CHAPTER III

MATERIALS AND METHODS

Animals and management

Twenty primiparous 87.5% crossbred Friesian cattle were used in the experiment. They were divided into two groups of ten animals each. One group was kept in evaporative cooling system (EVAP) that temperature reduced by using air force movement through cooling pad. Another group was kept in the open conventional housing system (NEVAP). An ambient temperature and relative humidity of both housing system were recorded for calculating the temperature humidity index (THI), for three consecutive days between 0700 to 1900 h by thermometer throughout the experimental period. The temperature humidity index (THI) was calculated according to NOAA, (1976) (Equation 1)

Equation 1

$$THI = [1.8(temp) + 32] - [0.55 - 0.0055(rh)][1.8(temp) - 26]$$

Diets were formulated to meet NRC requirements (National Research Council, 1989). All animals received feed in the form of Total Mixed Ration (TMR). The forage to concentrate ratio was 45:55 (DM basis). Ingredient compositions of feed were shown in Table 1. From the beginning to the end of experimental period, animals of both groups were fed the same ration. Food and water were available *ad libitum*. Cows were milked twice a day and milk yields were recorded by weighing from the postpartum to the 10^{th} wk of postpartum at 0600 and 1500 h. Body weight of individual animal was measured weekly throughout the experiment. From the 8^{th} wk to the 10^{th} wk postpartum, rectal temperature (RT) and respiration rate (RR) were measured every 2 h, between 0700 to 1900 h for three consecutive days. RT was recorded with digital electronic thermometer.

RR was measured by observing the movement of flank for 1 minute, three times and an average of respiration rate was calculated. Water consumption of each cow was measured once daily at 0600 h using individual water meter for three consecutive days. The protocol of the experiment is shown Figure 2.

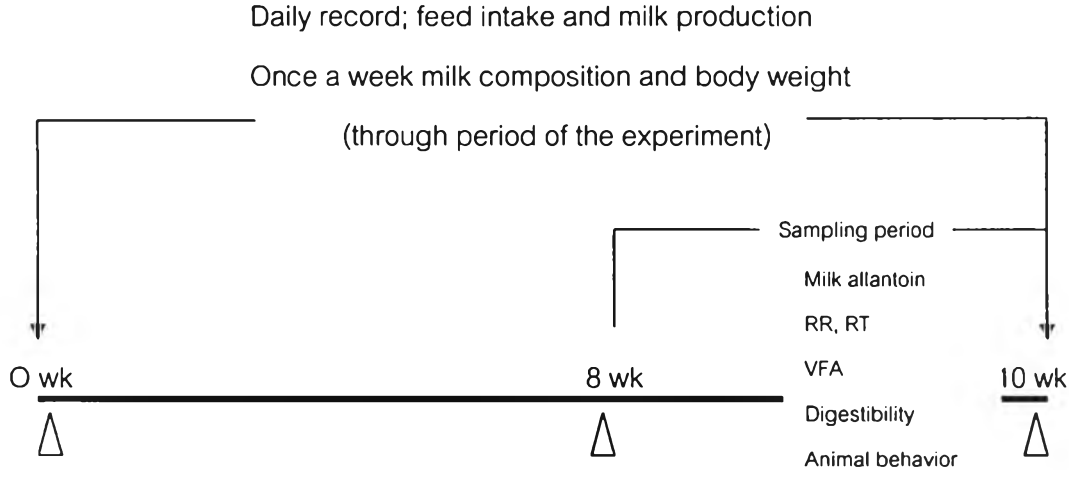

Figure 2. Protocol of the experiment

Table 1. Ingredient compositions of the TMR fed to the cow during the experimental period

Ingredient composition	Dry matter basis (%)
Corn-pine silage	44.8
Cassava	9.8
Rice bran	8.2
Soybean meal	17.8
Cottonseed	12.2
Bone meal	1.4
Dicalcium phosphate	3.7
Salt	1.4
Premix	0.6

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

Feed intake measurement and feed analysis

The DMI of each individual animal was measured daily from parturition to the 10th wk postpartum. The amount of offered feed and orts were weighed daily. Orts were removed in the morning before next feeding. Samples of feed were collected and immediately frozen at -20 °C for further analysis. Samples were composited, dried by a forced air oven at 55 °C for 72 h, and ground with a Wiley mill to pass through a 1-mm screen (cyclotec 1093 sample mill). NDF and ADF were analyzed according to Goering and Van Soest (1970), and crude protein (CP) using Kjeldahl N according to the Association of Official Analytical Chemists (AOAC, 1990).

Milk production and determinations

Milk production was recorded daily from parturition to the 10th wk of postpartum. Every week of the experimental period, milk samples were collected twice daily (a.m. and p.m.) and kept at -20 °C for milk composition analysis. During the 8th wk to the 10th wk postpartum, fresh milk samples (6 a.m. and 3 p.m.) was collected three consecutive days and kept at -20 °C for determination of milk allantoin concentration.

Milk lactose, fat and protein concentrations were analyzed using Milko scan 133B (N. FOSS ELECTRIC. DENMARK). Milk allantoin concentration was determined by method modified from Young and Conway (1942) using spectrophotometer (Model UV – 160A SHIMADZU). The absorbance was read at 522 nm.

Feed digestibility

Chromic oxide (Cr_2O_3) was used as an external marker to estimate digestibility of dry matter (DDM), NDF and ADF. Five grams of Cr_2O_3 in gelatin capsules was dosed twice daily at 0600 and 1600 h (10 g/d) during the 8th wk to the 10th wk postpartum for 10 days (Bargo et al., 2002). Dosing began 7 days prior to the start of fecal collection and

continued throughout the fecal sampling. The fecal grab sample from rectum was collected every 4 hours from d 7 to d 11 post dosing of the marker. Fecal collections were started at 0700, 1100, 1500, 1900, 2300. 0300 h. The fecal sampling was kept at -20 ^{o}C for chromium analysis by the method from Williams et al. (1962).

Frozen fecal samples were dried by a forced air oven at 60 ^{o}C for 72 h, and ground with a Wiley mill to pass through a 1-mm screen (cyclotec 1093 sample mill). Half grams (0.5 g) of composite sample per cow was ashed at 550 ^{o}C for 6 h, digested and diluted with distilled water. The supernatants were analyzed for the concentration of chromium using atomic absorption spectrophotometer (Model spectr AA - 300). The digestibility of NDF and ADF can be determined by application of the formula in equation 2 and DDM was calculated as according to equation 3 and 4.

Equation 2

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

Equation 3

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

Equation 4

$$\text{Dry matter digestibility} = \left[\frac{\text{DM intake} - \text{DM excreted}}{\text{DM intake}} \right] \times 100$$

Rumen passage rate

Immediately after the end of each digestibility, dairy was fed a Cr-free concentrate and rumen passage rate were carried out. Individual fecal grab samples were obtained from the rectum at 0, 4, 8, 12, 16, 20, 24, 30, 36, 44, 50, 62, 68, 74 and 96 h after the Cr-free concentrate was fed. Change in the concentration of Cr in the feces was used to estimate the passage rate. The fecal sample was kept at -20 ^{o}C for Cr analysis using method as described by Williams et al. (1962). After the analyses, results were plotted with marker concentration against sampling times. The points after the peak on the descending straight line were used for regression analysis, as a described by Grovum and Williams (1973).

Ruminal fluid collection and determination

At the end of experimental period, the oro-ruminal intubation was used for ruminal fluid collection after 2.5 hours feeding in the morning (Whitelaw et al., 1970). Ruminal content was sucked by the air pump. The ruminal content was strained immediately using two layers of cheesecloth and 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* hydrochloric acid and kept at -20 ^{o}C. Ruminal fluid was analyzed by the method modified from Erwin (1961).

Frozen ruminal fluid was thawed at room temperature and then 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).

The concentrations of VFA were determined by application of the following formula:

Equation 5

$$[\ VFA/C_x] \text{ (nM)} = [\text{std } C_x] \times \frac{\text{(A-sample) } C_x \times (\text{A-standard})_{\text{int std}}}{(\text{A-sample})_{\text{int std}} \times (\text{A-standard})_{cx}} \times 7/11$$

C_x = Volatile fatty acids at x carbon atom

Equation 6

Ratio of acetate to propionate = [acetate] / [propionate]

Equation 7

Total VFA] = [acetate] + [propionate] + [butyrate] + [valerate]

Animal behavior

During the 8th wk to the 10th wk of experimental period, the behavior of each animal in EVAP and NEVAP was observed 24 hours for two consecutive days by using video recorders. Tape records were considered eating, ruminating, total chewing time, ruminating/NDF and ruminating/DMI.

Statistical analysis

Data were reported as the mean value ± SD. The unpaired t-test was used to estimate the statistical significant difference of the values between groups. Significant differences were declared at $P < 0.05$.