CHAPTER III

MATERIALS AND METHODS

Animals and Feed Management

Six multiparous crossbred Friesian cows, 114 $\pm$ 24 d postpartum, were used in the experiment. They were divided into three groups of two cows each. Cows were kept individually in tie stall housing with solid floor and open sides.

Cows were fed ad libitum with TMR (Table 5). The TMR was the mixture of silage and concentrate at the ratio of 39:61 (DM basis). Silage was prepared by mixing baby corn stover and pineapple waste (Table 4) at the ratio of 2:1 and kept in plastic bag till feeding. A basal diet (control) and two treatment diets were the same except Na. Dietary Na was varied by using Na_2CO_3. The amount of Na_2CO_3 in the control diet and two treatment diets were 0%, 1% and 2% (DM basis), respectively.

Table 4 Nutrient of corn mixed with pineapple silages (dry matter basis)

Nutrient (%)	Composition (%)
CP	7.5
ADF	26.3
NDF	51.5
Na	0.15

Table 5 Active ingredients of TMR (dry matter basis)

Composition (%)	control	1%Na_2CO_3	2%Na_2CO_3
Silages	39.48	39.44	39.40
Soybean meal	17.15	17.20	17.20
Cassava	16.75	16.98	16.92
Rice bran	13.17	13.16	12.72
Cotton seed meal	6.91	7.20	7.65
Rice hull	2.43	0.91	0.00
Mono-dicalcium	1.16	1.16	1.16
Shell	1.16	1.16	1.16
Limestone	1.16	1.16	1.16
Bone meal	1.16	1.16	1.16
Premix*	0.33	0.33	0.33
Potassium chloride	0.26	0.26	0.26
Sodium carbonate	**0.00**	**1.00**	**2.00**

Premix 1 kg : Vitamin A 2,400,000 IU, Vitamin D_3 500,000 IU, Vitamin E 500 IU, Vitamin B_{12} mg, Manganese (Mn) 8 g, Zinc (Zn) 8 g, Iron (Fe) 10 g, Copper (Cu) 2 g, Magnesium (Mg) 26.4 g, Cobolt (Co) 400 mg, Iodine (I) 400 mg, Selenium (Se) 40 mg*

Physical and Chemical Properties of Sodium Carbonate

The commercially supplied Na_2CO_3 that was added in the experimental diet was a white powder with bitter taste with the following percentage composition: Na_2CO_3 99.4, CaO 0.02, MgO 0.01, Fe_2O_3 0.002; and loss on ignition, 0.138. The total concentration of Na in this compound was approximately 43.3% (by weight).

Experimental Design

A Latin square (3 x 3) design was used in this study. Six cows were assigned randomly into three groups for rotationally receiving three different treatments (control, 1%Na_2CO_3 and 2%Na_2CO_3) at different period of times (period I, II and III). Three consecutive periods were assigned to each treatment (Table 6). Each period was 14 days of the adjusting period and 7 days of the collecting period as shown in Figure 5.

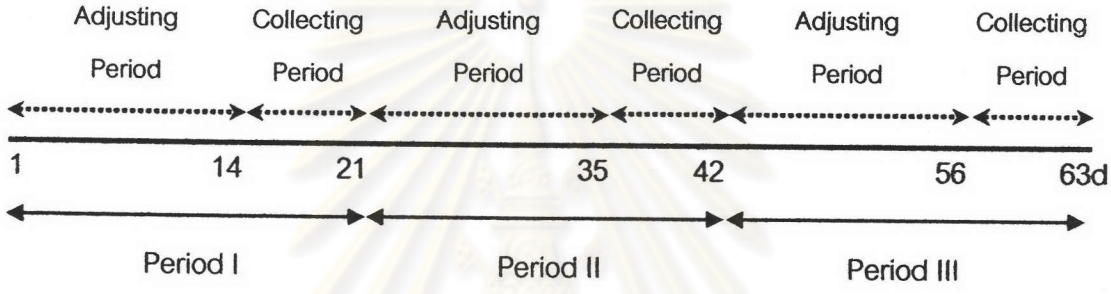

Figure 5 The experimental diagram

Table 6 The experimental design

	Number of cows		
	1 & 2	3 & 4	5 & 6
Period I	control	1%Na_2CO_3	2%Na_2CO_3
Period II	2%Na_2CO_3	control	1%Na_2CO_3
Period III	1%Na_2CO_3	2%Na_2CO_3	control

Measurements of dry matter intake, water intake, milk yield and related parameters

Dry matter intake

Feed offered and orts were measured daily during the experimental period. Feed samples of TMR were collected every other day during the collecting periods for the determination of the DM content. The DM content was used to calculate DMI.

Feed compositions

The feed TMR samples of each treatment were collected every other day during the collecting periods and pooled to one sample, dried at 60°C for 48 h in air-forced oven (Binder ED53), ground (1-mm screen) with a cutting mill (cyclotec 1093 sample mill), and kept at -20°C for analysis later. Feed samples were thawed, dried (100°C for 24h), and ashed (550°C for 4 h) for Na and K analysis. The Na and K concentrations in feed samples were dissolved in 3*N* HCl plus three drops of HNO_3 and diluted with deionized water. The diluted solution was analyzed for Na^+ and K^+ concentrations by Flame Photometry (Corning 410 C). The samples in feed for Cl^- analysis were dissolved in 25 ml of a 0.4N HNO_3 40% glacial acetic acid solution, shaken vigorously for 1 h, and then was determined by Chloridometer (Cl Analyzer 960). Feed N was determined by the Kjeldal method (AOAC, 2001), and crude protein was calculated as N x 6.25. Feed ADF and NDF analysis were determined by the method of Van Soest et al. (1991).

Body weight and water intake

Body weight of each cow was recorded at the beginning of the experiment and after treatment of diet in every week. The water intake of each cow was recorded individually on 4 consecutive days in each period at approximately 0200 p.m. The water sample was collected during the experiment and frozen at -20°C for subsequent analysis for electrolytes concentrations. The sample was thawed and analyzed for Na^+ and K^+ concentrations by Flame Photometry (Corning 410 C) and Cl^- concentration by Chloridometer (Cl Analyzer 960).

Milk yield and compositions

The total milk yield from individual cows were recorded and collected during milking both at 0500 a.m. and 0300 p.m. in each collection period. Two 60 ml of milk in plastic bottles were collected for 3 days (18^{th} to 20^{th} d) in each period. One sample was analyzed for concentrations of fat, protein, lactose, TS, and SNF via Milko Scan® 133B (N. FOSS ELECTRIC DENMARK). Milk component were calculated as yield and

concentrations according to the a.m. and p.m. milking for 3 days (at 18th to 20th d) in each experimental period. The other milk sample was frozen at -20°C for later electrolyte analysis.

Milk had been deproteinized with 10% trichloroacetic acid, vortex, and centrifuged at 2500 x g for 10 min; supernatant was harvested and diluted with deionized water, and then the concentrations of Na^+ and K^+ in milk were measured by Flame Photometry (Corning 410 C), the concentration of Cl^- in milk was determined by Chloridometer (Cl Analyzer 960).

Protocol for feed, milk, plasma, urine, and feces sample collection were described following a diagram:

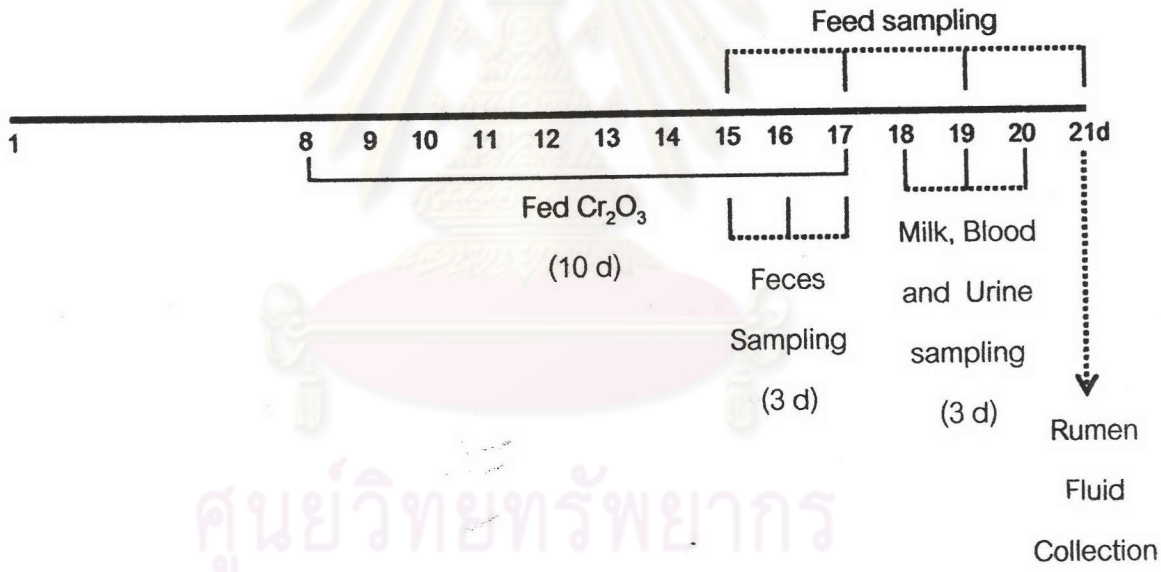

Figure 6 Diagram for samples collection

Plasma electrolytes

Blood samples from the external jugular vein were collected from each cow on 3 consecutive days in each period (18th to 20th d) at approximately 1400 h, by venipuncture with a G21 needle into a 20 U/ml heparinized vacuum tube. The sample was kept on ice, centrifuged post-sampling at 2500 x g for 20 min, and then the plasma was pipetted off and frozen at –20°C until electrolyte analysis. The Na^+, K^+ and Cl^- concentrations in

plasma were thawed and analyzed; Na^+ and K^+ concentrations in plasma were measured by Flame Photometry (Corning 410 C), Cl^- concentration in plasma was determined by Chloridometer (Cl Analyzer 960).

Urinary electrolytes

Urine sample was collected from each cow on 3 consecutive days in each period (18^{th} to 20^{th} d) at approximately 1400 h by manual stimulation. The urine was kept in a 30 ml glass container with 0.9 ml concentrate HCl as preservative and was frozen at -20°C until electrolytes and creatinine analysis. The concentrations of Na^+ and K^+ in urine were measured by Flame Photometry (Corning 410 C). Urinary Cl^- concentration was determined by the Chloridometer (Cl Analyzer 960). Urinary creatinine was determined using a procedure modified from Bartels et al. (1972).

Fecal electrolytes

Five grams of chromic oxide (Cr_2O_3) containing in gelatin capsule was fed twice daily at 0600 and 1600 h during 8^{th} to 17^{th} d of experimental period. Each cow was received 10 grams of Cr_2O_3 every day. On day 15 to 17 grab sampling of fecae per rectum, about 200 gm, was done 6 times per day at 0700, 1100, 1500, 1900, 2300, 0300h for 3 consecutive days (18^{th} to 20^{th} d). All of samples were frozen at -20°C before it was dried at 60°C for 48h, grinned to 1 mm, pooling to one sample, and then kept at –20°C until electrolytes analysis. Feces examination for DM, ADF, NDF, Na^+, K^+ and Cl^- concentrations were determined by the similar method as for feeds. The Cr concentration in fecal sampling was analyzed by the method from Williams et al. (1962).

Calculations for the digestibility of DM, ADF, NDF, Na, K and Cl were performed as following:

Equation 1

$$\text{Digestibility of nutrient (\%)} = 100 - \left[100 \times \frac{\%\text{Cr in feed}}{\%\text{Cr in feces}} \times \frac{\%\text{Nutrient in feces}}{\%\text{Nutrient in feed}} \right]$$

where

Cr = chromium

Equation 2

$$\text{Fecal output (kg/day DM)} = \frac{\text{Marker dose (g/day)}}{\text{Marker concentration in faces (g/kg DM)}}$$

Rumen fluid

On the last day of each period around 0900 h from 4 h post-feeding, ruminal fluid was collected from rumen by stomach tube with an electric vacuum pump. Ruminal fluid was strained through four layers of cheesecloth and then pH of the ruminal fluid was measured using pH meter (HI9025C). A 60 ml aliquot of the filtered ruminal fluid was preserved by adding 3 ml of *6 N* HCl (Younker et al.,1998) and kept at -20°C. Ruminal fluid was analyzed for VFA concentrations by the method modified from Erwin (1961).

Briefly, frozen ruminal fluid was thawed at room temperature. There was centrifuged at 9,000 rpm for 8 min and the supernatant aliquots were removed. The volume of 0.4 ml working internal standard solution (isocaproic acid, formic acid and 25% metaphosphoric acid) was mixed with 0.7 ml of the supernatant or standard solution. The aliquots were analyzed for the concentration of VFA using a gas chromatograph equipped with a hydrogen flame ionization detector. The column used for analysis (GL Sciences Inc) was treated with 1% (wt/wt) H_3PO_4 (length 2.1 m, ID 4 mm, OD 7 mm) and packed with 10% FFAP (80 – 100 mesh).

Determinations of the concentrations of VFA were calculated from the following formula:

Equation 3

$$\text{The concentrations of } VFA_{cx} = \frac{std_{cx} \times (\text{A-sample})_{cx} \times (\text{A-standard})_{\text{int std}} \times (7/11)}{(\text{A-sample})_{\text{int std}} \times (\text{A-standard})_{cx}}$$

where

VFA = volatile fatty acid

c = carbon

x = a number of carbon atom

std_{cx} = The standard of carbon atom

$A\text{-}sample_{cx}$ = The area of sample (carbon atom)

$A\text{-}sample_{int\ std}$ = The area of sample (internal standard)

$A\text{-}standard_{cx}$ = The area of standard (carbon atom)

$A\text{-}standard_{int\ std}$ = The area of standard (internal standard)

Equation 4

$$\text{Total [VFA]} = [C_2] + [C_3] + [C_4] + [C_5]$$

where

[VFA] = The concentration of volatile fatty acid (mmol/l)

$[C_2]$ = The concentration of acetic acid (mmol/l)

$[C_3]$ = The concentration of propionic acid (mmol/l)

$[C_4]$ = The concentration of butyric acid (mmol/l)

$[C_5]$ = The concentration of valeric acid (mmol/l)

Equation 5

$$\text{Acetate per propionate ratio} = \frac{[C_2]}{[C_3]}$$

Statistical Analysis

Statistical analyses were done by using procedures in SAS package (SAS, 1985). The linear model (statistical model) representing the relationship among the studied traits and factors was as follows;

$$y_{ijkl} = \mu + period_i + cow_j + day_k + period\,(day)_{i(k)} + treatment_l + e_{ijkl}$$

where

y_{ijkl} is value of an interested trait that collected from cow j, which received treatment l, at day k of the period i

μ is overall mean for the interested trait of the dataset

$period_i$ is the effect of period i (i = 1, 2, and 3) in the experiment

cow_j is the effect of the individual used cow j (j = 1, 2, 3, ..., and 6)

day_k is the effect of collecting day k in the tested period i

$period(day)_{i(k)}$ is the nested effect of collecting day k in the period i

$treatment_l$ is the effect of assigned treatment l (l = 1 is control, l = 2 is 1%Na_2CO_3, and l = 3 is 2%Na_2CO_3)

e_{ijkl} is effect of random error occurring from the other factors that not be included in the used statistical model

According to the studied traits that had incomplete records (missing values), least square mean and standard error were calculated. Differences among treatments' mean (control, 1%Na_2CO_3, and 2%Na_2CO_3) were done using t-test. The analyses for testing hypothesis were done by using Least Significant Differences at $P<0.05$ (SAS, 1985).