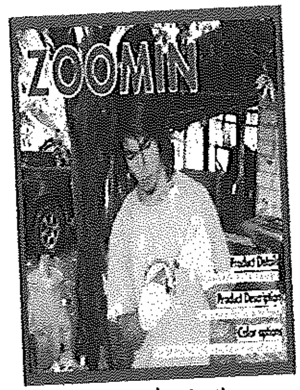
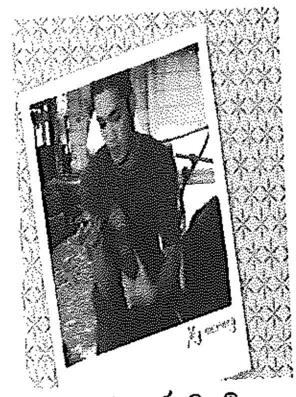
ภาคผนวก

คมกริช ลมชิต

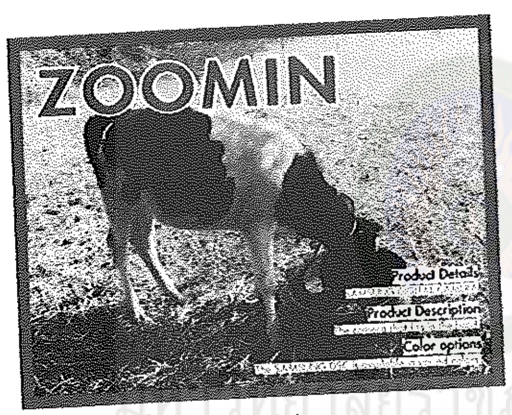


จักรพงษ์ ลีกวี

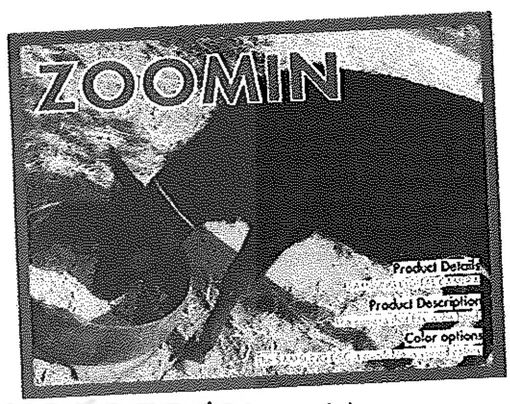


ฐาปกรณ์ สิทธิ

นักศึกษาวิจัยผู้ช่วย



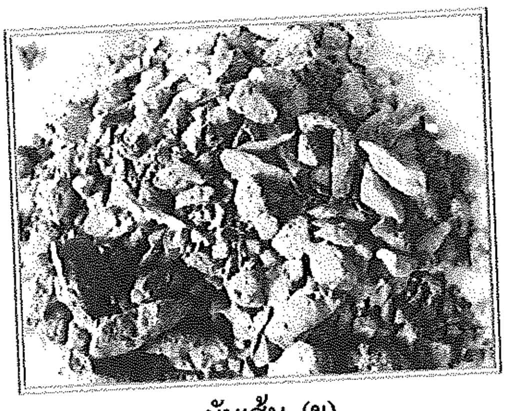
กลุ่มควบคุม (ก)



กลุ่มทดลอง (ข)

ภาพที่ 1 แสดงสัตว์ทดลองจำนวน 4 ตัวที่ใช้ในฟาร์มเกษตรกรอำเภอบรบือ

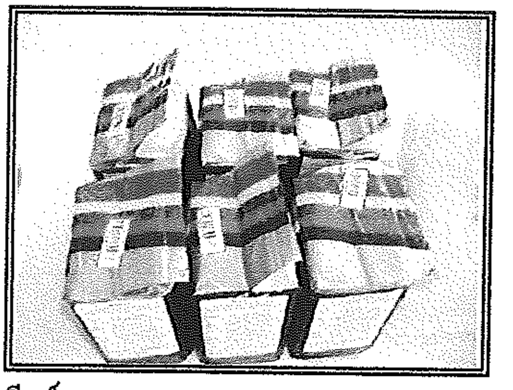
ระยอง 7 (ก)



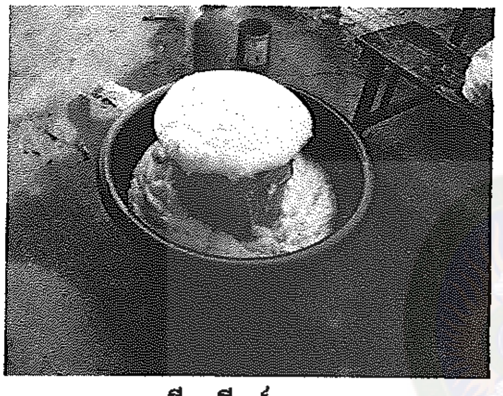
มันเส้น (ข)

ภาพที่ 2 แสดงวัตถุดิบการทำมันเส้นหมักยีสต์-มาเลท

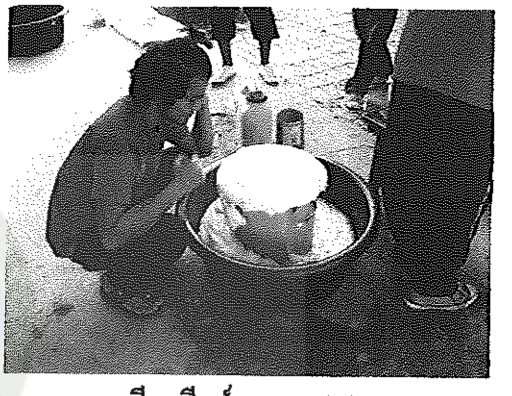
น้ำตาลทราย (ก)



เชื้อยีสต์ (*Saccharomyces cerevisiae*) **(ข)**

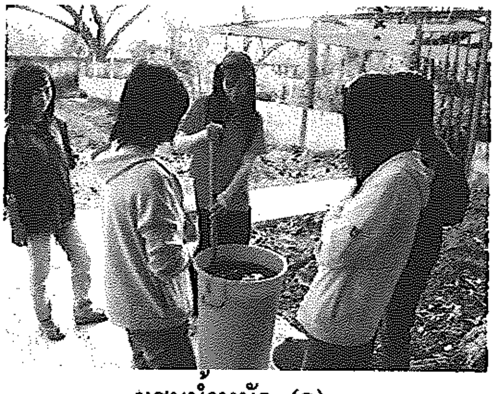


การเตรียมยีสต์ (ค)



การเตรียมยีสต์-มาเลท (ง)

ภาพที่ 3 แสดงขั้นตอนการเตรียม ยีสต์หมัก



ผสมน้ำหมัก (ก)

ผสมมันเส้นหมักยีสต์-มาเลท (ข)

ภาพที่ 4 แสดงขั้นตอนการทำน้ำหมักยีสต์

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Manipulation of Rumen Ecology by Yeast and Malate in Dairy Heifer

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Abstract: Four, one-year old of dairy heifers were randomly assigned according to a 2x2 Factorial arrangement in a 4x4 Latin square design to study supplementation of malate level at 500 vs 1,000 g with yeast at 1,000 vs 2,000 g in concentrate. The treatments were as follows: T1 = supplementation of malate at 500 g + yeast at 1,000 g; T2 = supplementation of malate at 500 g + yeast at 2,000 g; T3 = supplementation of malate at 1,000 g + yeast at 1,000 g; T4 = supplementation of malate at 1,000 g + yeast at 2,000 g in concentrate, respectively. The cows were offered the treatment concentrate at 1 %BW and ruzi grass was fed *ad libitum*. The results have revealed that rumen fermentation and blood metabolites were similar for all treatments. However, the concentration of volatile fatty acid was significantly different especially the concentration of propionic acid was slightly higher in heifer receiving T4 than T3, T2 and T1 (24.4, 22.9, 22.4 and 19.7%, respectively). The populations of protozoa and fungal zoospores were significantly different 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 with ruzi grass as a roughage could improved rumen ecology in dairy heifers.

Key words: Yeast, *Saccharomyces cerevisiae*, malate, rumen ecology, dairy heifer

INTRODUCTION

The rumen has been well recognized as an essential fermentation that is capable of preparing end-products particularly Volatile Fatty Acids (VFAs) and microbial protein synthesis as major energy and protein for the ruminant host, hence, the more efficient the rumen is, the optimum the fermentation end-products are being synthesized. In recent years, there have been increasing interests, researches conducted as well as reviews in relation to rumen studies, rumen ecology and rumen manipulation (Martin *et al.*, 1999; Wanapat, 2003; Khampa *et al.*, 2006). In the tropics, most of ruminants have been fed on low-quality roughages, agricultural crop-residues, industrial by - products which basically contained high levels of lingo-celluloses materials, low level of fermentable carbohydrate as well as low level of good-quality protein.

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 *S. ruminantium* uses this pathway (Gottschalk, 1986). The fact 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 studies 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 diets with yeast *(Saccharomyces cerevisiae)* increases 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 ruzi grass as a basal roughage on rumen ecology in dairy heifers.

MATERIALS AND METHODS

Animals, diets and experimental design: Four, one-year old of dairy heifers weighing at 150±10 kg. Cows were randomly assigned according to a 2x2 Factorial arrangement in a 4x4 Latin square design to study two levels malate at 500 vs 1,000 g with yeast *(Saccharomyces cerevisiae)* at 1,000 vs 2,000 g in concentrates supplementation on rume ecology. The dietary treatments were as follows: T1 = supplementation of malate at 500 g + yeast at 1,000 g; T2 = supplementation of malate at 500 g + yeast at

Table 3: Effects of malate and yeast on feed-intake and rumen fermentation in dairy heifers

Item	Treatments[1]				Contrast[2]			
	T1	T2	T3	T4	SEM	M	Y	MxY
DM intake (%BW)								
Ruzi grass	1.6	1.7	1.8	1.9	0.08	NS	NS	NS
Concentrate	1.5	1.5	1.5	1.5	-	NS	NS	NS
Total	3.1	3.2	3.3	3.4	0.12	NS	NS	NS
Ruminal temperature (°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
NH_3-N (mg%l)	16.2	17.4	18.5	18.8	1.98	NS	NS	NS
BUN (mg%)	9.6	10.7	12.6	13.2	2.69	NS	NS	NS
Total VFA (mmol/L)	107.2[a]	118.2[b]	119.2[b]	118.3[b]	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 MxY interaction.
* = $p<0.05$, NS = $p>0.05$.

Table 4: Effects of malate level and yeast on rumen microorganisms in dairy heifers

Total direct counts (cell/ml)	Treatments[1]				Contrast[2]			
	T1	T2	T3	T4	SEM	M	Y	MxY
Bacteria (x10[12])	5.1[a]	6.2[ab]	7.9[ab]	9.9[b]	1.22	*	NS	NS
Protozoa								
Holotric (x10[3])	3.3[a]	3.2[a]	2.7[ab]	2.3[b]	0.26	*	NS	NS
Entodiniomorph (x10[6])	9.3[a]	6.8[b]	3.1[c]	2.4[c]	0.71	*	*	NS
Fungal zoospores (x10[6])	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 MxY interaction.
* = $p<0.05$, NS = $p>0.05$.

dairy heifers. This result was in agreement with earlier work 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 finishing beef cattle and lactating dairy cows.

Characteristics of ruminal fermentation and blood metabolism: Rumen ecology parameters were measured for temperature, pH and NH_3-N, VFA (Table 4). In addition, BUN was determined to investigate their 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 microbial protein synthesis in the rumen. In addition, the result obtained was closer to optimal ruminal NH_3-N between at 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 with yeast supplement in concentrates on Volatile Fatty Acid (VFA) on 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 VFAs and propionate concentrations in the rumen were increased with increasing malate level and yeast in the diet ($p<0.05$). Especially, the concentration of propionic acid was slightly higher in T4 than T3, T2 and T1 respectively. However, it was found that total VFA concentration in all diets ranged from 70-130 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 level with yeast 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).

Rumen microorganisms populations: Table 4 presents rumen microorganism populations. The populations of fungal zoospores, protozoa and total bacteria direct counts were significantly different and populations of bacteria had higher numbers in heifer receiving diets T4 than T3, T2 and T1. In contrast, the present number of protozoa in the rumen was decreased by malate level 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 *et al.* (2007) reported that 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. 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 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*.

Conclusions: Based on this experiment, it could be concluded that supplementation of malate with yeast *(Saccharomyces 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 increase populations of bacteria, but decreased protozoal populations. These results suggest that the combined use of concentrates containing high level of cassava chip at 70% DM with malate at 1,000 g and yeast at 2,000 g in concentrate could highest improved rumen ecology in dairy heifers.

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