CHAPTER III

MATERIALS AND METHODS

Animals and management

Fourteen, non-pregnant, late lactating crossbred Holstein cattle, approximately 270 days postpartum, were used in the experiment. They were divided into two groups of seven animals each. Animals were housed in tie stall type shades, having a solid floor and open sides. An ambient temperature in the shed was recorded daily and averaged weekly during the experiments. The maximum temperature at noon and the minimum at night were 34±1°C and 26±1°C, respectively. The relative humidity was 68 ± 12%. Animals were fed with rice straw as a source of roughage throughout the experiment. All cows were fed twice a day with concentrate and rice straw to maintain their milk production and body score condition at 2.5. Water and mineral block were available for ad libitum intake. The composition of minerals in 1 kg lick block consists of Na, 136 gm; Ca, 140 gm; P, 60 gm; Mg, 20 gm; K, 25 gm; S, 12 gm; Fe, 1000 mg; Zn, 800 mg; Mn, 350 mg; Cu, 300 mg; Co, 80 mg; I, 245 mg; and Se, 20 mg. Cows were milked twice a day and milk yields were recorded by weight at 0600 and 1600 h. Body weight of individual animal was measured weekly through the period of an experiment.

Experimental procedures

Animals were divided into two groups, the control group and the experiment group. Two consecutive periods were assigned to each group, consisting of 2 weeks of the pretreated period and 8 weeks of the experimental period. In the pretreated

period, animals in both groups were allowed to adjust to the type of diet. In the experimental period, animals in the control groups were given similarity diets throughout the period of experiment while animals in the treated group were supplemented with monensin. The treated animal was given monensin orally with a slow-released intraruminal monensin capsule (Rumensin®; Elanco Animal Health, Wiri, Auckland, New Zealand), containing 32 gm of sodium monensin. The slow-released form has 100 days duration of action and it releases approximately 320 mg of monensin per day. Each capsule number was recorded and the animal was monitored for 20 minutes after treatment to ensure that the animal had been successfully treated. Mammary blood flow measurements, milk yield and ruminal fluid collections were performed on week 1, 2, 3, 4, 6 and 8 after treatment. The protocol of the experiment for six consecutive measurements in each animal is shown as following.

Protocol of the experiment

⇑ Monensin administration

Δ Sampling periods

Measurements of mammary blood flow

On the day of the experiment, measurements of mammary blood flow were measured in duplicate. Mammary blood flow for half of the udder was determined by the dye T-1824 dilution technique according to Chaiyabutr et al. (1997). The animal

was catheterized with two catheters (i.d. 1.0 mm., o.d. 1.3 mm., length 45 mm.) into either the left or right milk vein by using an intravenous polyethylene catheters (Jelco, Critikon; Johnson & Johnson, Berk, U.K.) under local anesthesia. One tip of the catheter was positioned near the sigmoid flexure, anterior to the point at which the vein leaves the udder. The other was positioned downstream approximately 20 cm from the first one. Both catheters were flushed with sterile heparinized normal saline and were left in place during the experiment. The dye (T-1824) was dissolved in sterile normal saline and diluted to the concentration of 100 mg/l. The diluted dye was infused into the milk vein by the peristaltic pump (Gilson Medical Electronics, Villiers-Le-Bel, France) at a constant rate of 80 ml/min for 1-2 minutes. Venous blood was collected before infusion from the catheterized milk vein. Two samples of venous blood were taken during dye infusion, placed seperately in heparinized tubes. All samples were stored in crushed ice for further determinations of mammary plasma flow. Mammary blood flow of half of the udder was calculated from the mammary plasma flow and the haematocrit value (Thomson and Thomson, 1977). The whole mammary blood flow was calculated by doubling the half-udder mammary blood flow since the yields of the two half of the udder were similar.

Determinations of mammary arteriovenous difference, mammary extraction ratio and uptake of glucose

Plasma from blood collected from both cocygeal artery and milk vein were used to determine plasma glucose by colorimetric method using the enzymatic oxidation in the presence of glucose oxidase. The formed hydrogen peroxide reacts under the catalysis of the peroxidase with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as the indicator. Arteriovenous concentration difference and mammary extraction ratio of glucose were calculated from both plasma glucose in

the artery and venous blood (equation 2 and 3). Mammary uptake of glucose was calculated using mammary plasma flow and glucose arteriovenous difference of the gland (equation 4).

Milk collection

Milk sample was collected by hand milking and divided into two portions, the fresh milk sample and formalinized milk sample (1 ml of 40% formalin in 60 ml of fresh milk). All milk samples were kept at –20 °C for determinations of lactose, milk fat and protein concentration from the formalinized milk samples. The allantoin concentration was determined from fresh milk samples.

Determinations of milk composition

Formalinized milk samples were analysed for protein, lactose and fat concentrations as followed.

The milk protein concentration was analyzed using micro Kjeldhal method. The total nitrogen of milk sample was calculated from equation 5.

The milk lactose concentration was analyzed by the colorimetric method. Tele's method (Teles et al., 1978).

The milk fat concentration was measured using microcapillary method described by Chaiyabutr (1994). Milk samples were warmed to 38 °C and filled in the capillary tube. The capillary tubes were centrifuged at 12500 rpm for 15 minutes. The percentage of packed fat volume was read by the haematocrit reader (Model 150, Baker instrument). Milk fat concentration was calculated as described by Chaiyabutr (1994) (equation 6).

The concentration of milk allantoin was analyzed from the fat and protein-free milk samples by the method modified from Young and Conway (1942). The fat and protein were removed from the milk sample by centrifugation and precipitation with 10% TCA, respectively. Milk allantoin was converted to glyoxylic acid by acid hydrolysis. The phenylhydrazone of glyoxylic acid was formed and oxidized by ferrycyanide to develop the chromophore. After standing at room temperature for 20 minutes, the absorbance was read at 522 nm.

Collection of ruminal fluid

The ruminal content was collected by oro-ruminal intubation in the morning after 2.5 h of morning feeding, since the total VFAs reached a maximum concentration approximately 2.5 h after each meal (Whitelaw et al., 1970). After intubation, the ruminal content was collected by suction force using the air pump. The ruminal content was strained immediately using two layers of cheesecloth, the ruminal fluid was collected and preserved by adding 3 ml of 6 N hydrochloric acid to 60 ml of ruminal fluid. The preserved ruminal fluid samples were freezed at –20 °C until ruminal VFA concentrations were analyzed.

Determinations of ruminal VFA concentrations

Ruminal VFA concentrations were analyzed by the method modified by Erwin (1961). Frozen ruminal fluid was thawed and centrifuged at 3,000 rpm for 10 minutes. The supernatant was used for analysis. The internal standards used in this method were meta-veleric acid and standard volatile fatty acid with 25% metaphospholic acid. The volume of 0.4 ml of standard solution was mixed with 0.7 ml of the supernatant of the ruminal fluid to meet the volume of 1.1 ml. These samples were measured for

the VFA concentration by a gas chromatography equipped with a hydrogen flame ionization detector. The column treated with 1% (wt/wt) H_3PO_4 (20 m x 4 mm (i.d.), 3 mm (o.d.)) packed with 10% AT-1200 (80-100 mesh), was used for analyzing. The concentration of individual VFA, expressed as mole/100 mole of the total VFA concentration, the ratio of acetate to propionate and the total concentration of VFA were calculated (equation 7,8,9).

Calculation

Equation 1

$$\text{MPF (ml/min)} = \frac{V_{infused} \times ([\text{ dye }]_{before} - [\text{ dye }]_v) \times 2}{([\text{ dye }]_{before} - [\text{ dye }]_a)}$$

$$\text{MBF (ml/min)} = \frac{\text{MPF} \times 100}{(100 - \text{Hct})}$$

Equation 2

$$[\text{Glu}]_{a\text{-}v} \text{ (mM)} = [\text{Glu}]_a - [\text{Glu}]_v$$

Equation 3

$$\text{Glucose extraction ratio (\%)} = \frac{[\text{Glu}]_a - [\text{Glu}]_v \times 100}{[\text{Glu}]_a}$$

Equation 4

$$\text{Mammary glucose uptake (}\mu\text{mole/min)} = \text{MPF X } [\text{Glu}]_{a\text{-}v}$$

Equation 5

$$\text{Total N} = \frac{\text{ml of } H_2SO_4 \text{ in titration x N of standard } H_2SO_4 \text{ x } 0.004\text{x}100\text{x}6.38}{\text{Gram of sample}}$$

Equation 6

Milk fat concentration (g%) = (0.75 x %fat) – 0.321

Equation 7

$$[\text{ VFA/C}_x\text{ }] \text{ (mM)} = \frac{[\text{std C}_x] \text{ x (A-sample)C}_x \text{ x (A-standard)}_{\text{int std}}}{\text{(A-sample)}_{\text{int std}} \text{ x (A-standard)}_{cx}} \text{ x } \frac{11}{7}$$

[VFA] (mole/100 mole) = $[C_x]$ x 100 / [Total VFA]

Cx =Volatile fatty acids at x carbon atom

Equation 8

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

Equation 9

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

Statistical analysis

Data were reported as the mean values ± S.D. The paired t-test was used to estimate the statistical significant difference of the values within group. The unpaired t-test was used to estimate the statistical significant difference of the values between groups.