CHAPTER III

MATERIALS AND METHODS

Animals and management

Twelve, non-pregnant, late lactating 87.5% crossbred Holstein cows, approximately 30 weeks postpartum were used in the experiment. They were divided into two groups of six animals each. Animals were housed in tie stall type shed, having a solid floor and open sides.

Animals individually received amount of concentrate and roughage related to milk production. Water and mineral block were available for *ad libitum* intake. Cows were milked twice a day and weighing at 0400 and 1600 h recorded daily milk yields.

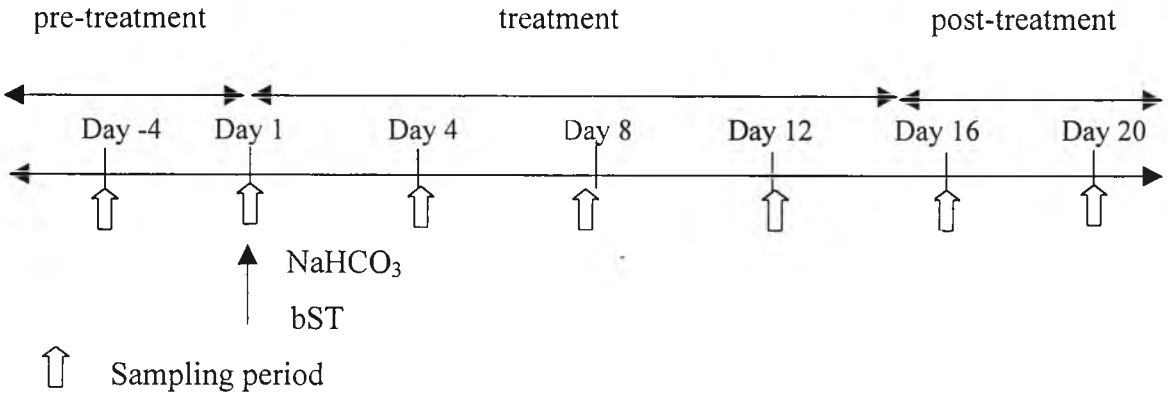
Experimental procedures

Animals were divided into two groups, the control group and the experimental group. Three consecutive periods were assigned to each group, consisting of 1 week of the pre-treated period, 2 weeks of the experimental period and 1 week of the post-treated period.

In the pre-treated period (day -4), animals in both groups were allowed to adjust to the type of diet. In the experimental period (day 1), animals in the control group were given sodium bicarbonate buffer (pH 9.4) while animals in the treated group were given recombinant bovine somatotropin (POSILAC®, Monsanto, USA). by subcutaneous injection at the tailhead depression (ischio-rectal fossa). The 14-day prolonged-release bST, contains 500 mg of sometribove zinc, which is equivalent to 36 mg of bST/cow/day. Measurements of mammary blood flow, milk yield and blood

collections were performed on day -4, 1, 4, 8, 12, 16 and 20 after bST treatment. The protocol of the experiment is shown as following.

Protocol of the experiment



Measurements of mammary blood flow

On the day of experiment, mammary blood flow for half of the udder was determined by Flow meter (Transonic systems Inc.).

Blood collection

Blood samples were collected from milk vein and coccygeal artery by venipuncture with a #21 needle into a heparinized tube. Blood samples were kept in crushed ice and then centrifuged at 3000 rpm for 30 min at 4 °C. Plasma samples from both coccygeal artery and milk vein were kept at -20 °C until measurements of the concentration of both plasma hormone and metabolites.

Plasma samples from both coccygeal artery and milk vein were used to determine the concentrations of glucose, triglyceride, β–hydroxybutyrate and acetate.

Determinations of the plasma glucose concentration, mammary arterio-venous difference, mammary extraction ratio and uptake of glucose.

The plasma glucose concentration was assayed by colorimetric method using 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 an indicator. The mammary extraction ratio of glucose was calculated by divided the arterio-venous concentration difference by arterial plasma glucose. Mammary uptake of glucose was calculated using mammary plasma flow multiplied by glucose arterio-venous difference (equation 1).

Determinations of the plasma triglyceride concentrations, mammary arterio-venous difference, mammary extraction ratio and uptake of triglyceride.

The plasma triglyceride concentration was assayed by an enzymatic method using enzymatic hydrolysis with lipases. The indicator is the quinoneimine formed from hydrogen peroxide, 4-aminoantipyrine and 4- chlorophenol under the catalytic influence of peroxidase. The arterio-venous concentration difference and mammary extraction ratio of triglyceride were calculated as described in equation 1.

Determinations of the plasma β–hydroxybutyrate concentration, mammary arterio-venous difference, mammary extraction ratio and uptake of β–hydroxybutyrate.

The plasma β–hydroxybutyrate concentration was assayed using an enzymatic reaction in the presence of β–hydroxybutyrate dehydrogenase (Sigma chemical). The arterio-venous concentration difference and mammary extraction ratio of β–hydroxybutyrate were calculated as described in equation 1.

Determinations of the plasma acetate concentration, mammary arterio-venous difference, mammary extraction ratio and uptake of acetate.

The plasma acetate concentration was assayed by an enzymatic method (Boehringer Mannhelm). The arterio-venous concentration difference and mammary extraction ratio of acetate were calculated as described in equation 1.

Milk collection

Milk was collected by hand milking and kept in formaldehydehyde. The formalinized milk sample (1 ml of 40% formalin in 60 ml of fresh milk) was kept at 4 °C for determinations of lactose, fat and protein concentrations.

Determination of milk composition

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

The milk protein concentration was analyzed using milkoscan.

The milk lactose concentration was analyzed by the colorimetric method, as described by Tele et al., 1978. The color development was based on the combined action of phenol, sodium hydroxide, picric acid and sodium bisulfite with lactose. The concentrations of samples were read the optical density at wavelength 520 by spectrophotometer.

The milk fat concentration was measured using microcapillary method. 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 hematocrit reader read the percentage of packed fat volume (Model 150, Baker instrument). Milk fat concentration was calculated as described by Chaiyabutr (1994) (equation 2).

Determination of the plasma IGF-I concentration.

Arterial plasma samples were used to determine the plasma IGF-I concentration using the OCTEIA® IGF-I kit. The OCTEIA® IGF-I kit is a two-site immunoenzymometric assay for the quantitative determination of the plasma IGF-I concentration. In brief, plasma samples were incubated with a reagent to inactivate binding proteins, and then diluted for assay. The pretreated, diluted sample was incubated, together with horseradish peroxidase labeled monoclonal anti-IGF-I, in antibody-coated wells for 2 hours at room temperature. The wells were washed and a single component chromogenic substrate (a formulation of tetramethyl-benzidine) was added to develop color. The absorbance of the stopped reaction mixture was read microtitre plate reader, color intensity developed being directly proportional to the amount of IGF-I present in the sample.

Calculations

Equation 1

$$\text{Arterio-venous difference (mM)} = A\text{-}V$$

$$\text{The extraction ratio (\%)} = \frac{A\text{-}V \times 100}{A}$$

$$\text{Mammary uptake (}\mu\text{mole/min)} = MPF \times A\text{-}V$$

A = arterial plasma metabolites concentration

V = venous plasma metabolites concentration

Equation 2

$$\text{Milk fat concentration} = (0.75 \times \%\ \text{fat}) - 0.321$$

Statistical analysis

All data were presented as the means $\pm$ SD. Statistical significant difference between period in the same group was determined by the student's paired t-test. The student's unpaired t-test was used to estimate the statistical significant difference between groups.