ผลของระดับการเสริมสารมาเลทและยีสต์ในอาหารข้นที่มีมันเส้นเป็นองค์ประกอบในระดับสูงต่อกระบวนการหมักในกระเพาะหมักและการย่อยได้ของโภชนะในโคนมสาว

Effects of Malate and Yeast Level in Concentrate Containing High Cassava Chip on Ruminal Fermentation and Digestibility of Nutrients in Dairy Heifers

สิทธิศักดิ์ คำผา[1]* พละ เชาวรัตน์[2] และ เมธา วรรณพัฒน์[3]

[1,2]อาจารย์ ภาควิชาสัตวศาสตร์ คณะเทคโนโลยีการเกษตร มหาวิทยาลัยราชภัฏมหาสารคาม จังหวัดมหาสารคาม 44000
[3]ศาสตราจารย์ ภาควิชาสัตวศาสตร์ คณะเกษตรศาสตร์ มหาวิทยาลัยขอนแก่น จังหวัดขอนแก่น 40002

บทคัดย่อ

ผลการศึกษาการเสริมระดับสารมาเลทและยีสต์ในอาหารข้นต่อกระบวนการหมักในกระเพาะหมักและความสามารถในการย่อยได้ของโภชนะในโคนมสาว พบว่าโคนมสาวที่ได้รับสารมาเลทและยีสต์ต่างกันมีผลต่อกระบวนการหมักของของเหลวในกระเพาะหมัก การย่อยได้ของโภชนะในอาหาร และประชากรของจุลินทรีย์ในกระเพาะหมักแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) ผลจากการทดลองครั้งนี้สรุปได้ว่าการเสริมอาหารข้นที่มีมันเส้นเป็นองค์ประกอบระดับสูง 70 เปอร์เซ็นต์ของวัตถุแห้งร่วมกับเสริมสารมาเลท 1,000 กรัม และยีสต์ 2,000 กรัมสามารถเพิ่มประสิทธิภาพกระบวนการหมักในกระเพาะหมักและความสามารถในการย่อยได้ของโภชนะในโคนมสาว

Abstract

The objective of this study was to determine the effects of malate level and yeast in concentrate on ruminal fermentation and digestibility of nutrients in heifers. The results revealed that rumen fermentation, ruminal microorganisms and digestibility of nutrients were significantly different ($P<0.05$) as affected by malate level and yeast. In conclusion, the combined use of concentrate containing high level of cassava chip at 70%DM with malate at 1,000 g and yeast at 2,000 g in concentrate could improve rumen fermentation and digestibility of nutrients in dairy heifers.

คำสำคัญ : มาเลท ยีสต์ อาหารข้น โคนมสาว

Keywords : Malate, Yeast, Concentrate, Heifer

*ผู้นิพนธ์ประสานงาน ไปรษณีย์อิเล็กทรอนิกส์ sittisak_k2003@yahoo.com โทร. 085-002-3075

1. Introduction

Cassava (*Manihot esculenta,* Crantz) production in tropical areas has a potential use in ruminant livestock nutrition and feeding. Cassava root contains high levels of energy and has been used as a source of readily fermentable energy in ruminant rations (Wanapat, 2003; Kiyothong and Wanapat, 2004; Promkot and Wanapat, 2005). One strategy for using high degradable carbohydrates is to use in combination with readily available Non-protein nitrogen (NPN) sources such as urea. Urea is commonly used as N source when highly soluble carbohydrates are fed and maintained (Wohlt *et al.,* 1978). However, efficient utilization of protein and NPN in ruminants depends upon knowledge of the basic principles underlying ruminal microbial N metabolism (Fernandez *et al.,* 1987). Moreover, ruminal pH has great impact on rumen fermentation efficiency (Wanapat, 2003).

Some strictly anaerobic bacteria use a reductive or reverse citric acid cycle known as the succinate-propionate pathway to synthesize succinate and (or) propionate. Both malate and fumalate are key intermediates in the succinate propionate pathway and *Selenomonas ruminantium* uses this pathway (Gottschalk, 1986). The fact that dicarboxylic acids, especially malate and fumalate, stimulate lactate utilization is consistent with the presence of this pathway in this ruminal anaerobe (Callaway and Martin, 1996). Previous study by Sanson and Stallcup (1984) reported that supplementation of malate in ruminant diets has been shown to increase nitrogen retention in sheep and steers, and to improve average daily gain and feed efficiency in bull calves. In addition, supplementing diet with yeast (*Saccharomyces cerevisiae*) increase milk production of dairy cows and weight gain of growing cattle (Brossard *et al.,* 2006). Production responses attributed to yeast are usually related to stimulation of cellulolytic and lactate-utilizing bacteria in the rumen, increased fiber digestion, and increased flow of microbial protein from the rumen which may be beneficial for feedlot cattle fed high-grain diets (Guedes *et al.,* 2007). However, the use of malate and yeast in cassava based-diets has not yet been investigated. Therefore, the objective of this experiment was to investigate the supplementation of malate and yeast in concentrates containing high level of cassava chip with urea-treated rice straw as a basal roughage on ruminal

fermentation and digestibility of nutrients in dairy heifers.

2. Materials and Methods

2.1 Animals, Diets and Experimental Design

Four, one-year old of dairy heifers weighing at 150 $\pm$ 10 kg. Heifers were randomly assigned according to a 2 x 2 Factorial arrangement in a 4 x 4 Latin square design to study two levels of malate at 500 vs. 1,000 g with yeast (*Saccharomyces cerevisiae*) at 1,000 vs. 2,000 g in concentrates supplementation on ruminal fermentation efficiency and digestibility of nutrients. The dietary treatments were as follows: T1 = supplementation of malate at 500 g with yeast at 1,000 g; T2 = supplementation of malate at 500 g with yeast at 2,000 g; T3 = supplementation of malate at 1,000 g with yeast at 1,000 g and T4 = supplementation of malate at 1,000 g with yeast at 2,000 g in concentrate, respectively. The composition of dietary treatments and urea-treated rice straw (UTS) used are shown in Table 1 and 2.

Heifers were housed in individual pens and individually fed concentrate at 1%BW. All heifers were fed ad *libitum* of UTS with water and a mineral-salt block. Feed intake of concentrate and roughage were measured separately and refusals were recorded. The experiment was run in four periods, each experimental period lasted for 21 days, the first 14 days were for treatment adaptation and for feed intake measurements whilst the last 7 days were for sample collections of rumen fluid and faeces. Body weights were measured daily during the sampling period prior to feeding.

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).

2.2 Data Collection and Sampling Procedures

UTS and concentrate were sampled daily during the collection period and were composited by period prior to analyses. Feed, fecal and urine samples were collected by rectal sampling whilst urine samples were collected by spot sampling during the last 7 days of each period. Composited samples were dried at 60 $^{\circ}$C and ground (1- mm screen using Cyclotech Mill, Tecator, Sweden) and were

then analyzed for dry matter (DM), ether extract (EE), ash and crude protein (CP) content (AOAC, 1985), neutral detergent fiber (NDF), acid dertergent fiber (ADF) and acid detergent lignin (ADL) (Goering and Van Soest, 1970) and acid insoluble ash (AIA). AIA was used to estimate digestibility of nutrients (Van Keulen and Young, 1977).

Rumen fluid samples were collected at 0, 2 and 4 h post-feeding at the end of each period. Approximately 200 ml of rumen fluid was taken from the middle part of the rumen by a stomach tube connected with a vacuum pump. Ruminal fluid was immediately measured for pH and temperature using HANNA instruments HI 8424 microcomputer after withdrawal. Ruminal fluid samples were then filtered through four layers of cheesecloth. Samples were divided into two portions. One portion was used for ammonia nitrogen (NH_3-N) analyses where 5 ml of H_2SO_4 solution (1M) was added to 50 ml of rumen fluid. The mixture was centrifuged at 16,000 g for 15 minutes and the supernatant stored at –20^{o}C prior to NH_3-N analysis using the micro Kjeldahl methods (AOAC, 1985) and to volatile fatty acids (VFAs) analyses using a HPLC according to Zinn and Owens (1986). Another portion was fixed with 10% formalin solution in normal saline (Galyean, 1989) for microbial determiantion.

The total count of bacteria, protozoa and fungal zoospores were made using the methods of Galyean (1989) based on the use of a haematocytometer (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 5,000 g for 10 minutes and stored at -20 ^{o}C until analysis of blood urea nitrogen (BUN) according to the method of Crocker (1967).

2.3 Statistical Analysis

All data obtained from the experiment were subjected to ANOVA for a 4 x 4 Latin square design with 2 x 2 Factorial arrangement of treatments using the General Linear Models (GLM) procedures of the Statistical Analysis System Institute (SAS, 1998). Treatment means were compared by Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980).

3. Results and Discussion

3.1 Chemical Composition of Feeds

The chemical compositions of UTS and concentrate diets fed in dairy heifers are presented in Table 2. Concentrate diets contained similar concentrations of DM, organic matter (OM), CP, NDF, ADF and total digestible of nutrients (TDN). Diets containing high level of cassava chip based diets had a slightly higher non-structural carbohydrate (NSC) and lower NDF due to increased level of cassava chip in the diets. Furthermore, the similar values for UTS have been similar to those reported by Wanapat (2000).

Table 1 Ingredients of concentrate used in the experiment (% DM basis)

Ingredient (%DM)	Dietary treatments			
	Concentrate I	Concentrate II	Concentrate III	Concentrate IV
Cassava chip	69	68	68.5	67.5
Palm meal	3	3	3	3
Soybean meal	10	10	10	10
Molasses	5	5	5	5
Coconut oil	4	4	4	4
Urea	3	3	3	3
Sulfur	1	1	1	1
Salt	1	1	1	1
Limestone	1	1	1	1
Mineral mix[1]	1.5	1.5	1.5	1.5
Malate (g)	500	500	500	500
Yeast (g)	1,000	1,000	1,000	1,000

[1] Minerals and vitamins (each kg contains): Vitamin A: 10,000,000 IU; Vitamin E: 70,000IU; Vitamin D: 1,600,000 IU; Fe: 50g; Zn: 40g; Mn: 40g; Co: 0.1g; Cu: 10g; Se: 0.1g; I: 0.5g.

Table 2 Chemical composition of concentrates and urea-treated rice straw (UTS) used in the experiment

Chemical compositions (%)	Dietary treatments[1]				
	Conc. I	Conc. II	Conc. III	Conc. IV	UTS
DM	88.7	89.4	88.7	89.4	55.8
OM	91.1	91.2	91.1	91.2	88.9
CP	16.2	16.1	16.2	16.1	7.9
NDF	13.7	12.9	13.7	12.9	73.2
ADF	8.8	8.9	8.8	8.9	52.3
TDN	79.5	79.7	79.5	79.7	55.1
ME, Mcal/kg (DM)[2]	2.9	2.9	2.9	2.9	1.9
Feed cost (US$/kg)	0.25	0.28	0.28	0.30	0.05

[1] Conc. = concentrate; UTS = urea-treated rice straw.

[2] Estimated: Metabolizable energy (ME, Mcal/kg, DM) = TDNx0.04409x0.82. (NRC, 1989).

3.2 Effect on Feed Intake and Digestibility of Nutrients

The effects of different levels of malate and yeast (*S. cerevisiae*) supplementation on feed-intake of dairy heifers are presented in Table 3. Feed intake was non-significantly different among treatments and tended to be higher in dairy heifers receiving T4 than T3, T2, T1 (2.7, 2.6, 2.6 and 2.5%BW, respectively). This data indicated that the level of malate and yeast supplementation had no effect on feed-intake in dairy heifers. This result was in agreement with earlier works by Sommart *et al.,* 2000 and Khampa *et al.,* 2006 which reported that inclusion of cassava chip in diets resulted in satisfactory animal performance and had no negative effects on animal health in fattening beef cattle and lactating dairy cows.

Apparent digestibility of DM, OM, CP, NDF and ADF were significant different ($P<0.05$), especially digestible nutrient intake of crude protein was higher for heifers fed cassava-based diets with T4 than T3, T2 and T1, respectively (Table 3). However, the slightly lower NDF digestibility of the cassava-based diets may have contributed to higher degradation in substantial decrease in fiber digestibility as reported by Hoover

(1986). Furthermore, in the experiment by Erdman (1988) reported that the sources of starch influence the rate of NDF digestion differently at pH 6.8 than 5.5. In addition, when ruminal pH was reduced below 6.3 in dairy cows, ADF digestion could be decreased at 3.6% unit per 0.1 pH and may result in depressed feed-intake. The average daily gain (ADG) was significantly different among treatment and highest in in dairy heifers receiving T4 than T3, T2 and T1 (295.2, 288.7, 279.1 and 272.1 g/d, respectively).

3.3 Characteristics of Ruminal Fermentation and Blood Metabolism

Rumen ecology parameters were measured for temperature, pH, and NH_3-N, VFAs (Table 4). In addition, BUN was determined to investigate it's relationships with rumen NH_3-N and protein utilization. Rumen pH at 0, 2 and 4 h post-feeding were unchanged by dietary treatments and the values were quite stable at 6.7-6.9, but all treatment means were within the normal range which has been reported as optimal for microbial digestion of fiber and also digestion of protein (6.0-7.0) (Hoover, 1986).

Ruminal NH_3-N and BUN concentrations were not altered by malate level and yeast supplement in diets containing high cassava-based diets. As NH_3-N is regarded as the most important nitrogen source for the microbial protein synthesis in the rumen. In addition, the result obtained here was closer to optimal ruminal NH_3-N (15-30 mg%, Wanapat and Pimpa, 1999; Chanjula *et al.,* 2003, 2004) for increasing microbial protein synthesis, feed digestibility and voluntary feed intake in ruminant fed on low-quality roughages.

The influence of malate level and yeast supplement in concentrates on total volatile fatty acids proportion of acetic acid, propionic acid and butyric acid and acetic to propionic ratio are shown in Table 4. The mean of total VFAs and propionate concentrations in the rumen were increased ($P<0.05$) with increasing malate level (112.7 vs. 118.8 mmol/L; 21.1 vs. 23.7 mol/100 mol, respevtively) and yeast in the diet. Especially, the concentration of propionic acid was slightly but significantly ($P<0.05$) higher in T4 than T3, T2, and T1 respectively. However, it was found that the total VFAs concentration in all diets ranged from 70 to 130 m*M*, the range suggested by France and Siddons (1993). Although the acetate to propionate ratio was decreased by the level of sodium dl-malate, but the supplementation of malate increased the

daily output of propionate without decreasing the production of acetate, and it was in agreement with the results reported by other authors (Callaway and Martin, 1996; Khampa *et al.,* 2006).

Table 3 Effects of malate level and yeast supplementation on feed-intake and digestibility of nutrients in dairy heifers

Item	Treatments[1]					Contrast[2]		
	T1	T2	T3	T4	SEM	M	Y	M x Y
DM intake (%BW)								
UTS	1.5	1.6	1.6	1.7	0.08	NS	NS	NS
Concentrate	1.0	1.0	1.0	1.0	-	NS	NS	NS
Total	2.5	2.6	2.6	2.7	0.09	NS	NS	NS
Apparent total-tract digestibility (%)								
DM	70.6^{a}	72.4^{ab}	75.5^{ab}	77.9^{b}	1.81	*	NS	NS
OM	75.2^{a}	75.9^{a}	77.6^{ab}	79.3^{b}	1.05	NS	*	NS
CP	68.7^{a}	72.4^{a}	71.0^{ab}	74.2^{b}	1.23	NS	*	NS
NDF	55.2^{a}	59.3^{b}	60.2^{b}	65.4^{c}	0.59	*	*	NS
ADF	50.2^{a}	50.6^{a}	54.3^{b}	54.5^{b}	1.11	NS	*	NS
ADG (g/d)	272.1^{a}	279.1^{a}	288.7^{b}	295.2^{b}	2.86	*	*	NS

[a,b,c] Values on the same row with different superscripts differ (*P< 0.05*).

[1] T1 = malate at 500 g with yeast at 1,000 g; T2 = malate at 500 g with yeast at 2,000 g; T3 = malate at 1,000 g with yeast at 1,000 g; T4 = malate at 1,000 g with yeast at 2,000 g.

[2] Probability of main effects of level malate (M) in concentrates (500 vs 1,000 g), levels of yeast (Y) (1,000 vs 2,000 g), or the M x Y interaction. * = *P<0.05,* NS = *P>0.05.*

Table 4 Effects of malate level and yeast supplementation on ruminal fermentation, blood metabolites and volatile fatty acids in dairy heifers

Item	Treatments[1]					Contrast[2]		
	T1	T2	T3	T4	SEM	M	Y	M x Y
Ruminal Temperature (^{0}C)	39.7	39.5	39.7	39.8	0.52	NS	NS	NS
Ruminal pH	6.7	6.7	6.8	6.9	0.14	NS	NS	NS
Ruminal NH_3-N (mg/dl)	16.2	17.4	18.5	18.8	1.98	NS	NS	NS
BUN (mg/dl)	9.6	10.7	12.6	13.2	2.69	NS	NS	NS
Glucose (mg/dl)	55.8	56.6	57.9	58.7	1.95	NS	NS	NS
Total VFA (mmol/L)	107.2a	118.2b	119.2b	118.3b	1.03	*	NS	NS
Molar proportion of VFA (mol/100mol)								
Acetate (C2)	71.2[a]	68.5[b]	67.2[b]	66.7[c]	0.37	**	NS	NS
Propionate (C3)	19.7[a]	22.4[b]	22.9[b]	24.4[c]	0.34	**	NS	NS
Butyrate (C4)	9.1	9.1	9.9	8.9	0.46	NS	NS	NS
C2:C3 ratio	3.6[a]	3.1[b]	3.0[b]	2.7[c]	0.04	**	NS	NS
C2+C4:C3 ratio	4.1[a]	3.5[b]	3.4[b]	3.1[c]	0.05	*	NS	NS

[a,b,c] Values on the same row with different superscripts differ (*P<0.05*).

[1] T1 = malate at 500 g with yeast at 1,000 g; T2 = malate at 500 g with yeast at 2,000 g; T3 = malate at 1,000 g with yeast at 1,000 g; T4 = malate at 1,000 g with yeast at 2,000 g.

[2] Probability of main effects of level malate (M) in concentrates (500 vs. 1,000 g), levels of yeast (Y) (1,000 vs. 2,000 g), or the M x Y interaction. * = *P<0.05,* NS = *P>0.05.*

Table 5 Effects of malate level and yeast on ruminal microorganisms in dairy heifers

Item	Treatments[1]					Contrast[2]		
	T1	T2	T3	T4	SEM	M	Y	M x Y
Total direct counts (cell/ml)								
Bacteria ($x10^{11}$)	5.1^{a}	6.2^{ab}	7.9^{ab}	9.9^{b}	1.22	*	NS	NS
Protozoa								
Holotric ($x10^{4}$)	3.3^{a}	3.2^{a}	2.7^{ab}	2.3^{b}	0.26	*	NS	NS
Entodiniomorph ($x10^{5}$)	9.3^{a}	6.8^{b}	3.1^{c}	2.4^{c}	0.71	*	*	NS
Fungal zoospores ($x10^{4}$)	2.2^{a}	3.4^{a}	5.3^{b}	6.8^{b}	0.51	*	*	NS

[a,b,c] Values on the same row with different superscripts differ (*P<0.05*).

[1] T1 = malate at 500 g with yeast at 1,000 g; T2 = malate at 500 g with yeast at 2,000 g; T3 = malate at 1,000 g with yeast at 1,000 g; T4 = malate at 1,000 g with yeast at 2,000 g.

[2] Probability of main effects of level malate (M) in concentrates (500 vs. 1,000 g), levels of yeast (Y) (1,000 vs. 2,000 g), or the M x Y interaction. * = *P<0.05,* NS = *P>0.05.*

3.4 Rumen Microorganisms Populations

Table 5 presents rumen microorganism populations. The populations of fungal zoospores, protozoa and total bacteria direct counts were significantly different and the populations of bacteria had higher numbers (*P<0.05*) in heifer receiving diets T4, T3, T2 than T1. In contrast, the present number of protozoa in the rumen was decreased by malate and yeast supplementation in high cassava-based diets. In the experiment by Newbold *et al.* (1996) has shown that feeding 100 mg of malate per day in sheep caused an increase in the number of total bacteria and tended to increase the population of cellulolytic bacteria. In agreement with these observations, Lopez *et al.* (1999) reported that fumalate (another intermediate in the succinate to propionate pathway) increased the number of cellulolytic bacteria almost three-fold during fermentation in the RUSITEC system. In addition Guedes (2007) reported that yeast are usually related to stimulation of cellulolytic and lactate-utilizing bacteria in the rumen, increased fiber digestion, and flow of microbial protein from the rumen which may be beneficial for feedlot cattle fed high-grain diets. As cassava chip can be readily degraded in the rumen and ruminal pH was decreased, malate could

stimulate lactate utilization by *S. ruminantium* and could improve pH in the rumen. It is possible that the supplementation of malate with yeast may play an important role in increasing bacterial populations. Moreover, Martin *et al.* (1999) reported that increasing dietary concentrations of malate might help to reduce problems associated with ruminal acidosis by stimulating lactate utilization by *S. ruminantium.*

4. Conclusions

Based on this experiment, it could be concluded that supplementation of malate with yeast (*S. cerevisiae*) in concentrate containing high level of cassava chip maintained could improved ruminal fermentation efficiency, increasing propionate production and decreased acetate to propionate ratio. Moreover, high level of cassava chip in diet resulted in increase in size of populations of bacteria, but decreased in protozoal populations size. These results suggest that the combined use of concentrate containing high level of cassava chip at 70%DM with malate at 1,000 g and yeast at 2,000 g could give the highest improvement ruminal fermentation efficiency and digestibility of nutrients.

5. Acknowledgements

The authors would like to express their most sincere gratitude and appreciation to the Rajabhat Mahasarakham University, Tropical Feed Resources Research and Development Center (TROFREC) and The National Research Council of Thailand for their financial support of research and the use of research facilities.

6. References

OAC. 1985. **Official Methods of Analysis.** Association of Official Analysis Chemists, DC, USA.

Brossard, L., Chaucheyras-Durand, F., Michalet-Doreau, B. and Martin, C. 2006. **Dose effect of live yeasts on rumen microbial communities and fermentations during butyric latent acidosis in sheep: newtype of interaction.** J. Anim. Sci. 82: 1-11.

Callaway, T.R. and Martin, S.A. 1996. **Effects of organic acid and monensin treatment on in vitro mixed ruminal microorganism fermentation of cracked corn.** J. Anim. Sci. 74: 1982-1989.

Chanjula, P., Wanapat, M., Wachirapakorn, C., Uriyapongson, S. and Rowlinson, P. 2003. **Ruminal degradability of tropical feeds and their potential**

use in ruminant diets. Asian-Aust. J. Anim. Sci. 16: 211-216.

Chanjula, P., Wanapat, M., Wachirapakorn, C., Uriyapongson, S. and Rowlinson, P. 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 Gomes, M.J. 1992. **Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives-an overview of the technical details.** International Feed Resources Unit, Rowel Research Institute, Aberdeen, UK.

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

Erdman, R.A. 1988. **Dietary buffering requirements of the lactating dairy cows. A review.** J. Dairy Sci. 71: 3246-3266.

Fernandez, J.M., Sahulu, T., Lu, C., Ivey, D. and Potchoiba, M.J. 1997. **Production and metabolic aspects of non-protein nitrogen incorporation in lactation rations of dairy goats.** Small Rum. Res. 26: 105-107.

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

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

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

Gottschalk, G. 1986. **Bateria metabolism (2nd Ed.).** Sparinger-Verlag. New York.

Guedes, C.M., Goncalves, D.M., Rodrigues, A.M. and Dias-da-Silva, A. 2007. **Effects of a *Saccharomyces cerevisiae* yeast on ruminal fermentation and fibre degradation of maize silages in cows.** Anim. Feed Sci. Technol. 145: 27-40.

Hoover, W.H. 1986. **Chemical factors involved in ruminal fiber digestion.** J. Dairy Sci. 69: 2755-2766.

Hoover, W.H. and Stokes, S.R. 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.

Khampa, S., Wanapat, M., Wachirapakorn, C., Nontaso, N. and Wattiaux, M. 2006. **Effect of levels of sodium dl-malate supplementation on ruminal fermentation efficiency in concentrates containing high levels of cassava chip in dairy steers.** Aisan-Aust. J. Anim. Sci. 19: 368-375.

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

Lopez, S., Newbold, C. and Wallace, R.J. 1999. **Influence of sodium fumarate addition on rumen fermentation in vitro.** Br. J. Nutr. 81: 59-64.

Martin, S.A., Streeter, M.N., Nisbet, D.J., G.M. Hill and Williams, E.E. 1999. **Effect of DL-malate on ruminal metabolism and performance of cattle fed a high concentrate diets.** J. Anim. Sci. 77: 1008-1015.

Newbold, C.J., Wallace, R.J. and McIntosh, F.M. 1996. **Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for ruminants.** Br. J. Nutr. 76: 249-261.

NRC. 1989. **Nutritional Requirements of Dairy Cattle.** National Academy Press, Washington, DC. USA.

Promkot, C. and Wanapat, M. 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.

Sanson, D.W. and Stallcup, O.T. 1984. **Growth response and serum constituents of Holstein bulls fed malic acid.** Nutr. Rep. Int. 30: 1261-1267.

SAS. 1998. **SAS/STAT User's Guide.** Version 6.12. SAS Inst. Inc., Cary, NC, USA.

Sommart, K., Wanapat, M., Parker, D.S. and Rowlinson, P. 2000. **Cassava chip as an energy source for lactating dairy cows fed rice straw.** Asian-Aust. J. Anim. Sci. 13: 1094-1101.

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

Van Keulen, J. and Young, B.A. 1977. **Evaluation of acid insoluble ash as a neutral marker in ruminant digestibility studies.** J. Anim. Sci. 44: 282-287.

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

Wohlt, J.E., Clark, J.H. and Blaisdell, F.S. 1978. **Nutritional value of urea versus preformed protein for ruminants. II. Nitrogen utilization by dairy cows fed corn based diets containing supplemental nitrogen from urea and/or soybean meal**. J. Dairy Sci. 61: 916-931.

Zinn, A.R. and Owen, F.N. 1986. **A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis.** Can. J. Anim. Sci. 66: 157-163.