed intake, rumen microbial fermentation, nutrient utilization and performance of lactating dairy cows. Asian-Aust. J. Anim. Sci., 17: 1400-1410.

Chen, X.B. and M.J. Gomes, 1995. Estimation of Microbial Protein Supply to Sheep and Cattle Based on Urinary Excretion of Purine Derivatives - An Overview of the Technical Details. Occasional Publication 1992. International Feed Resources Unit, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, UK.

Chen, X.B., D.J. Kyle and E.R. ørskov, 1993. Measurement of allantoin in urine and plasma by high-performance liquid chromatography with pre-column derivatization. J. Chromatogr., 617: 241-247.

Cheng, K.J., C.S. Stewart, D. Dinsdale and J.W. Costerton, 1984. Electron microscopy of bacteria involved in the digestion of plant cell walls. Anim. Feed Sci. Tec., 10: 93-120.

Crocker, C.L., 1967. Rapid determination of urea nitrogen in serum or plasma without deproteinzation. American J. Med. Tec., 33: 361-365.

France, J. and R.C. Siddons, 1993. Volatile fatty acid production. In: Quantilitive Aspects Ruminant Digestion and Metabolisim (Eds., J.M. Forbes and J. Frace). C.A.B. International, Willingford, UK.

Galyean, M., 1989. Laboratory Procedure in Animal Nutrition Research. Department of Animal and Life Science. New Mexico state University, USA.

Goering, H.K. and P.J. Van Soest, 1970. Forage Fiber Analysis (apparatus, reagent, procedures and some application). Agric. Handbook No. 379, ARS, USDA, Washington, D.C.

Hong, N.T.T., M. Wanapat, C. Wachirapakorn, P. Pakdee and P. Rowlinson, 2003. Effects of timing of initial cutting and subsequent cutting on yields and chemical compositions of cassava hay and its supplementation on lactating dairy cows. Asian-Aust. J. Anim. Sci., 16: 1763-1769.

Hoover, W.H. and S.R. Stokes, 1991. Balancing carbohydrate and proteins for optimum rumen microbial yield. J. Dairy Sci., 74: 3640-3644.

IAEA., 1997. Estimation of the rumen microbial protein production from purine derivatives in rumen. Animal production and healt section. Vienna, Austria.

Jouaney, J.P. and K. Ushida, 1999. The role of protozoa in feed digestion. Asian-Aust. J. Anim. Sci., 12: 113-126.

Khampa, S., M. Wanapat, C. Wachirapakorn, N. Nontaso and M. Wattiaux, 2005. Effect of levels of sodium dl-malate supplementation on ruminal fermentation efficiency in concentrates containing high levels of cassava chip in dairy steers. Asian-Aust. J. Anim. Sci., 19: 368-375.

Kiyothong, K. and M. Wanapat, 2004. Growth, hay yield and chemical composition of cassava and Stylo 184 grown under intercropping. Aisan-Aust. J. Anim. Sci., 17: 799-807.

Krebs, G. and R.A. Leng, 1984. The effect of supplementation with molasses/urea blocks on ruminal digestion. Proc. Aust. Soc. Anim. Prod., 15: 704-711.

Melaku, S., K.J. Peters and A. Tegegne, 2004. Microbial nitrogen supply, nitrogen retention and rumen function in Menz sheep supplemented with dried leaves of multipurpose trees, their mixtures or wheat bran. Small Rum. Res., 52: 25-36.

Mould, F.L. and E.R. ørskov, 1984. Manipulation of rumen fluid pH and its influence on cellulolysis in sacco, dry matter degradation and the rumen microflora of sheep offered either hay or concentrate. Anim. Feed Sci. Tec., 10: 1-14.

Perdok, H.B. and R.A. Leng, 1990. Effect of supplementation with protein meal on the growth of cattle given a basal diet of untreated or ammoniated rice straw. Asian-Aust. J. Anim. Sci., 3: 269-279.

Preston, R. L., D.D. Schnakanberg and W.H. Pander, 1965. Protein utilization in ruminants. I. Blood urea nitrogen as affected by protein intake. J. Nutr., 86: 281-287.

Promkot, C. and M. Wanapat, 2005. Effect of level of crude protein and use of cottonseed meal in diets containg cassava chips and rice straw for lactating dairy cows. Asian-Aust. J. Anim. Sci., 18: 502-511.

Robinson, P. H., S. Tamminga and A. M. Van Vuuren, 1986. Influence of declining level of feed intake and varying the proportion of starch in the concentrate on rumen fermentation in dairy cows. Livest. Prod. Sci., 15: 173-189.

Samuel, M., S. Sagathewan, J. Thomas and G. Mathen, 1997. An HPLC method for estimation of volatile fatty acids of ruminal fluid. Indian J. Anim. Sci., 69: 805-807.

SAS., 1998. User's Guide: Statistics, Version 7th Edition. SAS Inst. Inc., Cary, NC.

Satter, L.D. and L.L. Slyter, 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. Br. J. Nutri., 32: 199-208.

Sommart, K., D.S. Parker, M. Wanapat and P. Rowlinson, 2000. Fermentation characteristics and microbial protein synthesis in an in vitro system using cassava, rice straw and dried ruzi grass as substrates. Asian-Aust. J. Anim. Sci., 13: 1084-1093.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedure of Statistics. McGraw Hill Book Co. New York.

Sutton, J.D., S.V. Morant, J.A. Bines, D.J. Napper and D.I. Givens, 1993. Effect of altering the starch:fibre ratio in the concentrates on hay intake and milk production by Friesian cows. J. Agric. Sci. (Camb)., 120: 379-390.

Wanapat, M., 1990. Nutritional Aspects of Ruminant Production in Southeast Asia with Special Reference to Thailand. Funny Press, Ltd., Bangkok, Thailand.

Wanapat, M., 2000. Rumen manipulation to increase the efficient use of local feed resources and productivity of ruminants in the tropics. Asian-Aust. J. Anim. Sci., 13 (Suppl.): 59-67.

Wanapat, M., 2003. Manipulation of cassava cultivation and utilization to improve protein to energy biomass for livestock feeding in the tropics. Asian-Aust. J. Anim. Sci., 16: 463-472.

Wanapat, M. and O. Pimpa, 1999. Effect of ruminal NH_3-N levels on ruminal fermentaion, purine derivatives,digestibility and rice straw intake in swamp buffaloes. Asian-Aust. J. Anim. Sci., 12: 904-907.