CHAPTER V

DISCUSSION

It has been reported that milk yield of 87.5% crossbred Holstein cattle decreased rapidly coincided with the reduction of exogenous growth hormone and mammary blood flow (Chaiyabutr et al., 2000). The present study was designed to clarify whether the regulation of body fluid in lactation occurring during treatments of somatotropin in crossbred cattle. In the bST treated animal, milk yield increased after administration throughout the experimental period. Milk yield of the control animals receiving placebo slightly increased in the early period of lactation. The bST treated animals receiving bST for 45 days significantly increased milk yield by 19.8% in the early period of lactation. It is recognized that an increase in milk production is closed correlated to dry matter intake and dry matter intake to water consumption (Murphy, 1992). The study by Holter and Urban (1992) reported that total water intake was 2.6 kg per kilogram of milk produced. In the present study, total DM intake were not significantly different between control animals and bST treated animals throughout experimental period. However, the effect of bST administration significantly influenced the milk production efficiency. The ratio of dry matter intake to milk production was lower in bST treated animals as compared to those of control animals at treatment period of lactation. It indicates that the energy output in milk and for maintenance was greater than energy consumed in the food for the bST treated animals. The control animals were approximately in energy equilibrium, there being no change in the ratio of total DM intake to milk yield during period of study.

During lactation, dairy cattle consume more of water to make up the largest portion of milk and for evaporative cooling for heat dissipation mechanism. The bST treated animals increased water intake in the early period of lactation from 65 kg/day to 71 kg/day, which was about 9% accounted for 19.8% of an increase milk yield from the pretreatment period. This result shows that milk production affects water intake including body water turnover rate. The bST treated animals increased body fluid compartments throughout period of study i.e. TBW, EBW, blood volume and plasma volume, while the control animals decreased TBW with that of a higher milk secretion in the early period of lactation. EBW did not include water associated with gastrointestinal contents. The EBW increased in bST treated animals due to increased in ECF compartment, while ICF compartment did not change among period of lactation. Thiocyanate space does not include rumen water, therefore changes of ruminal fluid volume should not affect estimate of extracellular volume (Woodford et al., 1984). Gut water increase in animals treated bST as compared to those of control animals because the bST treated animals increased water intake in the early period of lactation. Martin and Ehle (1986) estimated gut and empty body water were 115 and 367 liters in lactating and 153 and 372 liters in nonlactating cows, respectively. The mechanisms responsible for the regulation of body fluid are not yet fully known in ruminant, although the expansion of ECF and TBW after bST administration was noted in growth hormone deficiency human (Janssen et al., 1997). The study by Janssen and coworker (1997) shown that GH deficiency (GHD) in adults was associated with a reduction total body water and extracellular water. Four weeks of GH treatment significantly increased body weight, TBW, ECF and ECF/TBW. Several other studies directly measured ECF, but not TBW, with variable reports of low ECF, a proportional decrease in ECF corresponding to a decrease in fat-free soft tissue mass (FFSTM). GH therapy appears to increased fat-free matter (FFM) and decreased fat mass (Janssen et al., 1997). An increase in both absolute TBW and ECF of bST treated animals throughout experimental period might be resulted from an increase in body weight. During treatment period, animals gained weight in both control and bST treated animals. However, a higher proportion increase in body weight of bST treated animals than those of control animals would consider to be the direct effect of somatotropin on the increased body cell mass and FFM. This may be attributed to an accumulation of body water. The sodium-retaining effect of somatotropin on the renal tubular reabsorption of sodium would be another explanation of an induction in expansion of both TBW and ECF. Further evidence has shown that GH (or IGF-1) may act directly on renal function relating to receptors of both GH and IGF-1 on the renal proximal tubular cell (Janssen et al., 1997). However, plasma osmolality remained constant during the course of lactation in both groups indicating that homeostasis was being maintained throughout the period of experiment.

A high TBW of bST treated animals may relate to the adaptation of animals to the tropical environment. Thus, a higher water reserve in animals given bST would not only provide a higher reservoir of soluble metabolites for biosynthesis of milk but was also useful in slowing down the elevation in body temperature during lactation in hot conditions (Chaiyabutr et al., 1997). The decrease in TBW of the 87.5% HF animals during the early stages of lactating period occurred rather rapidly which may be attributed to a relatively lower efficiency in the water retention mechanism although the estimated water intake was slightly higher (Chaiyabutr et al., 1997). In the present study, animals in both groups were not pregnant and were housed in the same shed under the same environment. Thus, changes in the water turnover rate of both groups were not influenced by the effect of either pregnancy or changes of environmental condition (Chaiyabutr et al., 1997). The bST treated animals showed no significant changes of the water turnover rate per body fat free wet weight ($kg^{0.82}$) and the biological half-life of tritium in all periods of experiment in comparison to those of control animals. This indicates that increased losses of water with increase in milk yield in bST treated animals might be compensated by a larger body water pool and animals could restore their body fluids to equilibrium in lactating period with no significant change of body water turnover rate and water half-life. These changes would be due to the fact that the process of lactation requires more water and more loss of water secretion in milk which is generally known about 87% would account for these phenomena.

During lactation, blood flow to the mammary gland is the major parameter controlling milk production. These changes effectively alter body fluid and thus circulatory distribution including the blood supply to the mammary gland. In the present study, a marked increase in mammary blood flow of bST treated animals in the treatment period could not be attributed to a change in blood volume and plasma volume, which remained nearly constant as a percent of body weight. Plasma volume has been reported to be proportional to body weight in beef cattle (Woodford et al., 1984). In lactating dairy cows, increase blood flow to the mammary gland may allow plasma volume to remain nearly constant despite loss of body weight (Woodford et al., 1984). In the present study, an increase in mammary blood flow was apparent in the bST treated animals during the treatment period. The ratio of mammary blood flow to the rate of milk yield did not significantly change through the experimental period in both groups. This result indicates that the marked increase in the mammary blood flow of bST treated animals at the early period of lactation correlated with an increase in milk yield.

Somatotropin is a homeorhetic control that results in a coordinated series of changes involving both nutrient supply and mammary utilization (Bauman and Currie, 1980). This coordination would include a diversion of cardiac output and increase in blood flow to the mammary gland which parallels the magnitude of the milk yield in response to exogenous bST (Davis et al., 1988). The present results confirmed the study in both cows and goats that the plasma IGF-1 level increased in response to growth hormone treatment. (Davis et al., 1988). The effect of bST to mammary circulation is indirect, mediated via insulin like growth factor-1 (Capuco et al., 2001). An arterial infusion of IGF-1 in to the mammary gland has also been shown to stimulate blood flow to the gland and increase in milk production (Etherton and Bauman, 1998). The first evidence implicated IGF-1 in bovine galactopoiesis including observations of chronically IGF-1 concentrations in blood and lactating mammary tissue during periods of bST administration (Glimm et al., 1988; Weber et al., 2000). An elevation of both plasma IGF-1 concentration and udder blood flow was also noted in late lactating crossbred cows treated with bST (Tanwattana et al., 2003).

Treatment with bST cause a dramatic increase in mammary uptake and utilization of nutrients for the synthesis of milk. It has been known that glucose is the major blood precursor of lactose. At least 85% of the carbon atom in milk lactose in derived from glucose. A decreases glucose uptake in skeletal muscle and adipose tissue occurred during lactation, whereas it increased glucose uptake in the mammary gland (Davis et al., 1988). In the present study, bST treated animals did not change the concentration of arterial plasma glucose but mammary glucose uptake increased in the treatment period. The study by Miller and coworker (1991) showed that glucose plasma arterial concentration increased 7% from early to mid lactation, whereas arterial-venous difference remained the same, indicating a 10% reduction in extraction percentage. It has been estimated for glucose extraction range from 16 to 29% (McDowell et al., 1988). The linear relationship between glucose uptake by the mammary gland and milk volume reflects increased lactose synthesis along with the dilution to maintain osmolality of milk. This indicates that glucose utilization by the mammary gland during lactation would be dependent on both the mammary blood flow and the activity of the mammary epithelial cell. The reduction in glucose oxidation during somatotropin treatment would provide approximately 30% of the additional glucose required for lactose synthesis. Animals may have been mobilizing some glycogen reserves, but normally such reserves would be very low in dairy cows at this stage of lactation. It is possible that the rates of gluconeogenesis from propionate were affected (Peel and Bauman, 1987).

In ruminant, the carbon sources used for fatty acid synthesis are acetate and β-hyroxybutyrate. Acetate seem to be an important carbon source for medium chain length of fatty acid. In the present study, the arterial plasma acetate concentration slightly increased during bST treatment. While, mammary A-V difference of acetate was not significantly changed by bST treatment, which was similar to the results of Miller and co worker (1991). The mammary acetate uptake in the present study was increased, which was similar to the results of Fullerton and coworker (1989). The study by Fullerton and coworker (1989) showed that the rates of acetate uptake were increased after 7 d of bST treatment and remained elevated for 10 d posttreatment. Acetate is involved in two critical aspects of mammary gland metabolism : 1) de novo synthesis of short- and medium-chain milk fatty acids and 2) generation of ATP (tricarboxylic acid cycle) and $NADPH_2$ (cytosolic isocitrate dehydrogenase) (Miller et al., 1991).

The other volatile fatty acid in the form of β-hyroxybutyrate arise mainly from butyrate in rumen. The present study, mammary A-V difference and extraction of β-hyroxybutyrate were not different throughout the experimental period. The mammary uptake of β-hyroxybutyrate of bST treated animals was increased during the treatment period, which was similar to the results of Miller and coworker (1991). The reduction in plasma β-hyroxybutyrate concentration observed as cows progressed from early to midlactation. The rise in the β-hyroxybutyrate plasma arterial concentration in early lactation would be likely the result of an increase in ketogenesis from NEFA. The correlation between the NEFA and β-hyroxybutyrate plasma arterial concentrations have been noted (Miller et al., 1991). An increase in the efficiency of mammary gland for extraction and net uptake of β-hyroxybutyrate during lactation indicate that β-hyroxybutyrate is used as a precursor for biosynthesis of normal milk fat during this time.

An extraction of triglyceride by the mammary gland has been reported to vary from 30 to 44% (McDowell et al., 1987). In the present study, the concentration of arterial plasma triglyceride, mammary A-V difference, extraction and mammary uptake were not changed in bST treated animals. Sechen and co worker (1989) demonstrated that during lactation, measurement of arteriovenous differences of FFA across the mammary gland together with mammary blood flow did not provide a quantitative estimation of their total uptake by mammary tissue, since there was release of FFA into venous blood due to triglyceride hydrolysis during the uptake of plasma triglyceride.

Milk fat content of bST treated animals was increased, while milk protein and milk lactose were not changed by bST treatment. Milk fat was synthesized in the mammary epithelial cells. The fatty acids used to synthesize the milk fat arise from both blood lipids and from de novo synthesis within the mammary epithelial cells. An increased fat content in milk due to bST injection has been observed previously (West et al., 1990). Milk fat content of cows in positive energy balance is not influenced by bST treatment, and milk fat yield follows the trend of milk production (West et al., 1990). However, an increase in milk fat after bST injection would relate to an increase in the mobilization of long-chain fatty acids from body reserves when cows are in negative energy balance (McDowell et al., 1987).

Peel and Bauman (1987) reported that administration of bST did not change milk protein percentage when cows were in positive nitrogen balance, but the milk protein percentage of cows in negative nitrogen balance tended to decline.

In conclusion, bST exerts it effect on an increase in both TBW and EBW. An increase in ECF compartment would be due to the increase in water intake during early lactation which correlated with an increase in water secretion in milk. Increased

ECF in bST treatment might be resulted from the decrease in fat mass during early lactation. The stimulatory effect of bST on milk producting in 87.5%HF animals involves changes in both mammary blood flow (extra-mammary factors) and mammary nutrients uptake (intra-mammary factors). The present results indicate that growth hormone affect mammary gland function is not mediated solely by the action of IGF-1. The action of IGF-1 on an increase in blood flow to mammary gland in bST treated animals may be due to an elevation of body fluid in distribution of milk precursors to the gland.