รายงานวิจัยฉบับสมบูรณ์

โครงการ

"กลไกการควบคุมการหลั่งน้ำนมระหว่างระยะการให้นมใน โคนมลูกผสม Holstein : บทบาทของ growth hormone

Control mechanisms for milk secretion during the lactating periods in crossbred Holstein cattle : role of growth hormone"

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ธันวาคม 2548

สัญญาเลขที่ BRG2/02/2545

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FINAL REPORT

for the Project

CONTROL MECHANISMS FOR MILK SECRETION DURING THE LACTATING PERIODS IN CROSSBRED HOLSTEIN CATTLE : ROLE OF GROWTH HORMONE

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CONTROL MECHANISMS FOR MILK SECRETION DURING THE LACTATING PERIODS IN CROSSBRED HOLSTEIN CATTLE : ROLE OF GROWTH HORMONE

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Executive Summary

Project Title: Control mechanisms for milk secretion during the lactating periods in crossbred holstein cattle : role of growth hormone

Project Code: BRG/02/2545

Project Objective: The overall objectives of the present study as included in the terms of reference were to:

- study the effects of recombinant bovine somatotropin (rbST) administration on water and mammary circulation in different periods of lactation in crossbred Holstein cattle
- study the effects of recombinant bovine somatotropin (rbST) administration on the plasma level of insulin like growth factor-1, insulin and plasma metabolites in different periods of lactation in crossbred Holstein cattle
- study the effects of recombinant bovine somatotropin (rbST) administration on the utilization of glucose in the mammary gland in different periods of lactation in crossbred Holstein cattle
- study the effects of recombinant bovine somatotropin (rbST) administration on cellular metabolites in milk secretion at different periods of lactation in crossbred Holstein cattle
- study the effects of recombinant bovine somatotropin (rbST) administration on plasminogen and plasmin system in the mammary gland in different periods of lactation in crossbred Holstein cattle

Materials and methods:

Ten, first lactating, non-pregnant, crossbred, 87.5% Holstein dairy cattle were selected for the experiment. They were divided into two groups of five animals each. Animals in each group were fed with rice straw treated with 5% urea as the source of roughage. All animals were housed in sheds and tethered in individual stalls and fed

twice daily. The maximum temperature in the shed at noon was 34±1°C and the minimum temperature at night was 26±1°C. The relative humidity was 68±12%. Animals received an average of 4 kg/day of roughage in combination with the same concentrated mixture (7 kg/day) to maintain a moderate body condition score (2.5, scale = 1 to 5). Each day, the food was given in equal portions at about 06.00 h and 17.00 h when the animals were milked. Animals had free access to water and were fed their respective rations throughout the experimental period.

Four consecutive periods of study were used for each group. These consisted of a pretreatment period (45 days postpartum before lactation peak) and three treatment periods of 105 days postpartum (early lactation), 165 days postpartum (mid- lactation) and 225 days postpartum (late lactation). During the treatment periods, animals in the experimental group, which had completed 60 days of lactation, were injected subcutaneously every 14 days until the end of study with 500 mg of recombinant bovine somatotropin (rbST). It was suspended in 792 mg of a prolonged-release formulation of sesame oil (POSILAC, Monsanto, USA). Animals in the control group were injected subcutaneously every 14 days with 800 mg of sterile sesame oil without rbST as placebo.

Effects of long-term administration of recombinant bovine somatotropin on mammary functions were carried out in crossbred Holstein cattle, where appropriate the experiments were divided into different series of studies to cover both intra- and extra-mammary functions.

Overview of results and conclusion:

The initial experiment was designed to evaluate the effect of exogenous bovine somatotropin on water metabolism in relation to mammary function in early lactation of crossbred Holstein cattle. In rbST-treated animals, milk yield increased 19.8% which coincided with significant increase in water intake. Water turnover rate significantly increased, while the biological half-life of water did not change in rbST-treated animals. Total body water (TBW) and total body water space (TOH)

significantly increased in rbST-treated animals, while it was decreased in the control animals. Absolute values of empty body water (EBW) markedly increased, which associated with an increase in the extracellular fluid (ECF) volume. An absolute values of plasma volume and blood volume were also significantly increased in rbST-treated animals. The increase in mammary blood flow in rbST-treated animals was proportionally higher than an increase in milk production. The plasma IGF-1 concentration was significantly increased in rbST-treated animals when compared with those of control animals during the treatment period. Milk fat concentration increased during rbST treatment in early lactation, while the concentrations of both protein and lactose in milk were not affected. The present results indicate that rbST exerts it effect on an increase in both TBW and EBW. An increased ECF in rbST-treated animals might be partly resulted from the decrease in fat mass during early lactation. The action of rbST on mammary blood flow might not be mediated solely by the action of IGF-1 for increase in blood flow to mammary gland. An elevation of body fluid during rbST treatment in early lactation may be partly caused an increase in mammary blood flow in distribution of milk precursors to the gland.

Further studies were designed to clarify whether the short lactation persistency occurring in the crossbred cattle in the tropics would be affected by a reduction in circulating growth hormone in association with changes of body fluid and mammary blood flow during stages of lactation. Four consecutive study periods were carried out in each group. The milk yield per day of rbST-treated animals increased in early lactation (19.8 %), mid-lactation (9.5%) and decreased in late lactation (-2.7%) when compared with the pretreatment period. Absolute values of total body water (TBW), extracellular water (ECW), plasma volume and blood volume were significantly increased during rbST treatment. The estimated value of intracellular water (ICW) of

the rbST-treated animals showed no differences, whereas it significantly decreased in the control animals during early and mid-lactation. The water turnover rate (WTO) of rbST-treated animals significantly increased in early and mid-lactation. Mammary blood flow (MBF) significantly increased during rbST administration in all stages of lactation. From these results it can be concluded that the rbST exerts its galactopoietic action, in part, through increases in both the TBW and ECW in association with an increase in MBF for milk production.

Effects of the long-term administration of recombinant bovine somatotropin (rbST) on circulating levels of IGF-I, insulin, mammary blood flow and other variables relevant to milk synthesis, were carried out. During the pre-treatment period, there were no significant differences in plasma concentrations of IGF-I, insulin and other parameters between the control animals and rbST-treated animals. The concentration of plasma IGF-I was significantly increased after rbST administration, which was higher than the control animals throughout lactation. Arterial plasma glucose, protein and triglyceride concentrations in each group remained unchanged throughout the study. The total daily dry matter intakes were not significantly different between the groups. Milk yield increased by 20% with rbST treatment and it was 22% greater than that of the control animals receiving placebo in early lactation. Milk yield of rbST treated animals rose to a peak in early lactation and then gradually declined. In late lactation, milk yield of rbST-treated animals was decreased to 19 % as compared with early lactation. Udder plasma flow and udder blood flow markedly increased with rbST treatment and there were no significant changes in the control animals. The ratio of udder blood flow to the rate of milk production increased to mid and late lactation in controls and the rbST treated animals. These findings suggest that a short persistency of lactation in rbST-treated animals was similar to the control animals receiving placebo. Changes in milk production during the progress of lactation in rbST-treated animals might not be controlled systemically but also locally within the mammary gland. The lack of effect of higher plasma IGF-I levels on persistency of lactation in rbST-treated animals, may

be due to changes in the pattern of IGF-I binding proteins and paracrine production inhibiting IGF-I action.

It is known that as lactation progresses gradual involution of the secretory tissue results in tight junction becoming leaky. A number of studies indicate that bST can delay involution of the mammary gland by reducing the activity of the plasmin-plasminogen system, an important initiator of tissue remodeling during lactation advance in dairy ruminant The present study was designed to clarify whether long-term administration of recombinant bovine somatotropin (rbST) suppresses milk plasmin-plasminogen activity within the mammary gland and allow a persistence of milk production during different stages of lactation in crossbred Holstein cattle. Animals receiving rbST gave greater milk yields than control animals in all stages of lactation The milk yield of rbST-treated animals significantly increased in early lactation, when compared with the pretreatment period. The peak milk yield in both groups declined from the early period of lactation as lactation advanced to mid and late lactation. Udder blood flow significantly increased during rbST administration, while there were no significant changes throughout lactation in the control animals. The concentration of milk lactose of both controls and rbST treated animals showed no significant changes throughout lactation, while the concentrations of milk protein and milk fat of rbST-treated animals increased during advanced lactation. The milk fat concentration of rbST-treated animals had a significantly greater than that of control animals in the early lactation. No significant changes for the concentration of milk Na and K including Na/K ratio in comparison with control animals at different stages of lactation. The concentration of milk Cl significantly increased during advanced lactation in the control animals, while the concentration of milk Cl of rbST-treated animals significantly decreased ($P<0.05$) in the early lactation. The plasminogen and plasmin activities increased during lactation advances in both groups. The concentration of plasmin in milk gradual increased, while milk plasminogen concentration significantly increased as lactation advances in both the controls and rbST-treated animals. The plasminogen : plasmin ratio decreased in the control animals while it increased in rbST-treated animals as lactation advances. These findings demonstrate that administration of rbST cause animals to maintain milk plasmin at low concentration throughout lactation. The decrease in milk secretion during the progress of lactation might not be controlled by changes in extra-

mammary factors but, in part, through changes within the mammary gland relating to the activity of the plasmin-plasminogen system.

The rate of milk production has been known to depend on function of number of secretory cells and their metabolic activity. During lactogenesis, changes in the concentrations of metabolites in the mammary secretion are apparent. The objective Further studies were designed to determine the effects of long-term treatment with recombinant bovine somatotropin (rbST) on the concentrations of cellular metabolites in the milk which could be interpreted in relation to the biochemical changes occurring within the mammary gland. During rbST administration, milk yield rose (+22%) to a peak in early lactation. Lactose and milk triacylglycerol secretion of rbST-treated animals significantly increased, which coincided with significant increase in the concentrations of milk glucose in early and mid-lactation as compared with pretreatment period. The concentrations of milk galactose markedly increased whereas the concentrations of milk UDP-glucose significantly decreased as lactation advances in both groups. The concentrations of milk citrate decreased while the concentrations of 2-oxoglutarate increased as lactation advances in both groups. The concentration of milk isocitrate significantly decreased at late lactation in the control animals. The concentration of milk G6P, milk G1P and cAMP markedly decreased as lactation advances in both groups. These findings indicate that the concentration of glucose in milk reflecting intracellular glucose concentrations, can be one of the factors regulate the rate of lactose production. The galactopoietic effect elicited by administration of rbST during early lactation depends on increased the conversion of glucose to intermediary metabolites in the lactose biosynthetic pathway.

Further studies were conducted to improve understanding the role of somatotropin on the mammary uptake of nutrients by using techniques for measuring mammary blood flow and combining with measurements of nutrient arterial concentrations and arteriovenous concentration differences for the mammary uptake during long-term administration of bST throughout lactation. Milk yield in animals given rbST was higher than that of the control animals given placebo and the persistency of production was higher in these animals throughout their lactation. The peak milk yield in both groups of animals declined from the early period of lactation

as lactation advanced to mid and late lactation. During early lactation, the milk yield of rbST treated animals was higher than those of the control animals. The rate of udder blood flow and plasma flow markedly increased during rbST administration. The mean arterial plasma concentrations for glucose, acetate, β-hydroxybutyrate and free glycerol were largely unchanged throughout periods of study in both controls and rbST-treated animals. The arteriovenous differences and extraction ratio of glucose across the mammary gland decreased as compared with pretreatment period in both groups. The net mammary uptake of glucose in early lactation of rbST-treated animals increased approximately 20%, whereas it decreased in mid- and late lactation as compared with the pretreatment period. The arteriovenous concentration differences, extraction ratio and mammary uptake of acetate were increased as lactation advances as compared with the pretreatment period in rbST-treated animals. The arteriovenous concentration differences, extraction ratio and mammary uptake for acetate of rbST-treated animals were significantly higher than those of the controls during early and mid-lactation. The arteriovenous differences and extraction ratio of β-hydroxybutyrate were not responsive to in either the controls or rbST-treatment. The mammary uptake for β-hydroxybutyrate of rbST-treated animals increased as lactation advances in comparison with pretreatment period while it remained constant through the course of lactation .in the control animals. The arteriovenous differences and extraction ratio of free glycerol across the mammary gland in both groups showed valiable which were affected to the net mammary uptake. The mean arterial plasma concentrations for free fatty acid (C_{16} to C_{18}) were elevated after rbST administration as compared with the pretreatment period and those of control animals. The values of arteriovenous differences and the net uptake by the mammary gland for FFA were variable during lactating periods in both groups. The arteriovenous differences, extraction ratio of triacylglycerol were unchanged as compared with pretreatment period in rbST-treated animals, but the net uptake of triacylglycerol across the mammary gland increased in rbST-treated animals in comparison with pretreatment period. There were no significant

differences of arteriovenous differences, extraction ratio and net uptake of triacylglycerol during lactation advance in control animals. These results indicate that the increased partition of nutrients to the mammary gland induced by rbST treatment would be facilitated by increased mammary blood flow.

The experiment was done to determine the effects of long-term administration with recombinant bovine somatotropin to the utilization of glucose in the mammary gland of crossbred Holstein cattle. The utilization of glucose in the mammary gland was determined by measuring rates of glucose uptake and the incorporation of glucose into milk components in both control animals and rbST-treated animals. In pretreatment period, there were no significant differences of the total glucose entry rate and glucose carbon recycling between the controls and rbST-treated animals. In early lactation, the glucose turnover rate of rbST-treated animals was decreased as compared with the pretreatment period, whereas there was no change in the control animals. Comparing for the mid-lactating period, rbST-treated animals showed an elevation of plasma glucose clearance and significant increases in the glucose turnover rate in comparison with pretreatment period. It was decreased during rbST administration in the early period of lactation. The percentages and values of non-mammary glucose utilization showed significantly increases during lactation advances to mid and late lactation as compared with pretreated period in rbST-treated animals

Animals treated with rbST showed significantly higher levels of mammary plasma flow and milk yield in early lactation than those of control animals. Milk lactose concentration showed no differences between groups of animals or among periods of lactation in the same group., The milk lactose secretion of rbST treated-animals significantly increased whereas milk citrate concentration were significantly decreased in early lactation as compared with pretreatment period. The milk citrate concentration decreased in both groups during lactation advances. Milk triacylglycerol concentration and triacylglycerol secretion of rbST-treated animals were markedly higher in early lactation than that of pretreatment period and it was still in a high level throughout lactation. A high milk lactose secretion and citrate secretion during early lactation were apparent in rbST-treated animals when

compared with those of control animals. The percentage of utilization of glucose carbon for synthesis of milk lactose was not significantly different between controls and rbST-treated animals. The utilization of glucose carbon for synthesis of milk citrate for rbST-treated animals was significantly higher than that of control animals during mid and late lactation. The utilization of glucose for synthesis of milk triacylglycerol was significantly higher during rbST administration throughout periods of lactation. As lactation advances, the intracellular glucose phosphorylated by the mammary gland were calculated to be completely metabolized via the pentose phosphate. Metabolism of glucose 6-phosphate via the Embden-Meyerhof pathway was calculated in term of the proportion of glucose metabolized, which there was considerable variation with advanced lactation of both groups. During early lactation, the NADPH formation from glucose that required for fatty acid synthesis *de novo* in the mammary gland of rbST treated-animals, which was significantly higher than that of the control animals. .The milk fatty acid concentrations with a chain length of C_{16} to C_{18} sinificantly increased during rbST administration in differnt stages of lactation as compared with those of control animals. From this result, it can be conclude that changes in the glucose utilization for biosynthetic pathways in the mammary gland of rbST-treated animals would depend on the sufficient pool of intracellular glucose concentration during rbST administration, which has effect on an increase in glucose 6-phosphate flux through the lactose synthesis and pentose cycle pathway.

Finally, the results of all these experiments are discussed in general term. The metabolic pathway involved the metabolism of precursors of milk lactose and milk fat in the mammary gland during rbST administration, is presented.

Abstract

The present study was designed to clarify whether a shorter lactation persistency of crossbred cattle containing 87.5%Holstein genes during lactation advance was due to the reduction of the growth hormone level or associated with some other mechanisms. Ten, first lactating, non-pregnant, crossbred, Holstein dairy cattle were divided into two groups of five animals each; an experimental group and a control group. Animals in each group were fed with rice straw, treated with 5% urea, as the source of roughage. Four consecutive study periods were carried out in each group. These consisted of a pretreatment period (45 days postpartum before lactation peak) and three treatment periods during early lactation (105 days postpartum), mid-lactation (165 days postpartum) and late lactation (225 days postpartum). During the treatment periods, animals that had completed 60 days of lactation were injected subcutaneously every 14 days with 500 mg of recombinant bovine somatotropin (rbST) (POSILAC, Monsanto, USA) in the experimental group, while animals in the control group were injected subcutaneously every 14 days, with 800 mg of sterile sesame oil, without rbST, as a placebo.

The present results show that the total daily dry matter intakes were not significantly different between the control animals and rbST-treated animals throughout the study. The milk yield per day of rbST-treated animals increased in early lactation (20 %), mid-lactation (9.5%) and decreased in late lactation (-2.7%) when compared with the pretreatment period. Milk yield increased in rbST treatment which was 22% greater than that of the control animals receiving placebo in early lactation. Milk yield of rbST treated animals rose to a peak in early lactation and then gradually declined. In late lactation, milk yield of rbST treated animals was decreased to 19 % as compared with early lactation. The study on the regulation body fluids showed significant increases in absolute values of total body water (TBW), extracellular water (ECW), plasma volume and blood volume during rbST treatment. The estimated value of intracellular water (ICW) of the rbST-treated animals showed no differences, whereas it significantly decreased in the control animals during early and mid-lactation. The water turnover rate (WTO) of rbST-treated animals significantly increased in early and mid-lactation. Udder plasma flow and udder blood flow markedly increased with rbST treatment in all stages of lactation and there were no significant changes in the control animals. During the treatment periods, the

increase in the concentration of plasma IGF-I in rbST treated animals was significantly higher than those of the control animals throughout the lactating period. Plasma glucose, protein and triglyceride concentrations in each group remained stable throughout the study.

The concentration of milk components for lactose of both controls and rbST treated animals showed no significant changes throughout lactation, while the concentrations of milk protein and milk fat of rbST-treated animals increased during advanced lactation. The milk fat concentration of rbST-treated animals had a significantly greater than that of control animals in the early lactation. No significant changes for the concentration of milk Na and K including Na/K ratio in comparison with control animals at different stages of lactation. The concentration of milk Cl significantly increased during advanced lactation in the control animals, while the concentration of milk Cl of rbST-treated animals significantly decreased in the early lactation. The plasminogen and plasmin activities increased during lactation advances in both groups. The plasminogen: plasmin ratio decreased in the control animals while it increased in rbST-treated animals as lactation advances.

The study on the mode of uptake of plasma nutrients by the mammary gland revealed that mean arterial plasma concentrations for glucose, acetate, β-hydroxybutyrate and free glycerol were largely unchanged throughout periods of study in both controls and rbST-treated animals. The arteriovenous differences and extraction ratio of glucose across the mammary gland decreased as compared with pretreatment period in both groups. The net mammary uptake of glucose in early lactation of rbST-treated animals increased approximately 20%, whereas it decreased in mid- and late lactation as compared with the pretreatment period. The arteriovenous concentration differences, extraction ratio and mammary uptake of acetate were increased as lactation advances as compared with the pretreatment period in rbST-treated animals, which were significantly higher than those of the control animals during early and mid-lactation. The arteriovenous differences and extraction ratio of β-hydroxybutyrate were not responsive to rbST-treatment. The mammary uptake for β-hydroxybutyrate of rbST-treated animals increased as lactation advances in comparison with pretreatment period while it remained constant through the course of lactation in the control animals. The arteriovenous differences and extraction ratio of free glycerol across the mammary gland in both groups showed valiable. The mean

arterial plasma concentrations for free fatty acid (C_{16} to C_{18}) were elevated after rbST administration as compared with the pretreatment period and those of control animals. The values of arteriovenous differences and the net uptake by the mammary gland for FFA were variable during lactating periods in both groups. The arteriovenous differences, extraction ratio of triacylglycerol were unchanged as compared with pretreatment period in rbST-treated animals, but the net uptake of triacylglycerol across the mammary gland increased in rbST-treated animals in comparison with pretreatment period. There were no significant differences of arteriovenous differences, extraction ratio and net uptake of triacylglycerol during lactation advance in control animals.

The utilization of glucose in the mammary gland was determined by measuring rates of glucose uptake and the incorporation of glucose into milk components in both control animals and rbST-treated animals. Lactose and milk triacylglycerol secretion of rbST-treated animals significantly increased which coincided with significant increase in the concentrations of milk glucose in early and mid-lactation as compared with pretreatment period. Milk triacylglycerol concentration and triacylglycerol secretion of rbST-treated animals were markedly higher in early lactation than that of pretreatment period and it was still in a high level throughout lactation. A high milk lactose secretion and citrate secretion during early lactation were apparent in rbST treated-animals when compared with those of control animals. The concentrations of milk galactose markedly increased whereas the concentrations of milk UDP-glucose significantly decreased as lactation advances in both groups. The concentrations of milk citrate decreased while the concentrations of 2-oxoglutarate increased as lactation advances in both groups. The concentration of milk isocitrate significantly decreased at the late lactation in the control animals. The concentration of milk G6P, milk G1P and cAMP markedly decreased as lactation advances in both groups.

The study on glucose metabolism relating to the utilization of glucose in the mammary gland revealed that there were no significant differences of the total glucose entry rate and glucose carbon recycling between the controls and rbST-treated animals in the pretreatment period. In early lactation, the glucose turnover rate of rbST-treated animals was decreased as compared with the pretreatment period, whereas there was no change in the control animals. Comparing for the mid-lactating period, rbST-treated animals showed an elevation of plasma glucose clearance and significant increases in the glucose turnover rate in comparison with pretreatment

period. The percentages and values of non-mammary glucose utilization showed significantly increases during lactation advances to mid and late lactation as compared with pretreatment period in rbST-treated animals. The utilization of glucose carbon for synthesis of milk citrate for rbST-treated animals was significantly higher than that of control animals during mid and late lactation.The utilization of glucose carbon for synthesis of milk triacylglycerol was significantly higher during rbST administration throughout periods of lactation. During early lactation, the NADPH formation from glucose that required for fatty acid synthesis *de novo* in the mammary gland of rbST treated-animals, which was significantly higher than that of the control animals. The milk fatty acid concentrations with a chain length of C_{16} to C_{18} significantly increased during rbST administration in different stages of lactation as compared with those of control animals.

From these results, it can be conclude that the mechanism by which rbST directly or indirectly affects mammary gland function that likely affect changes in the glucose utilization for biosynthetic pathways during early lactation. It affects the increase in the sufficient pool of intracellular glucose concentration, which has effect on an increase in glucose 6-phosphate flux through the lactose synthesis and pentose cycle pathway. The action of rbST on mammary blood flow might not be mediated solely by the action of IGF-1 for increase in blood flow to mammary gland. The rbST exerts its galactopoietic action, in part, through increases in both the TBW and ECW including blood volume. An elevation of body fluid during rbST treatment in early lactation may be partly caused an increase in mammary blood flow in distribution of milk precursors to the gland. The lack of effect of higher plasma IGF-I levels on persistency of lactation in rbST treated animals, may be due to changes in the pattern of IGF-I binding proteins and paracrine production inhibiting IGF-I action. The decrease in milk secretion during the progress of lactation might not be controlled by changes in extra-mammary factors but, in part, through changes within the mammary gland relating to the activity of the plasmin-plasminogen system. The increased partition of nutrients to the mammary gland induced by rbST treatment would be facilitated by increased mammary blood flow. The concentration of glucose in milk reflecting intracellular glucose concentrations, can be one of the factors regulate the rate of lactose production. The galactopoietic effect elicited by administration of rbST during early lactation depends on increased the conversion of glucose to intermediary metabolites in the lactose biosynthetic pathway. A short persistency of lactation in

rbST treated animals was similar to the control animals receiving placebo. Changes in milk production during the progress of lactation in rbST treated animals might not be controlled systemically but also locally within the mammary gland.

บทคัดย่อ

การศึกษาครั้งนี้มีจุดประสงค์เพื่ออธิบาย การคงระยะของการให้นมสูงที่มีช่วงสั้นกว่าปกติในโคนมลูกผสมสายเลือดโฮลสไตน์87.5% ในช่วงระยะของการให้นม ว่าเป็นผลจากการลดลงของระดับgrowth hormoneในเลือด หรือเกี่ยวข้องกับกลไกอื่นๆ ภายในร่างกาย การศึกษาใช้โคนมลูกผสมโฮลสไตน์ ที่ให้นมครั้งแรกและไม่ตั้งท้อง จำนวน 10 ตัว โดยแบ่งโคนมออกเป็น 2 กลุ่ม กลุ่มละ 5 ตัว คือ กลุ่มทดลองและกลุ่มควบคุม แต่ละกลุ่มให้กินฟางข้าวที่ผ่านการหมักด้วย 5% ยูเรียเป็นอาหารหยาบ ระยะการศึกษาแบ่งเป็น 4 ช่วงต่อเนื่องกันในแต่ละกลุ่ม คือ ช่วงก่อนการทดลอง (45 วันภายหลังคลอด ก่อนเข้าสู่ระยะการให้นมสูงสุด) ระยะต้นของการให้นม (105 วันภายหลังคลอด) ระยะกลางของการให้นม (165 วันภายหลังคลอด) และระยะท้ายของการให้นม (225 วันภายหลังคลอด) หลังจากที่โคนมทั้ง 2 กลุ่มคลอดลูกได้ 60 วันในโคนมกลุ่มทดลองทำการฉีด recombinant bovine somatotropin (rbST) ปริมาณ 500 มิลลิกรัมเข้าใต้ผิวหนังในทุกๆ 14 วัน ส่วนโคนมกลุ่มควบคุมจะทำการฉีด สารหลอก (placebo)ที่เป็นน้ำมันงา ปริมาณ 800 มิลลิกรัมเข้าใต้ผิวหนังทุกๆ 14 วัน ตลอดระยะการทดลอง

จากผลของการศึกษาพบว่าโคนมที่อยู่ในกลุ่มควบคุมและโคนมในกลุ่มที่ให้rbST อัตราการกินอาหารแห้ง (dry matter) ไม่แตกต่างกันตลอดระยะการศึกษา การบันทึกการหลั่งน้ำนมต่อวันของโคนมในกลุ่มที่ให้ rbST มีการเพิ่มขึ้นในระยะต้น (20%) และระยะกลางของการให้นม (9.5%) และจะลดลงในระยะท้ายของการให้นม (-2.7%) เมื่อเปรียบเทียบกับระยะก่อนการทดลอง กลุ่มสัตว์ที่ให้ rbST การให้น้ำนมต่อวันเพิ่มขึ้น 22% เมื่อเทียบกับสัตว์ที่อยู่ในกลุ่มควบคุมในระยะต้นของการให้นม ปริมาณการให้น้ำนมต่อวัน ของกลุ่มโคนมที่ให้ rbST เพิ่มถึงจุดสูงในระยะต้นของการให้นม หลังจากนั้นจะค่อยๆ ลดลงในระยะท้ายของการให้นมลดลงเป็น 19% เมื่อเทียบกับระยะต้นของการให้นม จากการศึกษาการควบคุมของเหลวภายในร่างกายพบว่าปริมาณน้ำทั้งหมดของร่างกาย ปริมาณน้ำนอกเซลล์ ปริมาตรพลาสมา และปริมาตรเลือดของโคนมในกลุ่มที่ให้ rbST จะเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ เมื่อวัดค่าของปริมาณน้ำภายในเซลล์ในกลุ่มที่ให้ rbST ไม่พบความแตกต่าง แต่ในสัตว์กลุ่มควบคุมพบว่ามีการลดลงอย่างมีนัยสำคัญในระยะต้นและระยะกลางของการให้นม ส่วนอัตราการหมุนเวียนของน้ำภายในร่างกายของกลุ่มโคนมที่ให้ rbST จะเพิ่มขึ้นอย่างมีนัยสำคัญในระยะต้นและระยะกลางของการให้นม อัตราการไหลของเลือดและของพลาสมาสู่ต่อมน้ำนมจะเพิ่มขึ้นอย่างชัดเจนในกลุ่มที่ให้ rbST ตลอดระยะของการให้นมแต่ไม่พบการเปลี่ยนแปลงในโคนมกลุ่มควบคุม

ระหว่างการทดลอง ความเข้มข้นของ IGF-1 ในพลาสมาจะเพิ่มขึ้นในกลุ่มที่ให้ rbST มากกว่าในกลุ่มควบคุมอย่างมีนัยสำคัญตลอดระยะของการให้นม ระดับความเข้มข้นของกลูโคส

โปรตีน และไตรกลีเซอไรด์ในพลาสมาของโคนมในแต่ละกลุ่มยังคงอยู่ในระดับเดิมตลอดระยะการศึกษา

การศึกษาความเข้มข้นของส่วนประกอบในน้ำนมสำหรับแลคโตสในโคนมกลุ่มควบคุมและกลุ่มที่ให้ rbST ไม่มีการเปลี่ยนแปลงตลอดระยะของการให้นม แต่ความเข้มข้นของโปรตีนและไขมันในน้ำนมของโคนมกลุ่มที่ให้ rbST จะเพิ่มขึ้นในระยะท้าย ๆ ของการให้นม ความเข้มข้นของไขมันนมในกลุ่มโคนมที่ให้ rbST จะเพิ่มมากกว่าในกลุ่มควบคุมอย่างมีนัยสำคัญในระยะต้นของการให้นม ความเข้มข้นโซเดียมและโพแทสเซียมในน้ำนม รวมทั้งอัตราส่วนระหว่างโซเดียมต่อโพแทสเซียมในแต่ละระยะของการให้นมไม่พบความแตกต่างอย่างมีนัยสำคัญ ความเข้มข้นของคลอไรด์ในน้ำนมจะเพิ่มขึ้นอย่างมีนัยสำคัญเมื่อเข้าสู่ระยะท้ายๆ ของการให้นมในโคนมกลุ่มควบคุม ในขณะที่กลุ่มโคนมที่ให้ rbST ความเข้มข้นของคลอไรด์ในน้ำนมจะลดลงอย่างมีนัยสำคัญในระยะต้นของการให้นม ระดับ plasminogen และ plasminในน้ำนมจะเพิ่มขึ้นในระยะท้าย ๆ ของการให้นมในสัตว์ทั้ง 2 กลุ่ม อัตราส่วนระหว่าง plasminogen ต่อ plasmin พบว่าลดลงในกลุ่มควบคุม แต่ในกลุ่มโคนมที่ให้ rbST จะเพิ่มขึ้น ในระยะท้ายๆ ของการให้นม

การศึกษาแนวทางการใช้สารอาหารจากพลาสมาโดยต่อมน้ำนมพบว่า ค่าเฉลี่ยความเข้มข้นในพลาสมาที่มาจากเลือดแดงสำหรับกลูโคส, acetate, β-hydroxybutyrate และ glycerol อิสระส่วนใหญ่ไม่มีการเปลี่ยนแปลงตลอดระยะการศึกษาทั้งกลุ่มโคนมที่ให้ rbST และโคนมกลุ่มควบคุม การศึกษาความแตกต่างของความเข้มข้นของสารอาหารระหว่างเลือดดำและเลือดแดงในต่อมน้ำนมและสัดส่วนของการแยกใช้ในต่อมน้ำนมสำหรับการใช้กลูโคสโดยต่อมน้ำนมในระยะต้นของการให้นมในกลุ่มโคนมที่ให้ rbST จะเพิ่มขึ้นประมาณ 20% และจะลดลงในระยะกลางและระยะท้ายของการให้นม ความแตกต่างของความเข้มข้นของสารอาหารระหว่างเลือดดำและเลือดแดงในต่อมน้ำนมและสัดส่วนของการแยกใช้ในต่อมน้ำนม สำหรับ acetate จะเพิ่มมากขึ้นในกลุ่มโคนมที่ให้ rbST ซึ่งสูงกว่าโคนมในกลุ่มควบคุมอย่างมีนัยสำคัญทั้งในระยะต้นและระยะกลางของการให้นม ความแตกต่างของความเข้มข้นของสารระหว่างเลือดดำและเลือดแดงในต่อมน้ำนมและสัดส่วนของการแยกใช้โดยต่อมน้ำนมสำหรับ β-hydroxybutyrate ไม่พบการเปลี่ยนแปลงในโคนมกลุ่มที่ให้ rbST การใช้ β-hydroxybutyrate ในต่อมน้ำนมในกลุ่มโคนมที่ให้ rbST จะเพิ่มขึ้นเมื่อเข้าสู่ระยะท้าย ๆ ของการให้นมแต่ในโคนมกลุ่มควบคุมไม่พบการเปลี่ยนแปลง ความแตกต่างของความเข้มข้นของสารอาหารระหว่างเลือดดำและเลือดแดงในต่อมน้ำนมและสัดส่วนของการแยกใช้โดยต่อมน้ำนมสำหรับ glycerol อิสระพบมีค่าแปรปรวนตลอดระยะการให้นมในโคนมทั้ง 2 กลุ่ม ความเข้มข้นของกรดไขมันอิสระ (C_{16}-C_{18}) ในพลาสมามีค่าเฉลี่ยเพิ่มขึ้นในกลุ่มโคนมที่ให้ rbST เมื่อเปรียบเทียบกับก่อนการทดลองและจะสูงกว่าในโคนมกลุ่มควบคุม ความแตกต่างของความเข้มข้นระหว่างเลือดดำและเลือดแดงในต่อมน้ำนมและการใช้ไขมันอิสระโดยต่อมน้ำนมมีค่าแปรปรวนในโคนมทั้ง 2 กลุ่ม ความเข้มข้นของสารอาหารระหว่างเลือดดำและเลือดแดงในต่อมน้ำนม

และสัดส่วนของการแยกใช้โดยต่อมน้ำนมของ triacylglycerol และสัดส่วนของการแยกใช้โดยต่อมน้ำนมไม่มีการเปลี่ยนแปลงในกลุ่มโคนมที่ให้ rbST เมื่อเทียบกับระยะก่อนทดลอง แต่การใช้ triacylglycerol โดยต่อมน้ำนมจะเพิ่มขึ้นภายหลังการให้ rbST ในโคนมกลุ่มควบคุมจะไม่พบข้อแตกต่างระหว่างความเข้มข้นของสารอาหารระหว่างเลือดดำและเลือดแดงในต่อมน้ำนมและสัดส่วนของการแยกใช้ triacylglycerol โดยต่อมน้ำนม

ผลการใช้กลูโคสร่วมไปกับการถูกใช้ไปเป็นส่วนประกอบของน้ำนมต่าง ๆ ภายในต่อมน้ำนมพบว่าอัตราการขับหลั่งแลคโตสและ triacylglycerol ในน้ำนมในกลุ่มโคนมที่ให้ rbST จะเพิ่มขึ้นอย่างมีนัยสำคัญร่วมไปกับการเพิ่มขึ้นของความเข้มข้นของกลูโคสในน้ำนมทั้งในระยะต้น และระยะกลางของการให้นม การขับหลั่ง triacylglycerol ในน้ำนมและความเข้มข้นของ triacylglycerol ในน้ำนมในกลุ่มโคนมที่ฉีด rbST จะเพิ่มสูงอย่างชัดเจนในระยะต้นของการให้นม และยังคงระดับสูงตลอดระยะการให้นม การขับหลั่งแลคโตสและซิเตรทพบสูงขึ้นในระยะต้นของการให้นมในกลุ่มโคนมที่ให้ rbST เปรียบเทียบกับโคนมในกลุ่มควบคุม ความเข้มข้นของกาแลคโตสในน้ำนมจะเพิ่มขึ้นอย่างชัดเจนในขณะที่ความเข้มข้นของ UDP-glucose ในน้ำนมจะลดลงอย่างมีนัยสำคัญเมื่อเข้าสู่ระยะท้าย ๆ ของการให้นมในโคนมทั้ง 2 กลุ่ม ความเข้มข้นของ citrate ในน้ำนมจะลดลงในขณะที่ความเข้มข้นของ 2-oxoglutarate จะเพิ่มขึ้นในระยะท้าย ๆ ของการให้นมในโคนมทั้ง 2 กลุ่ม ความเข้มข้นของ isocitrate ในน้ำนมจะลดลงในระยะท้ายของการให้นมในโคนมกลุ่มควบคุม ความเข้มข้นของ G6P, G1P และ cAMP ในน้ำนมจะลดลงอย่างชัดเจน เมื่อเข้าสู่ระยะท้ายของการให้นมในโคนมทั้ง 2 กลุ่ม

การศึกษาเมแทบอลิซึมของกลูโคสสัมพันธ์กับการใช้กลูโคสภายในต่อมน้ำนมพบว่าในระยะก่อนการทดลอง อัตราการหมุนเวียนการใช้กลูโคสทั้งหมดภายในร่างกายและการนำกลับมาใช้ของคาร์บอนอะตอมในกลูโคสไม่พบความแตกต่างระหว่างกลุ่มโคนมควบคุมกับกลุ่มโคนมที่ให้ rbST ในระยะต้นของการให้นม อัตราการหมุนเวียนของกลูโคสในกลุ่มโคนมที่ให้ rbST จะลดลงเมื่อเปรียบเทียบกับระยะก่อนการทดลอง แต่ไม่พบความเปลี่ยนแปลงในโคนมกลุ่มควบคุม ส่วนในระยะกลางของการให้นมของกลุ่มโคนมที่ให้ rbST การเคลียรานซ์กลูโคสจากพลาสมา รวมทั้งอัตราการหมุนเวียนกลูโคสจะเพิ่มขึ้นอย่างมีนัยสำคัญเมื่อเปรียบเทียบกับระยะก่อนการทดลอง สัดส่วนการใช้กลูโคสนอกต่อมน้ำนมพบว่าจะเพิ่มขึ้นเมื่อเข้าสู่ระยะกลางและระยะท้ายของการให้นมเมื่อเปรียบเทียบกับระยะก่อนการทดลองในกลุ่มโคนมที่ให้ rbST การใช้คาร์บอนอะตอมของกลูโคสไปในการสังเคราะห์ซิเตรทในน้ำนมจะมีมากกว่าในกลุ่มโคนมที่ให้ rbST เมื่อเทียบกับโคนมกลุ่มควบคุมในระยะกลางและระยะท้ายของการให้นม การใช้กลูโคสคาร์บอนไปในการสังเคราะห์ triacylglycerol ในน้ำนมจะพบว่าสูงขึ้นอย่างมีนัยสำคัญตลอดระยะการให้นมในกลุ่มโคนมที่ให้ rbST ในระยะต้นของการให้นม การสร้าง NADPH ที่มาจากกลูโคสสำหรับใช้ในการสังเคราะห์กรดไขมันภายในต่อมน้ำนมในกลุ่มโคนมที่ให้ rbST จะพบสูงกว่าอย่างมีนัยสำคัญ เมื่อ

เปรียบเทียบกับโคนมกลุ่มควบคุม ความเข้มข้นของกรดไขมันในน้ำนมที่ความยาวของโซ่อะตอมคาร์บอนที่ C_{16}-C_{18} จะเพิ่มขึ้นอย่างมีนัยสำคัญในระหว่างที่ให้ rbST ในระยะต่าง ๆ ของการให้น้ำนมเปรียบเทียบกับโคนมในกลุ่มควบคุม

จากผลของการศึกษาสามารถสรุปได้ว่ากลไกที่ rbST มีผลต่อการทำงานของต่อมน้ำนมโดยตรงหรือโดยอ้อมซึ่งมีผลต่อการเปลี่ยนแปลงของการใช้กลูโคสภายในเซลล์ต่อมน้ำนมไปในวิถีของการสังเคราะห์น้ำนมในช่วงระยะต้นของการให้นม จากการเพิ่มขึ้นของความเข้มข้นของระดับกลูโคสภายในเซลล์ต่อมน้ำนมจะมีผลไปเพิ่มการเคลื่อนของ G6P เข้าสู่กระบวนการสังเคราะห์แลคโตสและใช้ในวิถีเพนโตส การทำงานของ rbST ที่มีผลต่ออัตราการไหลของเลือดสู่ต่อมน้ำนมนอกจากจะผ่านการทำงานของ IGF-1 แล้ว การทำงานของ rbST ยังไปเพิ่มปริมาตรของน้ำทั้งหมดภายในร่างกายและปริมาตรน้ำนอกเซลล์ซึ่งเป็นส่วนหนึ่งร่วมไปกับการช่วยเพิ่มอัตราการไหลของเลือดสู่ต่อมน้ำนมโดยนำสารไปสู่ต่อมน้ำนมเพื่อการเพิ่มผลผลิตน้ำนม การเพิ่มระดับ IGF-1 ในพลาสมาไม่มีผลต่อการคงระยะของการให้นมสูงที่มีช่วงสั้นกว่าปกติในกลุ่มโคนมที่ให้ rbST อาจเนื่องมาจากมีการสร้างโปรตีนในพลาสมาที่จะไปจับกับ IGF-1 อิสระและยับยั้งการออกฤทธิ์ของ IGF-1 การลดลงของการหลั่งน้ำนมเมื่อเข้าสู่ระยะท้ายของการให้นมไม่ได้ถูกควบคุมโดยการเปลี่ยนแปลงของปัจจัยภายนอกต่อมน้ำนมเท่านั้น แต่ส่วนหนึ่งเป็นผลจากการเปลี่ยนแปลงภายในต่อมน้ำนมที่เกี่ยวข้องกับบทบาทของระบบ plasmin-plasminogen ภายในต่อมน้ำนมด้วย จากผลของการศึกษาครั้งนี้ชี้ให้เห็นว่าการเพิ่มสารอาหารไปสู่ต่อมน้ำนมในกลุ่มโคนมที่ให้ rbST เป็นผลจากการเหนี่ยวนำให้มีการเพิ่มอัตราการไหลของเลือดสู่ต่อมน้ำนม ความเข้มข้นของกลูโคสในต่อมน้ำนมจะสะท้อนถึงความเข้มข้นของกลูโคสภายในเซลล์ต่อมน้ำนม สามารถใช้เป็นปัจจัยหนึ่งที่จะควบคุมอัตราการสร้างแลคโตสในการใช้กลูโคสในวิถีการสังเคราะห์แลคโตส ในกลุ่มโคนมที่ให้ rbST ที่มีช่วงระยะสั้นของการคงระยะของการให้นมในระดับสูงเหมือนกับโคนมกลุ่มควบคุมที่ได้รับสารหลอก แสดงถึงการเปลี่ยนแปลงของผลผลิตน้ำนมในระยะท้ายของการให้นมจะถูกควบคุมจากปัจจัยที่มีการเปลี่ยนแปลงภายนอกต่อมน้ำนมและภายในต่อมน้ำนม

CHAPTER I

General Introduction

Chapter I

General Introduction

The major problem for the Thai dairy practices is low milk yield and short lactation period of either pure exotic or crossbred dairy cattle. The decrease in milk yield after peak lactation in dairy cattle has long been a biological conundrum for the mammary biologist, as well as a cause of considerable lost income for the dairy farmer. Many factors can affect milk production in dairy cattle in the tropics. For examples, high environmental temperature, less genetic potential for milk production of indigenous cattle and inadequate supply for foraging during summer months are noted. Several approaches have been attempted to try to improve dairy productivity in the tropics. Selecting the types of suitable crossbreeding of indigenous and exotic cattle for the tropics is practiced. During lactation, coordination between nutrient delivery and biosynthetic capacity are thought to be under endocrine control with homeorhetic mechanism. Milk secretion is a continuous process and requires a continuous supply of substrate for milk productions. An appearance of a shorter lactation persistency of crossbred Holstein cattle during transition period from early to mid-lactation has been reported to be due to the reduction of the growth hormone level (Chaiyabutr et al., 2000b). However, the mechanism of action for growth hormone on milk production in crossbred dairy cattle remains unclear. The aim of the work described in this report was to obtain quantitative data for improvement of milk production in crossbred dairy cattle. A study was performed in crossbred Holstein cattle containing 87.5% Holstein genes during long-term bovine somatropin administration on various physiological responses in relation to the mechanism response for the control of milk secretion at different stages of lactation.

It is known that lactating dairy cows metabolize large amounts of water and are affected rapidly by water deprivation. An increase in water intake during lactation closely matched to increase in water secreted in milk, which milk composition has about 87% of water. An

alteration in bodily function during lactation is apparent; for example, blood volume (Chaiyabutr et al., 1997) and cardiac output (Hanwell and Peaker, 1977) are increased. These changes may effectively alter body fluid and thus circulatory distribution including the blood supply to the mammary gland. During early lactation, nutrient partitioning relating to circulatory distribution are known to make a contribution of resources to the mammary gland for a high milk synthesis (Linzell, 1974). During early lactation, high producing cows cannot consume enough dry matter to meet nutrient requirements. Negative energy balance and body fat mobilization is usually apparent. During lactation, coordination between nutrient delivery and biosynthetic capacity are thought to be under endocrine control with homeorhetic mechanism. Several lines of evidence indicate that administration of growth hormone does not act directly on mammary gland. Since the receptors for growth hormone has not been demonstrated on epithelial cells of mammary tissue (Akers, 1985). Previous study has been shown that the levels of plasma bovine somatotropin of 87.5%HF cows rose in early period of lactation and markedly reduced in mid and late lactation (Chaiyabutr et al., 2000b). However, a few researches in crossbred dairy cattle have been conducted to study effects of bovine somatotropin on the interaction between body fluid, fat mass and mammary function during early to mid lactation. To study the physiological mechanism behind the result of the decrease in milk secretion with a concomitant decreases in both the level of circulating growth hormone and mammary blood flow during transition period of the early to mid lactation in 87.5% HF animals (Chaiyabutr et al., 2000b). The experiment was conducted with respect to the effect of recombinant bovine somatotropin (rbST) administration on body water metabolism, mammary function and other physiological parameters during peak yield in early lactation (Chapter III).

One study on the regulation of body fluids and mammary blood flow (MBF) in different types of crossbred Holstein cattle indicated that the 87.5%HF animals had lower efficiency in

water retention mechanism and poor adaptation to tropical environment, in comparison with 50%HF animals (Chaiyabutr et al., 1997; 2000a). A low persistency of lactation with a decrease in MBF during the transition period from early to mid-lactation, was noted in the 87.5%HF animals. Bovine somatotropin (bST) is known as a homeorhetic hormone connected with both growth and lactation. Although a number of reviews have been published on the relationship between the plasma bST concentration and milk yield in both normal and hot environments (West et al., 1991; Johnson et al., 1991). There are few studies on the mechanisms acting within the body of crossbred cattle concerning the role of bST on water metabolism, in relation to persistent lactation. Long-term treatment with rbST at different stages lactation were carried out to obtain a more complete picture (Chapter IV).

It has been demonstrated that receptors for bST are not apparent on secretory epithelial cells of mammary tissue (Akers, 1985). The mechanism of action of bST on milk production is still a controversial area. The effects of bST on milk production are thought to be indirectly mediated via nutrient partitioning effects or via insulin like growth factor-I (IGF-I) (Bauman, 1992). Some studies support this role. Infusion of IGF-I into the pudic artery of lactating goats has been shown to increase blood flow and milk production on the infused side (Prosser et al.1990; Prosser et al.1994), whereas infusion of bST into the mammary artery of sheep did not increase milk yield (Peel and Bauman, 1987). Several other reports, refuting the role of IGF-I as mediators of bST action, have been published (Barber et al.1992; Flint et al.1992; Plaut et al, 1993). It has been reported that bST can stimulate milk production under circumstances in which IGF-I does not (Prosser and Davis,1992). Chaiyabutr et al.(2000b) reported that the galactopoietic effect of bST is not associated with the plasma level of IGF-I as lactation advances in 87.5% HF animals. The plasma level of IGF-I has been shown to remain at the same level as lactation advances, despite declining circulating bST, mammary blood flow and milk yield (Chaiyabutr et al. 2004). These data did not support a role for IGF-I

in mediating the action of bST on milk production. However, an increase in plasma IGF-I level, with a concomitant increase in both mammary blood flow and milk yield in late lactation, was seen after exogenous administration of rbST in 87.5%HF animals (Tunwattana et al., 2003). Despite a number of studies looking at these differences, there have been few observations about the mechanism of short persistency of lactation in 87.5% HF dairy cattle. To understand this apparent paradox, long-term administration of rbST, throughout lactation, might lead to better understanding adaptability in crossbred cattle. The vivo relationship between long-term exogenous administrations of rbST, circulating levels of IGF-I, mammary blood flow and biological variables relevant to milk synthesis in 87.5% HF animals were determined. (Chapter V)

An advance of lactation is characterized by decrease in milk yield and concomitant decrease in blood ST concentration (Hart et al., 1980). A number of studies indicate that bST can delay involution of the mammary gland by reducing the activity of the plasmin-plasminogen system, an important initiator of tissue remodeling during lactation advance in dairy ruminants (Baldi et al., 1997; Politis et al., 1990). There is evident that the progressive loss of milk synthesis capacity by mammary epithelial cells occurs during mammary involution, although substrate supply to the mammary gland is often adequate to maintain the maximum rate of milk synthesis. As lactation advances, a leaky of cell tight junctions is also apparent during involution of the secretory tissue. In the process of the proteolysis, the proteinase responsible is plasmin, which is transferred from blood into milk as an inactive precursor (plasminogen) and then converted to active plasmin by plasminogen activators, which are produced in quantity within the mammary gland especially in the late lactation. Increases in plasmin production in milk are important in determining milk production and initiate the onset of involution within the mammary gland (Ossowski et al., 1979). Little is known about responsible for this proteolysis relating to the role of growth hormone on the

persistency of lactation in crossbred cattle. Thus, the relationship between milk plasmin-plasminogen and milk yield including milk compositions during long-term administration of rbST were carried out at different stages of lactation in 87.5% HF animals (ChapterVI).

It is known that milk production is dependent upon its blood supply to provide substrates at appropriate rates to sustain milk synthesis. The rate of supplying to mammary gland is determined by substrate concentration in the plasma and mammary blood flow. Milk production is the result of coordination between nutrient delivery to and biosynthetic capacity of the mammary glands. There is evident that substrate supply to the mammary gland is often inadequate to maintain the maximum rate of milk synthesis (Linzell ,1974). The mammary gland may be producing milk at a rate below its potential. However, the rate of milk production depends on function of number of secretory cells and their metabolic activity. The delivery of nutrients to the mammary gland is dependent on the physiological state of the animal by homeostatic and homeorhetic mechanisms (Bauman,1992). During early lactation nutrients are partitioned from peripheral tissues to the mammary gland to support the requirements for milk synthesis during peak lactation. Genetic selection has intensified the physiological demand on peripheral tissues to provide nutrients to the mammary gland partially because of the limitation of energy intake during peak lactation. The crossbred cattle containing 87.5% Holstein genes decreased in milk yield, which was related to reductions in mammary blood flow and circulating bST as lactation advances to mid- and late lactation (Chaiyabutr et al., 2000a, 2000b). It is not known which factors are the cause and which factors are the effect for the short persistency of lactation in crossbred Holstein cattle especially about the function of mammary tissue and the utilization of substrate in the mammary gland. The present experiment was conducted to improve understanding by using techniques for measuring mammary blood flow and combining these with measurements of nutrient arterial concentrations and arteriovenous concentration differences for the mammary

uptake of nutrients during long-term administration of exogenouse bST throughout lactation (ChapterVII).

The rate of milk production is known to depend on function of number of secretory cells and their metabolic activity. There is evident that major changes in the concentrations of metabolites in the mammary secretion are apparent during lactogenesis. Previous studies in vivo have shown that changes occur in the metabolism of the mammary gland between different types of the crossbred dairy cattle. During lactation advance to mid and late lactation, the rate of lactose synthesis decreases and milk yield falls in 87.5%HF animals (Chaiyabutr et al., 2000a). A poorer lactation persistency in 87.5% HF animals has been shown to relate to a decrease in the lactose biosynthetic pathway with a reduction in the percentage of metabolism of glucose 6-phosphate to galactose moiety of lactose (Chaiyabutr et al., 2000c); at the same time both the level of plasma bovine somatotropin and blood flow to the mammary gland decreased during the transition period from early to mid-lactation (Chaiyabutr et al., 2000a, Chaiyabutr et al., 2000b). These findings were obtained in vivo using radiotracer techniques and by measuring arteriovenouse concentration differences of metabolites across the gland. However, detailed information on the changes in the concentrations of metabolites within the mammary gland corresponding to a short persistency of lactation is not available in the crossbred dairy cattle. Long-term treatment with recombinant bovine somatotropin (rbST) at different stages of lactation were carried out to obtain a more complete picture of the role of bovine somatotropin in changes in the concentrations of metabolites in milk which could be interpreted in relation to the biochemical changes occurring within the mammary gland in crossbred dairy cattle (ChapterVIII).

It is known that dairy herds in tropical countries are mixed exotic breeds and crossbreeds. The low milk production of both exotic and crossbred cattle is still the main problem in dairy farming in the tropics. Many factors affecting the rate and quality of milk

secretion by altering the metabolic endocrinology of dairy cattle are extensively reviewed The mechanism by which this is thought to be achieved is complex and involves a number of events. In crossbred cattle, mechanisms of milk secretion are known to be inherited and are thought to be among the causes of differences in metabolic parameters. Few data are available concerning the utilization of glucose and glucose metabolism in the udders of crossbred Holstein dairy cattle in vivo during bovine somatotropin administration. Our previous study has shown that the glucose utilization for biosynthetic pathways in the mammary gland of 50% HF animals was maintained in a similar pattern throughout the periods of lactation, while a short persistency of lactation in 87.5% HF animals has been shown to relate to a decrease in the lactose biosynthetic pathway (Chaiyabutr et al., 2000c). The apparent discrepancies raise the question, whether the differences would be the effects of physiological state, genetic potential and endocrine regulation. Therefore, the present experiment was conducted to obtain the above information. Specifically, we examined the effects of long-term adminitration of rbST on glucose metabolism and the efficiency of utilization of glucose by the mammary gland in different stages of lactation in 87.5% HF Holstein cattle (ChapterIX).

The overall responses of long-term administration of rbST in different stages of lactation in 87.5% HF Holstein cattle for the physiological changes in both extra-mammary tissue and intra-mammary tissue are discussed in Chapter X.

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CHAPTER II

Materials and methods

Chapter II

Materials and Methods

Methodological details relevant for this report are presented or referred to in the separate publications (Chapter III to IX). This chapter is limited to the experimental protocol.

Animals and management

Ten, first lactating, non-pregnant, crossbred, 87.5% Holstein dairy cattle were selected for the experiment. They were divided into two groups of five animals each. Animals in each group were fed with rice straw treated with 5% urea as the source of roughage. All animals were housed in sheds and tethered in individual stalls and fed twice daily. The maximum temperature in the shed at noon was 34±1°C and the minimum temperature at night was 26±1°C. The relative humidity was 68±12%. Animals received an average of 4 kg/day of roughage in combination with the same concentrated mixture (7 kg/day) to maintain a moderate body condition score (2.5, scale = 1 to 5). Each day, the food was given in equal portions at about 06.00 h and 17.00 h when the animals were milked. Animals had free access to water and were fed their respective rations throughout the experimental period.

The urea treated rice straw was prepared by mixing the urea solution with dry straw (5 kg urea dissolved in 100 litter water per 100 kg dry rice straw). Rice straw sprayed with urea solution was mixed thoroughly and stored under airtight conditions in a cement pit for 21 days. A continuous supply of treated rice straw was made available by using a 2 pit x 21 day system of urea treatment. After 21 days, the rice straw treated with 5% urea was offered to the animals.

Experimental procedures

Animals were divided into the control (n=5) and experimental (n=5) groups. Four consecutive periods of study were used for each group. These consisted of a pretreatment period (45 days postpartum before lactation peak) and three treatment periods of 105 days postpartum (early lactation), 165 days postpartum (mid- lactation) and 225 days postpartum (late lactation). During the treatment periods, animals in the experimental group, which had completed 60 days of lactation, were injected subcutaneously every 14 days until the end of study with 500 mg of recombinant bovine somatotropin (rbST). It was suspended in 792 mg

of a prolonged-release formulation of sesame oil (POSILAC, Monsanto, USA). Animals in the control group were injected subcutaneously every 14 days with 800 mg of sterile sesame oil without rbST. Injection in each animal was administered at the tail head depression (ischiorectal fossa). From the pretreatment to the end of the treatment periods, animals of both groups were fed the same ration starting before parturition until the completion of the study. The dry matter intake of each animal was measured by weighing the concentrate and roughage offered and refused each day. Animals were normally milked at around 0600 h and 1700 h using a milking machine and milk production was recorded daily. Measurements of the udder blood flow were carried out in the afternoon. At around 1100 h, an arterial blood sample was collected from the coccygeal artery, by venipuncture with a #21 needle and into a heparinized tube. Blood samples were kept in crushed ice and then centrifuged at 3000 rpm for 30 min at 4°C. Arterial plasma samples were collected for measurements of the level of hormones and metabolites. Plasma samples in aliquots were collected and frozen at -40°C until the time of the assays. Animals were weighed after collecting a milk sample in each period.

Preparation for mammary blood flow measurements

On the day before the experiment began and in each period of the experiment, two catheters (i.d. 1.0 mm, o.d. 1.3 mm, L 45 mm) were inserted into either the left or right milk vein using a intravenous polymer catheter (Jelco, Critikon; Johnson & Johnson, U.K.), under local anesthesia. This was done on the standing animal for the measurement of mammary blood flow. The tip of the catheter was positioned near the sigmoid flexure, anterior to the point at which the vein leaves the udder. The other catheter was positioned downstream, about 20 cm from the first one. All catheters were flushed with sterile heparinized normal saline and were left in place during the experiment. Blood flow through half of the udder was determined by measuring the dilution of dye T-1824 (Evans blue) by a short term continuous infusion. Briefly, a dye (T-1824) was dissolved in sterile normal saline and diluted to a concentration of 100mg/L. The solution was infused by a peristaltic pump (Gilson Medical Electronics, France), at a constant rate of 80 ml/min into the milk vein for 1-2 min. Before infusion, blood was drawn from downstream in the milk vein as a pre-infusion sample. About 10 seconds after starting infusion, 10 ml of blood was drawn from downstream in the milk vein at a constant rate into a heparinized tube. Two consecutive plasma samples were taken during dye infusion. Blood flow of half of the udder was calculated from plasma samples. In lactating cows, quarter milking showed that the yields of the two halves of the udder were

similar. Udder blood flow was therefore calculated by doubling the flow measured in one milk vein. Packed cell volume was measured after centrifugation of the blood in a micro-capillary tube for calculation of blood flow.

Determinations of water intake and milk yield

The measurement of daily water consumption of each animal was calculated by weighing the individual water bowl of each animal. The daily water intake per animal in each period of lactation was recorded by averaging over seven days. Animals were normally milked at around 0600 h and 1700 h using a milking machine and milk production was recorded daily. Milk yield per day per animal was recorded at each period of lactation. Animals were weighed after collecting the milk sample in each specified day.

Determinations of plasma volume, blood volume, extracellular water, total body water and water turnover rate

In each animal per period, the water turnover rate and total body water were determined by tritiated water dilution techniques using a single dose injection of 3,000 μci per animal of carrier free tritiated water (TOH) in normal saline. The equilibrium time was determined by taking blood samples for 3 days after the injection. Blood samples for measurements of water turnover rate, biological half-life of tritium, TOH space and total body water were performed.

Determinations of extracellular water (ECW) and plasma volume were carried out using sodium thiocyanate and the Evans blue dye (T-1824). The injection of 20 ml of sodium thiocyanate solution (10 g/100 ml normal saline) and 20 ml of the Evans blue dye (T-1824) (0.5 g/100 ml normal saline) was given via a ear vein catheter to estimate ECW volume and the plasma volume, respectively. Venous blood samples from the jugular vein were taken at 20, 30, 40 and 50 min after dye injection. Dilution of dye at zero time was determined by using a semi logarithmic concentration on time extrapolation. Blood volume was calculated from the plasma volume and packed cell volume. Intracellular water (ICW) was calculated by subtracting ECW from TBW. Plasma osmolality was measured using the freezing point depression method (Advance Osmometer model 3, U.S.A.).

Glucose turnover measurements

Glucose kinetic studies of each animal in each experimental period were carried out. Briefly, at about 1100h a priming dose of radioactive glucose in 20 ml of sterile NSS

containing 60 μCi(3-^{3}H) glucose and 40 μCi(U-^{14}C) glucose was administered intravenously via the ear vein catheter and followed by a constant infusion of 1 ml/min of sterile saline (0.9%) containing 2 μCi(U-^{14}C) glucose and 3 μCi(3-^{3}H) glucose for 4h (Peristaltic pump; EYLA Model 3). During the final 1 hour (1400-1500h) of infusion, three sets of blood samples were collected at 20 min. intervals. A venous blood sample was collected from the milk vein via a catheter while an arterial blood sample was collected from the coccygeal artery by venipuncture with a #21 needle. Blood samples in heparinized tubes were kept in crushed ice for chemical studies.

Determinations of other parameters

Plasma and milk samples from each experimental period were kept at -40°C and -20°C respectively for chemical, biochemical enzymes assay, plasma hormones and milk components measurements.

Statistical analysis

All the results were statistically analyzed by a paired t-test for variables within a treatment which were compared against the pretreatment values in the same group. Mean values of variables within a period were compared across treatments between group by an unpaired t-test. Mean values are presented as mean±S.D. In some cases a further comparison of consistent changes was made using Wilcoxon's signed-rank test.

CHAPTER III

Relationship of Early Lactation and Bovine Somatotropin on Water Metabolism and Mammary Circulation of Crossbred Holstein Cattle

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(Running head: rbST treatment and water metabolism in early lactation)

Relationship of Early Lactation and Bovine Somatotropin on Water Metabolism and Mammary Circulation of Crossbred Holstein Cattle

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ABSTRACT : The objective of the study was carried out to evaluate the effect of exogenous bovine somatotropin on water metabolism in relation to mammary function in early lactation of crossbred Holstein cattle. Ten, 87.5% crossbred Holstein cattle were divided into two groups of 5 animals each. At day 60 of lactation, the control group was given placebo while animals in the experimental group were given recombinant bovine somatotropin (rbST) by subcutaneous injection with 500 mg of rbST (14-days prolong-release rbST). In rbST-treated animals, milk yield increased 19.8% which coincided with significant increase in water intake ($P<0.01$), while DM daily intake was not different when compared to the control animals. Water turnover rate as absolute values significantly increased ($P<0.05$), while the biological half-life of water did not change in rbST-treated animals. Total body water (TBW) and total body water space (TOH) as absolute values significantly increased ($P<0.01$) in rbST-treated animals, while it was decreased in the control animals. Absolute values of empty body water (EBW) markedly increased ($P<0.05$), which associated with an increase in the extracellular fluid (ECF) volume. An absolute values of plasma volume and blood volume were also significantly increased ($P<0.05$) in rbST-treated animals. The increase in mammary blood flow in rbST-treated animals was proportionally higher than an increase in milk production. The plasma IGF-1 concentration was significantly increased ($P<0.01$) in rbST-treated animals when compared with those of control

animals during the treatment period. Milk fat concentration increased during rbST treatment, while the concentrations of both protein and lactose in milk were not affected. The present results indicate that rbST exerts it effect on an increase in both TBW and EBW. An increased ECF compartment in rbST-treated animals might be partly resulted from the decrease in fat mass during early lactation. The action of rbST on mammary blood flow might not be mediated solely by the action of IGF-1 for increase in blood flow to mammary gland. An elevation of body fluid during rbST treatment in early lactation may be partly caused an increase in mammary blood flow in distribution of milk precursors to the gland.

Key Words: rbST, Mammary blood flow, Total body water, Early lactation, Cross bred Holstein cattle.

INTRODUCTION

It is known that lactating dairy cows metabolize large amounts of water and are affected rapidly by water deprivation. An increase in water intake during lactation closely matched to increase in water secreted in milk (Woodford et al., 1984), which milk composition has about 87% of water (Murphy, 1992). An alteration in bodily function during lactation is apparent; for example, blood volume (Chaiyabutr et al., 1997) and cardiac output (Hanwell and Peaker, 1977), are increased. These changes may effectively alter body fluid and thus circulatory distribution including the blood supply to the mammary gland. The lactating mammary gland receives signals from the rest of body in forms of nutrient and hormones from blood. Mammary blood flow is thus a major parameter controlling milk production in a way to carry milk precursors to the mammary gland at the process of milk synthesis.

During lactation, coordination between nutrient delivery and biosynthetic capacity are thought to be under endocrine control with homeorhetic mechanism. However, the mechanism of action for growth hormone on milk production remains unclear. Several lines of evidence indicate that administration of growth hormone does not act directly on mammary gland. Since the receptors for growth hormone has not been demonstrated on epithelial cells of mammary tissue (Akers, 1985). It has been reported that mammary blood flow and milk secretion of crossbred cattle containing 87.5% Holstein (HF) genes were significantly higher during early lactation and markedly declined when lactation advances. The levels of plasma growth hormone of 87.5%HF cows also rose in early period of lactation and markedly reduced in mid and late lactation (Chaiyabutr et al., 2000). Water turnover rate and total body water as percentage of body weight of 87.5% HF animals showed a poor adjustment to the tropical environment (Chaiyabutr et al., 1999). During early lactation, high producing cows cannot consume enough dry matter to meet nutrient requirements. Negative energy balance and body fat mobilization is usually apparent. A few researches in crossbred dairy cattle have been conducted to study effects of bovine somatotropin on the interaction between body fluid, fat mass and mammary function during early to mid lactation. Although, a decrease in fat-free mass coinciding to a decrease in ECF, but not TBW, has been reported in growth hormone deficient human, and it appears to increased fat-free mass and decreased fat mass during GH therapy (Janssen et al., 1997). An appearance of a shorter lactation persistency of crossbred cattle containing 87.5% Holstein genes during transition period from early to mid-lactation has also been reported to be due to the reduction of the growth hormone level (Chaiyabutr et al., 2000). During early lactation, nutrient partitioning relating to circulatory distribution are known to make a contribution of resources to the mammary gland for a high milk synthesis (Linzell,

1974) Changes in body fluid have not been evaluated for responsibility of the rapid decreasing milk secretion during lactation advances in 87.5% HF animals. Therefore, the objective of this study is to determine the physiological mechanism behind the result of the decrease in milk secretion, with a concomitant decreases in both the level of circulating growth hormone and mammary blood flow during advanced lactation in 87.5% HF animals (Chaiyabutr et al., 2000), may be related in part to changes in body fluid. The present experiment was conducted with respect to the effect of administration of rbST on body water metabolism, mammary function, mammary blood flow and other physiological parameters during peak yield in early lactation.

MATERIALS AND METHODS

Animals and management

Ten, late pregnancy 87.5% crossbred Holstein cattle were used in the experiment. They were divided into two groups of five animals each. All animals were housed in tie stall type sheds, having a solid floor and open sides. . The maximum temperature in the shed at noon was 34±1°C and the minimum temperature at night was 26±1°C. The relative humidity was 68±12%. Animals in each group were fed with rice straw treated with 5% urea as the source of roughage throughout the experiment. Animals individually received an average of a 4 kg/day of roughage in combination with the same concentrated mixture (7kg/day) to maintain a moderate body condition score (2.5 scale = 1 to 5). The chemical composition of feeds is presented in Table 1. Concentrate formulation was prepared in fresh weight (kg/100 kg) which consisted of soy bean meal 26.3 kg, cotton seed 37 kg, cassava 28.5 kg, rice bran 3.3 kg, limestone 1.3, dicalcium phosphate 1.5 kg, sodium bicarbonate 1.1 kg, potassium chloride 0.8 kg and premix 0.2 kg. The urea

treated rice straw was prepared by rice straw sprayed with urea solution was mixed thoroughly and stored under airtight conditions in a cement pit for 21 days (5 kg urea dissolved in 100 litres water per 100 kg dry rice straw). After 21 days, the rice straw treated with 5% urea was offered to the animals. A continuous supply of treated rice straw was made available by using a two pit x 21 day system of urea treatment. Food was given in equal portions at about 06.00 h and 17.00 h when animals were milked. All samples of urea treated rice straw and concentrate were analyzed for dry matter, crude protein and ash using procedures described by AOAC (1984). ADF and NDF were analyzed according to Van Soest and Robertson (1980). Water was available for *ad libitum* intake.

Experimental procedures

Animals were divided into control (n = 5) and experimental (n = 5) groups. Two consecutive period of experiments were carried out in each group, consisting of the pretreatment period (45 days postpartum), and treatment periods of 105 days postpartum (early lactation). The start of treatment period at day 60 of lactation, rbST-treated animals were injected subcutaneously every 14 days until the end of experiment with 500 mg of recombinant bovine somatotropin (rbST) suspended in 792 mg of a prolonged-release formulation in sesame oil (POSILAC, Monsato, USA), while animals in the control group were injected subcutaneously every 14 days with 800 mg of sterile sesame oil without rbST as placebo. An injection was administered at post scapular.

From the beginning of pretreatment to the end of treatment period, animals of both groups were fed the same ration from before parturition through the completion of experiment. The dry matter intake of each animal was determined by measuring

both the concentrate and roughage offered and refused each day. On the day of experiment in each period, measurements of the total body water, water turnover rate, mammary blood flow, plasma volume and extracellular fluid were carried out. The rate of milk secretion was recorded by hand milking in the afternoon and measurement of mammary blood flow was carried out. In each period of study animals were weighed after collecting the milk sample.

Animals preparation

On the day before the experiment in each period, two catheters (i.d. 1.0 mm, o.d. 1.3 mm, length 45 mm) were inserted into either the left or right milk vein by using intravenous polymer catheter (Jelco, Critikon; Johnson & Johnson, U.K.) under local anesthesia. This was carried out on standing animal for measurement of mammary blood flow. The tip of the catheter was positioned near the sigmoid flexure anterior to the point at which the vein leaves the udder. The other catheter was positioned downstream about 20 cm from the first one. The catheter for both isotope and dye injection was inserted into and ear vein under local anesthesia. All catheters were flushed with sterile heparinized normal saline and were left in place during the experiment.

Determinations of water intake, total body water, water turnover and empty body water

Estimation the rate of water intake values of each animal in each period of experiments was recorded by an averaged over seven days from weighing daily water consumption in water bowl. The water turnover rate and total body water were determined in each animal by tritiated water dilution techniques. The animal was injected intravenously via the ear vein with carrier free tritiated water in normal

saline at a single dose of 2,500 μCi per animal. The equilibration time was determined by taking blood samples for 3 days after the injection. Blood samples were collected 4, 8, 20, 26, 32, 44, 50, 56, 68 and 74 h subsequent to the injection for water turnover measurements (Chaiyabutr et al., 1997). The preparation for sample counting was achieved by the internal standardization technique as described by Vaughan and Boling (1961). The corrected activity of the samples, in disintegrate per minute (d.p.m.), were plotted on semi-logarithmic paper against time, in hours after dosing, and the extrapolated activity at theoretical zero time of complete mixing of radio-isotope was used to determine the total body water space (TOH). The TOH space was calculated:

TOH space (ml) = [standard count (dis/min) x dose (ml)] / [radio activity counts at zero time (dis/min)].

The biological half-life of tritium labelled water (T1/2) was determined from the slope of the linear regression line obtained from plot on semi-logarithmic paper of the activity of the samples taken over the period of 3 days against time.

The water turnover rate was calculated from the equation: Water turnover rate (l/d) = 0.693 x TOH space / biological half-life. The total body water was calculated by using the corrected factor (1 – fraction of plasma solids) x TOH space (Chaiyabutr et al., 1997).

Empty body water (EBW) did not include water associated with gastrointestinal contents or the water in the fetus. The EBW was estimated from the disappearance curve of tritium in blood plasma for each animal. Two compartment open system model was used to estimate the EBW (Shipley and Clark, 1972). The exponential equation describing the two compartment model was calculated from the equation:

$$Y = Ae^{-k1t} + Be^{-k2t}$$

Where, Y = concentration of tritium in plasma at time t

A = plasma concentration intercept of the fast phase of the plasma curve

B = plasma concentration intercept of the slow phase of the plasma curve

k1= first order rate constant of the fast phase

k2= first order rate constant of the slow phase

t = time in minutes

Determinations of plasma volume, extracellular fluid and intracellular fluid

In each period of study, plasma volume was measured by dye dilution technique using of Evans blue dye (T-1824) (E. Merck, Darmstadt, Germany) and extracellular fluid volume (ECF) was measured using sodium thiocyanate (NaSCN). The injection of 20 ml of the 0.5% T-1824 (0.5 g/100 ml normal saline), and 20 ml of the 10% NaSCN solution (10 g/100 ml normal saline) were given into the ear vein catheter. Venous blood samples from the jugular vein were taken at 20, 30, 40 and 50 min after dye injection. The dilution of dye at zero time was determined by using semi logarithmic concentration on time extrapolation. Blood volume was calculated from the plasma volume and packed cell volume (PCV). Intracellular fluid (ICF) was calculated by subtracting ECF from TBW. Plasma osmolality was measured using the freezing point depression method (Advance Osmometer model 3, Massachusetts, USA). The plasma solids concentration was determined by a refractometer.

Determination of mammary blood flow

Blood flow through half of the udder was determined by measuring the dilution of dye T-1824 (Evans blue) by a short term continuous infusion as described by Chaiyabutr et al. (1997).

Milk collection and determinations of milk compositions

Milk was collected by hand milking and kept in formaldehyde. The formalinized milk sample (300 µl of 40% formalin in 30 ml of fresh milk) was kept at 4°C for determinations of lactose (Tele et al., 1978); fat and protein concentrations by the colorimetric method using Gerber methods (Clunie Harvey and Hill,1967) and Milkoscan (Milkoscan 4,000, Foss Electrique), respectively.

Determination of the plasma IGF-1 concentration

The arterial plasma IGF-1 concentration was determined using the automated chemiluminescent immunoassays with alkaline phosphatase conjugated polyclonal rabbit anti-IGF-1 antibody in an IMMULITE® Analyzer (IMMULITE IGF-1, Diagnostic Products Corporation, Los Angeles, CA). The arterial plasma samples were processed in duplicate. All samples were included in the same assay to eliminate interassay variation. Intraassay variation for CV of samples was 6.7%.

Statistical analysis

All the data obtained were presented as the means ± SD. Statistical significant differences between periods in the same group were determined by the student's paired t-test. The student's unpaired t-test was used to estimate the statistical significant differences between groups (Snedecor and Cochran, 1989).

RESULTS

Daily dry matter intake, water intake and milk yield (table 2)

No significant differences in the total daily dry matter intake (DM) or DM as a percent body weight were apparent between control and rbST-treated animals. Daily water intake of rbST-treated animals significantly increased ($P<0.01$), which

coincided with increase in milk production. Animals given rbST in early lactation, milk yield increased from 13.4 to 16.0 kg/d/animal ($P<0.05$). In contrast to rbST-treated animals, milk yield of the control animals were not significantly different between pretreatment and treatment periods although the peak of milk yield occurred at week 10 in both groups. An evaluation of the dry matter intake and milk yield revealed that during the treatment period the mean ratios of total DM intake to milk yield of rbST-treated animals were lower than those of control animals. The mean ratios of dry matter intake to milk yield decreased significantly ($P<0.05$) in the treatment period of rbST-treated animals. The body weight significantly increased ($P<0.05$ and $P<0.01$) in treatment period as compared with those of the pretreatment period in both control animals and rbST-treated animals.

Plasma volume, blood volume, plasma osmolality and packed cell volume (Table 3)

In the pretreatment period, there were no significant differences of the plasma volume and blood volume as absolute values or as percentages of body weight between control animals and rbST-treated animals. Plasma volume and blood volume as absolute values significantly increased ($P<0.05$) during treated with rbST when compared with the pretreatment period. There were no significantly different of the packed cell volume and plasma osmolality throughout period of studies in both groups.

The water turnover rate, biological half-life and total body water (Table 4)

In the pretreatment period, there were no significant differences for the water turnover rate between control and rbST- treated animals. In the treatment period, water turnover rate as absolute values significantly increased ($P<0.05$) in animals

given rbST when compared with the control animals or with the pretreatment period in the same group. An average of water turnover rate as a percent of body weight and the water turnover rate per body fat free wet weight ($kg^{0.82}$) were not significantly different between controls and rbST-treated animals throughout the study period. There were no changes in the biological half-life of tritiated water between control animals and animals given rbST. The TOH space and total body water vary with the size of animals. The TOH space and total body water as absolute values was not significantly different between control animals and rbST-treated animals in the pretreatment period. The TOH space and total body water as absolute values of rbST-treated animals significantly increased ($P<0.01$) than those of control animals in the treatment period. The TOH space and total body water as absolute values in the treatment period were significantly increased ($P<0.01$) than that of pretreatment period in rbST-treated animals. In the control animals, total body water and TOH space period were significantly decreased than in the treatment those in the pretreatment period. No significant differences in TOH space and total body water as a percentage of body weight between control animals and animals given rbST.

Empty body water, gut water, extracellular fluid and intracellular fluid (Table 5)

There were no significant differences of the EBW as absolute values or as percentages of body weight between control animals and rbST-treated animals in the pretreatment period. In the treatment period, the EBW as absolute values of rbST-treated animals was higher ($P<0.05$) when compared with control animals. The value of gut water of rbST-treated animals significantly increased ($P<0.01$), while it significantly decreased ($P<0.05$) in control animals in the treatment period.

The ECF volume of rbST-treated animals was significantly higher ($P<0.05$) in the treatment period than those in the pretreatment period. An absolute value of ICF volume was significantly increased ($P<0.01$) in animals given rbST when compared with control animals in the treatment period. An absolute values of the ICF volume of the control animals showed significant decrease ($P<0.01$) in treatment period when compared with pretreatment period. No significant differences in intracellular fluid volume as percentage of body weight in both groups in comparison between the treatment period and the pretreatment period.

Mammary circulation and the plasma concentration of IGF-1 (Table 6)

Mammary plasma flow and mammary blood flow significantly increased ($P<0.05$) in rbST-treated animals than those of control animals. An increase in mammary blood flow coincided with an increase in milk yield in rbST-treated animals. The ratio of mammary blood flow to milk yield showed no significant differences between control animals and rbST-treated animals. The plasma IGF-1 concentration was significantly increased ($P<0.01$) in rbST-treated animals when compared with those of control animals during the treatment period.

Effects of rbST administration on milk composition (Table 7)

There were no significant differences of the concentration of protein and lactose concentration in milk between the control and rbST-treated animals. Milk fat concentration significantly ($P<0.05$) increased in rbST-treated animals when compared with the control animals. The concentration of milk fat of rbST-treated animals was significantly increased ($P<0.05$) in the treatment period when compared with the pretreatment period.

Table 1: Chemical composition of feed components (% on dry matter basis).

Particulars	Urea-treated rice straw	Concentrate
Dry matter	57.7	89.4
Crude protein	7.0	17.2
Acid detergent fibre	43.5	20.7
Neutral detergent fibre	70.2	28.4

Table 2: Dietary dry matter intake, water intake and milk yield in the control animals and animals treated with rbST. Values are means ± SD. (n = 5).

	Period of experiments	Control group	rbST group	Control vs rbSTgroup[1]
Dry matter intake:				
Total DM intake (kg/d)	Pretreatment	11.41 ± 0.66	12.30 ± 0.76	NS
	Treatment	11.64 ± 1.11	13.01 ± 1.67	NS
Total DM intake	Pretreatment	3.41 ± 0.38	3.40 ± 0.36	NS
(kg/100kg)	Treatment	3.26 ± 0.10	3.32 ± 0.27	NS
Water intake (l/d)	Pretreatment	58.66 ± 13.16	65.20 ± 10.57	NS
	Treatment	60.22 ± 12.31	70.89 ± 12.43**	NS
Milk yield (kg/d)	Pretreatment	12.98 ± 1.53	13.37 ± 2.66	NS
	Treatment	13.11 ± 1.85	16.02 ± 3.99*	NS
DM intake/Milk yield	Pretreatment	0.89 ± 0.12	0.96 ± 0.25	NS
	Treatment	0.90 ± 0.12	0.85 ± 0.22*	NS
Body weight (kg)	Pretreatment	336.9 ± 31.1	363.6 ± 27.1	NS
	Treatment	357.1 ± 34.0**	391.2 ± 35.6*	NS

P-values by paired t-test: * $P<0.05$, ** $P<0.01$, with respect to the pretreated period in the same group.

[1]Statistical analysis of treatment differences, NS = Nonsignificant ($P>0.05$).

Table 3: Plasma volume, blood volume and packed cell volume in the control animals and animals treated with rbST. Values are means ± SD. (n = 5).

	Period of experiments	Control group	rbST group	Control vs rbSTgroup[1]
Plasma volume:				
(l)	Pretreatment	16.0 ± 1.3	16.6 ± 1.4	NS
	Treatment	17.4 ± 1.9	19.4 ± 3.2*	NS
(l/100kg)	Pretreatment	4.7 ± 0.4	4.6 ± 0.2	NS
	Treatment	4.8 ± 0.2	4.9 ± 0.6	NS
Blood volume:				
(l)	Pretreatment	22.3± 2.0	23.3± 2.0	NS
	Treatment	24.6 ± 3.2	26.9 ± 4.7*	NS
(l/100kg)	Pretreatment	6.6 ± 0.5	6.4 ± 0.3	NS
	Treatment	6.9 ± 0.3	6.9 ± 0.8	NS
Hct (%)	Pretreatment	28.1 ± 1.5	28.6 ± 0.9	NS
	Treatment	29.2 ± 2.5	27.9 ± 1.4	NS
Plasma osmolality	Pretreatment	280 ± 4	274 ± 6	NS
(mOsm/kg)	Treatment	280 ± 5	276 ± 3	NS

P-values by paired t-test: * $P<0.05$, with respect to the pretreated period in the same group.

[1]Statistical analysis of treatment differences, NS = Nonsignificant ($P>0.05$).

Table 4: Changes in water turnover rate and total body water in the control animals and animals with rbST. Values are means ± SD. (n = 5).

	Period of experiments	Control group	rbST group	Control vs rbSTgroup[1]
Water turnover rate:				
(l/d)	Pretreatment	60.00 ± 13.57	69.63 ± 18.53	NS
	Treatment	60.65 ± 10.06	85.20 ± 19.35*	P<0.05
(l/100kg/d)	Pretreatment	17.52 ± 3.31	19.56 ± 5.50	NS
	Treatment	17.03 ± 2.70	22.11 ± 5.90	NS
(ml/$kg^{0.82}$/d)	Pretreatment	499.7 ± 97.7	564.4 ± 156	NS
	Treatment	490.0 ± 75.9	645.50 ± 168	NS
Biological half-life (d)	Pretreatment	3.25 ± 0.6	2.94 ± 0.78	NS
	Treatment	3.03 ± 0.44	2.60 ± 0.71	NS
TOH space:				
(l)	Pretreatment	268.7 ± 16.42	283.0 ± 8.7	NS
	Treatment	260.7 ± 14.75*	304.6 ±12.23**	P<0.01
(l/100kg)	Pretreatment	79.95 ± 3.04	78.13 ± 5.37	NS
	Treatment	73.23 ± 3.09**	78.22 ± 5.91	NS
Total body water:				
(l)	Pretreatment	246.6 ± 13.2	259.3 ± 7.5	NS
	Treatment	238.7 ± 11.6**	278.3 ± 11.3**	P<0.01
(l/100kg)	Pretreatment	73.42 ± 3.2	71.57 ± 5.01	NS
	Treatment	67.11 ± 3.4**	71.48 ± 5.49	NS

P-values by paired t-test: * P<0.05, ** P<0.01, with respect to the pretreated period in the same group.

[1]Statistical analysis of treatment differences, NS = Nonsignificant (P>0.05).

Table 5: Empty body water, gut water, extracellular fluid and intracellular fluid in the control animals and animals treated with rbST. Values are means ± SD. (n = 5).

	Period of experiments	Control group	rbST group	Control vs rbST group[1]
Empty body water:				
(l)	Pretreatment	145.84 ± 12.3	148.25 ± 11.21	NS
	Treatment	147.05 ± 7.02	163.81 ± 12.65	P<0.05
(l/100kg)	Pretreatment	43.34 ± 1.74	40.82 ± 2.41	NS
	Treatment	41.37 ± 2.75	42.09 ± 4.54	NS
Gut water:				
(l)	Pretreatment	100.76 ± 7.88	111.0 ± 7.52	NS
	Treatment	91.69 ± 6.77	114.5 ± 6.50	P<0.01
(l/100kg)	Pretreatment	30.08 ± 3.15	30.75 ± 4.10	NS
	Treatment	25.74 ± 1.44*	29.39 ± 2.25	P<0.05
Extracellular fluid:				
(l)	Pretreatment	76.55 ± 7.53	77.74 ± 9.25	NS
	Treatment	82.92 ± 11.18	88.61 ± 10.95*	NS
(l/100kg)	Pretreatment	22.87 ± 3.08	21.37 ± 1.79	NS
	Treatment	23.38 ± 3.96	22.65 ± 1.95	NS
Intracellular fluid:				
(l)	Pretreatment	170.1 ± 14.96	181.5 ± 9.26	NS
	Treatment	155.8 ± 16.06**	189.7 ± 4.61	P<0.01
(l/100kg)	Pretreatment	50.55 ± 2.95	50.20 ± 5.22	NS
	Treatment	43.73 ± 3.71	48.83 ± 4.87	NS

P-values by paired t-test: * P<0.05, ** P<0.01, with respect to the pretreated period in the same group.

[1]Statistical analysis of treatment differences, NS = Nonsignificant (P>0.05).

Table 6: Changes in mammary circulation and the plasma concentration of IGF-1 in the control animals and animals treated with rbST. Values are means ± SD. (n = 5).

	Period of experiments	Control group	rbSTgroup	Control vs rbSTgroup[1]
Mammary plasma flow	Pretreatment	2438 ± 331	2549 ± 342	NS
(ml/min)	Treatment	2730 ± 357	3927 ± 1203*	NS
Mammary blood flow	Pretreatment	3286 ± 461	3548 ± 463	NS
(ml/min)	Treatment	3817 ± 616	5310 ± 1620*	NS
Mammary blood flow/	Pretreatment	364 ± 25	397 ± 111	NS
Milk yield	Treatment	420 ± 32*	491 ± 152	NS
IGF-1 (ng/ml)	Pretreatment	40 ± 15	50 ± 29	NS
	Treatment	48 ± 16	209 ± 42**	P<0.01

P-values by paired t-test: * P<0.05,** P<0.01, with respect to the pretreated period in the same group.

[1]Statistical analysis of treatment differences, NS = Nonsignificant (P>0.05).

Table 7: Milk compositions in the control animals and animals treated with rbST.

Values are means ± SD. (n= 5).

	Period of experiments	Control group	rbSTgroup	Control vs rbSTgroup[1]
Milk composition:				
Protein (gm%)	Pretreatment	3.15 ± 0.21	3.16 ± 0.16	NS
	Treatment	3.27 ± 0.15	3.16 ± 0.25	NS
Fat (gm%)	Pretreatment	3.60 ± 0.76	3.90 ± 0.60	NS
	Treatment	3.60 ± 0.25	4.70 ± 0.77*	P<0.05
Lactose (gm%)	Pretreatment	4.49 ± 1.02	4.90 ± 0.24	NS
	Treatment	4.52 ± 0.55	4.79 ± 0.49	NS

P-values by paired t-test: * P<0.05, with respect to the pretreated period in the same group.

[1]Statistical analysis of treatment differences, NS = Nonsignificant (P>0.05).

DISCUSSION

The present study was designed to clarify whether a shorter lactation persistency of crossbred cattle containing 87.5%Holstein genes during lactation advance was due to the reduction of the growth hormone level (Chaiyabutr et al., 2000) or associated with some other mechanisms. We found that in the rbST-treated animal, milk yield over the 6 wk of the experiment significantly increased (by 19.8%) in the rbST - treated animals. Milk yield of the control animals receiving placebo slightly increased in the early period of lactation. Mishra and Shkla (2004) also reported higher milk yield of 25 % due to exogenouse administration of rbST after 60 days of postpartum in lactating buffalo. 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). In the present study, total DM intake was not significantly different between control animals and rbST-treated animals throughout experimental period. However, the effect of rbST administration significantly influenced the milk production efficiency. The ratio of dry matter intake to milk production was lower in rbST-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 rbST-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 rbST-treated animals increased water intake in the early period of lactation from 65 to 71 kg/day/animal, 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 rbST-treated animals increased body fluid compartments i.e. TBW, EBW and plasma volume, while the control animals decreased TBW with that of a higher milk secretion in the early period of lactation. An increase in the EBW in rbST-treated animals would be due to an increase in ECF compartment, while ICF compartment did not change through the period of study. Thiocyanate space does not include rumen water; therefore changes of ruminal fluid volume would not affect an estimation of extracellular volume (Woodford et al., 1984). An increase in water intake with rbST treatment in early lactation would contribute to an elevation of gut water content.

An increase in both absolute TBW and ECF of rbST-treated animals agrees with the report in GH deficient human treated with GH (Janssen et al., 1997). An elevation of body weight with rbST treatment would be the direct effect of somatotropin on increases in body cell mass and fat free mass. High milk yield during early lactation usually occurs with negative energy balance with body fat mobilization causing a decrease in fat mass. This may be attributed to an increase in body water with rbST treatment. 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). The sodium retaining by the effect of somatotropin on the renal tubular reabsorption of sodium would be another explanation for an expansion of both TBW and ECF.

A higher water reserve in animals given rbST 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 control animals from early to mid-lactation occurred rather rapidly which may be attributed to a relatively lower efficiency in the water retention mechanism in crossbred cattle containing

87.5%Holstein genes although the estimated water intake was slightly higher (Chaiyabutr et al., 1997). In the present study, the rbST-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 control animals. This indicates that increased losses of water with increase in milk yield with rbST treatment might be compensated by a larger body water pool, which animals could restore their body fluids to equilibrium in lactating period with no significant change of body water turnover rate and water half-life. Short persistency of lactation may not be occurred in rbST-treated animals during transition period from early to midlactation. This is a case in which a response pattern in milk yield of rbST-treated animals differed from that in early lactation of crossbred cattle containing 87.5%Holstein genes (Chaiyabutr et al 1999).

In the present study, increases in mammary blood flow to the udder of rbST-treated animals agree with several reports in both cows and goat (Davis et al., 1988; Mepham et al; 1984). A marked increase in mammary blood flow of rbST-treated animals could not be attributed to a change in blood volume and plasma volume, which remained nearly constant as a percent of body weight. 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).

The present results confirm the study in both cows and goats that the plasma IGF-1 level increased in response to growth hormone treatment. (Davis et al., 1988; Gulay et al., 2004). Several investigations show the effect of rbST on mammary circulation was indirect, mediated via IGF-1 (Capuco et al., 2001), whereas other works have demonstrated the direct effect of IGF-1 on an increase in the mammary blood flow and increase in milk production (Etherton and Bauman, 1998). An elevation of both plasma IGF-1 concentration and udder blood flow was also noted in

late lactating crossbred cows treated with rbST (Tanwattana et al., 2003). The present study confirms that mammary blood flow is a major determining factor for supply of nutrients for milk synthesis and follows the pattern of changes of milk yield.

Milk fat content of rbST-treated animals was increased, while milk protein and milk lactose were not changed by rbST 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 rbST injection has been observed previously (West et al., 1990). Milk fat content of cows in positive energy balance is not influenced by rbST treatment, and milk fat yield follows the trend of milk production (West et al., 1990). However, an increase in milk fat after rbST 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 rbST 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, rbST 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 rbST-treated animals might be partly resulted from the decrease in fat mass during early lactation. The present results indicate that growth hormone affecting mammary gland function might not be mediated solely by the action of IGF-1 on an increase in blood flow to mammary gland. The lack of effect of higher plasma IGF-1 levels in regulating mammary blood flow and milk yield in crossbred dairy cattle has also been noted (Chaiyabutr et al., 2003). An elevation of body fluid particularly blood volume (+15 %) despised large increases in mammary blood flow (+50 %)

during rbST treatment. These observations could suggest that a marked increase in blood flow through the mammary glands resulting from rbST administration would be achieved in part by local vasodilatation (Linzell, 1974), causing in distribution of milk precursors to the gland.

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CHAPTER IV

Effects of Long-term Exogenous Bovine Somatotropin on Water Metabolism and Milk Yield in Crossbred Holstein Cattle

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(Running head : SOMATOTROPIN AND WATER METABOLISM)

Effects of Long-term Exogenous Bovine Somatotropin on Water Metabolism and Milk Yield in Crossbred Holstein Cattle

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ABSTRACT

This study was designed to clarify whether the short lactation persistency occurring in the crossbred cattle in the tropics would be affected by a reduction in circulating growth hormone in association with changes of body fluid and mammary blood flow. Ten, first lactation, 87.5% Holstein Friesian (HF) animals were chosen and divided into two groups of 5 animals each. Four consecutive study periods were carried out in each group. These consisted of a pretreatment period (45 days post-partum)(pre-peak lactation) and three treatment periods during early lactation(105 days post-partum, mid-lactation (165 days post-partum) and late lactation (225 days post-partum). After 60 days of lactation, animals were injected sub-cutaneously biweekly intervals until the end of study with 500 mg of recombinant bovine somatotropin (rbST). The milk yield per day of rbST-treated animals increased in early lactation (19.8 %), mid-lactation (9.5%) and decreased in late lactation (-2.7%) when compared with the pretreatment period. Absolute values of total body water (TBW), extracellular water (ECW), plasma volume and blood volume were significantly increased during rbST treatment. The estimated value of intracellular water (ICW) of the rbST-treated animals showed no differences, whereas it significantly decreased in the control animals during early and mid-lactation. The water turnover rate (WTO) of rbST-treated animals significantly increased in early and mid-lactation. Mammary blood flow (MBF) significantly increased during rbST administration in all stages of lactation. These data demonstrated that the rbST exerts its galactopoietic action, in part, through increases in both the TBW and ECW in association with an increase in MBF for milk production.

(**Key words:** exogenous bovine somatotropin, water metabolism, crossbred Holstein cattle,milk yield)

Abbreviation key: HF = Holstein Friesian, **MBF** = mammary blood flow, **TBW** = total body water, **WTO** = water turnover rate, **ECW** = extracellular water, **ICW** = intracellular water, **rbST** = recombinant bovine somatotropin.

INTRODUCTION

Many factors can affect milk production in dairy cattle in the hot-humid tropics including high environmental temperatures, lower genetic potential for milk production in indigenous cattle and inadequate supply of food during the dry summer months. Several approaches have been attempted to try to improve dairy productivity in the tropics. Crossbreeding of indigenous and exotic cattle for tropical use has been exploited as an efficient tool for blending the adaptability of tropical cattle with the high milking potential of exotic breeds, resulting in increased milk production. There is still a need to identify the types of crossbred cattle that are the most suitable for the tropics. During one study on the regulation of body fluids and mammary blood flow (**MBF**) in different types of crossbred Holstein Friesians (**HF**) cattle (Chaiyabutr et al., 1997; 2000a), it was noted that 50%HF animals showed differences in the distribution of their body fluids and MBF from 87.5%HF animals during late pregnancy and different stages of lactation. The 87.5%HF animals had lower efficiency in water retention mechanism and poor adaptation to tropical environment, in comparison to 50%HF animals (Chaiyabutr et al., 2000a). A low persistent lactation yield, with a decrease in MBF during the transition period from early to mid-lactation, was noted in the 87.5%HF animals. MBF has been known to be a

major determinant for the rate of substrate supply for milk synthesis (Davis and Collier, 1985). The control mechanism for MBF in different stages of lactation in crossbred dairy cattle has not been fully elucidated. Differences between animals partitioning abilities are known to be inherited and are thought to be under endocrine control with a homeorrhetic principle in bovine lactation. Bovine somatotropin (bST) is known as a homeorrhetic hormone connected with both growth and lactation. The importance of bST for maintaining milk output in ruminant is well established (Bauman, 1992). Although a number of reviews have been published on the relationship between the plasma bST concentration and milk yield in both normal and hot environments (West et al., 1991; Johnson et al., 1991), the role of bST in body water regulation, in relationship to persistent lactation in crossbred dairy cattle in the tropics is not yet clear.

During lactation, an alteration in many bodily functions is apparent; for example, blood volume and cardiac output are increased (Chaiyabutr et al., 1997; Hanwell and Peaker, 1977) and blood flow in many parts of body is increased including MBF. It was reported that a decrease in milk yield was related to reductions in MBF and circulating bST as lactation advances to mid- and late lactation in 87.5%HF animals (Chaiyabutr et al., 2000a, 2000b). It is not known which factors are the cause and which factors are the effect for such a reduction and whether a high level of bST increases the metabolic rate (Tyrrell et al.,1988); as such an effect would make thermoregulation in a tropical environment more difficult as lactation advances. These changes were not apparent in crossbred dairy cattle containing 50%Holstein genes (Chaiyabutr et al., 2000b). There are few studies on the mechanisms acting within the body of crossbred cattle concerning the role of bST on water metabolism, in relation to persistent lactation, although an elevation

of total body water (**TBW**) and extracellular water (**ECW**) was noted in humans deficient in growth hormone, after taking injections of human somatotropin (Janssen et al., 1997).

To provide some of this information, the present experiment was carried out to determine whether recombinant bovine somatotropin (**rbST**) played an important role in maintaining milk yield in association with changes of body fluids and MBF, in crossbred dairy cattle containing 87.5%Holstein genes. Long-term treatment with rbST at different stages lactation were carried out to obtain a more complete picture of the role of somatotropin on lactation persistency, in crossbred dairy cattle in the tropics.

MATERIALS AND METHODS

Animals and Management

Ten, first lactation, non-pregnant, 87.5%HF dairy cattle were selected for the experiment. They were divided into two groups, five animals in each. Animals in each group were fed with rice straw treated with 5% urea, as the source of roughage throughout the experiments. All animals were housed in sheds, tethered in individual stalls and fed twice daily. The ambient temperature was recorded by a dry bulb thermometer. The relative humidity was calculated from the reading of dry and wet bulb thermometers. The maximum temperature in the shed at noon was 34±1°C and the minimum temperature at night was 26±1°C. The relative humidity was 68±12%. Animals received an average of 4 kg/d of roughage in combination with a concentrated mixture (7kg/d), to maintain a moderate body condition score 2.5 during the experiment, (scale = 1 to 5)(Wildman et al.,1982). The chemical composition of the feed is presented in Table 1. The dry matter intake (DMI) of each animal was determined by measuring both the concentrate and roughage offered and subtracting the amount refused each day. Urea

treated rice straw was offered four times a day at 08.oo, 12.00, 16.00 and 20.00h. Concentration was fed two times at 0800 and 1400h. Each day, during feeding trial, sub-sample of both feed was collected for dry matter determination. Feed sample was collected every day and kept at -20 C for chemical analysis. Animals had free access to water and were fed their respective rations throughout the experimental period.

Experimental Procedures

Animals were divided into control (n=5) and experimental (n=5) groups. Four consecutive study periods were carried out in each group. These consisted of a pretreatment period (45 days post-partum)(pre-peak lactation) and three treatment periods during early lactation(105 days post-partum), mid-lactation (165 days post-partum) and late lactation (225 days post-partum). After 60 days of lactation, animals were injected sub-cutaneously biweekly intervals until the end of study with 500 mg of recombinant bovine somatotropin (rbST) suspended in 792 mg of a prolonged-release formulation of sesame oil (POSILAC, Monsanto, USA). Animals in the control group were injected subcutaneously biweekly intervals with 800 mg of sterile sesame oil without rbST, as a placebo. Injections were administered at the tail head depression (ischiorectal fossa). Animals of both groups were fed the same ration, from before parturition and throughout the study. The measurement of daily water consumption of each animal was calculated by weighing the individual water bowl of each animal. The daily water intake per animal in each period of lactation was recorded by averaging over seven days. Animals were normally milked at around 0600 h and 1700 h using a milking machine and milk production was recorded daily. Milk yield per day per animal was recorded at each period

of lactation. Animals were weighed after collecting the milk sample in each specified day.

To measure MBF and to collect venous blood, cows were cannulated on the specified day before the experiment began at each period. While the cow was standing, two catheters (i.d. 1.0 mm, o.d. 1.3 mm, L 45 mm) were inserted into either the left or right milk vein using a intravenous polymer catheter (Jelco, Critikon; Johnson & Johnson, U.K.), under local anesthesia. The tip of the catheter was positioned near the sigmoid flexure anterior to the point at which the vein leaves the udder. The other catheter was positioned downstream about 20 cm from the first one. The catheter for isotope infusion and dye injection was inserted into an ear vein, under local anesthesia. All catheters were flushed with sterile, heparinized, normal saline (heparin 25 i.u./ml normal saline) and were left in place during the experiment.

Determinations of MBF and Water Metabolism

MBF through half of the udder was determined by measuring the dilution of dye T-1824 (Evans blue) using short term continuous infusion and adapted from the method of measuring blood flow in the milk veins of cattle as previously described (Chaiyabutr et al., 1997).

The water turnover rate (**WTO**) and TBW were determined by tritiated water dilution techniques using a single dose intravenous injection of 3,000 μci per animal, of carrier free tritiated water in normal saline. The equilibrium time was determined by taking blood samples for 3 days after the injection. Blood samples for measurements of WTO, biological half-life of tritiated water, TBW and the total body water space (**TOH**), using a correction factor from the plasma solids concentration, were performed as previously

described (Chaiyabutr et al., 1997). The plasma solids concentration was determined by a refractometer.

In each animal per period, the injection of 20 ml of sodium thiocyanate solution (10 g/100 ml normal saline) and 20 ml of the Evans blue dye (T-1824) (0.5 g/100 ml normal saline) was given via a ear vein catheter to estimate ECW volume and the plasma volume, respectively. Venous blood samples from the jugular vein were taken at 20, 30, 40 and 50 min after dye injection. Dilution of dye at zero time was determined by using a semi logarithmic concentration on time extrapolation. Blood volume was calculated from the plasma volume and packed cell volume (Chaiyabutr et al., 1980). The measurement method for ECW was modified from the method used by Medway and Kare (1959). Intracellular water (**ICW**) was calculated by subtracting ECW from TBW. Plasma osmolality was measured using the freezing point depression method (Advance Osmometer model 3, U.S.A.).

Statistical Analyses

The experimental results were examined statistically by a paired t-test for variables within a treatment which were compared against the pretreatment values in the same group. Mean values of variables within a period were compared across treatments between group by an unpaired t-test. Mean values are presented as mean±S.D.

RESULTS

DM intake, water intake, milk yield and body weight in the controls and rbST-treated animals are shown in Table2. The total, daily, DM intakes were not significantly different between the controls and rbST-treated animals, during the experimental periods. No significant increases occurred in the daily water intake in both early and mid-lactation of

the control animals but rose in late lactation, when compared with the pre-treatment period. In contrast, the daily water intake increased significantly stepwise as lactation advanced in the rbST-treated animals. Animals receiving rbST for 45 days, significantly increased ($P<0.01$) their milk yield from 13.4±2.6 kg/d during the pre-treatment period to16.0±2.1 kg/d (19.8 %) over early lactation and for the 105 days period, milk yield increased by 9.5% in mid-lactation, while animals received rbST for 165 days, milk yield decreased by 2.7%, all in comparison with the pre-treatment period. Milk yield of the rbST-treated animals increased significantly ($P<0.05$) above the milk yield of the control animals in the early period of lactation and continued at a high level throughout lactation. However, peak yields occurred during the early period of lactation; thereafter yields declined in both groups as lactation advanced. A DMI : Milk yield ratio was calculated and used as indicator of the efficiency of conversion of nutrients to milk. The mean ratio of total DMI to milk yield of rbST-treated animals were significantly decreased ($P<0.05$) after rbST administration in the early period of lactation. The mean ratio of DMI to milk yield of the control animals showed no significant changes throughout lactation. Body weights of both control animals and rbST-treated animals significantly rose ($P<0.01$) in a stepwise fashion above their initial weights in the pretreatment period but rbST-treated animals had a greater percentage change than those of the control animals throughout the lactation (by average 7.8 vs. 5.9%, 13.3 vs. 9.6% and 15.6 vs. 11.6 % for early, mid- and late lactation, respectively).

The control animals showed no significant changes in plasma volume or blood volume either in terms of absolute values or the relative values as a percentage of body weight, throughout the course of their lactation (Table 3). The absolute value of plasma volume and blood volume of rbST-treated animals significantly increased ($P<0.05$) but the

relative values as a percentage of body weight were unchanged during the course of treatment. The packed cell volume and plasma osmolality of both the control animals and rbST-treated animals were unchanged throughout lactation. An increase ($P<0.05$) in the absolute values of ECW was observed in rbST-treated animals, while there were no significant changes in the control animals throughout lactation. The value of ECW as a percentage of body weight in both the control and the rbST-treated animals was unchanged throughout periods of study. The estimated value of ICW in the control animals significantly decreased ($P<0.05$) in the early and mid lactation periods, while the rbST-treated animals showed no differences during the course of all treatments. The ICW of the rbST-treated animals increased significantly ($P<0.001$) above the ICW of the control animals in the early period of lactation and continued at a high level throughout lactation.

The average WTO and the WTO per fat free, wet, body weight ($kg^{0.82}$)(MacFarlane and Howard,1972) were significantly higher, while the biological half-life of tritiated water was significantly shorter in mid and late lactation as compared with the pre-treatment period in the control animals (Table 4). The rbST-treated animals showed significantly increased ($P<0.05$) WTO during mid- and late lactation. In the treated animals, receiving rbST for 45 days, the WTO was significantly higher ($P<0.05$) when compared with the control animals over a similar period. The WTO per fat free, wet, body weight ($kg^{0.82}$) and the biological half-life of tritiated water in rbST-treated animals were unchanged throughout lactation. In both early and mid-lactation periods, the control animals showed significant reductions($P<0.05$) in both TOH and TBW as the percentage of body weight. In contrast to the control animals, absolute values of TOH and TBW of rbST-treated animals were significantly higher ($P<0.01$) than those of the control animals over similar

periods during early and mid lactation. The values of TOH and TBW as a percentage of body weight of rbST-treated animals showed no significant differences during all periods of lactation.

MBF increased significantly ($P<0.05$) during rbST administration in both early and mid lactation, while there were no significant changes in all periods of lactation in the control animals (Table 5). The ratio of MBF to milk yield slightly increased as lactation advance in both the control animals and the rbST-treated animals.

Table 1. Chemical composition of feeds used in the experiment (% on dry matter basis)

Particulars	Urea-treated rice straw	Concentrate
Dry matter	58.0	89.4
Crude protein	8.9	17.8
Acid detergent fibre	61.2	21.5
Neutral detergent fibre	67.2	28.8
Lignin	8.8	7.0
Ash	16.8	5.6

Concentrate formulation: fresh weight (kg/100 kg) consisted of soy bean meal 30 kg, cotton seed 25 kg, cassava 25 kg, rice bran 15 kg, di-calcium phosphate 2 kg, sodium bicarbonate 1.7 kg, potassium chloride 0.7 kg and vitamin/mineral premix 0.6 kg.

The urea treated rice straw was prepared by mixing a urea solution (5 kg urea dissolved in 100 litres water per 100 kg dry rice straw) with dry straw and stored in airtight conditions in a cement pit for 21 days before being offered to the animals.

Table 2. Means ± SD of DMI, water intake, milk yield and body weight in different stages of lactation of 87.5%HF animals for the control group[1] and rbST-treated group[1].

Measurement	Lactation period[2]	Control Group	rbST Group	Contrasts[3]
DMI, kg/d	Pretreated	11.4±0.7	12.3±0.8	NS
	Early	11.6±1.1	13.0±1.7	NS
	Mid	12.2±1.8	13.9±1.3	NS
	Late	12.3±1.8	13.4±1.7	NS
Water intake, kg/d	Pretreated	58.7±13.2	65.2±10.6	NS
	Early	60.2±12.3	70.9±12.4**	NS
	Mid	73.7±16.2	74.2±8.9*	NS
	Late	74.1±19.4*	75.3±12.4**	NS
Milk yield, kg/d	Pretreated	13.0±1.5	13.4±2.7	NS
	Early	13.1±1.8	16.0±2.1**	P<0.05
	Mid	12.9±1.5	14.6±1.9	NS
	Late	11.5±1.0	13.0±1.3	NS
DMI/Milk yield, kg/kg	Pretreated	0.9±0.12	1.0±0.25	NS
	Early	0.9±0.12	0.9±0.22*	NS
	Mid	1.0±0.13	1.0±0.06	NS
	Late	1.1±0.19	1.0±0.12	NS
Body weight, kg	Pretreated	337±31	364±27	NS
	Early	357±34**	391±36**	NS
	Mid	370±34**	412±36***	NS
	Late	379±29**	420±43**	NS

[1]For the control group, n =5; for rbST- treated group, n=5.

[2]Statistical test of P-values between periods of lactation in each group using paired t-test of * (P<0.05), ** (P<0.01), *** (P<0.001), with respect to pretreated period.

[3]Contrasts : comparison of P-values of control group vs. rbST-treated group using unpaired t-test, NS=not significant.

Table 3. Means ± SD of plasma volume, blood volume, packed cell volume, plasma osmolality (Posm), extracellular water (ECW) and intracellular water (ICW) in different stages of lactation of 87.5%HF animals for the control group[1] and rbST-treated group[1].

Measurement	Lactation period[2]	Control Group	rbST Group	Contrasts[3]
Plasma volume, L	Pretreated	16.0±1.3	16.6±1.4	NS
	Early	17.4±1.9	19.4±3.2*	NS
	Mid	17.1±2.0	20.2±2.0**	P<0.05
	Late	17.0±0.8	20.5±3.4**	P<0.05
Plasma volume, L/100 kg	Pretreated	4.7±0.4	4.6±0.2	NS
	Early	4.8±0.2	4.9±0.5	NS
	Mid	4.6±0.3	4.9±0.6	NS
	Late	4.5±0.3	4.8±0.6	NS
Blood volume, L	Pretreated	22.2±2.0	23.3±2.0	NS
	Early	24.6±3.2	26.9±4.7*	NS
	Mid	24.3±3.0	28.3±2.8**	NS
	Late	24.0±1.0	28.4±5.1*	NS
Blood volume, L/100 kg	Pretreated	6.6±0.5	6.4±0.3	NS
	Early	6.8±0.3	6.8±0.7	NS
	Mid	6.6±0.6	6.9±0.8	NS
	Late	6.3±0.4	6.8±0.8	NS
Packed cell volume, %	Pretreated	28.1±1.5	28.6±0.9	NS
	Early	29.2±2.5	27.9±1.4	NS
	Mid	29.5±2.3	28.6±0.5	NS
	Late	28.9±1.1	27.6±0.9	NS
Posm, mOsm/kg	Pretreated	280±4	274±6	NS
	Early	280±5	276±3	NS
	Mid	280±2	277±6	NS
	Late	286±1	279±5	P<0.01
ECW, L	Pretreated	76.5±7.5	77.7±9.3	NS
	Early	82.9±11.2	88.6±10.9*	NS
	Mid	81.8±12.4	97.5±18.5*	NS
	Late	86.6±11.5*	106.1±22.2*	NS
ECW, L/100 kg	Pretreated	22.8±3.1	21.4±1.8	NS
	Early	23.4±3.9	22.6±2.0	NS
	Mid	22.2±3.5	23.5±3.0	NS
	Late	22.8±2.1	25.1±3.7	NS
ICW, L	Pretreated	170.0±14.9	181.5±9.3	NS
	Early	155.8±16.1**	189.7±4.6	P<0.001
	Mid	163.6±19.2	194.4±27.9	NS
	Late	173.5±29.2	183.9±31.7	NS
ICW, L/100 kg	Pretreated	50.6±3.0	50.2±5.2	NS
	Early	43.7±3.7**	48.8±4.9	NS
	Mid	44.4±5.4*	47.4±7.7	NS
	Late	45.7±6.1	43.8±6.9	NS

[1]For the control group, n=5; for rbST-treated group, n=5.

[2]Statistical test of P-values between periods of lactation in each group using paired t-test of * (P<0.05), ** (P<0.01), wi respect to pretreated period.

[3]Comparison of P-values of control group vs. rbST-treated group using unpaired t-test, NS= not significant.

Table 4. Means±SD of the water turnover rate(WTO), total body water (TBW), total body water space (TOH) and the biological half-life of tritiated water in different stages of lactation of 87.5%HF animals for the control group[1] and rbST-treated group[1].

Measurement	Lactation period[2]	Control Group	rbST Group	Contrasts[3]
WTO, L/d	Pretreated	60.0±13.5	70.6±18.5	NS
	Early	60.6±10.1	85.2±19.4*	P<0.05
	Mid	71.5±11.5***	95.7±36.7*	NS
	Late	73.4±16.3*	92.6±32.9	NS
WTO, L/100kg/d	Pretreated	17.5±3.3	19.6±5.5	NS
	Early	17.0±2.7	22.1±5.9	NS
	Mid	19.3±2.1	23.4±9.0	NS
	Late	19.2±3.4	21.8±6.8	NS
WTO, ml/kg$^{0.82}$/d	Pretreated	499.7±97.7	564.4±156.0	NS
	Early	490.0±75.9	645.5±168.1	NS
	Mid	558.6±64.0*	690.5±265.1	NS
	Late	560.9±103.6*	647.4±205.9	NS
Biological half-life, d	Pretreated	3.2±0.6	2.9±0.8	NS
	Early	3.0±0.4	2.6±0.7	NS
	Mid	2.6±0.4**	2.6±0.9	NS
	Late	2.7±0.5*	2.6±0.7	NS
TOH space, L	Pretreated	268.7±16.4	283.0±8.7	NS
	Early	260.7±14.7*	304.6±12.2**	P<0.001
	Mid	268.6±12.4	320.9±34.6*	P<0.01
	Late	286.2±33.9*	318.0±38.8	NS
TOH space, L/100 kg	Pretreated	79.9±3.0	78.13±5.4	NS
	Early	73.2±3.1**	78.22±5.1	NS
	Mid	72.9±4.7*	78.0±7.5	NS
	Late	75.4±4.6	75.6±5.4	NS
TBW, L	Pretreated	246.6±13.2	259.2±7.5	NS
	Early	238.7±11.6**	278.3±11.3**	P<0.001
	Mid	245.4±12.4	291.9±32.7*	P<0.01
	Late	260.1±31.7	290.0±35.1	NS
TBW, L/100 kg	Pretreated	73.4±3.2	71.6±5.0	NS
	Early	67.1±3.4***	71.5±5.5	NS
	Mid	66.6±4.1**	70.9±7.0	NS
	Late	68.5±4.5	68.9±4.9	NS

[1]For the control group, n=5; for rbST-treated group, n=5

[2]Statistical test of P-values between periods of lactation in each group using paired t-test of * (P<0.05), ** (P<0.01), ***(P<0.001) with respect to pretreated period.

[3]Comparison of P-values of control group vs. rbST-treated group using unpaired t-test, NS= not significant.

Table 5. Means±SD of mammary plasma flow(MPF), mammary blood flow(MBF), and the ratio MBF/ Milk yield in different stages of lactation of 87.5%HF animals for the control group[1] and rbST-treated group[1].

Measurement	Lactation period[2]	Control Group	rbST Group	Contrasts[3]
MPF, ml/min	Pretreated	2438±331	2594±342	NS
	Early	2730±357	3927±1203*	NS
	Mid	2698±319	3983±1183*	NS
	Late	2692±290	3533±1055	NS
MBF, ml/min	Pretreated	3286±461	3548±463	NS
	Early	3817±616	5310±1620*	NS
	Mid	3821±533	5458±1627*	NS
	Late	3750±476	4814±1464	NS
MBF/ Milk yield, L/kg	Pretreated	364±25	397±111.	NS
	Early	420±32	491±152	NS
	Mid	433±89	539±156	NS
	Late	471±69	539±168	NS

[1]For the control group, n=5; for rbST-treated group, n=5.

[2]Statistical test of P-values between periods of lactation in each group using paired t-test of * ($P<0.05$), with respect to pretreated period.

[3]Comparison of P-values of control group vs. rbST-treated group using unpaired t-test, NS= not significant.

DISCUSSION

The present study was designed to clarify whether poorer lactation persistency in crossbred cattle containing 87.5%Holstein genes was affected by a reduction in circulating growth hormone in association with changes of body fluid and mammary circulation. Long-term treatment with rbST was administered to 87.5%HF animals that had completed 60 days of lactation prior to the experiment. The 500 mg of rbST used biweekly intervals in the present study is the dose rate recommended for *Bos taurus* cows. This treatment of rbST was initiated at the earlier stage of lactation, milk yield increased in early lactation (+19.8 %) and in mid-lactation (+9.5%), but it decreased by 2.7% during late lactation in comparison with the pretreatment period. Low responses in milk yield during rbST treatment in the later stage of lactation are similar as previously reported in dairy crossbred cattle (Phipps et al., 1991). A rapid decline of yield resulting the shorter persistency of lactation of rbST-treated animals seems to be similar to those which occur in higher yielding cows (Chase, 1993). These results indicated that an increase in milk yield of dairy crossbred cattle, in response to rbST administration, will not be sustained for long, being influenced by stage of lactation.

Animals in both groups were fed ad libitum and total DMI were not significantly different between control animals and rbST-treated animals, throughout the experimental periods. However, the ratio of DMI to milk yield of rbST-treated animals, was lower in early lactation when compared with the pretreatment period but animals still gained weight throughout the experiment in both groups. It has been known that the support of milk secretion would come through provision of substrate and stimulation of mammary cell activity. Unfortunately, the present studies on the mammary cell activity were not available. The rbST increased milk yield relating to mammary cell activity appears

contradictory. Whereas some studies show no mammogenic effect of bST (Binelli et al., 1995), other studies show a possible mammogenic effect when cattle are administered bST (Knight et al., 1992). It indicates that the increased milk yield with rbST treatment in the present study is rather dependent upon the adequacy of the nutritional provision than the mobilization of body stores. A marked increase in milk yield with rbST treatment without loss of body weight, especially during early lactation, may be due to the fact that the animals were well fed to allow an adequate replacement of body reserves. Milk yield in the first lactation of crossbred animals in the present study would be lesser than those of multiparous cows (Sullivan et al. 1992), which is possibly related to the continued weight increase observed in animals during their first lactation. These results provide the physiological differences between crossbred animals and exotic breeds in partitioning ability, which would be inherited and capacity for milk production. Thus, the metabolic demands of lactation of the crossbred HF animals would be met by dietary intake during early lactation. In our previous report of the same line experiment (Chaiyabutr et al., 2005), no mobilization of body tissues as indicated by no alteration of the plasma levels of both triglyceride and glucose was noted in crossbred HF animals treated with rbST. Triglyceride has been known to restore during period of excess energy availability and are mobilized during periods of energy deprivation. No significant change in the plasma triglyceride concentration could be attributed to the higher milk production in the rbST- treated animals as diversion of surplus nutrient from diet for milk synthesis.

The rbST-treated animals increased water intake in the early period of lactation, from 65.2 kg/d to 70.9 kg/d, which is about 9%, accounting for 19.8% of their pre-treatment milk yield. An increase in milk yield which general contain 87% of water would account

for most of the increased water intake as lactation advanced in the rbST-treated animals. This result shows that milk production affects water intake, including the body water turnover rate. The rbST-treated animals increased body fluid compartments throughout all periods of study i.e. TBW, ECW and blood volume, while the control animals decreased TBW in comparison to pretreatment values in the early period of lactation. An increase in ECW would be influenced by an increase in voluntary intake (MacFarlane et al., 1959), which has been reported to occur after a few weeks of rbST administration (Coghlan et al., 1977). However, the ECW compartment did not include rumen water; thus any changes of ruminal fluid volume should not affect the determination of extracellular fluid volume. These results indicate that somatotropin plays an important role in water regulation and probably relating to the galactopoietic effect. Although the mechanisms responsible for water regulation are not yet fully known in ruminants, the expansion of ECW and TBW after growth hormone administration has been noted in growth hormone deficient humans (Janssen et al., 1997). As lactation advances, animals gained more live weight in both the control and the rbST-treated animals. However, a greater percentage increase in live weight of rbST-treated animals could be considered, at least in part, to be the direct effect of somatotropin on the increased body cell mass. This would be attributable to an accumulation of body water. The sodium retention effect of somatotropin on the renal tubular reabsorption of sodium (Wyse et al.,1993) while retaining constant plasma osmolality in the present result, would be another explanation for explaining water retention in the ECW compartment.

The high body water content of rbST-treated animals seems to be related to the adaptation of the animals to a tropical environment. An increase in both metabolic activity and heat production has been reported in bST-treated cows (West et al., 1991).

However, it was suggested that even though bST increases heat production, it also increases heat dissipation (Johnson et al., 1991, West et al., 1994). In the present study, the higher TBW and ECW of animals receiving rbST would not only provide a higher reservoir of soluble metabolites for biosynthesis of milk but also slow down any elevation of body temperature during lactation in hot conditions. In the present study, animals in both groups were not pregnant and were housed in the same shed in the same environment. Thus, a change in the water turnover rate of both groups of crossbred cattle was not influenced by the effect of pregnancy (Chaiyabutr et al., 1997) or changes in environmental conditions (Ranjhan et al., 1982). However, the rbST-treated animals showed no significant changes in the water turnover rate per fat free, wet, body weight ($kg^{0.82}$) and the biological half-life of tritiated water, in any periods measured in the current experiment, in comparison to the control animals. This indicates that water loss with the increase in milk yield of the rbST-treated animals might be compensated by a larger body water pool, which restores their body fluids to equilibrium, with no *significant changes of body water turnover rate and water half-life.* In contrast to the rbST-treated animals, the biological half-life of tritiated water in the control animals was significantly shorter, while the water turnover rate was significantly higher as lactation advanced to mid and late lactation. These changes would be due to the process of lactation requiring more water and more loss of water secretion in milk, which is generally known to be about 87% and would account for these phenomena. The control animals being 87.5%HF were genetically similar to the exotic *bos taurus* breed which might lead to poor adjustment in a tropical environment (Chaiyabutr et al., 2000a; Nakamura et al., 1993). The TBW and ICW of the control animals showed to be decreased during advanced lactation; it should be assumed that these changes are the

factors influencing lactation persistency. Animals could not maintain their body fluids which resulted in the rapid approach of the end of their normal short lactation.

The marked increase in the MBF was apparent in rbST-treated animals throughout lactation. This result supported other findings showing increases in MBF and milk secretion in both goats and cows given exogenous growth hormone (Hart et al., 1980; Davis et al., 1988). An increase in MBF has been shown to be the effect of an increase in cardiac output perfusing to the udder without any alteration in heart rate during growth hormone treatment (Davis et al., 1988). In the present results, an increase in both blood volume and plasma volume in rbST-treated animals would provide a greater venous return and stroke volume for increase in cardiac output, resulting in increased the blood supply to the mammary gland. Thus, the rate at which the milk yield elevated after the peak period when compared with the control animals, could have been due primarily to an increased availability of substrates for the mammary gland. However, observations in both the control animals and rbST-treated animals showed an increase in a ratio of MBF/milk yield as lactation advanced. The resultant progressive decline in milk yield of rbST-treated animals with still a higher level of either MBF or ECW, could be accounted for by changes in intra-mammary factors. Since it has been reported that the effect of somatotropin on MBF occurs by a mechanism which does not involve the direct action of somatotropin on the udder (Collier et al., 1984). In addition, study in vitro suggests that bST does not directly stimulate mammary secretory function (Gertler et al., 1983). The indirect action of rbST on mammary function may occur through some other agent e.g. insulin like growth factor-I, as administration of rbST in late, lactating, crossbred cows elevated milk yield, which coincided with increased plasma IGF-I concentration and udder blood flow (Tanwattana et al., 2003).

CONCLUSIONS

These experiments demonstrated that the rbST exerts its galactopoietic action through increases in both the TBW and ECW in association with an increase in MBF, which partitions the distribution of nutrients to the mammary gland for milk production. The data also suggest that as the lactation advances, the action of rbST does not prevent the decrease in the mammary function which still had a progressive decline in milk yield. Further studies are needed to determine the mechanisms by which bovine somatotropin influence mammary gland metabolism during lactation advance in crossbred cattle in the tropics.

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CHAPTER V

Effects of long-term administration of recombinant bovine somatotropin on milk production and insulin like growth factor-I, plasma levels of insulin and it's metabolites in crossbred Holstein cattle

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Effects of long-term administration of recombinant bovine somatotropin on milk production and insulin like growth factor-I, plasma levels of insulin and it's metabolites in crossbred Holstein cattle

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SUMMARY

The objective of this study was to determine the in vivo relationship between the long-term administration of recombinant bovine somatotropin (rbST), circulating levels of IGF-I and insulin, mammary blood flow and other variables relevant to milk synthesis, in crossbred, Holstein cattle. Ten, first lactating, non-pregnant, crossbred, Holstein dairy cattle were divided into two groups of five animals each; an experimental group and a control group. Animals in each group were fed with rice straw, treated with 5% urea, as the source of roughage. Four consecutive study periods were carried out in each group. These consisted of a pretreatment period (45 days postpartum before lactation peak) and three treatment periods during early lactation (105 days postpartum), mid-lactation(165 days postpartum) and late lactation (225 days postpartum). During the treatment periods, animals that had completed 60 days of lactation were injected subcutaneously every 14 days with 500 mg of recombinant bovine somatotropin (rbST) (POSILAC, Monsanto, USA) in the experimental group, while animals in the control group were injected subcutaneously every 14 days, with 800 mg of sterile sesame oil, without rbST, as a placebo. During the pre-treatment period, there were no significant differences in plasma concentrations of IGF-I, insulin and other parameters between the control group and the experimental group. During the treatment periods, the increase in the concentration of plasma IGF-I in rbST treated animals was significantly higher than in the control animals throughout the lactating period. Plasma glucose, protein and triglyceride concentrations in each group remained stable throughout the study. The total daily dry matter intakes were not significantly different between the groups. Milk yield increased by 20% with rbST

treatment and it was 22% greater than that of the control animals receiving placebo in early lactation. Milk yield of rbST treated animals rose to a peak in early lactation and then gradually declined. In late lactation, milk yield of rbST treated animals was decreased to 19 % as compared with early lactation. Udder plasma flow and udder blood flow markedly increased with rbST treatment and there were no significant changes in the control animals. The ratio of udder blood flow to the rate of milk production increased to mid and late lactation in controls and the rbST treated animals. These findings suggest that a short persistency of lactation in rbST treated animals was similar to the control animals receiving placebo. Changes in milk production during the progress of lactation in rbST treated animals might not be controlled systemically but also locally within the mammary gland. The lack of effect of higher plasma IGF-I levels on persistency of lactation in rbST treated animals, may be due to changes in the pattern of IGF-I binding proteins and paracrine production inhibiting IGF-I action.

INTRODUCTION

It is known that crossbreeding *Bos taurus* and *Bos indicus* has been an efficient tool for blending the adaptability of tropical cattle with the high milk potential of exotic breeds and thus increasing milk production. There is still a need to discover which crossbred cattle are most suitable for the tropics. It is not only the genetics that have to be considered but many other factors which affect the signals received by the mammary gland. Many factors including the concentration of plasma growth hormone (GH), have pronounced effects on the rate of milk secretion. It has been reported that the concentration of GH in 87.5% crossbred Holstein cattle, decreased rapidly as lactation progressed to mid and late lactation. This decrease could contribute to a reduction in milk yield and mammary blood flow (Chaiyabutr et al. 2000a). However, little is known about the other circulating factors that are involved in regulating mammary blood flow, a major parameter controlling milk production (Davis & Collier, 1985).

Bovine GH is known as a homeorrhetic hormone concerned with both growth and lactation, but the mechanism of action of bovine GH on milk production is a controversial area. Receptors for GH have not been demonstrated on secretory epithelial cells of mammary tissue (Akers, 1985). The effects of GH on milk production

are thought to be indirectly mediated *via* nutrient partitioning effects or *via* insulin like growth factor-I (IGF-I) (Bauman, 1992). There has been discussion as to whether IGF-I mediates the galactopoietic effects of growth hormone. Some studies support this role. Infusion of IGF-I into the pudic artery of lactating goats has been shown to increase blood flow and milk production on the infused side (Prosser et al.1990; Prosser et al.1994). Infusion of GH into the mammary artery of sheep did not increase milk yield (Peel & Bauman, 1987). Several other reports, refuting the role of IGF-I as mediators of GH action, have been published (Barber et al.1992; Flint et al.1992; Plaut, Ideda &Vonderhaar, 1993). It has been reported that GH can stimulate milk production under circumstances in which IGF-I does not (Prosser & Davis,1992). Chaiyabutr et al. (2000b) reported that the galactopoietic effect of GH is not associated with the plasma level of IGF-I as lactation advances in 87.5% HF animals. The plasma level of IGF-I has been shown to remain at the same level as lactation advances, despite declining circulating GH, mammary blood flow and milk yield (Chaiyabutr et al. 2004). These data did not support a role for IGF-I in mediating the action of GH on milk production. However, an increase in plasma IGF-I level, with a concomitant increase in both mammary blood flow and milk yield in late lactation, was seen after exogenous administration of rbST in 87.5%HF animals (Tunwattana et al., 2003).

Despite a number of studies looking at these differences, there have been few observations about the mechanism of short persistency of lactation in 87.5% HF dairy cattle. This could relate to the role of GH or a mechanism other than the circulating level of GH. To understand this apparent paradox we studied primarily the short persistency of lactation. Although GH has been known to be a major stimulus for the production of IGF-I and IGF-I is believed to play a role both in mammary development and milk production by mediating the effects of GH (Bauman,1992). Circulating concentrations of IGF-I are also sensitive to the nutritional status in many animal species (see reviews Clemmons & Underwood, 1991). The objective of the present study was to determine the in vivo relationship between long-term exogenous administration of bST, circulating levels of IGF-I, mammary blood flow and biological variables relevant to milk synthesis in 87.5% HF animals. Long-term administration of rbST, throughout lactation, might lead to better understanding adaptability in crossbred cattle. This could provide information about choosing suitable crossbred dairy cattle for increased milk production in the tropics.

MATERIALS AND METHODS

Animals and managements

Ten, first lactating, non-pregnant, crossbred, 87.5% Holstein dairy cattle were selected for the experiment. They were divided into two groups of five animals each. Animals in each group were fed with rice straw treated with 5% urea as the source of roughage. All animals were housed in sheds and tethered in individual stalls and fed twice daily. The maximum temperature in the shed at noon was 34±1°C and the minimum temperature at night was 26±1°C. The relative humidity was 68±12%. Animals received an average of 4 kg/day of roughage in combination with the same concentrated mixture (7 kg/day) to maintain a moderate body condition score (2.5, scale = 1 to 5). The chemical composition of the feed is presented in Table 1. Each day, the food was given in equal portions at about 06.00 h and 17.00 h when the animals were milked. Animals had free access to water and were fed their respective rations throughout the experimental period.

The urea treated rice straw was prepared by mixing the urea solution with dry straw (5 kg urea dissolved in 100 litter water per 100 kg dry rice straw). Rice straw sprayed with urea solution was mixed thoroughly and stored under airtight conditions in a cement pit for 21 days. A continuous supply of treated rice straw was made available by using a 2 pit x 21 day system of urea treatment. After 21 days, the rice straw treated with 5% urea was offered to the animals.

Experimental procedures

Animals were divided into the control (n=5) and experimental (n=5) groups. Four consecutive periods of study were used for each group. These consisted of a pretreatment period (45 days postpartum before lactation peak) and three treatment periods of 105 days postpartum (early lactation), 165 days postpartum (mid- lactation) and 225 days postpartum (late lactation). During the treatment periods, animals in the experimental group, which had completed 60 days of lactation, were injected subcutaneously every 14 days until the end of study with 500 mg of recombinant bovine somatotropin (rbST). It was suspended in 792 mg of a prolonged-release formulation of sesame oil (POSILAC, Monsanto, USA). Animals in the control group were injected subcutaneously every 14 days with 800 mg of sterile sesame oil without rbST. Injection in each animal was administered at the tail head depression

(ischiorectal fossa). From the pretreatment to the end of the treatment periods, animals of both groups were fed the same ration starting before parturition until the completion of the study. The dry matter intake of each animal was measured by weighing the concentrate and roughage offered and refused each day. Animals were normally milked at around 0600 h and 1700 h using a milking machine and milk production was recorded daily. Measurements of the udder blood flow were carried out in the afternoon. At around 1100 h, an arterial blood sample was collected from the coccygeal artery, by venipuncture with a #21 needle and into a heparinized tube. Blood samples were kept in crushed ice and then centrifuged at 3000 rpm for 30 min at 4°C. Arterial plasma samples were collected for measurements of the level of hormones and metabolites. Plasma samples in aliquots were collected and frozen at -40°C until the time of the assays. Animals were weighed after collecting a milk sample in each period.

Mammary blood flow measurements

On the day before the experiment began and in each period of the experiment, two catheters (i.d. 1.0 mm, o.d. 1.3 mm, L 45 mm) were inserted into either the left or right milk vein using a intravenous polymer catheter (Jelco, Critikon; Johnson & Johnson, U.K.), under local anesthesia. This was done on the standing animal for the measurement of mammary blood flow. The tip of the catheter was positioned near the sigmoid flexure, anterior to the point at which the vein leaves the udder. The other catheter was positioned downstream, about 20 cm from the first one. All catheters were flushed with sterile heparinized normal saline and were left in place during the experiment. Blood flow through half of the udder was determined by measuring the dilution of dye T-1824 (Evans blue) after a short term, continuous infusion, adapted from a method of measuring blood flow in the milk veins of cattle as previously described (Chaiyabutr et al.1997).

Determination of plasma hormones and metabolite concentration

The plasma IGF-I concentration was determined using Automated Chemiluminescent Immunoassays of IGF-I in an IMMULITE® Analyzer (IMMULITE IGF-1, Diagnostic Products Corporation, Los Angeles, CA). The plasma insulin concentration was quantified using a radio immunoassay (RIA) kit

(Coat a Count® Insulin, Diagnostic Products Corporation, Los Angeles, CA). Arterial plasma glucose concentrations were measured using enzymatic oxidation in the presence of glucose oxidase. Plasma triglyceride and total protein concentrations were measured by using an enzymatic colorimetric test and the Biuret test, respectively (Biotecnica Instruments,s.P.A.,Italy).

Statistical analysis

Data were compared between the periods of lactation in each group using a paired t-test. Between group trials and mean differences were examined statistically by an un-paired t-test. Mean values are presented as mean±S.D.

RESULTS

Changes in plasma concentrations of IGF-I and insulin and plasma metabolites (Table 2)

There was no significant difference in plasma IGF-I concentrations during the pre-treatment period between control animals and the rbST treated animals. The concentration of plasma IGF-I in rbST treated animals was significantly higher ($P<0.001$) than that of the control animals throughout all lactating periods. As this advanced to mid- and late lactation, the mean levels of both plasma IGF-I and insulin in the control animals remained constant and similar to the pre- treatment period. During mid- and late lactation, the plasma insulin levels significantly increased ($P<0.05$) over that seen during the pre-treatment period in rbST treated animals. Plasma glucose and protein concentrations remained stable throughout all periods of study, while the plasma triglyceride concentration slightly increased during mid- and late lactation in both groups.

Changes in dietary dry matter intake, milk yield, udder blood flow and body weight (Table 3)

The total daily dry matter intakes were not significantly different between the control and the rbST treated animals. Studies during the pre-treatment period of both groups were started 45 days post partum. The enhancement of milk yield in animals given rbST was higher than that of the control animals receiving placebo throughout their lactation. The peak milk yield in both groups declined from the early period of lactation as lactation advanced to mid and late lactation. Compared with the pre-

treatment value, the actual increases in milk yield during the different lactating periods was 20%, 10% and -2% for animals receiving the rbST over 45, 105 and 165 days, respectively. In early lactation, milk yield of rbST treated animals was 22% greater ($P<0.05$) than that of the control animals receiving placebo. An evaluation of the dry matter intake and milk yield revealed that the mean ratios of total dry matter intake to milk yield in rbST treated animals was significantly less ($P<0.05$) in the early period of lactation. The mean ratio of total dry matter intake to milk yield showed no significant changes throughout lactation in the control animals. The body weights of both control animals and rbST treated animals significantly increased stepwise as compared with the pre-treatment period, while rbST treated animals had a higher weight gain than control animals receiving placebo throughout the lactation. The rate of udder blood flow markedly increased during rbST administration. The udder blood flow of rbST treated animals increased from 3548 to 5310 and 5458 ml/min ($P<0.05$) in early and mid lactation, respectively, while there were no significant changes in the control animals receiving placebo. The ratio of udder blood flow to the rate of milk yield increased as lactation advanced in both the control and the rbST treated animals.

Table 1. *Chemical composition of feed components (% on dry matter basis)*

Particulars	Urea-treated rice straw	Concentrate
Dry matter	58.0	89.4
Crude protein	8.9	17.8
Acid detergent fibre	61.2	21.5
Neutral detergent fibre	67.2	28.8
Lignin	8.8	7.0
Ash	16.8	5.6

Concentrate formulation: fresh weight (kg/100 kg) consisted of soy bean meal 30 kg, cotton seed 25 kg, cassava 25 kg, rice bran 15 kg, dicalcium phosphate 2 kg, sodium bicarbonate 1.7 kg, potassium chloride 0.7 kg and vitamin/mineral premix 0.6 kg. .

Table 2. *The Plasma concentrations of the insulin like growth factor 1 (IGF-1), insulin, glucose, protein and triglyceride in different stages of lactation in the control and rbST treated animals (n = 5 in each group)*

	Period of lactation	Control Group	rbST Group	Control VS rbST Group
PlasmaIGF-1(ng/ml)	Pretreated	40±15	50±29	ns
	Early	48 ±16	209±42***	P<0.001
	Mid	47 ±13	202±55***	P<0.001
	Late	55±16	151±63***	P<0.01
Plasma insulin (µg/l)	Pretreated	0.32±0.23	0.34±0.31	NS
	Early	0.33±0.29	0.64±0.37	NS
	Mid	0.29±0.14	1.04±0.53*	P<0.01
	Late	0.42±0.32	0.83±0.89*	NS
Plasma glucose (mg/dl)	Pretreated	69±4	68±2	NS
	Early	70±7	69±4	NS
	Mid	65±3	69±3	NS
	Late	67±1	66±3	NS
Plasma triglyceride (mg/dl)	Pretreated	11.4±3.4	13.7±4.7	NS
	Early	11.3±4.4	11.2±1.2	NS
	Mid	12.7±3.9	15.7±6.3	NS
	Late	13.6±6.9	14.5±3.9	NS
Plasma protein (g/dl)	Pretreated	8.14±0.64	8.29±0.46	NS
	Early	8.05±0.91	7.72±0.42	NS
	Mid	7.75±0.95	7.99±0.41	NS
	Late	8.39±0.69	7.75±0.46	NS

P-values by paired t-test: * P<0.05, *** P<0.001 with respect to the pretreated period in each group.

P-values by unpaired t-test between the control animals and rbST treated animals.

Table 3. *The changes in dietary dry matter (DM) intake, milk yield, udder blood flow and body weight in different stages of lactation in the control and rbST treated animals (n = 5 in each group).*

	Period of lactation	Control Group	rbST Group	Control VS rbST Group
Dry matter intake (kg/d)	Pretreated	11.41±0.66	12.30±0.76	NS
	Early	11.64±1.11	13.01±1.67	NS
	Mid	12.22±1.76	13.91±1.28	NS
	Late	12.29±1.80	13.37±1.70	NS
Milk yield (kg/d)	Pretreated	13.0±1.5	13.3±2.7	NS
	Early	13.1±1.9	16.0±2.1**	P<0.05
	Mid	12.9±1.5	14.6±1.9	NS
	Late	11.5±1.0	13.0±1.3	NS
DM intake/Milk yield	Pretreated	0.89±0.12	0.96±0.25	NS
	Early	0.90±0.12	0.85±0.22*	NS
	Mid	0.95±0.13	0.95±0.06	NS
	Late	1.07±0.19	1.03±0.12	NS
Udder blood flow (ml/min)	Pretreated	3286±461	3548±463	NS
	Early	3817±616	5310±1620*	NS
	Mid	3821±533	5458±1627*	NS
	Late	3750±476	4814±1464	NS
Udder blood flow/ Milk yield	Pretreated	364±25	397±111	NS
	Early	420±32*	491±152	NS
	Mid	433±89	539±156	NS
	Late	471±69	539±168	NS
Body weight (kg)	Pretreated	336.9±31.1	363.6±27.1	NS
	Early	357.1±34.0**	391.2±35.6**	NS
	Mid	369.8±33.8**	412.4±35.5***	NS
	Late	379.2±29.8**	420.9±43.5**	NS

P-values by paired t-test: * P<0.05, ** P<0.01, *** P<0.001 with respect to the pretreated period in each group.

P-values by unpaired t-test between control animals and rbST treated animals.

DISCUSSION

Dairy herds in tropical countries are of mixed exotic breeds and crossbreeds. The potential for milk production of most indigenous cattle in the tropics is less than that of dairy cattle in temperate countries, while indigenous cattle have resistance to tropical diseases and a high level of heat tolerance (Nakamura et al., 1993). Exotic Bos taurus breeds have higher milk production but they also have inherent disadvantageous traits. Crossbreeding has been exploited as an efficient tool for blending the adaptability of tropical cattle with the high milking production of exotic breeds. We found that different types of crossbred Holstein Friesians (HF) showed differences in persistency of lactation and mammary circulation. Crossbred cattle containing 87.5% Holstein genes as compared to 50%HF animals, had a low persistency of lactation. We noted a quick decrease in the peak rate of decline with rapid decreases in both mammary blood flow and the concentration of plasma growth hormone (GH)(Chaiyabutr et al. 2000a). However, milk synthesis in ruminant is complex and dynamic depending on several factors including stage of lactation, energy balance and nutrition management . The present study was designed to clarify whether short lactation, occurring in crossbred cattle containing 87.5% Holstein genes (Chaiyabutr et al. 2000b), could be attributed to a decrease in the circulating level of GH or some other mechanism. Long-term administration of recombinant bovine somatotropin (rbST) was undertaken in 87.5%HF animals that had completed 60 days of lactation prior to treatment. The 500 mg of rbST used in the present study and given twice weekly was the dose recommended for Bos taurus cows. Animals treated with rbST showed increased milk yields and circulating levels of IGF-I throughout lactation. These findings were similar to those of previous studies on lactating cows showing that the injection of GH, elevated plasma IGF-I concentrations (Davis et al. 1987; Tunwattana et al. 2003). A number of studies indicated that GH increased milk yield by a mechanism which did not involve the direct action of GH on the mammary gland (Collier et al. 1984). The indirect effects of GH on milk production are thought to be mediated either via IGF-I or nutrient partitioning effects (Bauman,1992).

The synthesis and release of IGF-I is mainly by the liver (Granner, 1996). However, little is known about the regulation of synthesis and secretion of IGF-I in the liver of ruminant. Mechanisms for regulating the plasma IGF-I level are known to be dependent on the availability in the liver of both GH and some nutritional factors (Clemmons and Underwood, 1991). From the present data, the increase in IGF-I

secretion throughout the study would appear to be maintained by the availability of exogenous rbST in the liver. Exogenous rbST administration in the present study was sufficient to achieve a satisfactory stimulation of IGF-I (Collier et al.,1988). GH is a key regulator of the hepatic expression of circulating IGF-I, and circulating concentrations of IGF-I are sensitive to nutritional factors in many species of animals. No differences in the nutritional status between the controls and the rbST treated animals were apparent in all lactating periods. Animals in both groups were equally well-fed. Animals with a lower nutritional state having a lower basal level of IGF-I (Hodgkinson, Bass & Gluckman, 1991) or a negative energy balance, have reduced hepatic IGF-I production (Weller et al. 1994; Ketelslegers et al. 1995), would not be expected to occur in the present study.

The rbST had no effect on plasma levels of triglyceride, glucose and protein throughout lactation, although GH has been known to elevate concentrations of fat (free fatty acids) and glucose in the blood (Vernon & Finley, 1988).. These results could not be a factor in limiting IGF-I release from the liver (McGuire et al. 1995). However, an increase in extracellular water compartments including the plasma volume in animals treated with exogenous rbST was observed (Chaiyabutr et al., unpublished data). These responses could be attributed to an increase in the plasma pool of circulating substrates (plasma volume x concentration), facilitating the partitioning of nutrients for milk synthesis and IGF-I secretion.

During mid- and late lactation, plasma insulin levels have been shown to increase over those seen during the pre-treatment period in rbST treated animals. This suggests that an increase in IGF-I secretion would be dependent on the availability to the liver of both GH and insulin (Luo & Murphy, 1991). The relationship between GH and insulin was not apparent for rbST treated animals in early lactation. However, maintaining the plasma concentration of glucose with high concentrations of insulin was apparent in rbST treated animals. This indicates that in later lactation, elevated plasma concentrations of exogenous GH decreased the responsiveness of peripheral tissues to high concentrations of insulin. This would spare glucose for insulin insensitive tissues, particularly the mammary gland.

The milk yield of the control animals receiving placebo, slightly increased after treatment started. Peak yield were smaller in the controls than in rbST treated animals and decreased as lactation advanced to mid and late lactation. Milk yield responses at 45 days of rbST treatment in early lactation were significantly greater (+20%

compared with pre-treatment) than to the 105 days of rbST treatment in mid lactation(+9.5%) and to 165 days of rbST treatment in late lactation (-2.%) in late ; thus, rbST affects the shape of the lactation curve. These results confirm the finding that an increase in milk yield in response to rbST administration will not be sustained indefinitely (Bauman, 1992), and that it is influenced by the stage of lactation (Phipps et al.1991). The low potential for extended persistency of lactation in rbST treated animals appears similar to that which occurs in higher yielding cows (Chase, 1993). However, the effect of rbST administration significantly influenced milk production efficiency. The ratio of total dry matter intake to milk production was lower in rbST treated animals when compared to that of the control animals consuming similar DM at a similar period of lactation. It indicated that rbST is one of the factors capable of stimulating mammary gland synthetic capacity in crossbred lactating animals.

However, animals in both groups gained weight throughout the experiment. A marked increase in milk yield with rbST treatment without loss of body weight, especially during early lactation, may be due to the fact that the animals were fed to allow an adequate replacement of body reserves between lactations. Milk yield in the first lactating crossbred animals in the present study were not as great as that of multiparous cows (Sullivan et al., 1992). This is possibly related to the continued weight increase observed in animals during their first lactation. These results provide the physiological differences between crossbred animals and exotic breeds in partitioning ability, which would be inherited. During early lactation, the metabolic demands of lactation in both groups of the crossbred HF animals were met by dietary intake, thus not causing mobilization of body tissues as indicated by no alteration of the levels of both triglyceride and glucose.

During lactation, the blood flow to the mammary gland is the major parameter controlling milk production. In the present study, an increased mammary blood flow was concomitant with an increase in IGF-I in the rbST treated animals. We focused on the effect of IGF-I on mammary blood flow and whether it increased the availability of substrates to the mammary gland. There were indications that GH plays a role, requiring IGF-I as a mediator, which in turn stimulates milk yield. The present results support previous studies on goats (Hart, Lawrence & Mepham 1980) and cows (Davis et al., 1988), which also reported an increase in mammary blood flow during administration of exogenous growth hormone at different periods of lactation. The ratio of udder blood flow to the rate of milk yield increased as lactation advanced to

mid and late lactation in both the control and the rbST treated animals. A greater decrease in milk secretion, with minimal changes in mammary blood flow, caused a high ratio for the mammary blood flow to the rate of milk yield as lactation advanced in both groups. The question then arises as to whether the mammary metabolism influences mammary blood flow or mammary blood flow influences mammary metabolism, during rbST administration. This issue needs to be investigated further. Other circulatory factors, due to the effect of rbST, might affect mammary blood flow by a mechanism which did not involve direct action of GH on the udder (Collier et al. 1984). It seems that the effect of GH on mammary circulation is indirect and mediated via IGF-I, although a number of studies have demonstrated that similar increases in milk secretion and mammary blood flow occurred during growth hormone treatment in goats and cows (Davis et al. 1988; Hart et al., 1980). Injection of rbST in late lactating crossbred cows elevated both plasma IGF-I concentrations and udder blood flow (Tanwattana et al. 2003).

In the present study, during long-term administrations of rbST, milk yield rose to a peak in early lactation and then gradually declined over 32 weeks of the experiment to 19 % as compared with early lactation, whilst the plasma concentration of IGF-I and the mammary blood flow did not decrease in the rbST treated animals. These findings suggest that the stimulatory effect of recombinant bovine GH on milk production is not mediated solely by IGF-I. Changes in milk production during the progress of lactation in rbST treated animals might not be controlled systemically but also locally within the mammary gland. There are a number of possible explanations for this apparent finding. It probably involves greater synthesis of plasma IGF-I binding proteins as lactation advances which combines with IGF-I in the blood and so modulates the level of free IGF-I before it reached the mammary gland. It has been reported that approximately 95% of the infused IGF-I is bound by IGF binding proteins (Davis et al. 1989). Mammary tissue is itself capable of synthesizing an IGF-binding protein (e.g.IGFBP-5) during mammary gland involution in late lactation and this could inhibit IGF-mediated cell survival (Tonner et al. 1997; Flint & Knight, 1997) and initiate involution and a decrease in milk yield.

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CHAPTER VI

Effects of Long-term Administration with Recombinant Bovine Somatotropin on the Plasminogen-Plasmin System and Milk Compositions in Crossbred Holstein Cattle

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Effects of Long-term Administration with Recombinant Bovine Somatotropin on the Plasminogen-Plasmin System and Milk Compositions in Crossbred Holstein Cattle

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ABSTRACT

The present study was designed to clarify whether long-term administration of recombinant bovine somatotropin (rbST) suppresses milk plasmin-plsminogen activity within the mammary gland and allow a persistence of milk production during different stages of lactation in crossbred Holstein cattle. Ten, first lactation, 87.5%HF animals were divided into two groups of 5 animals each. Four consecutive periods of study were carried out in each group, a pretreatment period (45 days postpartum) and three consecutive treatment periods. In the treatment periods, the rbST-treated animals, which had completed 60 days of lactation, were injected subcutaneously every 14 days with 500 mg of rbST (POSILAC, Monsanto, USA) until the end of the study, while the control animals were injected subcutaneously every 14 days with 800 mg of sterile sesame oil, as a placebo. The treatment periods were carried out in early lactation (105 days postpartum), mid-lactation (165 days postpartum) and late lactation (225 days postpartum). Animals receiving rbST gave greater milk yields than control animals in all stages of lactation The milk yield of rbST-treated animals significantly increased in early lactation ($P<0.01$), when compared with the initial pretreatment period. The peak milk yield in both groups declined from the early period of lactation as lactation advanced to mid and late lactation. Udder blood flow significantly increased during rbST administration, while there were no significant changes throughout lactation in the control animals. The concentration of milk lactose of both controls and rbST treated animals showed no significant changes throughout lactation, while the concentrations of milk protein and milk fat of rbST-treated animals increased during advanced lactation. The milk fat concentration of rbST-treated animals had a significantly greater ($P<0.05$) than that of control animals in the early lactation. No significant changes for the

concentration of milk Na and K including Na/K ratio in comparison with control animals at different stages of lactation. The concentration of milk Cl significantly increased during advanced lactation in the control animals, while the concentration of milk Cl of rbST-treated animals significantly decreased ($P<0.05$) in the early lactation. The plasminogen and plasmin activities increased during lactation advances in both groups. The concentration of plasmin in milk gradual increased, while milk plasminogen concentration significantly increased as lactation advances in both the controls and rbST-treated animals. The plasminogen : plasmin ratio decreased in the control animals while it increased in rbST-treated animals as lactation advances. These findings demonstrate that administration of rbST cause animals to maintain milk plasmin at low concentration throughout lactation. The decrease in milk secretion during the progress of lactation might not be controlled by changes in extra-mammary factors but, in part, through changes within the mammary gland relating to the activity of the plasmin-plasminogen system.

Key words : rbST, Milk Yield, Plasminogen - Plasmin activity, Crossbred Holstein Cattle

INTRODUCTION

Bovine growth hormone or somatotropin (bST) is known as a homeorrhetic hormone connected with both growth and lactation. The relationship between plasma ST concentration and milk yield has been defined. An advance of lactation is characterized by decrease in milk yield and concomitant decrease in blood ST concentration (Hart et al., 1980). The importance of bST for enhancing and maintaining milk production in dairy ruminants is well established (Bauman, 1999). Administration of a slow-release formulation of bST to dairy ruminants improves lactation persistency by slowing down the post peak rate of decline (Gallo et al 1997). The milk production above peak using sustained release of bST in cows beginning at 60 days postpartum by Bauman et al.(1989) showed no increase in milk production and controversial. A few data are available for the role of bST for short persistency of lactation in crossbred dairy cattle in the tropics, although Chaiyabutr et al.(2000) reported that the concentration of bST of lactating crossbred cattle containing 87.5%Holstein genes markedly decreased as lactation advances to mid- and late lactation; this decrease could attribute to decreases in milk yield and mammary blood flow.

A number of studies indicate that bST can delay involution of the mammary gland by reducing the activity of the plasmin-plasminogen system, an important initiator of tissue remodeling during lactation advance in dairy ruminants (Baldi et al., 1997; Politis et al., 1990). There is evident that the progressive loss of milk synthesis capacity by mammary epithelial cells occurs during mammary involution, although substrate supply to the mammary gland is often adequate to maintain the maximum rate of milk synthesis. As lactation advances, a leaky of cell tight junctions is also apparent during involution of the secretory tissue. In the process of the proteolysis, the proteinase responsible is plasmin, which is transferred from blood into milk as an inactive precursor (plasminogen) and then converted to active plasmin by plasminogen activators, which are produced in quantity within the mammary gland especially in the late lactation. Increases in plasmin production in milk are important in determining milk production and initiate the onset of involution within the mammary gland (Ossowski et al., 1979). Little is known about responsible for this proteolysis relating to the role of growth hormone on the persistency of lactation in crossbred cattle. Thus, the objective of the present study was to determine the relationship between milk plasmin-plasminogen and milk yield including milk compositions during long-term administration of rbST in different stages of lactation in 87.5% HF animals.

MATERIALS AND METHODS

Animals and managements

Ten, first lactating, non-pregnant crossbred 87.5%Holstein dairy cattle were selected for the experiment. They were divided into two groups of five animals each. Animals in each group were fed with rice straw treated with 5% urea as the source of roughage throughout the experiments. All animals were housed in sheds and tethered in individual stalls and fed twice daily. The maximum temperature in the shed at noon was 34±1°C and the minimum temperature at night was 26±1°C. The relative humidity was 68±12%. Animals individually received an average of 4 kg/day of roughage in combination with the same concentrated mixture (7 kg/day) to maintain a moderate body condition score (2.5, scale = 1 to 5). The chemical composition of feeds is presented in Table 1. Each day, the food was given in equal portions at about 06.00 h

and 17.00 h when animals were milked. Animals had free access to water and animals were fed their respective rations throughout the experimental period.

The urea treated rice straw was prepared by mixing the urea solution with dry straw (5 kg urea dissolved in 100 litter water per 100 kg dry rice straw). Rice straw sprayed with urea solution was mixed thoroughly and stored under airtight conditions in a cement pit for 21 days. A continuous supply of treated rice straw was made available by using a 2 pit x 21 day system of urea treatment. After 21 days, the rice straw treated with 5% urea was offered to the animals.

Experimental procedures

Animals were divided into control (n=5) and experimental (n=5) groups. Four consecutive periods of study were carried out in each group, consisting of the pretreatment period (45 days postpartum), and treatment periods of 105 days postpartum (early lactation), 165 days postpartum (mid- lactation) and 225 days postpartum (late lactation). In the treatment period, animals in the experimental group which had completed 60 days of lactation, were injected subcutaneously every 14 days until the end of study with 500 mg of recombinant bovine somatotropin (rbST) suspended in 792 mg of a prolonged-release formulation in sesame oil (POSILAC, Monsanto, USA), while animals in the control group were injected subcutaneously every 14 days with 800 mg of sterile sesame oil without rbST as placebo. Injection in each animal was administered at the tail head depression (ischiorectal fossa). From the beginning of pretreatment to the end of treatment period, animals of both groups were fed the same ration from before parturition through the completion of study. The dry matter intake of each animal was determined by measuring both the concentrate and roughage offered and refused each day.

Milk sampling and determinations of milk compositions

Animals were normally milked at around 0600 h and 1700 h by a milking machine and daily milk yield (kg/day) was recorded and weekly average of each animal was calculated. Milk was collected in the afternoon of specified day and devided to two portions. One was kept in fresh milk for determination of the plasmin-plasminogen concentration and other portion was kept in formalinized milk. The formalinized milk sample (300 µl of 40% formaldehyde in 30 ml of fresh milk) was kept at 4°C for lactose, fat and protein concentrations by the colorimetric method (Tele et al.,1978), Gerber method and infrared method using Milkoscan, respectively. The concentrations

of electrolytes in aqueous phase of milk were estimated for sodium (Na) and Potassium (K) using Flame photometry, Chloride (Cl) concentration by Chloridometer (Corning).

Plasmin and plasminogen determination

The concentrations of plasmin and plasminogen in milk or casein fractions were determined the method of Korycka-Dahl et al (1983) with a slight modification. Briefly, The plasmin activity was performed by measuring the rate of hydrolysis of the chromogen substrate (H-D-valyl-L-leucyl-L-lysine-p-nitroanilide dihydrochloride, S-2251,Chromogenix Instrumentation Laboratory, Italy). Formation of p-nitroanilide resulting from substrate cleavage by plasmin was measured spectrophotometrically at 405nm. 1 unit of activity of plasmin and plasminogen was defined as the amount of enzyme that produced a change in absorbance at 405 nm of 0.001 in 1 min at pH 7.4, 37 C when p-nitroanilide was produced from S-2251 substrate.

Udder blood flow measurements

On the specified day in each period, measurements of the udder blood flow were carried out in the afternoon. Udder blood flow measurements were performed in duplicate. Blood flow through half of the udder was determined by measuring the dilution of dye T-1824 (Evans blue) by a short term continuous infusion as described by Chaiyabutr et al. (1997 Udder blood flow was calculated by doubling the flow measured in one milk vein (Bickerstaffe et al., 1974). Packed cell volume was measured after centrifugation of the blood in a microcapillary tube.

Statistical analysis

Values were compared between the periods of lactation in each group using a paired t-test. Between group trials and mean differences were examined statistically by an un-paired t-test. Mean values are presented as mean ±SD.

RESULTS

Udder blood flow and milk yield during different stages of lactation

An increase in milk yield in animals given rbST was higher than that of the control animals receiving placebo throughout their lactation (Table 2). The peak milk yield in both groups declined from the early period of lactation as lactation advanced to mid and late lactation. Compared with the pre-treatment value, the actual increases in milk yield during the different lactating periods was 20%, 10% and -2% for animals receiving the rbST over 45, 105 and 165 days, respectively. In early lactation, milk yield of rbST treated animals was 22% greater ($P<0.05$) than that of the control animals receiving placebo. The rate of udder blood flow markedly increased during rbST administration. The udder blood flow of rbST-treated animals increased from 3.55 to 5.31 and 5.46 L/min ($P<0.05$) in early and mid lactation, respectively, while there were no significant changes in the control animals receiving placebo. The ratio of udder blood flow to the rate of milk yield increased as lactation advanced in both the control and the rbST-treated animals.

Milk compositions at different stages of lactation

Both the control animals and rbST-treated animals showed no significant changes in the concentration of milk lactose throughout the course of their lactation (Table 3). The concentrations of milk protein and milk fat of rbST-treated animals increased during the course of treatment. The milk fat concentration of rbST-treated animals had a significantly greater than those of control animals in the early lactation ($P<0.05$). The milk Na; K concentrations of both groups showed no significant changes during advanced lactation, but the Na/K ratio of rbST-treated animals had significantly lower than those of control animals in the early period of lactation ($P<0.05$). When lactation advanced to mid- and late lactation, the Na/K ratio of both groups increased as compared with the pretreated period. In the present results, milk Cl concentration was significantly higher in control animals during lactation advanced to mid- and late lactation, while milk Cl concentration of rbST-treated animals significantly decreased in early lactation.

The milk plasminogen and plasmin activity in different stages of lactation

The plasminogen and plasmin activities were increased during lactation advances in both control and rbST-treated animals (Table 4). Milk plasmin concentrations gradual increased, while milk plasminogen significantly increased as lactation advances in both the controls and rbST-treated animals. The milk plasminogen concentrations were not significantly different between rbST-treated animals and control animals. The

plasminogen: plasmin ratio decreased in the control animals while it increased in rbST-treated animals as lactation advances.

TABLE I.

Chemical composition of experimental diet and nutrient analysis as a percentage of dry matter.

	Urea treated rice straw	**Concentrate**
Dry matter	58.0	89.4
Crude protein	8.9	17.8
Acid detergent fibre	61.2	21.5
Neutral detergent fibre	67.2	28.8
Lignin	8.8	7.0
Ash	16.8	5.6

Concentrate formation: ingredients by fresh weight (100 kg $^{-1}$) consisted of soy bean meal (30 kg), cotton seed (25 kg), cassava (25 kg), rice bran (15 kg), dicalcium phosphate (2 kg), sodium bicarbonate (1.7 kg), potassium chloride (0.7 kg) and premix (0.6 kg).

TABLE II.
Udder blood flow and milk yield during in different stages of lactation in the controls and rbST-treated animals (n = 5 in each group)

	Period of experiment	Control Group	rbST Group	Control VS rbST Group
Udder blood flow	Pretreated	3.29±0.46	3.55±0.46	NS
(L/min)	Early	3.82±0.62	5.31±1.62*	NS
	Mid	3.82±0.53	5.46±1.63*	NS
	Late	3.75±0.48	4.81±1.43	NS
Milk yield	Pretreated	13.0±1.5	13.3±2.7	NS
(kg/day/animal)	Early	13.1±1.9	16.0±2.1*	P<0.05
	Mid	12.9±1.5	14.6±1.9	NS
	Late	11.5±1.0	13.0±1.3	NS
MBF/ Milk yield	Pretreated	364±25	397±111	NS
	Early	420±32*	491±152	NS
	Mid	433±89	539±156	NS
	Late	471±69	539±168	NS

P-value by paired t-test with respect to the the pretreated period in the same group, (* P<0.05)

P-values by unpaired t-test between the control animals and rbST- treated animals.

TABLE III.

Milk compositions at different stages of lactation in the controls and rbST- treated animals (n = 5 in each group)

	Period of experiment	Control Group	rbST Group	Control VS rbST Group
Protein (gm%)	Pretreated	3.15±0.21	3.16±0.16	NS
	Early	3.27±0.15	3.16±0.25	NS
	Mid	3.45±0.19	3.51±0.17*	NS
	Late	3.69±0.05**	3.65±0.12**	NS
Fat (gm%)	Pretreated	3.60±0.76	3.90±0.60	NS
	Early	3.60±0.25	4.70±0.77*	P<0.05
	Mid	3.9±0.66	4.30±0.64	NS
	Late	4.0±0.77	4.70±0.79**	NS
Lactose(gm%)	Pretreated	4.49±1.02	4.90±0.24	NS
	Early	4.52±0.55	4.79±0.49	NS
	Mid	4.90±0.40	4.62±0.56	NS
	Late	4.81±0.29	4.79±0.38	NS
Milk Na^+ (mM)	Pretreated	29.4±2.3	28.6±1.1	NS
	Early	29.8±1.3	27.4±1.8	P<0.05
	Mid	29.6±3.2	29.2±2.3	NS
	Late	29.4±1.5	33.4±5.6	NS
Milk K^+ (mM)	Pretreated	37.5±4.0	39.6±1.9	NS
	Early	37.9±3.8	39.7±2.1	NS
	Mid	37.1±4.2	40.5±1.6	NS
	Late	36.2±4.2	39.1±1.8	NS
Milk Cl^-(mM)	Pretreated	25.6±4.7	25.0±1.9	NS
	Early	25.2±3.0	22.2±0.8*	P<0.05
	Mid	29.0±4.3***	25.4±4.2	NS
	Late	29.4±2.3*	28.8±5.0	NS
Milk Na/K ratio	Pretreated	0.79±0.09	0.73±0.03	NS
	Early	0.79±0.10	0.69±0.02	P<0.05
	Mid	0.81±0.12	0.72±0.04	NS
	Late	0.82±0.07	0.85±0.15	NS

P-value by paired t-test with respect to the pretreated period in the same group, (* P<0.05, **P<0.01, *** P<0.001).

P-values by unpaired t-test between the control animals and rbST- treated animals.

TABLE IV.

The milk plasminogen and plasmin activity in different stages of lactation in the controls and rbST-treated animals (n = 5 in each group)

	Period of experiment	Control Group	rbST Group	Control VS rbST Group
Plasmin	Pretreated	2.9±1.9	3.1±1.5	NS
(Units/ml milk)	Early	5.8±7.4	4.5±2.5	NS
	Mid	5.6±5.9	5.3±3.0	NS
	Late	6.7±6.4	7.0±4.1	NS
Plasminogen activity	Pretreated	123.8±24.3	125.7±32.0	NS
(Units/ml milk)	Early	161.2±60.8	175.3±36.2*	NS
	Mid	152.0±25.0	211.7±51.1*	P<0.05
	Late	181.2±32.0*	172.7±58.6*w	NS
Plasmin and Plasminogen	Pretreated	126.7±25.1	128.2±32.7	NS
(Units/ml milk)	Early	168.9±72.4	178.8±37.8*	NS
	Mid	157.6±28.8	215.9±51.2*	P<0.05
	Late	187.9±35.8*	180.2±55.0*w	NS
Plasminogen/Plasmin	Pretreated	59.0±41.7	52.9±35.9	NS
	Early	51.5±30.7	54.8±38.0	NS
	Mid	45.6±25.2	64.0±53.6	NS
	Late	40.2±18.6	56.3±49.4	NS

P-value by paired t-test with respect to the pretreated period in the same group, (* P<0.05, *w P<0.05 by Wilcoxon signed rank test).

P-values by unpaired t-test between the control animals and rbST- treated animals.

DISCUSSION

The previous study showed a short persistency of lactation in crossbred cattle containing 87.5% Holstein genes as compared with 50%HF animals. We noted a rapid decrease in the peak rate of decline with decreases in both mammary blood flow and the concentration of plasma growth hormone (GH)(Chaiyabutr et al. 2000). The present study was designed to understand the mechanism focusing primarily on this declining lactation in 87.5%HF cattle, could be attributed to a decrease in the circulating level of bovine somatotropin or some other mechanisms. The present results show that milk yield was significantly greater (by 22 %) in animals treated with rbST than the control animals in early lactation. As advanced lactation, rbST administration does not affect the shape of the lactation curve since the pattern of reduction in milk yield of rbST-treated animals was similar as in the control animal. In the present results, the milk protein and milk fat concentrations were significantly greater in rbST-treated animals than those of control animals during the treatment period. However, it has been noted that concentrations of these components may or may not change in animals treated with bovine somatotropin (Baldi, et al., 1997). Thus, the total yield of fat and protein of rbST-treated animals would be higher than those of the controls by the effect of increased milk yield during the treatment period.

The plasminogen and plasmin activities were increased during lactation advances in both rbST-treated animals and control animals. The plasminogen-plasmin system has been known to involve in the tissue remodeling associated with the declining phase of lactation and mammary gland involution in several dairy ruminant. Milk plasminogen concentrations are important in determining milk production by affecting the state of involution within the mammary gland. Increasing plasmin concentration in milk as lactation advances has been reported previously by Politis et al.(1989). Long-term administration of bST in dairy cows has been shown to prevent an increase in milk plasmin activity during late lactation, suggesting that bST acts to delay mammary gland involution (Politis et al.,1990). However, in the present results, the effect of rbST on prevention of an increase in milk plasmin activities was not apparent. A different pattern of this enzymatic system in crossbred dairy cattle would be suspected. In both the controls and rbST-treated animals showed gradual increase in milk plasmin concentrations as lactation advances. Milk plasmin is known to be influenced by the availability of plasminogen and the plasminogen activators. As plasminogen is ubiquitous in the body, thus, the plasminogen concentration in milk in animals treated

with rbST would not be expected to be limiting in the present study. Milk plasminogen concentrations were not significantly different between rbST treated animals and control animals given placebo as lactation advances which was similar to that of findings in cows by Politis et al., (1990). However, the plasminogen: plasmin ratio fell in the control animals while it increased in rbST-treated animals as lactation advances. The plasminogen : plasmin ratio is a useful index of plasminogen activation. This measurement is independent of milk volume. It indicates that massive activation of plasminogen and production of plasmin occured in the control animals than rbST-treated animals. Therefore, it do not exclude the possibility that rbST is involved in maintenance of the tissue function in the present results.

The mechanism of ion transport in the mammary cell has been proposed as either occurring by transcellular route or a paracellular route (Linzell and Peaker, 1971). Milk from rbST treated animals had no significant changes on milk Na and K concentrations but the Na/K ratio had significantly lower than milk from control animals in the early period of lactation. During advanced lactation to mid- and late lactation, the Na/K ratio of both groups increased as compared to the pretreated period. Milk Cl concentration was significantly higher in control animals during lactation advanced to mid- and late lactation, while milk Cl from rbST treated animals significantly decreased in early lactation after rbST administration. During the involution of the mammary gland, tissue permeability is markedly increased and breakdown of junctions between adjacent epithelial cells is thought to be a cause of this (Nguyen and Neville, 1998). Therefore, the present results do not exclude the possibility that rbST is involved in maintenance of tissue integrity in the mammary gland.

A number of studies indicated that bST increased milk yield by a mechanism which did not involve the direct action of bST on the mammary gland (Collier et al. 1984). There are no high affinity receptors for bST within the mammary gland (Akers, 1985). The indirect effects of bST on milk production are thought to be mediated either *via* IGF-I or nutrient partitioning effects (Bauman, 1992). In the present studies, during long-term administrations of rbST, milk yield rose to a peak in early lactation and then gradually declined over a period of months. This did not involve the plasma concentration of IGF-I and the mammary blood flow which has been shown to increase in the rbST treated crossbred dairy cattle (Chaiyabutr et al., 2005). These findings suggest that the decrease in milk secretion during the progress of lactation might not be controlled by changes in extra-mammary factors but, in part, through changes within the mammary gland relating to the activity of the plasmin-plasminogen system.

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CHAPTER VII

Effects of long term exogenous bovine somatotropin on nutrients uptake by mammary glands of crossbred Holstein cattle

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Effects of long term exogenous bovine somatotropin on nutrient uptake by mammary glands of crossbred Holstein cattle

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Abstract: Ten, first lactation, 87.5%HF dairy cattle were used to investigate effects of long-term administration of exogenouse bST and stage of lactation by using techniques for measuring mammary blood flow and combining these with measurements of nutrient plasma arterial concentrations and arterial-venous differences across the mammary gland for the mammary uptake. On day 60 and continuing until day 225 of lactation, animals in experimental groups were injected subcutaneously biweekly intervals until the end of study with 500 mg of recombinant bovine somatotropin (rbST) suspended in 792 mg of a prolonged-release formulation of sesame oil (POSILAC, Monsanto, USA). Animals in the control group were injected subcutaneously biweekly intervals with 800 mg of sterile sesame oil without rbST, as a placebo. Arterial and venous plasma samples were collected in each specified day on day 45 (pretreatment), 105 (early), 165 (mid) and 225 (late) of lactation Milk yield in animals given rbST was higher than that of the control animals given placebo and the persistency of production was higher in these animals throughout their lactation. The peak milk yield in both groups of animals declined from the early period of lactation as lactation advanced to mid and late lactation. During early lactation, the milk yield of rbST treated animals was higher than those of the control animals ($P< 0.05$). The rate of udder blood flow and plasma flow markedly increased during rbST administration. The mean arterial plasma concentrations for glucose, acetate,β-hydroxybutyrate and free glycerol were largely unchanged throughout periods of study in both controls and rbST-treated animals. The arteriovenous differences and extraction ratio of glucose across the mammary gland decreased as compared with pretreatment period in both groups. The net mammary uptake of glucose in early lactation of rbST-treated animals increased approximately 20%, whereas it decreased in mid- and late lactation as compared with the pretreatment period. The arteriovenous concentration differences, extraction ratio and mammary uptake of acetate were increased as lactation advances as compared with the pretreatment period in rbST-treated animals. The arteriovenous concentration differences, extraction ratio and mammary uptake for acetate of rbST-treated animals were significantly higher than those of the controls during early and mid-lactation.

The arteriovenous differences and extraction ratio of β-hydroxybutyrate were not responsive to in either the controls or rbST-treatment. The mammary uptake for β-hydroxybutyrate of rbST-treated animals increased as lactation advances in comparison with pretreatment period while it remained constant through the course of lactation .in the control animals. The arteriovenous differences and extraction ratio of free glycerol across the mammary gland in both groups showed valiable. The mean arterial plasma concentrations for free fatty acid (C_{16} to C_{18}) were elevated after rbST administration as compared with the pretreatment period and those of control animals. The values of arteriovenous differences and the net uptake by the mammary gland for FFA were variable during lactating periods in both groups. The mean arterial plasma concentrations for triacylglycerol(C_{16} to C_{18}) showed no significant differences after rbST administration throughout lactation. The arteriovenous differences, extraction ratio of triacylglycerol were unchanged as compared with pretreatment period in rbST-treated animals, but the net uptake of triacylglycerol across the mammary gland increased in rbST-treated animals in comparison with pretreatment period. There were no significant differences of arteriovenous differences, extraction ratio and net uptake of triacylglycerol during lactation advance in control animals. These results indicate that the increased partition of nutrients to the mammary gland induced by rbST treatment would be facilitated by increased mammary blood flow.

Key Words: rbST, Nutrients, Mammary Gland Uptake, Crossbred Holstein Cattle

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INTRODUCTION

It is known that milk production is dependent upon its blood supply to provide substrates at appropriate rates to sustain milk synthesis. The rate of supplying to mammary gland is determined by substrate concentration in the plasma and mammary blood flow. Milk production is the result of coordination between nutrient delivery to and biosynthetic capacity of the mammary glands. There is evident that substrate supply to the mammary gland is often inadequate to maintain the maximum rate of milk synthesis (Linzell and Mepham 1974). The mammary gland may be producing milk at a rate below its potential. However, the rate of milk production depends on function of number of secretory cells and their metabolic activity. The delivery of nutrients to the mammary gland is dependent on the physiological state of the animal by homeostatic and homeorhetic mechanisms (Bauman and Currie,1980). During early lactation nutrients are partitioned from peripheral tissues to the mammary gland to support the requirements for milk synthesis during peak lactation. Bovine somatotropin (bST) is known as a homeorrhetic hormone connected

with both growth and lactation, but the mechanism of action of bST on milk production is a controversial area, as receptors for bST have not been demonstrated on secretory epithelial cells of mammary tissue (Akers, 1985). Although a number of reviews have been published on the relationship between the plasma bST concentration and milk yield in ruminant (Bauman, 1992) in both normal and hot environments (West et al., 1991; Johnson et al., 1991), the role of bST in relationship to persistent lactation in dairy cattle in the tropics is not yet clear. Genetic selection has intensified the physiological demand on peripheral tissues to provide nutrients to the mammary gland partially because of the limitation of energy intake during peak lactation. It has been reported that in the crossbred cattle containing 87.5% Holstein genes decreased in milk yield, which was related to reductions in mammary blood flow and circulating bST as lactation advances to mid- and late lactation (Chaiyabutr et al., 2000a, 2000b). It is not known which factors are the cause and which factors are the effect for the short persistency of lactation in crossbred Holstein cattle especially about the function of mammary tissue and the utilization of substrate in the mammary gland. Therefore, the present experiment was conducted to improve understanding by using techniques for measuring mammary blood flow and combining these with measurements of nutrient plasma arterial concentrations and arterial-venous differences for the mammary uptake of nutrients during long-term administration of exogenouse bST throughout lactation.

MATERIALS AND METHODS

Animals and Management.

Ten, first lactation, non-pregnant, 87.5%HF dairy cattle were selected for the experiment. They were divided into two groups, five animals in each. Animals in each group were fed with rice straw treated with 5% urea, as the source of roughage throughout the experiments. All animals were housed in sheds, tethered in individual stalls and fed twice daily. Animals received an average of 4 kg/d of roughage in combination with a concentrated mixture (7kg/d), to maintain a moderate body condition score 2.5 during the experiment, (scale = 1 to 5)(Wildman et al.,1982). The chemical composition of the feed is presented in Table 1. The dry matter intake (DMI) of each animal was determined by measuring both the concentrate and roughage offered and subtracting the amount refused each day. Urea treated rice straw was offered four times a day at 08.00, 12.00, 16.00 and 20.00h. Concentration was fed two times at 0800 and 1400h. Each day, during feeding trial, sub-sample of both feed was collected for dry matter determination. Feed sample was collected every day and kept at -20 C for chemical analysis. Animals had free access to

water and were fed their respective rations throughout the experimental period. Details of the preparation of diets was described previously (Chaiyabutr et al., 2005) Briefly, the urea treated rice straw was prepared by mixing urea solution with dry straw (5 kg urea dissolved in 100 litres water per 100 kg dry rice straw). Rice straw sprayed with urea solution was mixed thoroughly and stored under airtight conditions in a cement pit for 21 days. A continuous supply of treated rice straw was made available by using a 2 pit x 21 day system of urea treatment. After 21 days, the treated rice straw with 5% urea was offered to the animals.

Experimental Procedures

Animals were divided into control (n=5) and experimental (n=5) groups. Four consecutive study periods were carried out in each group. These consisted of a pretreatment period (45 days post-partum)(pre-peak lactation) and three treatment periods during early lactation(105 days post-partum), mid-lactation (165 days post-partum) and late lactation (225 days post-partum). After 60 days of lactation, animals were injected sub-cutaneously biweekly intervals until the end of study with 500 mg of recombinant bovine somatotropin (rbST) suspended in 792 mg of a prolonged-release formulation of sesame oil (POSILAC, Monsanto, USA). Animals in the control group were injected subcutaneously biweekly intervals with 800 mg of sterile sesame oil without rbST, as a placebo. Injections were administered at the tail head depression (ischiorectal fossa). Animals of both groups were fed the same ration, from before parturition and throughout the study. Animals were normally milked at around 0600 h and 1700 h using a milking machine and milk production was recorded daily. Milk yield per day per animal was recorded at each period of lactation. Animals were weighed after collecting the milk sample in each specified day.

On the day of the experiment at around 1100 h, mammary blood flow (MBF) through half of the udder was determined and blood samples were taken from the milk vein and from the coccygeal artery by venipuncture with a #21 needle into a heparinized tubes. Blood samples were kept in crushed ice and then centrifuged at 3000 rpm for 30 min at 4°C. Plasma from both venous and arterial blood samples in aliquots at –40°C until nutrient concentrations were assayed.

Udder blood flow measurements. Udder blood flow measurements were performed in duplicate. Blood flow through half of the udder was determined by measuring the dilution of dye T-1824 (Evans blue) by a short term continuous infusion as described by

Chaiyabutr et al. (1997). Briefly, a dye (T-1824) was dissolved in sterile normal saline and diluted to a concentration of 100 mg/L. The solution was infused by a peristaltic pump (Gilson Medical electronics) at a constant rate of 85 ml/min into the milk vein for 1 min which could produce adequate mixing of dye with blood. Before infusion, blood was drawn from downstream in the milk vein as a pre-infusion sample. About 10 seconds after starting the infusion, 10 ml of blood was drawn from downstream in the milk vein at a constant rate into a heparinized tube. Two consecutive plasma samples were taken during each dye infusion at about 5 min intervals. Blood flow of half of the udder was calculated from plasma samples using the equation derived by Thompson and Thomson (1977). Quarter milking showed that the yields of the two halves of the udder were similar. Udder blood flow was therefore calculated by doubling the flow measured in one milk vein (Bickerstaffe et al., 1974). Packed cell volume was measured after centrifugation of the blood in a microcapillary tube.

Metabolite Determinations. Plasma glucose concentrations were measured using enzymatic oxidation in the presence of glucose oxidase. Plasma free fatty acid (FFA, C_{16}-C_{18}) concentrations were measured by using gas chromatography (Shimazu GC-7AG Gas Chromatograph) in comparison with the internal standard. The internal standard of triheptadecanoate and heptadecanoic acid was used for estimation of plasma triacylglycerol and FFA respectively as described by Thompson et al. (1975). Plasma β–hydroxybutyrate concentrations were assayed using an enzymatic reaction in the presence of β–hydroxybutyrate dehydrogenase (Sigma Chemical Co.). Plasma acetate concentrations were determined by chromatographic method. Plasma glycerol concentrations were determined by enzymatic method.

Statistics. Mean values are presented as mean ± SD. Values were compared among periods in each group using the paired t-test. Between group trials and mean differences were examined statistically by an un-paired t-test.

RESULTS

Changes in milk yield, udder blood flow and body weight (Table 1)

Studies of the pre-treatment period of both groups were started 45 days after the start of lactation. After 60 days of lactation, animals were injected sub-cutaneously biweekly intervals of rbST. The enhancement of milk yield in animals given rbST was higher than that of the control animals given placebo and the persistency of production was higher in these animals throughout their lactation. The peak milk yield in both groups of animals

declined from the early period of lactation as lactation advanced to mid and late lactation. Compared with the pre-treatment period, the actual increases in milk yield during the different lactating periods was 20%, 10% and -2% for animals receiving the rbST over 45, 105 and 165 days, respectively. During early lactation, the milk yield of rbST treated animals was higher than those of the control animals ($P< 0.05$). The body weights of both control animals and rbST treated animals significantly increased stepwise as compared with the pretreated period, while rbST treated animals had a higher weight gain than control animals throughout the lactation. The rate of udder blood flow and plasma flow markedly increased during rbST administration. The udder blood flow of rbST treated animals increased from 3548 to 5310 and 5458 ml/min ($P<0.05$) in both early and mid lactation, respectively, as compared with pretreatment period., while there were no significant changes in in the control animals. The values of both udder plasma flow and udder blood flow were significantly higher than those of the control during early and mid lactation. The ratio of udder blood flow to the rate of milk yield increased as lactation advanced in both the control and the rbST treated animals.

Arterial plasma concentration, arteriovenous concentration differences and mammary uptakes of glucose and acetate (Table 2).

The mean arterial plasma glucose concentrations were largely unchanged throughout periods of study in both controls and rbST-treated animals. However, during lactation advances, the arteriovenous differences and extraction ratio of glucose across the mammary gland decreased as compared with pretreatment period in both groups and the large extent of the decreases were apparent in rbST-treated animals as compared with the control animals. The net mammary uptake of glucose in early lactation of rbST-treated animals increased approximately 20%, whereas it decreased in mid- and late lactation as compared with the pretreatment period. The arterial plasma acetate concentrations were largely unchanged throughout periods of study in both controls and rbST-treated animals. The arteriovenous concentration differences, extraction ratio and mammary uptake of acetate were increased as lactation advances as compared with the pretreatment period in rbST-treated animals. The arteriovenous concentration differences, extraction ratio and mammary uptake for acetate of rbST-treated animals were significantly higher than those of the controls during early and mid-lactation.

Arterial plasma concentration, arteriovenous concentration differences and mammary uptakes of β-hydroxybutyrate and glycerol (Table 3).

The mean arterial plasma concentrations for β-hydroxybutyrate and free glycerol were unchanged throughout experimental periods in both groups. The arteriovenous differences and extraction ratio of β-hydroxybutyrate were not responsive to in either the controls or rbST-treatment. The mammary uptake for β-hydroxybutyrate of rbST-treated animals increased as lactation advances in comparison with pretreatment period while it remained constant through the course of lactation .in the control animals. The arteriovenous differences and extraction ratio of free glycerol across the mammary gland in both groups showed valiable which were affected to the net mammary uptake.

Arterial plasma concentration, arteriovenous concentration differences and mammary uptakes of free fatty acid and triacylglycerol (Table 4).

The mean arterial plasma concentrations for free fatty acid (C_{16} to C_{18}) of rbST-treated animals were elevated after rbST administration as compared with the pretreatment period and those of control animals. The significant differences of free fatty acid concentration between control animals and rbST-treated animals were apparent in the early lactation The values of arteriovenous differences and the net uptake by the mammary gland for FFA were variable during lactating periods in both groups. The mean arterial plasma concentrations for triacylglycerol(C_{16} to C_{18}) showed no significant differences after rbST administration throughout lactation. The arteriovenous differences, extraction ratio of triacylglycerol were unchanged as compared with pretreatment period in rbST-treated animals, but the net uptake of triacylglycerol across the mammary gland increased in rbST-treated animals in comparison with pretreatment period. There were no significant differences of arteriovenous differences, extraction ratio and net uptake of triacylglycerol during lactation advance in control animals.

Table 1. Means±SD of mammary plasma flow (MPF), mammary blood flow(MBF), and the ratio MBF/ Milk yield in different stages of lactation of the control animals and rbST-treated animals.

Measurement	Lactation period	Control Group	rbST Group	Control VS rbST Group
MPF, ml/min	Pretreated	2438±331	2594±342	NS
	Early	2730±357	3927±1203*	P<0.05
	Mid	2698±319	3983±1183*	P<0.05
	Late	2692±290	3533±1055	NS
MBF, ml/min	Pretreated	3286±461	3548±463	NS
	Early	3817±616	5310±1620*	P<0.05
	Mid	3821±533	5458±1627*	P<0.05
	Late	3750±476	4814±1464	NS
Milk yield, kg/d	Pretreated	13.0±1.5	13.3±2.7	NS
	Early	13.1±1.9	16.0±2.1**	P<0.05
	Mid	12.9±1.5	14.6±1.9	NS
	Late	11.5±1.0	13.0±1.3	NS
MBF/ Milk yield, L/kg	Pretreated	364±25	397±111	NS
	Early	420±32	491±152	NS
	Mid	433±89	539±156	NS
	Late	471±69	539±168	NS

For the control group, n=5; for rbST-treated group, n=5.

Statistical test of P-values between periods of lactation in each group using paired t-test of * (P<0.05), with respect to pretreated period.

Comparison of P-values of control group vs. rbST-treated group using unpaired t-test, NS= not significant.

Table 2. Arterial plasma concentrations, mammary arteriovenous differences and mammary uptake for glucose and acetate in different stages of lactation of the control animals and rbST-treated animals

	Period of experiment	Control Group	rbST Group	Control VS rbST Group
Glucose :				
Arterial concentrate	Pretreated	3.78±0.23	3.72±0.14	NS
(μmol/ml)	Early	3.83±0.37	3.80±0.24	NS
	Mid	3.60±0.14	3.77±0.15	NS
	Late	3.67±0.05	3.62±0.19	NS
A-V (μmol/ml)	Pretreated	0.93±0.30	0.98±0.15	NS
	Early	0.90±0.12	0.76±0.07*	P<0.05
	Mid	0.78±0.12	0.59±0.21**	NS
	Late	0.73±0.21	0.62±0.21**	NS
Extraction (%)	Pretreated	25±7	26±3	NS
	Early	24±4	20±3*	NS
	Mid	22±3	15±6**	P<0.05
	Late	20±6	17±6**	NS
Udder Uptake	Pretreated	2323±963	2502±496	NS
(μmol/min)	Early	2459±472	3010±941	NS
	Mid	2087±284	2201±433	NS
	Late	1979±625	2018±369	NS
Acetate :				
Arterial concentrate	Pretreated	1102±160	1171±109	NS
(μmol/l)	Early	1180±212	1238±101	NS
	Mid	1009±157	1129±130	NS
	Late	1251±124	1199±154	NS
A-V (μmol/l)	Pretreated	479±305	575±146	NS
	Early	458±160	624±57	P<0.05
	Mid	357±109	628±223	P<0.05
	Late	477±200	552±172	NS
Extraction (%)	Pretreated	42±24	49±16	NS
	Early	38±8	51±7	P<0.05
	Mid	35±8	55±14	P<0.05
	Late	37±13	46±13	NS
Udder Uptake	Pretreated	1111±617	1448±372	NS
(μmol/min)	Early	1281±543	2464±873	P<0.05
	Mid	964±340	2317±389**	P<0.001
	Late	1310±616	1858±620	NS

For the control group, n=5; for rbST-treated group, n=5.
Statistical test of P-values between periods of lactation in each group using paired t-test of * (P<0.05), with respect to pretreated period.
Comparison of P-values of control group vs. rbST-treated group using unpaired t-test, NS= not significant.

Table 3. Arterial plasma concentrations, mammary arteriovenous differences and mammary uptake for β-hydroxybutyrate and glycerol in different stages of lactation of the control animals and rbST-treated animals.

	Period of experiment	Control Group	rbST Group	Control VS rbST Group
β- hydroxybutyrate :				
Arterial concentrate	Pretreated	1604±735	1136±303	NS
(μmol/l)	Early	1777±563	1248±384	NS
	Mid	1608±447	1420±408	NS
	Late	1510±258	1172±213	P<0.05
A-V (μmol/l)	Pretreated	688±293	458±116	NS
	Early	761±239	462±220	NS
	Mid	636±191	582±175	NS
	Late	528±165	441±238	NS
Extraction (%)	Pretreated	44±7	42±11	NS
	Early	44±11	37±12	NS
	Mid	40±5	41±9	NS
	Late	36±15	37±17	NS
Udder Uptake (μmol/min)	Pretreated	1679±736	1156±269	NS
	Early	2116±835	1986±1344	NS
	Mid	1710±572	2403±1272	NS
	Late	1421±488	1370±500	NS
Glycerol :				
Arterial concentrate	Pretreated	37±6	40±7	NS
(μmol/l)	Early	39±14	39±5	NS
	Mid	36±3	31±4*	P<0.05
	Late	33±5	31±5*	NS
A-V (μmol/l)	Pretreated	4±6	-1±6	NS
	Early	3±26	-4±4	NS
	Mid	6±5	0.5±9	NS
	Late	2±5	-6±7	NS
Extraction (%)	Pretreated	10±15	-5±16	NS
	Early	-2±64	-12±12	NS
	Mid	17±12	0.2±28	NS
	Late	6±14	-24±30	NS
Udder Uptake (μmol/min)	Pretreated	11±14	-2±16	NS
	Early	9±61	-16±16	NS
	Mid	17±12	-0.2±40	NS
	Late	7±12	-19±18	P<0.05

For the control group, n=5; for rbST-treated group, n=5.
Statistical test of P-values between periods of lactation in each group using paired t-test of * (P<0.05), with respect to pretreated period.
Comparison of P-values of control group vs. rbST-treated group using unpaired t-test, NS= not significant.

Table 4. Arterial plasma concentrations, mammary arteriovenous differences and mammary uptake for free fatty acid and triacylglycerol in different stages of lactation of the control animals and rbST-treated animals.

	Period of experiment	Control Group	rbST	Control VS rbST Group
Free fatty acid (C16-18) :				
Arterial concentrate	Pretreated	437.3±109.9	402.7±83.4	NS
(μmol/l)	Early	375.3±34.0	546.1±104.0	P<0.01
	Mid	394.3±62.2	485.4±185.5	NS
	Late	371.5±57.3	430.3±58.8	NS
A-V (μmol/l)	Pretreated	-27.6±70.9	-12.5±9.1	NS
	Early	-71.9±87.3	-16.4±81.8	NS
	Mid	-21.3±14.7	-27.5±63.6	NS
	Late	-31.8±38.9	-12.1±18.7	NS
Extraction (%)	Pretreated	-6.4±14.3	-3.4±2.4	NS
	Early	-20.0±23.7	-3.7±16.7	NS
	Mid	-6.0±4.6	-8.0±14.6	NS
	Late	-8.1±10.1	-2.8±4.0	NS
Udder Uptake	Pretreated	-70.5±186.5	-30.5±22.5	NS
(μmol/min)	Early	-210.9±274.1	-87.2±280.2	NS
	Mid	-54.1±33.6	-88.1±240.4	NS
	Late	-90.1±109.6	-28.2±50.1	NS
Triacylglycerol(C16-18) :				
Arterial concentrate	Pretreated	148±50	143±49	NS
(μmol/l)	Early	128±50	128±14	NS
	Mid	145±45	178±72	NS
	Late	155±79	166±45	NS
A-V (μmol/l)	Pretreated	54±49	53±34	NS
	Early	52±53	36±24	NS
	Mid	65±48	98±64	NS
	Late	74±62	82±52	NS
Extraction (%)	Pretreated	33±24	33±16	NS
	Early	35±22	27±15	NS
	Mid	42±18	49±17	NS
	Late	41±23	45±21	NS
Udder Uptake	Pretreated	138±135	136±87	NS
(μmol/min)	Early	130±121	164±137	NS
	Mid	166±105	348±222	NS
	Late	188±150	263±151	NS

For the control group, n=5; for rbST-treated group, n=5.
Statistical test of P-values between periods of lactation in each group using paired t-test of * (P<0.05), with respect to pretreated period.
Comparison of P-values of control group vs. rbST-treated group using unpaired t-test, NS= not significant.

DISCUSSION

In the present results, there were marked changes in average blood flow to the udder during long-term rbST administration. There was positive correlation between mammary blood flow and milk yield during early lactation in rbST-treated animals. The relationship between mammary blood flow and milk yield showed an increase in the ratio of mammary blood flow:milk yield as lactation advances during administration of rbST. Similar estimates made in dairy cows also indicated an increase in this ratio during administration of rbST(McDowell et al., 1987) and it was assumed that the mass of mammary tissue was not affected by the somatotropin. The increase in blood flow to the mammary gland more than milk yield would be partially attributable to an increase in cardiac output which has been reported in lactating cows injected daily with bST (Soderholm et al.,1988). In the present results, the conclusions for the mammary uptake of substrates are not based on changes in arteriovenous concentration differences and extraction ratio between the controls and rbST-treated animals. During early lactation in rbST-treated animals, the rate of blood flow to the udder would be a major determinant of the rate of glucose uptake by the udder which coincided with an increase in the mammary blood flow. Several investigations of mammary gland substrate uptake indicated by an increase in the arterial glucose concentration with bST administration (Fullerton et al.,1989; Sandles et al., 1988) whereas other works have demonstrated no difference (McDowell et al., 1987). Results in the present study support the latter observations regarding plasma glucose concentration. However, the low values of arteriovenous concentration differences and as lactation advances in either controls or rbST-treated animals whereas arterial glucose concentration remained the same, indicating that glucose uptake by the mammary gland was affected by stage of lactation and the activity of the mammary epithelial cell. The great extent of reduction in arteriovenous concentration differences and the extraction ratio of glucose as lactation advances in rbST-treated animals indicates that glucose transport by the mammary cell was rate limiting step. The high blood flow to the mammary gland during rbST administration would decrease the transit time of glucose, thereby prolonging the contact time between glucose in blood and mammary epithelial cell. Specific glucose transporters in mammary cell membranes has been also detected (Prosser, 1988). Therefore, the increased partition of nutrients to the mammary gland induced by rbST treatment would be facilitated by increased mammary blood flow.

It has been known that volatile fatty acid in the form of acetate are the major of energy source of normal fed ruminants. In the present study, mammary arteriovenous

concentration differences, extraction ratio and mammary uptake of acetate increased in different stages of lactation as compared with pretreatment period in rbST-treated animals. An elevation of mammary acetate uptake during rbST administration is explainable in light of the high energy and substrate demands for milk synthesis, because acetate is involved in mammary gland metabolism in either de novo synthesis of short and medium-chain milk fatty acids or generation of ATP and NADPH. The distribution of short and medium chain milk fatty acids in milk fat was not altered by rbST treatment (data not presented), indicating that acetate was partially redirected from oxidation to de novo fatty acid synthesis. Acetate uptake was also critically dependent upon rate of mammary blood flow. Circulating β-hydroxybutyrate arise mainly from rumen butyrate in the fed animal (Leng and West, 1969). In the present result, levels of arteriovenous concentration differences and extraction ratio of β-hydroxybutyrate across the mammary gland including the arterial plasma concentration, were not affected during rbST administration. It indicates that the utilization by the mammary tissue was not obvious in during rbST administration in 87.5%HF feeding on urea treated rice straw. Although the principal effect of bST is to partition nutrients that lipid accretion is reduced and free fatty acids made available for oxidation (McCutcheon and Bauman, 1986). The greater energy requirement resulting in increased hepatic ketogenesis due to greater mobilization of fat reserves (Schultz, 1974) were not apparent in rbST-treated animals.

In the present experiment, the mean values for arterial plasma concentration of free fatty acid but not for triacylglycerol increased in rbST-treated animals and were more sensitive to alteration than other blood substrates, this phenomenon has been proposed as an indication of under-nutrition (Reid and Hinks, 1962). However, animals in both groups gained weight throughout the experiment. A marked increase in milk yield with rbST treatment without loss of body weight, especially during early lactation, may be due to the fact that the animals were fed to allow an adequate replacement of body reserves between lactations. Milk yield in the first lactating crossbred animals in the present study were not as great as that of multiparous cows (Sullivan et al., 1992). This is possibly related to the continued weight increase observed in animals during their first lactation. These results provide the physiological differences between crossbred animals and exotic breeds in partitioning ability, which would be inherited. During early lactation, the metabolic demands of lactation in both groups of the crossbred HF animals were met by dietary intake, thus not causing mobilization of body tissues as indicated by no alteration of the levels of both triglyceride and glucose. A significant increase in the concentration of FFA was apparent in rbST-treated animals as compared with the control animals during early

lactation. Thus, the lipolytic activity would be a function of bST treatment per se in stead of the associated changes in energy balance. The 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 is the release of FFA into venous blood due to triacylglycerol hydrolysis during the uptake of plasma triacylglycerol as in lactation (West et al.,1967). The net uptake of triacylglycerol by the mammary gland did not significantly increase in lactating period in comparison to the pretreatment period in both groups. It is possible that changes for releasing of FFA which are a result of changes of enzymatic activity of lipoprotein lipase in the mammary tissue. This enzyme activity has been reported to be higher in lactating bovine mammary tissue relative to other tissue (Shirley et al., 1973).

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CHAPTER VIII

Effects of long-term administration of recombinant bovine somatotropin on cellular metabolites in the milk secretion at different stages of lactation in crossbred Holstein cattle

(Submitted for publication in Br.J. Nutri.)

(**Running head**: Somatotropin and milk cellular metabolites)

Effects of long-term administration of recombinant bovine somatotropin on cellular metabolites in the milk secretion in crossbred Holstein cattle

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Abstract

The objective of this study was to determine the effects of long-term treatment with recombinant bovine somatotropin (rbST) on the concentrations of cellular metabolites in the milk. Ten crossbred Holstein cattle were used, divided into two groups of five animals each. Four consecutive study periods were carried out for the pretreatment period, early, mid and late lactation. Animal that had completed 60 days of lactation was injected subcutaneously every 14 days throughout lactation with 500 mg of rbST. Milk yield of rbST-treated animals rose (+22%) to a peak in early lactation. Lactose and milk triacylglycerol secretion of rbST-treated animals significantly increased ($P<0.05$) which coincided with significant increase in the concentrations of milk glucose in early and mid-lactation as compared with pretreatment period. The concentrations of milk galactose markedly increased whereas the concentrations of milk UDP-glucose significantly decreased ($P<0.05$) as lactation advances in both groups. The concentrations of milk citrate decreased while the concentrations of 2-oxoglutarate increased as lactation advances in both groups. The concentration of milk isocitrate significantly decreased at late lactation in the control animals. The concentration of milk G6P, milk G1P and cAMP markedly decreased as lactation advances in both groups. These findings indicate that the concentration of glucose in milk reflecting intracellular glucose concentrations, can be one of the factors regulate the rate of lactose production. The galactopoietic effect elicited by administration of rbST during early lactation depends on increased the conversion of glucose to intermediary metabolites in the lactose biosynthetic pathway.

Bovine somatotropin: Milk cellular metabolite : Crossbred Holstein cattle

Introduction

The rate of milk production depends on function of number of secretory cells and their metabolic activity. There is evident that major changes in the concentrations of metabolites in the mammary secretion are apparent during lactogenesis. The decrease in milk yield after peak lactation in dairy cattle has long been a biological conundrum for the mammary biologist, as well as a cause of considerable lost income for the dairy farmer. Previous studies in vivo have shown that changes occur in the metabolism of the mammary gland between different types of the crossbred dairy cattle. During lactation advance to mid and late lactation, the rate of lactose synthesis decreases and milk yield falls in 87.5%HF animals (Chaiyabutr et al., 2000a). A poorer lactation persistency in 87.5% HF animals has been shown to relate to a decrease in the lactose biosynthetic pathway with a reduction in the percentage of metabolism of glucose 6-phosphate to galactose moiety of lactose (Chaiyabutr et al., 2000a); at the same time both the level of plasma bovine somatotropin and blood flow to the mammary gland decreased during the transition period from early to mid-lactation in 87.5%HF animals in comparison with 50%HF animals (Chaiyabutr et al., 2000b, Chaiyabutr et al., 2000c). These findings were obtained in vivo using radiotracer techniques and by measuring arterio-venouse concentration differences of metabolites across the gland. However, detailed information on the changes in the concentrations of metabolites within the mammary gland corresponding to a short persistency of lactation is not available in the crossbred dairy cattle.

Lactose is synthesized in the mammary secretory cell from glucose derived from the blood and involves the conversion of glucose to glucose 6-phosphate, glucose 1-phosphate, UDP-glucose and then UDP-galactose in the cytsol. UDP-galactose passes into the Golgi vesicle where it combines with glucose to form lactose and UDP. The contents of the Golgi vesicle are secreted into alveolar lumen by exocytosis (Kuhn et al. 1980). Thus, glucose is an important intermediary of metabolism in general and is particularly important for lactation. As milk yield increases in early lactation and decreased as lactation advance, changes in the concentration of some metabolites may represent a rate of secretion of the metabolite into milk volume.

Therefore, the purposes of this study were to determine the concentration of a variety of cellular metabolites in milk if there are changes with the decrease in the level of plasma bovine somatotropin and milk yield as lactation advances. Long-term treatment

with recombinant bovine somatotropin (rbST) at different stages of lactation were carried out to obtain a more complete picture of the role of bovine somatotropin in changes in the concentrations of metabolites in milk which could be interpreted in relation to the biochemical changes occurring within the mammary gland in crossbred dairy cattle.

Materials and methods

Animals and their management

The experiment was conducted on ten, first lactating, non-pregnant, crossbred, 87.5% Holstein dairy cattle. They were divided into two groups of five animals each. Animals in each group were fed with rice straw treated with 5% urea as the source of roughage throughout the experiments. All animals were housed in sheds and tethered in individual stalls and fed twice daily. The maximum temperature in the shed at noon was 34±1°C and the minimum temperature at night was 26±1°C. The relative humidity was 68±12%. Animals individually received an average of 4 kg/day of roughage in combination with the same concentrated mixture (7 kg/day) to maintain a moderate body condition score (2.5, scale = 1 to 5). Each day, the food was given in two parts to the animal separately at the time of milking (morning 06.00 h and evening 17.00 h). The chemical composition of the feed has been previously described (Chaiyabutr et al.2005). Animals had free access to water throughout the day.

Experimental procedures

Animals were divided into control (n=5) and experimental (n=5) groups. Four consecutive periods of study were undertaken for each group, consisting of a pretreatment period (at day 45 postpartum), and treatment periods of 105 days postpartum (early lactation), 165 days postpartum (mid- lactation) and 225 days postpartum (late lactation). During the treatment periods, animals in the experimental group were injected subcutaneously starting at day 60 pospartum, every 14 days until the end of study, with 500 mg of recombinant bovine somatotropin (rbST), suspended in 792 mg of a prolonged-release formulation of sesame oil (POSILAC, Monsanto, USA), while animals in the control group were injected subcutaneously in every 14 days with 800 mg of sterile sesame oil as a placebo. Injection in each animal was administered at the tail head depression (ischiorectal fossa). From the beginning of

pretreatment to the end of the treatment periods, animals of both groups were fed the same ration, from before parturition until the completion of the study. Animals were normally milked using a milking machine and milk production was recorded daily.

Collection of milk sample and estimation of milk metabolites

On the day of study in each period, milk sample was collected from each animal in the evening milking. Sample was kept at 4°C until prepared for metabolites analysis. The samples of milk were defatted and deproteinized, and analysed for metabolites. Briefly, milk was centrifuged at 50,000g for 45 min at 4°C. The aqueous phase below the solidified fat layer was removed and deproteinized with 5M-perchloric acid. The precipitated protein was removed by centrifugation at 2500 g for 10 min. The supernatant fraction was neutralized with 5M potassium hydroxide and precipitated potassium perchlorate was removed by centrifugation. The resulting supernatant fraction using directly for the measurements of isocitrate (Beutler,1985), 2-oxoglutarate (Burlina,1985), galactose (Beutler, 1985), uridine 5'-diphosphoglucose (UDP-glc) and uridine5'-diphosphogalactose(UDP-gal) (Keppler & Decker, 1985) glucose1-phosphate (G1P) (Michal, 1984a) and glucose6-phosphate (G6P) (Michal, 1984b), were performed according to spectrophotometric methods. Milk glucose was determined using glucose oxidase. Milk citrate and lactose concentrations were determined colorimetrically as described by White and Davis (1963) and Teles et al. (1978), respectively. The concentration of cyclic adenosine 3,5'monophosphate (cAMP) in milk was determined using cAMP(3H) assay System TRK432 (Amersham Biosciences UK Limited, UK). Milk triglyceride fatty acid composition (C_6 to C_{18}) was determined by gas chromatography after extraction by chloroform and methanol (Christopherson & Glass, 1969).

The rate of lactose secretion was determined from the milk yields and the lactose concentrations in milk. The rate of fatty acid secretion in milk was calculated from the milk yield and the concentrations of the medium chain fatty acids .

Chemicals and biochemicals.

All coenzymes and the enzymes, UDP glucose dehydrogenase (EC1.1.1.22), UDP glucose pyrophosphorylase (EC2.7.7.9) and UDP glucose 4-epimerase (EC 5.1.3.2) were purchased from CalBiochem.,U.K. Phosphoglucomutase (EC 2.7.5.1) and B-

galactose dehydrogenase (EC 1.1.1.48) were purchased from Roche Diagnostics Asia Pacific Ple Ltd. Singapore. Glucose oxidase, (EC 1.1.3.4), G6P dehydrogenase (EC 1.1.1.49), Isocitrate dehydrogenase (EC 1.1.1.42) and Glutamate dehydrogenase (EC 1.4.1.3) were obtained from Sigma Chemical Co., U.S.A. All other chemicals were analytical grade.

Mammary blood flow measurements

On the day the experiment, blood flow through half of the udder was determined by measuring the dilution of dye T-1824 (Evans blue) after a short term, continuous infusion, adapted from a method of measuring blood flow in the milk veins of cattle as previously described (Chaiyabutr et al.1997).

Statistical analysis

Values were compared between the periods of lactation in each group using a paired t-test. Between group trials and mean differences were examined statistically by an un-paired t-test. Mean values are presented as mean±S.D.

Results

The present results show that milk yield responses at 45 days of rbST treatment during early lactation were significantly greater (+20% compared with pre-treatment) than to the 105 days of rbST treatment in mid lactation (+9.5%) and to 165 days of rbST treatment in late lactation (-2.%) (Table1). Milk yields in animals treated with rbST were higher than those of the control animals receiving placebo throughout their lactation. In early lactation, milk yield of rbST treated animals was 22% greater ($P<0.05$) than that of the control animals. The peak milk yield in both groups declined as lactation advanced to mid and late lactation. In late lactation, milk yield of rbST treated animals decreased to 19 % as compared with the pre-treatment value. The mean values of both of lactose secretion and milk triacylglycerol secretion in rbST-treated animals was significantly increased ($P<0.05$) in the early period of lactation. The udder blood flow of rbST treated animals significantly increased in early and mid lactation ($P<0.05$), while there were no significant changes in the control animals receiving placebo.

Table 2 shows significant increase in the concentrations of glucose in milk during rbST administration in early and mid-lactation in comparison to pretreated period,

while it remained constant throughout lactation in the control animals. The concentrations of galactose in milk markedly increased as lactation advances over that seen during the pre-treatment period in both controls and rbST treated animals. The concentrations of UDP-glucose in milk appeared to significant decrease ($P<0.05$) with slight decrease in the concentrations of UDP-galactose in milk as lactation advances in both the controls and rbST treated animals. UDP-galactose : UDP-glucose ratio was constant throughout different stages of lactation in both controls and rbST treated animals. The concentrations of G6P in milk of the control animals markedly decreased as lactation advances, while it remained constant in early lactation during rbST administration and it significantly decreased in mid- and late lactation in comparison with pretreated period ($P<0.05$). The concentrations of G1P in milk appeared to significant decrease ($P<0.05$) as lactation advances in both the controls and rbST treated animals. A marked decrease in the concentrations of cAMP in milk was seen as lactation advances in both groups.

Table 3 shows no significant differences in the concentrations of isocitrate in milk of rbST-treated animals at different stages of lactation. In the control animals, the concentrations of milk isocitrate significantly decreased at the late of lactation. The concentrations of citrate in milk decreased whereas the concentrations of 2-oxoglutarate increased as lactation advances in both groups. The value of isocitrate: 2-oxoglutarate ratio had a tendency to decrease as lactation advances in the control animals while it remained constant in the rbST-treated animals.

Table 1. Changes in Udder blood flow, milk secretion of lactose and triacyglycerol at different stages of lactation in the controls and rbST treated animals (Mean ± SD).

	Period of experiment	Control Group	rbST Group	Control VS rbSTGroup
Udder blood flow	Pretreated	3.38±0.46	3.55±0.46	NS
(l/min)	Early	3.82±0.62	5.31±1.62*	NS
	Mid	3.82±5.33	5.46±1.63*	NS
	Late	3.75±0.48	4.81±1.46	NS
Milk yield (kg/d)	Pretreated	12.98±1.53	13.37±2.66	NS
	Early	13.11±1.85	16.02±2.11**	P<0.05
	Mid	12.89±1.47	14.64±1.89	NS
	Late	11.53±1.00	13.01±1.34	NS
Milk lactose secretion	Pretreated	1113±245	1259±239	NS
(μmol/min)	Early	1139±197	1469±363*	NS
	Mid	1215±166	1303±229	NS
	Late	1068±107	1202±158	NS
Milk triacyglycerol	Pretreated	397.2±59.5	469.1±151.6	NS
secretion (μmol/min)	Early	457.5±157.3	794.5±223.6*	P<0.05
	Mid	493.6±107.0	664.4±259.8	NS
	Late	465.7±127.0	662.6±214.6	NS

P-values by paired t-test: * P<0.05, ** P<0.01 with respect to the pretreated period in each group.

P-values by unpaired t-test between the control animals and rbST treated animals.

Table 2. Changes in the concentrations of glucose, galactose, UDP-glucose, UDP-galactose, cAMP, G-6-P and G-1-P in crossbred Holstein's milk at different stages of lactation in the controls and rbST treated animals (Mean ± SD).

	Period of experiment	Control Group	rbST Group	Control VS rbST
Milk Glucose(mmol/l)	Pretreated	0.49±0.16	0.49±0.10	NS
	Early	0.54±0.28	0.63±0.12***	NS
	Mid	0.56±0.29	0.62±0.05*	NS
	Late	0.58±0.26	0.41±0.14	NS
Milk Galactose	Pretreated	243±60	296±145	NS
(µmol/l)	Early	419 ± 140*	460±107*	NS
	Mid	500 ±150*	593±61**	NS
	Late	502± 147*	570±67*	NS
Milk UDP-glucose	Pretreated	12.6 ±3.1	13.0±1.5	NS
(µmol/l)	Early	10.8 ± 2.7	11.0±2.5*	NS
	Mid	10.0 ±1.8 *	11.3±1.4*	NS
	Late	9.5 ± 1.8*	11.0±2.0*	NS
Milk UDP-galactose	Pretreated	18.6 ± 3.7	20.3±7.3	NS
(µmol/l)	Early	15.8 ± 2.2	18.0±5.6	NS
	Mid	14.7 ± 4.3	19.6±6.5	NS
	Late	14.9± 4.6	17.7±8.8	NS
UDP-gal/ UDP-glc	Pretreated	1.52±0.25	1.66±0.81	NS
	Early	1.56±0.54	1.74±0.86	NS
	Mid	1.48±0.44	1.78±0.78	NS
	Late	1.56±0.33	1.72±1.19	NS
Milk cAMP(nmol/ml)	Pretreated	7.20±1.04	7.65±2.04	NS
	Early	4.87±1.77**	3.94±2.24**	NS
	Mid	4.18±1.81**	2.13±0.74**	P<0.05
	Late	3.28±1.21***	1.21±0.61**	P<0.01
Milk G-6-P (µmol/l)	Pretreated	75.3 ±8.8	76.4 ±10.3	NS
	Early	38.2±17.4*	60.4±21.8	NS
	Mid	24.0±18.5**	23.7±19.3*	NS
	Late	22.0 ±18.8**	27.0±26.8*	NS
Milk G-1-P (µmol/l)	Pretreated	155.3 ±93.3	146.5±58.4	NS
	Early	43.5±26.1*	71.7±34.2*	NS
	Mid	27.5±28.1*	40.7±55.3*	NS
	Late	31.0±36.2*	10.2±3.5**	NS

P-values by paired t-test: * P<0.05, ** P<0.01, *** P<0.001 with respect to the pretreated period in each group.

P-values by unpaired t-test between the control animals and rbST treated animals.

Table 3. Changes in the concentrations of isocitrate, 2-oxoglutarate and citrate in crossbred Holstein's milk at different stages of lactation in the controls and rbST treated animals (Mean ± SD).

	Period of experiment	Control Group	rbST Group	Control VS rbST
Milk isocitrate (µmol/l)	Pretreated	29.3±3.8	29.9±4.8	NS
	Early	30.9±9.1	30.4±5.6	NS
	Mid	26.5±5.9	28.1±7.9	NS
	Late	23.0±3.5*	28.4±5.9	NS
Milk 2-oxoglutarate (µmol/l)	Pretreated	63.4±26.9	57.5±30.7	NS
	Early	95.1±49.1	87.0±32.5	NS
	Mid	92.4±36.0	92.7±12.9	NS
	Late	92.9±28.6	88.0±15.9	NS
Isocitrate /2-oxoglutarate	Pretreated	0.54±0.23	0.43±0.18	NS
	Early	0.45±0.41	0.37±0.09	NS
	Mid	0.34±0.20	0.31±0.10	NS
	Late	0.26±0.06	0.33±0.09	NS
Milk-citrate(mmol/l)	Pretreated	8.4±0.9	8.2±0.8	NS
	Early	7.8±1.6	7.3±1.0*	P<0.05
	Mid	7.7±0.8	7.2±0.8	NS
	Late	7.2±1.1	6.8±1.1	NS

P-values by paired t-test: * P<0.05 with respect to the pretreated period in each group.

P-values by unpaired t-test between the control animals and rbST treated animals.

Discussion

In the tropics, crossbreeding of indigenous and exotic cattle for tropical use have been exploited as an efficient tool for blending the adaptability of tropical cattle with the high milking potential of exotic breeds resulting in increased milk production. Chaiyabutr et al.(2000b) reported that the concentration of bovine somatotropin of lactating crossbred containing 87.5%Holstein genes decreased as lactation progressed to mid- and late lactation; this decrease could attribute to a reduction in milk yield. The role of bovine somatotropin on physiological changes in relation to short persistency of lactation in crossbred dairy cattle in the tropics is not yet clear. The concentration of metabolites in milk can be interpreted in terms of changes in metabolic activity of the mammary secretory cell and it may provide an insight into biochemical processes without using tissue samples (Chaiyabutr et al.1981; Faulkner & Clapperton, 1981).

The present results show that the peak milk yield of rbST-treaed animals declined as lactation advanced to mid and late lactation. Thus, rbST also affects the shape of the lactation curve. These results confirm the finding that an increase in milk yield in response to rbST administration will not be sustained indefinitely (Bauman, 1992), and that it is influenced by the stage of lactation (Phipps et al.1991).

An increase in milk yield during bST administration is thought to be determined primarily by lactose secretion (Linzell and Peaker, 1971). Lactose is synthesized in the mammary secretory cell from glucose derived from the blood. The concentration of milk glucose significantly increased which coincided with an increase in milk yield during rbST administration in both early and mid-lactation. This would reflect to the intracellular glucose concentration (Kuhn and White,1975; Faulkner et al,1981), since glucose freely permeates across Golgi vesicles and apical membranes of the mammary secretory cells (Faulkner & Peaker, 1987). Mammary cell cannot synthesize free glucose because they lack glucose-6-phosphatase activity (Threadgold & Kuhn, 1979). It is likely that the high concentrations of milk glucose in rbST-treated animals are related to a high rate of glucose uptake by the mammary gland, consistent with the higher mammary blood flow to the mammary gland during rbST administration (Chaiyabutr et al., 2005). The present results show that the concentration of milk glucose coincided with an increase in lactose secretion in rbST-treated animals. This suggests that the rate of entry of glucose into mammary cell would be partly

responsible for the lactose synthesis. Lactose secretion can draw water osmotically from the inside of the cell into the vescicle and into milk. This is believed to be a mechanism for increasing milk yield by which bulk water movement occurs into milk (Linzell & Peaker, 1971). The rate of lactose synthesis has been known to be controlled by the regulation of the activity of lactose synthase, then an accumulation of intermediary substrates would be expected. The synthesis of lactose involves combination of glucose and UDP-galactose; the UDP-galactose originates from G6P (Ebner & Schanbacher, 1974). In early lactating period, the high concentration of G6P in milk of animals treated with rbST would reflect the availability of intracellular G6P in the cell which is sufficient to account for the process of cytosolic lactose synthesis.

In contrast to milk glucose, an increase in the concentration of milk galactose as lactation advances in both groups is thought to be either cytosolic origin via Golgi vesicles permeate the apical membrane directly from the cytosol (Faulkner et al.,1985), or from the hydrolysis of lactose and other acid-labile compounds such as UDP-galactose or galactose 1-phosphate in the luminal milk (Kuhn & White,1975).

The concentration of milk UDPglucose fell more than that of UDPgalactose as lactation advances in rbST-treated animals. It indicates that the equilibrium between these two metabolites was not maintained in the cytosol during rbST administration. The metabolites UDPglucose and UDPgalactose are both synthesized in the cytosol with a high concentration. It is possible that there was either an increase in the conversion of UDPglucose to UDPgalactose by catalysing UDPglucose 4-epimerase (Babad & Hassid,1966), or a decrease in UDPglucose transport into Golgi vesicle during rbST administration, since the aqueous phase of milk is derived mainly from the fluid of the Golgi vesicles (Linzell & Peaker, 1971). The high level of UDPgalactose in milk would reflect its concentration in the Golgi vesicles rather than in the cytosol. However, the concentrations of UDPgalactose in the Golgi vesicles have been shown to be a balance between its facilitated transport into the Golgi vesicle (Kuhn & White,1975), and its utilization for lactose synthesis. In contrast to rbST-treated animals, the decreases in milk UDP-galactose concentration as lactation advances in the control animals may reflect an initial decrease in the cytosolic G6P concentration if regulation of glucose uptake by the udder precedes the decreased rate of lactose synthesis. Therefore, it may be coincidental that the proportional decreases between UDPglucose and UDPgalactose concentrations in milk are in similar manner in the control animals. The metabolites of G6P and G1P are thought to be of cytosolic

origin and are generally in lower concentration than those metabolites of Golgi vesicle origin. This may reflect their greater utilization within cytosol and their lower uptake and secretion via Golgi vesicle.

Bovine somatotropin is known as a homeorrhetic hormone concerned with both growth and lactation, but the mechanism of action of bovine somatotropin on milk fat production is a controversial area, as receptors for bovine somatotropin have not been demonstrated on secretory epithelial cells of mammary tissue (Akers, 1985). The effects of bovine somatotropin on milk fat production would be indirectly mediated either *via* nutrient partitioning effects or *via* fatty acid synthesis *de novo* (Bauman, 1992). Since, administration of exogenouse bST has been known to reduce lipid accretion and fatty acids made available for oxidation and precise coordination with an increase in milk fat yields (McCutcheon & Bauman, 1986). During prolonged treatment with rbST, an increase in the rate of milk triacylglycerol secretion, indicating an increase in fatty acid synthesis *de novo* in the mammary gland. This increase would be expected to result in an increased rate of utilization of reducing equivalents in the form of NADPH in the mammary gland. One of the source of NADPH in the cytosol is involved the enzyme isocitrate dehydrogenase which is an important alternative pathway for NADPH regeneration in ruminants (Bauman et al.,1970). The activity of the enzyme, isocitrate dehydrogenase is high in ruminant mammary gland and the reaction catalysed which involves the simultaneous oxidation of isocitrate to 2-oxoglutarate and reduction NADP to NADPH (Bauman et al.,1970). Thus, when rates of fatty acid increase, the utilization of NADPH also increases, flux through the isocitrate dehydrogenase reaction increases producing decreased isocitrate concentrations and increased 2-oxoglutarate concentrations. The results from the present experiment indicate that, the concentrations of milk isocitrate decreased in stepwise with an increase in the concentration of 2-oxoglutarate as lactation advances in control animals. The values of milk isocitrate: 2-oxoglutarate ratio also decreased in stepwise as lactation advances, which were related inversely to the rate of fatty acid synthesis in the mammary gland. The decreases in the values of isocitrate:2-oxoglutarate ratio can be used to follow changes in NADPH and NADP in the cytosol (Veech et al,1969).. The more conversion of isocitrate to 2-oxoglutarate in the cytosol of the cell occurred in the control animals than those of rbST-treated animals. Therefore, any changes in isocitrate:2-oxoglutarate in milk appear to reflect the expected changes in the corresponding cytosolic values. Administration rbST did not

affect isocitrate : 2-oxoglutarate ratio by changes in the concentrations of isocitrate and 2-oxoglutarate which may alter cytosolic citrate concentrations by interconversion of citrate and isocitrate and thus provide an explanation for the decrease in the citrate concentration in milk.

The decrease in the concentrations of cAMP was seen as lactation advance in both groups. Degradation of cAMP within the cell is achieved by the action of cAMP-phosphodiesterase enzymes. cAMP has been shown to be both mitogenic and morphogenic in the gland (Silberstein et al, 1984) and it has also been suggested that cAMP-mediated processes might influence apoptosis of mammary epithelial cells (Marti et al, 1994) . The cAMP concentration in milk altered with milk yield in both the control and rbST treated animals, being high with high milk yield and low with low milk yield. Similar results have been obtained in the goat (Blatchford et al, 1984). The presence of concentration of cAMP in milk in both groups in different stages of lactation may indicate the role of cAMP as a mediator of cellular function as lactation advances.

Conclusion

The present study has shown that rbST administration to crossbred dairy cattle in different stages of lactation can increase milk yield coinciding with an increase in both lactose output and milk glucose concentration. An increase in mammary blood flow would be partly responsible for the rate of entry of glucose into mammary cell. Milk glucose concentrations reflect intracellular glucose concentrations during rbST administration, can be one of the factors regulate the rate of lactose production. During early lactation, a large portion of the conversion of intracellular glucose to intermediary metabolites of rbST-treated animals, was mainly used in the lactose biosynthetic pathway, when compared with controls. These findings illustrate that rbST administration exerts its galactopoietic action, in part, through both extra-mammary and in-tramammary effects.

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CHAPTER IX

Effects of recombinant bovine somatotropin (rbST) administration on the utilization of glucose in the mammary gland in different periods of lactation in crossbred Holstein cattle

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Effects of recombinant bovine somatotropin (rbST) administration on the utilization of glucose in the mammary gland in different periods of lactation in crossbred Holstein cattle

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ABSTRACT : The experiment was done to determine the effects of long-term administration with recombinant bovine somatotropin to the utilization of glucose in the mammary gland of crossbred Holstein cattle. Ten crossbred Holstein cattle were used, divided into two groups of five animals each. Four consecutive study periods were carried out for the pretreatment period, early, mid and late lactation. Animals were injected subcutaneously with 500 mg of rbST (POSILAC) beginning 60 days postpartum in every 14 days throughout lactation. The utilization of glucose in the mammary gland was determined by measuring rates of glucose uptake and the incorporation of glucose into milk components in both control animals and rbST-treated animals.

In pretreatment period, there were no significant differences of the total glucose entry rate and glucose carbon recycling between the controls and rbST-treated animals. In early lactation, the glucose turnover rate of rbST-treated animals was decreased as compared with the pretreatment period, whereas there was no change in the control animals. Comparing for the mid-lactating period, rbST-treated animals showed an elevation of plasma glucose clearance and significant increases in the glucose turnover rate in comparison with pretreatment period. The percentages and values of non-mammary glucose utilization showed significantly increases during lactation advances to mid and late lactation as compared with pretreated period in rbST-treated animals

Animals treated with rbST showed significantly higher levels of mammary plasma flow and milk yield in early lactation than those of control animals. Milk lactose concentration showed no differences between groups of animals or among periods of lactation in the same group., The milk lactose secretion of rbST treated-animals

significantly increased whereas milk citrate concentration were significantly decreased in early lactation as compared with pretreatment period. The milk citrate concentration decreased in both groups during lactation advances. Milk triacylglycerol concentration and triacylglycerol secretion of rbST-treated animals were markedly higher in early lactation than that of pretreatment period and it was still in a high level throughout lactation. A high milk lactose secretion and citrate secretion during early lactation were apparent in rbST treated-animals when compared to those of control animals. The percentage of utilization of glucose carbon for synthesis of milk lactose was not significantly different between controls and rbST-treated animals. The utilization of glucose carbon for synthesis of milk citrate for rbST-treated animals was significantly higher than that of control animals during mid and late lactation. The utilization of glucose for synthesis of milk triacylglycerol was significantly higher during rbST administration throughout periods of lactation. As lactation advances, the intracellular glucose phosphorylated by the mammary gland was calculated to be completely metabolized via the pentose phosphate. Metabolism of glucose 6-phosphate via the Embden-Meyerhof pathway was calculated in term of the proportion of glucose metabolized, which there was considerable variation with advanced lactation of both groups. During early lactation, the NADPH formation from glucose that required for fatty acid synthesis *de novo* in the mammary gland of rbST treated-animals, which was sinificantly higher than that of the control animals. The milk fatty acid concentrations with a chain length of C_{16} to C_{18} sinificantly increased during rbST administration in differnt stages of lactation as compared with those of control animals. From this result, it can be conclude that the mechanism by which rbST directly or indirectly affects mammary gland function likely affect changes in the glucose utilization for biosynthetic pathways during early lactation. This affects the increase in the sufficient pool of intracellular glucose concentration, which has effect on an increase in glucose 6-phosphate flux through the lactose synthesis and pentose cycle pathway.

Key Words : rbST, Glucose Metabolism, Mammary Gland, Lactation

INTRODUCTION

It is known that dairy herds in tropical countries are mixed exotic breeds and crossbreeds. Exotic Bos taurus breeds have higher milk production but they also have inherent disadvantageous traits. They have low heat torelance with a higher heat load which causes a decrease in milk production. Bos indicus cattle have low genetic potential for milk production but are well adapted to the environment. Therefore, exotic Bos taurus breeds are used mainly for crossbreeding with native and other Bos indicus cows. There is still a need to answer the question of the type of crossbred cattle most suitable for the tropics and the management necessary for efficient dairy production in a hot climate. The low milk production of both exotic and crossbred cattle is still the main problem in dairy farming in the tropics. Many factors affecting the rate and quality of milk secretion by altering the metabolic endocrinology of dairy cattle are extensively reviewed. Many studies have shown that the administration of growth hormone to ruminants increased rates of productive efficiency (Breier et al., 1991; Burton et al .,1994). The mechanism by which this is thought to be achieved is complex and involves a number of events. It has been reported that the concentration of bovine somatotropin of the crossbred cattle containing 87.5% Holstein genes decreased rapidly as lactation progressed to mid and late lactation; this decrease could attribute to a reduction in milk yield and mammary blood flow (Chaiyabutr et al. 2000). However, little is known on the other circulating factors that involved in regulating mammary blood flow which is a major parameter controlling milk production.

An increase in production, espectially with respect to milk production in dairy cow, necessitates a substantial increase in the glucose requirement of the animal. Glucose is an important intermediary of metabolism in general and is particularly important for lactation. Glucose is utilized by the mammary gland for the biosynthesis of lactose, triacylglycerol and citrate. This has been studied in lactating ruminants *in vivo* and in the isolated perfused udder ( ). The role of glucose in regulating milk secretion has been formulated in the theory that lactose secretion can draw water osmotically from the inside of the mammary cells to milk (Linzell and Peaker, 1971). This is believed to be a mechanism for increasing milk yield by which bulk water movement occurs into milk.

Metabolism of glucose in mammary glands is also important in providing the reducing equivalents required for the *de novo* synthesis of fatty acids.

The supply of glucose is a principal determinant of the milk yield response to growth hormone. It has been reported that the whole body utilization of glucose is increased and whole body oxidation of glucose is decreased during bovine somatotropin treatment (Bauman et al.,1988). In crossbred cattle, mechanisms of milk secretion are known to be inherited and are thought to be among the causes of differences in metabolic parameters. Few data are available concerning the utilization of glucose and glucose metabolism in the udders of crossbred Holstein dairy cattle *in vivo* during bovine somatotropin administration. Our previous study has shown that the glucose utilization for biosynthetic pathways in the mammary gland of 50% HF animals was maintained in a similar pattern throughout the periods of lactation, while a short persistency of lactation in 87.5% HF animals has been shown to relate to a decrease in the lactose biosynthetic pathway (Chaiyabutr et al., 2000). The apparent discrepancies raise the question, whether the differences would be the effects of physiological state, genetic potential and endocrine regulation. Therefore, the present experiment was conducted to obtain the above information. Specifically, we examined the effects of prolonged adminitration of rbST on glucose metabolism and the efficiency of utilization of glucose by the mammary gland in different stages of lactation in 87.5% HF Holstein cattle.

MATERIALS AND METHODS

Animals and managements

Ten, first lactating, non-pregnant crossbred 87.5%Holstein dairy cattle were selected for the experiment. They were divided into two groups of five animals each. Animals in each group were fed with rice straw treated with 5% urea as the source of roughage throughout the experiments. All animals were housed in sheds and tethered in individual stalls and fed twice daily. The maximum temperature in the shed at noon was 34±1°C and the minimum temperature at night was 26±1°C. The relative humidity was 68±12%. Animals individually received an average of 4 kg/day of roughage in combination with the same concentrated mixture (7 kg/day) to maintain a moderate body condition score (2.5, scale = 1 to 5). The chemical composition of feeds is presented in Table 1. Each

day, the food was given in equal portions at about 06.00 h and 17.00 h when animals were milked. Animals had free access to water and animals were fed their respective rations throughout the experimental period.

The urea treated rice straw was prepared by mixing the urea solution with dry straw (5 kg urea dissolved in 100 litter water per 100 kg dry rice straw). Rice straw sprayed with urea solution was mixed thoroughly and stored under airtight conditions in a cement pit for 21 days. A continuous supply of treated rice straw was made available by using a 2 pit x 21 day system of urea treatment. After 21 days, the rice straw treated with 5% urea was offered to the animals

Experimental procedures

Animals were divided into control (n=5) and experimental (n=5) groups. Four consecutive periods of study were carried out in each group, consisting of the pretreatment period (45 days postpartum), and treatment periods of 105 days postpartum (early lactation), 165 days postpartum (mid-lactation) and 225 days postpartum (late lactation). In the treatment period, animals in the experimental group which had completed 60 days of lactation, were injected subcutaneously every 14 days until the end of study with 500 mg of recombinant bovine somatotropin (rbST) (POSILAC, Monsanto, USA), while animals in the control group were biweekly injected subcutaneously every 14 days with 800 mg of sterile sesame oil without rbST as placebo. Injection in each animal was administered at the tail head depression (ischiorectal fossa). Animals were normally milked at around 0600 h and 1700 h by a milking machine and milk production was recorded daily. On the day of study in each period, measurements of the udder blood flow were carried out in the afternoon. At around 1100 h, an arterial blood sample was collected from the coccygeal artery by venipuncture with a #21 needle into heparinized tube. Blood samples in heparinized tube were kept in crushed ice and then centrifuge at 3000 rpm for 30 min at 4°C. Arterial plasma samples were collected and frozen at -40°C in aliquots until time of assays for measurements the level of metabolites.

Glucose turnover measurements.

The study on glucose kinetics and efficiency of utilization of glucose by the mammary gland using both (U-^{14}C)-glucose and (3-^{3}H)-glucose infusions in crossbred animals was performed at different stages of lactation: pretreatment, early, mid- and late lactation, during treatment with rbST through the period of the experiment. Glucose kinetic studies of each animal in each lactating period were carried out as described previously by Chaiyabutr et al. (1998). Briefly, at about 1100h of the specified day, a priming dose of radioactive glucose in 20 ml of sterile NSS containing 60 μCi[3-^{3}H]glucose and 40 μCi [U-^{14}C]glucose was administered intravenously via the ear vein catheter and followed by a constant infusion of 1 ml/min of sterile saline (0.9%) containing 2 μCi [U-^{14}C]glucose and 3 μCi[3-^{3}H]glucose for 4h (Peristaltic pump; EYLA Model 3). During the final 1 hour (1400-1500h) of infusion, three sets of blood samples were collected at 20 min. intervals. A venous blood sample was collected from the milk vein via a catheter while an arterial blood sample was collected from the coccygeal artery by venipuncture with a #18 needle. Blood samples in heparinized tubes were kept in crushed ice for chemical studies. Milk secretion was recorded for the final 1 hour of infusion. Milk samples were used for measurement of radioactive glucose incorporation into other milk components.

Udder blood flow measurements

Measurements of udder blood flow through half of the udder were performed in duplicate by measuring the dilution of dye T-1824 (Evans blue) by a short term continuous infusion as described by Chaiyabutr et al. (1997). In brief, a dye (T-1824) was dissolved in sterile normal saline and diluted to a concentration of 100 mg/L. The solution was infused by a peristaltic pump (Gilson Medical Electronics) at a constant rate of 85 ml/min into the milk vein for 1 min which could produce adequate mixing of dye with blood. Before infusion, blood was drawn from downstream in the milk vein as a pre-infusion sample. About 10 seconds after starting the infusion, 10 ml of blood was drawn from downstream in the milk vein at a constant rate into a heparinized tube. Two consecutive plasma samples were taken during each dye infusion at about 5 min intervals. Blood flow of half of the udder was calculated from plasma samples using the equation derived by Thompson and Thomson (1977). Quarter milking showed that the yields of the two halves of the udder were similar. Udder blood flow was therefore calculated by doubling the flow measured in one milk vein (Bickerstaffe et al., 1974).

Packed cell volume was measured after centrifugation of the blood in a microcapillary tube. Lactating cows were hand milked before start of infusion and milked again before the final 1 hour (1400-1500) of infusion. Milk was collected during the final 1 hour of infusion for measurement of radioactive glucose incorporation into lactose, milk citrate and milk fat. Milk yield was recorded by weight.

Chemical methods

Plasma glucose concentrations were measured using enzymatic oxidation in the presence of glucose oxidase (Human GmBH, Germany). Plasma triacylglycerol (TG, C_{16}-C_{18}) and plasma free fatty acids (FFA,C_{16}-C_{18}) were measured by using gas chromatography (Shimazu GC-7AG Gas Chromatograph) in comparison with the appropriate internal standard. The internal standards of triheptadecanoate and heptadecanoic acid for estimation of plasma TG and FFA, respectively, were as described by Thomson et al (1979). The specific activity of labelled plasma glucose was determined by the method described by Chaiyabutr and Buranakarl (1989). Radiochemicals for [U-^{14}C]glucose and [3-^{3}H]glucose were obtained from the Radiochemical Center, Amersham Bucks, U.K. The isotopes were dissolved in sterile pyrogen free saline (0.9% NaCl). The radioactivity in blood bicarbonate was measured by acidifying 2 ml of blood with an equal volume of 6% perchloric acid. $^{14}CO_2$ was liberated and trapped as $K^{14}CO_3$ in a plastic cup which contained 0.1 ml 40% KOH.

The concentration of milk lactose was determined by spectrophotometry (Teles et al., 1978). Lactose radioactivity was determined after isolation by the hydrolysis method (Wood et al., 1965). Milk triglyceride fatty acid composition (C_6 to C_{18}) was determined by gas chromatography after extraction by chloroform and methanol (Christopherson and Glass, 1969). Milk fat was isolated by centrifugation at 50,000 g for 1h at 3°C. The solidified top layer of lipid was assayed for radioactivity after extraction by chloroform and methanol. The concentration of milk citrate was determined by spectrophotometry from tricarboxylic acid filtrate (White and Davies, 1963). Citrate radioactivity was determined after isolation by anion exchange chromatography (Hardwick et al., 1963).

Calculations

Glucose turnover in the whole animal (T), expressed as μmol/min, was calculated from the equation

$$T = I/G_A$$

Where I = rate of infusion of U-^{14}Cglucose or 3-^{3}Hglucose (μCi/min) and G_A= specific activity of ^{14}C- or ^{3}H-glucose in arterial plasma at equilibrium (μCi/μmol).

Recycling of glucose carbon in the whole animal, expressed as % glucose turnover, was calculated from the equation:

$$\text{Recycling} = (T_3 - T_{14})x100/T_3$$

where T_3 = reversible turnover of glucose calculated from 3-^{3}H glucose and T_{14} = irreversible turnover of glucose calculated from U-^{14}C glucose.

The metabolic glucose clearance rate in the whole animal (C_G), expressed as ml/min, was calculated from the equation:

$$C_G = T_3/P_{AG}$$

where T_3 = reversible turnover of glucose calculated from 3-^{3}H glucose (μmol/min) and P_{AG} = arterial plasma glucose concentration (μmol/ml).

Uptake of substrates by the udder (U_M), expressed as μmol/min, was calculated from the equation:

$$U_M = Q_P \times (P_A - P_V)$$

where Q_P = udder plasma flow (ml/min), P_A = concentration of substrate in coccygeal arterial plasma (μmol/ml) and P_V = concentration of substrate of plasma from milk vein(μmol/ml).

Milk substrate output (MO), expressed as μmol/min, was calculated from the equation: $MO = Ms \times Cs/1000$

where Ms = milk secretion rate (ml/min) and Cs = concentration of substrate in milk (μ mol/l).

Release (R) of $^{14}CO_2$ into mammary venous blood, expressed as μmol glucose incorporated into CO_2 per min, was calculated from the equation:

$$R_{CO2} = Q_B \times (^{14}CO_{2V} - ^{14}CO_{2A})/G_A$$

where Q_B = udder blood flow (ml/min), $^{14}CO_{2A}$ = arterial blood $^{14}CO_2$ (μCi/ml), $^{14}CO_{2V}$ = mammary venous blood $^{14}CO_2$ (μCi/ml) and G_A = specific activity of ^{14}C-glucose in arterial plasma at equilibrium (μCi/μmol).

Incorporation (A) of radioactivity from glucose into milk components was calculated from the equation:

$$A = M_A/G_A \times t$$

where A = incorporation of radioactivity from glucose into milk components (μmol/min), M_A = total activity of ^3H or ^{14}C in the milk components (μCi), G_A = specific activity of ^{14}C- or ^3H-glucose in arterial plasma at equilibrium (μCi/μmol) and t = time of infusion (min).

Requirement of NADPH for fatty acid synthesis (P) in the mammary gland, expressed as μmol/min, was calculated from the equation:

$$P_{NADPH} = \Sigma[FFA_n \times (n-2)]$$

where n = chain length of the fatty acid (6 to 16) and FFA_n = output in milk of fatty acid chain length n (μmol/min).

Values for FFA_n were calculated from all medium chain length fatty acids and 30% of C_{16}-fatty acids (Annison and Linzell, 1964).

Net metabolism of glucose phosphorylation (G_{6p}), expressed as μmol/min, was calculated from the equation:

$$G_{6p} = U_G - L$$

where U_G = mammary glucose uptake (μmol/min) and L = output of lactose in milk(μ mol/min).

Net metabolism of glucose (B) to the galactose or glucose moiety of lactose, expressed as μmol/min, was calculated from the equation:

$$B = L$$

Where L = output of lactose in milk (μmol/min).

Metabolism of glucose via the pentose phosphate pathway (PC) was calculated from the equation:

$$Y = 3\,PC/(1+2PC)$$

where Y = specific yield of $^{14}CO_2$ from (1-^{14}C) glucose via the pentose phosphate pathway (Katz and Wood, 1963).

If the NADPH formed via PC were used exclusively for reductive biosynthesis of fatty acids, the ^3H-incorporation from (3-^{3}H) glucose into fatty acids would equal the $^{14}CO_2$

released from (1-^{14}C) glucose via the pentose phosphate pathway (Katz et al., 1974). Metabolism of glucose via PC was therefore calculated from the equation:

$$Z = 3\,PC/(1+2PC)$$

where Z = (Total ^{3}H in milk fatty acid)/t x G_A x (U_G - L)

Net metabolism of glucose 6-phosphate via (G_{PC}), expressed as μmol/min, was calculated from the equation:

$$G_{PC} = G_{6p} \times PC$$

Net metabolism of glucose 6-phosphate via the Embden-Meyerhof pathway (G_E), expressed as μmol/min, was calculated from the equation:

$$G_E = G_{6p} - (B + G_{PC})$$

Table 1. Chemical composition of experimental diet and nutrient analysis as a percentage of dry matter.

	Urea treated rice straw	**Concentrate**
Dry matter	58.0	89.4
Crude protein	8.9	17.8
Acid detergent fibre	61.2	21.5
Neutral detergent fibre	67.2	28.8
Lignin	8.8	7.0
Ash	16.8	5.6

Concentrate formation: ingredients by fresh weight (100 kg^{-1}) consisted of soy bean meal (30 kg), cotton seed (25 kg), cassava (25 kg), rice bran (15 kg), dicalcium phosphate (2 kg), sodium bicarbonate (1.7 kg), potassium chloride (0.7 kg) and premix (0.6 kg).

Table 2. Glucose turnover rate, related variables and body weight at different stages of lactation of crossbred Holsteins during treatment with rbST.

	Period of lactation	Control Group	rbST Group	Control vs rbST
Glucose turnover rate				
(3-^{3}H) glucose	Pretreated	3917±723	4485±855	NS
(μmol/min)	Early	4161±193	4402±848	NS
	Mid	4384±768	5512±823	P<0.05
	Late	4286±706	4868±1164	NS
(U-^{14}C)glucose	Pretreated	3609±745	4197±652	NS
(μmol/min)	Early	3929±528	3588±953	NS
	Mid	3922±828	5394±938	P<0.05
	Late	3993±1341	4163±768	NS
Glucose-C recycling	Pretreated	8.1±3.6	8.5±4.9	NS
(%)	Early	10.5±3.6	9.5±2.1	NS
	Mid	11.9±6.6	11.4±6.4	NS
	Late	9.6±3.2	13.5±9.1	NS
Plasma glucose	Pretreated	1041±208	1201±205	NS
clearance (ml/min)	Early	1090±73	1155±189	NS
	Mid	1220±198	1470±274	NS
	Late	1167±188	1318±215	NS
Non mammary	Pretreated	1751±1266	1990±849	NS
Glucose utilization	Early	1715±634	1612±1312	NS
(μmol/min)	Mid	2297±596	3079±824*	NS
	Late	2307±1070	3068±1163*	NS
Non mammary	Pretreated	40.8±27.0	43.4±12.2	NS
Glucose utilization	Early	40.6±14.0	34.0±23.0	NS
(%)	Mid	52.0±6.0	57.8±9.5*	NS
	Late	52.4±17.0	62.4±16.0**	NS
Body weight (kg)	Pretreated	336.9±31.1	363.6±27.1	NS
	Early	357.1±34.0**	391.2±35.6**	NS
	Mid	369.8±33.8**	412.4±35.5***	NS
	Late	379.2±29.8**	420.9±43.5**	NS

P- values by paired t-test: * P<0.05, ** P<0.01, *** P<0.001 with respect to the pretreated period in each group.

P-values by unpaired t-test between control animals and rbST treated animals.

Table 3. Udder blood flow, milk yield and milk components in different stages of lactation of crossbred Holstein cattle during treatment with rbST.

	Period of lactation	Control Group	rbST Group	Control vs rbST
Udder plasma flow	Pretreated	2438±331	2594±342	NS
(ml/min)	Early	2730±357	3927±1203*	P<0.05
	Mid	2698±319	3983±1183*	P<0.05
	Late	2692±290	3533±1055	NS
Milk yield (kg/d)	Pretreated	12.98±1.53	13.37±2.66	NS
	Early	13.11±1.85	16.02±3.99*	P<0.05
	Mid	12.89±1.47	14.64±1.89	NS
	Late	11.53±1.00	13.01±1.34	NS
Lactose in milk	Pretreated	136.5±9.1	136.0±6.6	NS
(mmol/l)	Early	131.7±7.1	133.0±13.6	NS
	Mid	135.5±10.8	130.3±15.7	NS
	Late	133.4±7.9	133.0±10.6	NS
Citrate in milk	Pretreated	8.4±0.9	8.2±0.8	NS
(mmol/l)	Early	7.8±1.6	7.3±1.0*	P<0.05
	Mid	7.7±0.8	7.2±0.8*	NS
	Late	7.2±1.1	6.8±1.1*	NS
Triacylglycerol in	Pretreated	48.26±5.87	50.94±15.84	NS
milk (mmol/l)	Early	49.81±13.34	70.98±8.22*	P<0.05
	Mid	54.65±7.40	64.77±21.33	NS
	Late	57.81±14.15	72.44±18.28*	NS

P-values by paired t-test: * P<0.05, ** P<0.01, *** P<0.001 with respect to the pretreated period in each group.

P-values by unpaired t-test between control animals and rbST treated animals.

Table 4. The secretion of milk components at different stages of lactation of crossbred Holstein cattle during treatment with rbST.

	Period of lactation	Control Group	rbST Group	Control vs rbST
Milk lactose secretion	Pretreated	1108±240	1259±239	NS
(μmol/min)	Early	1130±184	1469±363*	NS
	Mid	1211±166	1302±229	NS
	Late	1068±107	1202±158	NS
Milk citrate secretion	Pretreated	74.9±9.6	76.4±19.8	NS
(μmol/min)	Early	71.0±18.0	81.0±22.5	NS
	Mid	68.8±12.5	72.7±9.7	NS
	Late	57.5±7.7	61.9±12.3	NS
Milk triacyglycerol	Pretreated	397.2±59.5	469.1±151.6	NS
secretion (μmol/min)	Early	457.5±157.3	794.5±223.6*	P<0.05
	Mid	493.6±107.8	664.4±259.8	NS
	Late	465.7±127.0	662.6±214.6	NS

P-values by paired t-test: * P<0.05, ** P<0.01, *** P<0.001 with respect to the pretreated period in each group.

P-values by unpaired t-test between control animals and rbST treated animals.

Table 5. Utilization of glucose carbon in the udder at different stages of lactation of crossbred Holstein cattle during treatment with rbST.

	Period of lactation	Control Group	rbST Group	Control vs rbST
	[14C]Glucose incorporation (μmol/min) into:			
milk lactose	Pretreated	1540.8±318.8	1827.3±117.6	NS
	Early	1797.8±486.3	2035.8±350.5	NS
	Mid	1856.5±326.8	1934.7±280.9	NS
	Late	1603.2±355.2	1850.8±420.6	NS
milk citrate	Pretreated	11.7±4.5	13.1±4.5	NS
	Early	10.9±3.8	16.5±5.5	NS
	Mid	12.1±2.7	18.7±5.4	P<0.05
	Late	11.3±3.8	17.9±2.5	P<0.05
milk triacylglycerol	Pretreated	16.3±5.7	18.3±3.9	NS
	Early	21.7±9.3	31.7±6.4**	NS
	Mid	27.7±7.8*	30.6±6.2**	NS
	Late	27.3±15.4	33.4±3.4**	NS
venous blood CO2	Pretreated	292.0±128.3	222.4±52.9	NS
	Early	257.4±244.5	239.1±57.4	NS
	Mid	376.8±217.2	557.1±284.6	NS
	Late	267.0±43.2	372.8±113.8	NS
	Percentage of glucose carbon appearing as:			
milk lactose	Pretreated	72.3±20.1	75.5±15.3	NS
	Early	78.9±20.1	73.6±27.8	NS
	Mid	88.5±5.2	88.9±8.2	NS
	Late	77.1±16.9	91.3±11.8*	NS
milk citrate	Pretreated	0.51±0.15	0.65±0.23	NS
	Early	0.46±0.19	0.60±0.31	NS
	Mid	0.58±0.14	0.84±0.12	P<0.05
	Late	0.64±0.33	0.90±0.16	NS
milk triacylglycerol	Pretreated	0.73±0.25	0.74±0.18	NS
	Early	1.10±0.50	1.15±0.44	NS
	Mid	1.35±0.49*	1.45±0.51*	NS
	Late	1.03±0.52	1.70±0.40***	P<0.05
venous blood CO2	Pretreated	14.2±9.9	8.4±2.3	NS
	Early	11.1±10.2	8.3±2.0	NS
	Mid	18.4±11.5	26.9±15.1	NS
	Late	14.9±6.2	18.6±5.6	NS

P-values by paired t-test: * P<0.05, ** P<0.01, *** P<0.001 with respect to the pretreated period in each group.

P-values by unpaired t-test between control animals and rbST treated animals.

Table 6. Rates of pathways of glucose metabolism in the udder at different stages of lactation of crossbred Holsteins during treatment with rbST.

Period of lactation	Control Group	rbST Group	Control vs rbST
Flux through the pentose phosphate pathway calculated as ^3H incorporation into milk fatty acid (equivalent μmol of glucose/min)			
Pretreated	67.1±25.5	58.8±20.7	NS
Early	94.0±44.6	158.2±103.8	NS
Mid	130.5±58.6	119.0±51.4*	NS
Late	103.9±54.1	108.8±35.1*	NS
Corrected ^3H incorporation into milk fatty acid (equivalent μmol of glucose/min)			
Pretreated	71.4±21.5	60.9±21.3	NS
Early	94.3±44.9	164.2±99.3*	NS
Mid	146.2±65.2*	121.7±57.8*	NS
Late	112.9±45.0	132.1±46.8**	NS
Net metabolism of glucose 6-phosphate via the pentose phosphate pathway (μmol/min)			
Pretreated	84±34	68±26	NS
Early	149±96	152±101	NS
Mid	162±89	125±51	NS
Late	104±27	161±89	NS
Net metabolism of glucose 6-phosphate via the pentose phosphate pathway (%)			
Pretreated	11±7	7±2	NS
Early	9±5	18±24	NS
Mid	14±7	10±5	NS
Late	11±5	15±9	NS
Metabolism of glucose 6-phosphate via the galactose moiety of lactose (μmol/min)			
Pretreated	1214±881	1243±647	NS
Early	1328±423	1542±913	NS
Mid	876±270	899±331	NS
Late	911±655	817±472	NS
Metabolism of glucose 6-phosphate via the galactose moiety of lactose (%)			
Pretreated	77±41	73±23	NS
Early	64±13	74±35	NS
Mid	76±10	78±11	NS
Late	72±27	76±17	NS
Metabolism of glucose 6-phosphate via Embden-Meyerhof pathway (%)			
Pretreated	-2±65	-28±52	NS
Early	-3±28	-58±121	NS
Mid	-36±27	-42±35	NS
Late	-40±69	-38±46	NS

P-values by paired t-test: * $P<0.05$, ** $P<0.01$, *** $P<0.001$ with respect to the pretreated period in each group.

P-values by unpaired t-test between control animals and rbST treated animals.

Table 7. NADPH production from glucose in the udder at different stages of lactation of crossbred Holsteins during treatment with rbST.

Period of lactation	Control Group	rbST Group	Control vs rbST
Requirement of all NADPH for fatty acid synthesis (μmol/min)			
Pretreated	1317±295	1666±682	NS
Early	1677±616	2820±868	P<0.05
Mid	1838±525	2470±979	NS
Late	1725±542	2459±1024	NS
Requirement of all NADPH formation from glucose via the pentose phosphate pathway (%)			
Pretreated	30±7	24±5	NS
Early	39±8	34±19	NS
Mid	41±11	29±5	NS
Late	37±17	29±9	NS

P-values by paired t-test: * P<0.05, ** P<0.01, *** P<0.001 with respect to the pretreated period in each group.

P-values by unpaired t-test between control animals and rbST treated animals.

Table 8. Fatty acid composition of milk fat in the udder at different stages of lactation of crossbred Holsteins during rbST administration.

		Fatty acid chain length (μmol/ml milk)			
		Pretreatment	Early lactation	Mid lactation	Late lactaion
Control group	C6	0.86±0.47	1.69±2.02	1.13±0.49	1.27±0.76
	C8	0.63±0.24	0.80±0.28	0.95±0.39	0.94±0.35
	C10	1.25±0.28	1.57±0.56	1.82±0.96	1.79±0.66
	C12	1.19±0.36	1.44±0.43	1.87±0.89	1.76±0.57
	C14	3.98±1.31	4.68±0.84	6.15±2.04	5.94±1.44
	C16:0	16.83±1.81	20.74±7.85	20.37±2.09	23.73±7.28
	C16:1	1.00± 0.50	1.01±0.49	1.00±0.49	1.53±0.60
	C18:0	8.06±0.70	7.71±1.37	8.73±2.54	8.10±2.10
	C18:1	11.63±2.38	11.19±2.04	13.63±1.13	14.28±2.11
	C18:2	0.80±0.20	0.83±0.48	0.70±0.34	0.90±0.40
	Total	46.06±5.87	51.65±13.34	56.35±7.40	60.24±14.15
rbST treated	C6	0.98±0.10	1.56±0.48	1.40±0.69	1.48±0.50
	C8	0.59±0.24	1.03±0.31	1.01±0.23	1.03±0.36
	C10	1.31±0.58	2.10±0.63	2.05±0.45	2.16±1.00
	C12	1.44±0.71	2.04±0.61	2.11±0.61	2.40±1.05
	C14	5.17±2.38	7.43±2.14	7.65±1.85	8.78±4.12
	C16:0	20.49±7.54	26.88±2.75	24.01±12.41	25.86±6.10
	C16:1	1.06±0.60	1.02±0.32	1.20±0.51	1.60±0.60
	C18:0	7.53±1.82	9.40±0.70*	8.05±1.96	9.53±1.70
	C18:1	13.65±3.24	20.50±2.55***	18.49±4.49*	21.21±4.43**
	C18:2	0.81±0.30	1.01±0.37	1.44±0.43	1.10±0.66
	Total	52.81±15.84	73.01±8.22*	67.41±21.33	75.14±18.28

P-values by paired t-test: * P<0.05, ** P<0.01, *** P<0.001 with respect to the pretreated period in each group.

P-values by unpaired t-test between control animals and rbST treated animals.

Table 9. $^{3}H/^{14}C$ ratios in plasma glucose and related producted at different stages of lactation of crossbred Holstein cattle during treatment with rbST.

	Period of lactation	Control Group	rbST Group	Control vs rbST
Plasma glucose	Pretreated	0.92±0.11	0.92±0.06	NS
	Early	0.99±0.12	0.83±0.18	NS
	Mid	0.89±0.08	0.99±0.04	NS
	Late	0.91±0.18	0.88±0.19	NS
Milk lactose	Pretreated	0.85±0.03	0.89±0.21	NS
	Early	0.84±0.04	0.83±0.19	NS
	Mid	0.91±0.08	0.88±0.04	NS
	Late	0.89±0.04	0.79±0.12	NS
Milk triacylglycerol	Pretreated	3.17±1.72	3.15±1.24	NS
	Early	3.59±1.67	3.63±1.97	NS
	Mid	4.09±1.21	3.85±1.23	NS
	Late	3.58±0.98	2.81±0.97	NS
Milk citrate	Pretreated	0.42±0.07	0.59±0.16	NS
	Early	0.58±0.16	0.46±0.13	NS
	Mid	0.44±0.05	0.46±0.11	NS
	Late	0.42±0.11	0.36±0.06	NS

P-values by paired t-test: * $P<0.05$, ** $P<0.01$, *** $P<0.001$ with respect to the pretreated period in each group.

P-values by unpaired t-test between control animals and rbST treated animals.

RESULTS

Glucose turnover, related variables and body weight (Table 2)

The glucose turnover rate in both the controls and rbST-treated animals was determined by making simultaneous estimates of the total glucose entry rate using 3-[^{3}H] glucose infusion and the utilization rate of glucose using [U-^{14}C]glucose infusion. All values of glucose turnover rates in different stages of lactation for both groups are expressed as absolute values. In pretreatment period, there were no significant differences of the total glucose entry rate and glucose carbon recycling between the controls and rbST-treated animals. However, in early lactation, the utilization glucose turnover rate of rbST-treated animals was decreased as compared with the pretreatment period, whereas there was no change in the control animals. Comparing for the mid-lactating period, rbST-treated animals showed an elevation of plasma glucose clearance and significant increases in the glucose turnover rate ($P<0.05$) in comparison with control animals. Both absolute values and percentages of utilization of glucose by tissues other than the mammary gland were calculated from the total rate of glucose synthesis and the rate of glucose uptake by the mammary gland. It was decreased during rbST administration in the early period of lactation. The percentages and values of non-mammary glucose utilization showed significantly increases during lactation advances to mid and late lactation in as compared with pretreated period in rbST-treated animals. During the course of lactation there were significant increases of body weight in both groups. Elevations of body weights were not different between groups at each period of lactation.

Udder plasma flow, milk yield and milk composition (Tables 3 ,4)

In animals treated with rbST, mammary plasma flow and milk yield initially showed significantly higher levels ($P<0.05$) in early lactation than that of control animals. The trends for persistency were observed as for udder plasma flow in rbST treated-animals throughout lactation. The values of milk lactose concentration showed no differences between groups of animals or among periods of lactation in the same group. In rbST treated-animals, the milk lactose secretion significantly increased in early lactation as

compared with pretreatment period. In rbST-treated animals, mean values of milk citrate concentration during early lactation were significantly decreased ($P<0.05$) as compared with pretreatment period. During lactation advances, the milk citrate concentration decreased in both groups. Milk triacylglycerol concentration and triacylglycerol secretion of rbST-treated animals were markedly higher in early lactation than that of pretreatment period and it was still in a high level throughout lactation.

Utilization of glucose carbon in the udder (Table 5)

A high milk lactose secretion and citrate secretion during early lactation were apparent in rbST treated-animals when compared to those of control animals. These differences were primarily due to differences in milk secretion rates. However, the percentage of utilization of glucose carbon for synthesis of milk lactose was not significantly different between controls and rbST-treated animals. The utilization of glucose carbon for synthesis of milk citrate for rbST-treated animals was significantly higher than that of control animals during mid and late lactation. The utilization of glucose for synthesis of milk triacylglycerol was significantly higher ($P<0.01$) during rbST administration throughout lactation. The ^{3}H from C-3 of glucose was recovered in milk fat. The major portion of this ^{3}H was associated with the fatty acid fraction of the saponified triacylglycerol. Less than 2% of radioactive carbon was present in triacylglycerol in both groups. The amount of ^{14}C-glucose incorporated to CO_2 in the venous blood of rbST-treated animals increased in mid and late lactation.

Rates of pathways of glucose metabolism in the udder (Table 6)

Data for glucose metabolism via the pentose phosphate pathway show that the incorporation of ^{3}H from [3-^{3}H]glucose into fatty acids and the flux through the pentose phosphate pathway was calculated to be increased as lactation advances in both groups. Correction of the lower $^{3}H/^{14}C$ ratio likely to be present in intracellular glucose 6-phosphate gave significant flux values of 164, 121 and 132 μmol/min for early, mid and late lactation of rbST-treated animals, respectively, in comparison with pretreatment period. The results of the net metabolism of glucose 6-phosphate via the pentose phosphate pathway (PC) has been calculated according to the equation:

glucose 6-phosphate ⟶ glyceraldehyde 3-phosphate + $3CO_2$ (Katz and Wood, 1963)

Complete metabolism of one molecule of glucose 6-phosphate according to this equation would require three cycles of the pentose phosphate pathway. Therefore, the flux through the pathway should be three times the net rate of glucose metabolized in the pentose phosphate pathway. From the results , as lactation advances, the intracellular glucose phosphorylated by the mammary gland were calculated to be completely metabolized via the pentose phosphate pathway in terms of absolute values and the percentages were higher when compared with pretreatment period of both groups. The percentages of metabolism of glucose 6-phosphate to the galactose moiety of lactose were slightly higher during early lactation in rbST treated-animals when compared to control animals and during lactation advance, these values decreased in both groups. Metabolism of glucose 6-phosphate via the Embden-Meyerhof pathway was calculated in term of the proportion of glucose metabolized, which there was considerable variation with advanced lactation of both groups.

NADPH production from glucose (Table 7)

It can be calculated from the milk fat compositions and output in the present experiment that the requirements for NADPH for fatty acid synthesis increased during administration of rbST in different stages of lactation. During early lactation, the NADPH formation from glucose accounted for 2820 μmol/min of that required for fatty acid synthesis *de novo* in the mammary gland of rbST treated-animals, which was sinificantly higher than that of the value of 1677 μmol/min for the control animals.

Milk fatty acid concentrations (Table 8)

During pretreatment period, the milk fatty acid concentrations with a chain length of C_6 to C_{18} for both groups of animals were not different. During rbST administration in differnt stages lactation, the milk fatty acid concentration, particularly with a chain length of C_{16} to C_{18}, significantly increased as compared with those of control animals..

The $^3H/^{14}C$ ratios in glucose and related products (Table 9)

The $^3H/^{14}C$ ratio in arterial plasma glucose was lower than that of the infusion in both groups of crossbred HF cattle. These values were not different between the control and rbSt treated-animals, indicating some recycling of glucose-C in the whole animal.

A slight decrease in the $^{3}H/^{14}C$ ratio was seen in milk lactose, whereas the $^{3}H/^{14}C$ ratio of milk triacylglycerol was slightly higher in both groups. The ^{3}H and ^{14}C from glucose were also shown to be incorporated into milk citrate. The $^{3}H/^{14}C$ ratio of milk citrate was slightly low in both groups as lactation advances.

DISCUSSION

The supply of glucose is a principal determinant of the milk yield, since glucose requirement is used for lactose production. The administration of rbST elicited a marked increase in the milk production of crossbred dairy cattle in the present study. The absolute milk yield response to rbST administration started before the peak of lactation in early lactation and it was significantly higher than those of the control animals throughout periods of lactation. Elevated responses did not maintain for the duration of the treatment period in rbST treated animals. These results confirm the finding that an increase in milk yield in response to rbST administration will not be sustained indefinitely (Bauman, 1992), and that it is influenced by the stage of lactation (Phipps et al.1991). The low potential for extended persistency of lactation in rbST treated animals appears similar to that which occurs in higher yielding cows (Chase, 1993). However, it has been reported that the whole lactational response to somatotropin might be reduced if treatment begins very early in lactation (Bauman and Vernon, 1993; Burton et al., 1994). A marked increase in milk yield without an alteration in lactose content during early lactation in rbST treated animals indicates that this requires a substantial increase in supply of glucose to the mammary gland (Bauman and McCutcheon, 1986). Glucose is essential for milk secretion and glucose moiety of lactose arises directly from plasma glucose (Ebner & Schanbacher, 1974). In the present results, the milk secretion of animals in both controls and rbST treated animals was not dependent on the blood glucose level, since the plasma glucose concentrations remained constant over a wide range at different stages of lactation. The marked increase in the udder blood flow of rbST treated animals in the present results will support the previous conclusion from a

study in cows or goats by Linzell (1973) that glucose uptake is determined mainly by mammary blood flow.

Gluconeogenesis in ruminants has been known to be the main source of glucose production (Lindsay, 1970). In the present studies, animals were maintained on a similar concentrate intake. Relatively constant plasma glucose concentrations in both groups indicate that steady state conditions between the rate of irreversible loss of glucose and the rate of gluconeogenesis existed in the body pool of glucose. The present experiment showed that rbST treatment did not significantly affect the reversible turnover of [3-^{3}H]glucose throughout stages of lactation, while the irreversible turnover of [U-^{14}C]glucose was reduced during the early lactation but not for mid-and late lactation. Our previous experiments showed that the insulin level increased during rbST treatment in different stages of lactation in crossbred HF animals (Chaiyabutr et al., 2005). It indicates that rbST administration during early lactation antagonizes whole body turnover of glucose stimulated by insulin. Growth hormone is thought to be antagonistic to the action of insulin in tissues that are sensitive to insulin (Rose and Obara,1995), preventing the uptake of glucose by pheripheral tissue and thus sparing glucose to mammary gland , which are insensitive to insulin (McGuire et al., 1995). It also noted that glucose clearance which stimulated by insulin was also reduced during early lactation. This speculative sparing glucose utilization in tissues sensitive to insulin would partially allow for increase in lactose synthesis and milk yield. As lactation advances, the irreversible turnover of [U-^{14}C]glucose of rbST treated animals was increased in mid lactation, which was significantly higher than that of control animals. The reversible turnover of [3-^{3}H]glucose may represent the total glucose turnover rate as the ^{3}H is not recycled from products of partial glucose degradation (Katz et al.,1965). Thus one way of estimating ^{14}C-recycling is by simultaneously injecting [3-^{3}H]glucose and [U-^{14}C]glucose as in the present experiments. There were no differences for an increased recycling of glucose-C between the controls and rbST treated animals during advanced lactation suggests that a constant level of tricarbon units originally derived from glucose being again reincorporated into glucose was not affected by rbST treatment .

Glucose is known to be used for the synthesis of lactose and other milk components in the process of milk synthesis (Linzell and Peaker, 1971; Bauman and Davis, 1975). In general an increasee in milk yield can be attributed to an increase in the rate of lactose synthesis (Linzell & Peaker, 1971). However, an increase in the lactose

yield during rbST administration was not related to the lactose concentration in milk, which largely unchanged. These results can be attributed to a difference in the activity of the mammary epithelial cells between controls and rbST treated animals. The synthesis of lactose involves a combination of glucose and UDP-galactose. The UDP-galactose originates from glucose 6-phosphate (Ebner and Schanbacher,1974). An administration rbST showed increases in both milk yield and glucose uptake by mammary gland, which were accompanied by an increase in milk glucose secretion (Chaiyabutr et al., unpublished data, Charpter VIII). These results would coincide with the calculated of metabolism of glucose 6-phosphate to the galactose moiety of lactose in rbST treated animals which was higher than that of control animals in early lactation. The availability of cytosolic glucose 6-phosphate in the cells of rbST treated animals in early lactation would be sufficient to account for the cytosolic lactose synthesis. Decreases in the metabolism of glucose 6-phosphate to the galactose moiety of lactose as lactation advances in both groups would affect the lactose synthesis and milk production.

A low enzymatic activity for lactose synthesis might be expected to appear as lactation advances in the crossbred animal. However, lactose synthesis is a complex process (Kuhn et al.,1980). There is still a need for more information to elucidate the changes in enzymatic activity in this particular system. The quantitative utilization of the glucose taken up by the mammary gland is used directly in the synthesis of lactose, and in other portions is metabolized via the pentose phosphate pathway, Embden-Meyerhof pathway and the tricarboxylic acid cycle. Glucose carbon was used by the mammary cell to produce lactose, citrate and triacylglycerol for milk secretion. The data obtained for the utilization of glucose carbon for the synthesis of lactose, triacylglycerol and citrate during mid and late lactation were higher in rbST treated animals as comparison with the control animals. The differences in these results between the controls and rbST treated animals without a reduction in feed intake may be explained by the difference of nutrient partition or utilization in the mammary gland. In addition to the use of glucose carbon for milk synthesis, the hydrogen from glucose has been shown to be incorporated into milk fat. Studies *in vitro* have shown that glucose metabolism via the pentose phosphate pathway may not be as important for NADPH production as in the rat. Fatty acid synthesis from acetate can occur in the absence of glucose in sheep mammary-tissue slices (Balmain et al., 1952) and the perfused goat udder (Hardwick et al., 1963). In the present studies, estimates of the contribution of the pentose phosphate pathway in

providing NADPH for fatty acid synthesis *in vivo* have been based on the assumption that all the glucose that was oxidized to CO_2 was metabolized via the pentose phosphate pathway. The calculation of the metabolism of glucose 6-phosphate via the Embden-Meyerhof pathway or the pentose phosphate pathway has been estimated in the goat udder *in vivo* (Chaiyabutr et al., 1980). However, few data have been available from the *in vivo* study of crossbred lactating cows. In the present studies glucose 6-phosphate metabolized via the pentose phosphate pathway gave percentage values of 9-11% throughout lactation in control animals while it increasd from 7 % in pretreatment to 18 % in early lactation after rbST administration. These estimations are in contrast to experiments in the isolated perfused cow udder by Wood and co-workers (1965), in which about 23% to 30% of the glucose was metabolized via the pentose phosphate pathway. The difference in estimation is probably due to no consideration of the recycling of glucose 6-phosphate which occurs when glucose is metabolized via the pentose cycle in the udder with the consequent loss of ^3H from glucose 6-phosphate (Davis and Bauman, 1974). However, the net proportion of the metabolism of glucose 6-phosphate via the pentose cycle pathway during different stages of lactation in rbST treated animals was higher than those of control animals. Metabolism of glucose via the pentose phosphate pathway yields 2 molecules of NADPH per molecule of glucose, only one of which could be labelled with ^3H in the present experiments. The data presented here provided evidence that 24% to 34% of the NADPH was required during early lactation for fatty acid synthesis *de novo* from glucose metabolism in the udder of rbST treated animals, while 30% to 39 % was required in the control animals. If there is a common pool of glucose 6-phosphate which is available for both lactose synthesis and pentose phosphate metabolism, then the recycling of glucose 6-phosphate within the udder would result in too low a value for NADPH production from glucose. The net metabolism of glucose in the pentose phosphate pathway can be calculated from the incorporation of ^3H from [3-^3H]glucose in fatty acids assuming that the NADPH formed is used exclusively for biosynthesis of fatty acids (Katz et al.,1974). This technique has been used to study the *in vitro* metabolism of rat mammary and adipose tissue (Katz and Wals 1970,1972; Katz et al., 1966) and it was also used for the study of the *in vivo* metabolism of goat mammary tissue (Chaiyabutr et al.,1980). Based on the techniques and calculations of Katz and co-workers (1974) and assuming that cytosolic NADPH is used only for fatty acid synthesis, it has been shown that the glucose phosphorylated by

the udder of rbST treated animals was metabolized via the pentose phosphate pathway which was higher than those of control animals. In rbST treated animals, a high proportion of the glucose taken up by the udder which was oxidized in the tricarboxylic acid cycle would be apparent in mid- and late lactations. High values of both the proportion and absolute amount of glucose carbon incorporation to milk citrate and milk triacylglycerol of rbST treated animals during mid- and late lactation are evidences supporting an increased proportion of glucose 6-phosphate metabolized via the Embden-Meyerhof pathway. It has been shown that metabolism of glucose 6-phosphate by the Embden-Meyerhof pathway can result in ^{3}H being retained in glycerol if the triose phosphate isomerase reaction is not at equilibrium (Katz and Rognstad, 1976). Metabolism of glucose 6-phosphate by the pentose phosphate pathway usually results in the loss of all ^{3}H from [3-^{3}H]glucose in lactating cows. During advanced lactation, whether an increased disequilibrium of the triose phosphate isomerase reaction occurs in the udder of rbST treated animals as compared with the control animals and causes a higher level of $^{3}H/^{14}C$ ratio in milk triacyglycerol needs to be further investigated. The high metabolism of glucose 6-phosphate in early lactation of rbST treated animals appeared to be due primarily to a high flux through the lactose synthesis and to pentose phosphate pathway, probably reflecting the high milk production during rbST treatment. Tritium and carbon-14 were also shown to be incorporated into milk citrate which provided 17 μmol/min in rbST-treated animals and 11 μmol/min in control animals for the carbon skeleton of citrate in the early lactating period as compared with pretreatment period. It has been postulated that milk citrate could be synthesized from 2-oxoglutarate via the NADP-dependent isocitrate dehydrogenase reaction (Hardwick,1965). In addition ^{3}H is lost to NADPH or water in metabolism via the pentose phosphate pathway or glycolytic pathway, so it is likely that ^{3}H incorporation into milk citrate was also via NADP^{3}H. It is possible that the incorporation of ^{3}H into milk citrate may occur in different manners in the exchange reaction of the cytosolic NADP-dependent isocitrate dehydrogenase. Both fatty acid synthesis and the NADP-dependent isocitrate dehydrogenase reaction between control animals and rbST-treated animals may have different mechanisms with a common pool of cytosolic NADPH. A significant increase in the concentration of FFA in milk was apparent in rbST-treated animals as compared with the control animals in early lactation. A similar result for an increase in milk fat content due to rbST injection has also been observed previously (West et al., 1991). It has

been known that milk fat is 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. However, an increase in milk fat after rbST injection was associated with the increased yield of long-chain fatty acids characteristic of plasma free fatty acids and body fat. Thus, the lipolytic activity would be a function of bST treatment per se in stead of the associated changes in energy balance.

In conclusion, the data presented here represent the estimation *in vivo* of glucose metabolism in the udder and its distribution to lactose synthesis, the pentose phosphate pathway and the Embden-Meyerhof pathway during rbST administration in 87.5% HF animals. Of the glucose taken up by the udder of rbST treated animals during early lactation, an average 18% and 21% were metabolized in the pentose phosphate pathway and contributed to NADPH production, respectively. The sufficient pool of intracellular glucose concentration during rbST administration, has effect on an increase in glucose 6-phosphate which increased flux through the lactose synthesis and pentose cycle pathway. Although we know a great deal of differences that occur between the control animals and rbST treated animals, we do not know the different enzymatic activities during rbST administration in different stages of lactation which affect the rate of metabolic pathways. There is still a need for more information, for example,on whether the high enzymatic activity of fructose 1-6 diphosphatase or the lower enzymatic activity of pyruvate dehydrogenase occurs in rbST treated animals throughout the stages of lactation or occurs during early lactation which causes an increase in the metabolism of glucose 6-phosphate via the Embden-Meyerhof pathway and tricarboxylic acid cycle.

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CHAPTER X

General discussion

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General Discussion

The present study was designed to clarify whether a shorter lactation persistency of crossbred cattle containing 87.5%Holstein genes during lactation advance was due to the reduction of the growth hormone level (Chaiyabutr et al., 2000b) or associated with some other mechanisms. The results presented in this report indicate that administration of rbST affects bodily functions both intra-mammary and extra-mammary functions. The rbST-treated animals increased body fluid compartments throughout all periods of study i.e. TBW, ECW and blood volume, while the control animals decreased TBW in comparison to pretreatment values in the early period of lactation(Charpter III and IV). The treatment of rbST being initiated at the earlier stage of lactation exerts it effect on an increase in empty body water (EBW). An increase in the EBW in rbST-treated animals would be due to an increase in ECF compartment, while ICF compartment did not change through the period of study. Increased ECF in rbST-treated animals might be partly resulted from the decrease in fat mass during early lactation. These results are agreed with the report in human that an expansion of both ECW and TBW was apparent after growth hormone administration in growth hormone deficient patients (Janssen et al., 1997). There are a number of possible explanations for this apparent finding. An increase in TBW and ECW would be influenced by an increase in voluntary intake (MacFarlane et al., 1959), which has been reported to occur after a few weeks of rbST administration (Coghlan et al., 1977). A greater percentage increase in live weight of rbST-treated animals could be considered, at least in part, to be the direct effect of somatotropin on the increased body cell mass and fat free mass. This would be attributable to an accumulation of body water. The sodium retention effect of somatotropin on the renal tubular reabsorption of sodium (Wyse et al.,1993), would be another explanation for water retention in the ECW compartment.

The higher TBW and ECW of animals receiving rbST would not only provide a higher reservoir of soluble metabolites for biosynthesis of milk but also slow down any elevation of

body temperature during lactation in hot conditions. An increase in both metabolic activity and heat production has been reported in bST-treated cows (West et al., 1991). It was suggested that even though bST increases heat production, it also increases heat dissipation (Johnson et al., 1991, West et al., 1994). However, the rbST-treated animals showed no significant changes in the water turnover rate per fat free, wet, body weight ($kg^{0.82}$) and the biological half-life of tritiated water, in any periods measured in the experiment, in comparison to the control animals. This indicates that water loss with the increase in milk yield of the rbST-treated animals might be compensated by a larger body water pool, which restores their body fluids to equilibrium, with no significant changes of body water turnover rate and water half-life. In contrast to the rbST-treated animals, the biological half-life of tritiated water in the control animals was significantly shorter, while the water turnover rate was significantly higher as lactation advanced to mid and late lactation. These changes would be due to the process of lactation requiring more water and more loss of water secretion in milk, which is generally known to be about 87% and would account for these phenomena. The control animals being 87.5%HF were genetically similar to the exotic *bos taurus* breed which might lead to poor adjustment in a tropical environment (Chaiyabutr et al., 2000a; Nakamura et al., 1993). The TBW and ICW of the control animals showed to be decreased during advanced lactation; it should be assumed that these changes are the factors influencing lactation persistency. Animals could not maintain their body fluids which resulted in the rapid approach of the end of their normal short lactation.

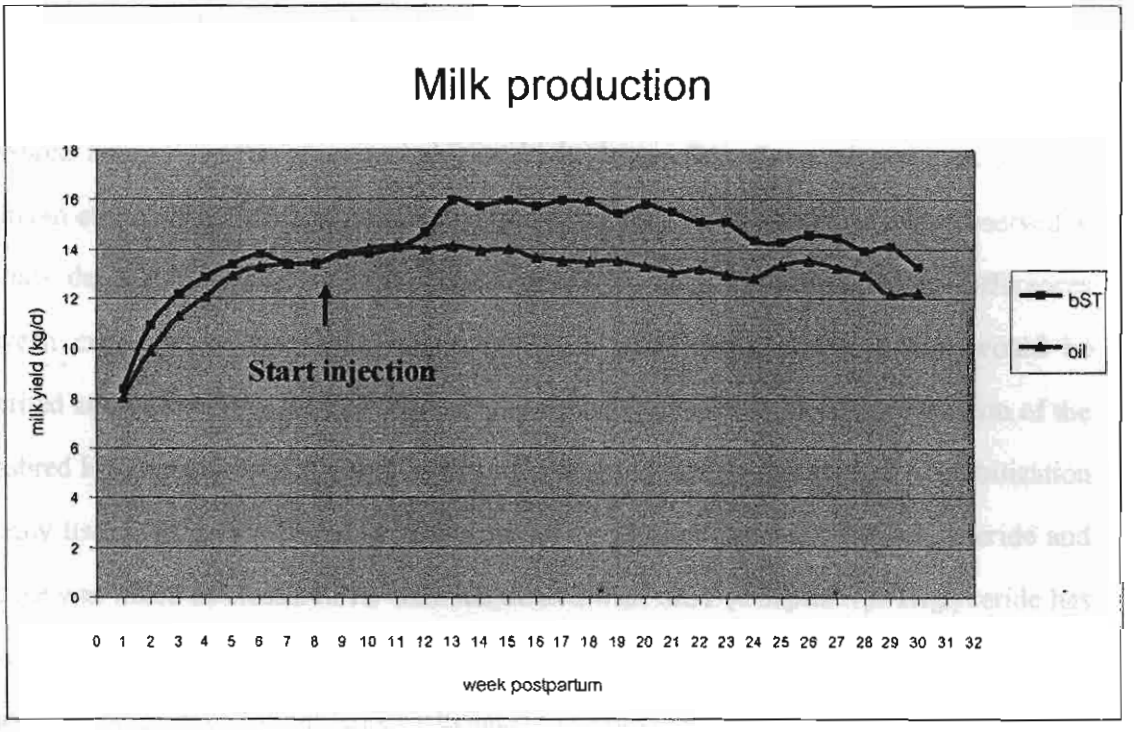
In the present study, increases in mammary blood flow to the udder of rbST-treated animals agree with several reports in both cows and goat (Davis et al., 1988; Gulay et al., 2004; Mepham et al; 1984). A marked increase in mammary blood flow of rbST-treated animals could not be attributed to a change in blood volume and plasma volume, which remained nearly constant as a percent of body weight. In lactating dairy cows, increase in blood flow to the mammary gland may allow plasma volume to remain nearly constant despite loss of body weight (Woodford et al., 1984). Several investigations show the effect

of rbST on mammary circulation was indirect, mediated via IGF-I (Capuco et al., 2001), whereas other works have demonstrated the direct effect of IGF-I on an increase in the mammary blood flow and increase in milk production (Etherton and Bauman, 1998). An elevation of both plasma IGF-I concentration and udder blood flow was also noted in late lactating crossbred cows treated with rbST (Tanwattana et al., 2003). The present study confirms that mammary blood flow is a major determining factor for supply of nutrients for milk synthesis and follows the pattern of changes of milk yield.

The treatment of rbST was initiated at the earlier stage of lactation, milk yield increased in early lactation (+19.8 %) and in mid-lactation (+9.5%), but it decreased by 2.7% during late lactation in comparison with the pretreatment period (Figure 1). Low responses in milk yield during rbST treatment in the later stage of lactation are similar as previously reported in dairy crossbred cattle (Phipps et al., 1991). A rapid decline of yield resulting the shorter persistency of lactation of rbST-treated animals seems to be similar to those which occur in higher yielding cows (Chase, 1993). These results indicated that an increase in milk yield of dairy crossbred cattle, in response to rbST administration, will not be sustained for long, being influenced by stage of lactation.

The mechanism by which rbST directly or indirectly affects mammary gland function likely involves the DMI. The ratio of DMI to milk yield of rbST-treated animals, was lower in early lactation when compared with the pretreatment period but animals still gained weight throughout the experiment in both groups. It has been known that the support of milk secretion would come through provision of substrate and stimulation of mammary cell activity. Unfortunately, the present studies on the mammary cell activity were not available. The rbST increased milk yield relating to mammary cell activity appears contradictory. Whereas some studies show no mammogenic effect of bST (Binelli et al., 1995), other studies show a possible mammogenic effect when cattle are administered bST (Knight et al., 1992). It indicates that the increased milk yield with rbST treatment in the present study is

Figure 1. Milk yield in the controls and rbST-treated animals during prolonged treatment with placebo or rbST, respectively.



rather dependent upon the adequacy of the nutritional provision than the mobilization of body stores. A marked increase in milk yield with rbST treatment without loss of body weight, especially during early lactation, may be due to the fact that the animals were well fed to allow an adequate replacement of body reserves. Milk yield in the first lactation of crossbred animals in the present study would be lesser than those of multiparous cows (Sullivan et al. 1992), which is possibly related to the continued weight increase observed in animals during their first lactation. These results provide the physiological differences between crossbred animals and exotic breeds in partitioning ability, which would be inherited and capacity for milk production. Thus, the metabolic demands of lactation of the crossbred HF animals would be met by dietary intake during early lactation. No mobilization of body tissues as indicated by no alteration of the plasma levels of both triglyceride and glucose was noted in crossbred HF animals treated with rbST (Chapter V). Triglyceride has been known to restore during period of excess energy availability and are mobilized during periods of energy deprivation. No significant change in the plasma triglyceride concentration supports the interpretation that the extra energy to support increased milk yield arose from surplus nutrient of DMI rather than from greater mobilization of body reserves. In the present study, milk fat content of rbST-treated animals was increased, while milk protein and milk lactose were not changed by rbST treatment. Peel and Bauman (1987) reported that administration of rbST 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. A significant increase in the concentration of FFAin milk was apparent in rbST-treated animals as compared with the control animals in early lactation. A similar result for an increase in milk fat content due to rbST injection has also been observed previously (West et al., 1990). It has been known that milk fat is 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. Milk fat content of cows in positive energy balance is not influenced by rbST treatment, and milk fat yield follows the trend of milk production (West et al., 1990). However, an increase in milk

fat after rbST injection was associated with the increased yield of long-chain fatty acids characteristic of plasma free fatty acids and body fat (Chapter VII and IX). Thus, the lipolytic activity would be a function of bST treatment per se in stead of the associated changes in energy balance.

During early lactation, an elevation of body fluid particularly blood volume (+15 %) despised large increases in mammary blood flow (+50 %) during rbST treatment. These observations could suggest that a marked increase in blood flow through the mammary glands resulting from rbST administration would be achieved in part by local vasodilatation (Linzell, 1974), causing in distribution of milk precursors to the gland. An increase in MBF has been shown to be the effect of an increase in cardiac output perfusing to the udder without any alteration in heart rate during growth hormone treatment (Davis et al., 1988). In the present results, an increase in both blood volume and plasma volume in rbST-treated animals would provide a greater venous return and stroke volume for increase in cardiac output, resulting in increased the blood supply to the mammary gland. Thus, the rate at which the milk yield elevated after the peak period when compared with the controls, could have been due primarily to an increased availability of substrates for the mammary gland. The progressive decline in milk yield of rbST-treated animals with still a higher level of either MBF or ECW, could be accounted for by changes in intra-mammary factors. Since it has been reported that the effect of somatotropin on MBF occurs by a mechanism which did not involve the direct action of somatotropin on the udder (Collier et al., 1984). In addition, study in vitro suggests that bST does not directly stimulate mammary secretory function (Gertler et al., 1983). The indirect action of rbST on mammary function may occur through some other agent e.g. insulin like growth factor-I, as administration of rbST in late, lactating, crossbred cows elevated milk yield, which coincided with increased plasma IGF-I concentration and udder blood flow (Tanwattana et al., 2003).

The experiment in Chapter V showed an increase in milk yields and circulating levels of IGF-I throughout lactation in animals treated with rbST. These findings were similar to those of previous studies on lactating cows showing that the injection of somatotropin, elevated

plasma IGF-I concentrations (Davis et al.1987; Tunwattana et al. 2003). Somatotropin increased milk yield by a mechanism which did not involve the direct action on the mammary gland (Collier et al.1984). The indirect effects of somatotropin on milk production are thought to be mediated either via IGF-I or nutrient partitioning effects (Bauman,1992). In the present study, during long-term administrations of rbST, milk yield rose to a peak in early lactation and then gradually declined over 32 weeks of the experiment, whilst the plasma concentration of IGF-I and the mammary blood flow did not decrease in the rbST treated animals. These findings suggest that the stimulatory effect of recombinant bovine somatotropin on milk production is not mediated solely by IGF-I. Changes in milk production during the progress of lactation in rbST treated animals might not be controlled systemically but also locally within the mammary gland. There are a number of possible explanations for this apparent finding. It probably involves greater synthesis of plasma IGF-I binding proteins as lactation advances which combines with IGF-I in the blood and so modulates the level of free IGF-I before it reached the mammary gland. It has been reported that approximately 95% of the infused IGF-I is bound by IGF binding proteins (Davis et al. 1989). Mammary tissue is itself capable of synthesizing an IGF-binding protein (e.g.IGFBP-5) during mammary gland involution in late lactation and this could inhibit IGF-mediated cell survival (Tonner et al. 1997; Flint & Knight, 1997) and initiate involution and a decrease in milk yield.

The expriment in Chapter VI for the plasminogen and plasmin activities indicate that the plasminogen-plasmin system involved in the tissue remodeling associated with the declining phase of lactation and mammary gland involution. Milk plasminogen concentrations are important in determining milk production by affecting the state of involution within the mammary gland. Increasing plasmin concentration in milk as lactation advances has been reported previously by Politis et al.(1989). Long-term administration of bST in dairy cows has been shown to prevent an increase in milk plasmin activity during late lactation, suggesting that bST acts to delay mammary gland involution (Politis et al.,1990). However, in the present results, the effect of rbST on prevention of an increase in milk plasmin

activities was not apparent. A different pattern of this enzymatic system in crossbred dairy cattle would be suspected. In both the controls and rbST-treated animals showed gradual increase in milk plasmin concentrations as lactation advances. Milk plasmin is known to be influenced by the availability of plasminogen and the plasminogen activators. As plasminogen is ubiquitous in the body, thus, the plasminogen concentration in milk in animals treated with rbST would not be expected to be limiting in the present study. Milk plasminogen concentrations were not significantly different between rbST treated animals and control animals given placebo as lactation advances which was similar to that of findings in cows by Politis et al., (1990). However, the plasminogen: plasmin ratio fell in the control animals while it increased in rbST-treated animals as lactation advances. The plasminogen : plasmin ratio is a useful index of plasminogen activation. This measurement is independent of milk volume. It indicates that massive activation of plasminogen and production of plasmin occured in the control animals than rbST-treated animals. Therefore, it do not exclude the possibility that rbST is involved in maintenance of the tissue function in the present results.

The effect of long-term rbST administration for the fate of nutrients uptake by the mammary gland is mentioned (Chapter VII). The supply of glucose is a principal determinant of the milk yield, since glucose requirement is used for lactose production. A marked increase in milk yield without an alteration in lactose content during early lactation in rbST treated animals indicates that this requires a substantial increase in supply of glucose to the mammary gland (Bauman and McCutcheon, 1986). Glucose is essential for milk secretion and glucose moiety of lactose arises directly from plasma glucose (Ebner & Schanbacher, 1974). In the present study, the milk secretion of animals in both groups was not dependent on the blood glucose level, since the plasma glucose concentrations remained constant over a wide range at different stages of lactation. The marked increase in the udder blood flow of rbST treated animals in the present results will support the previous conclusion from a study in cows or goats by Linzell (1973) that glucose uptake is determined mainly by mammary blood flow.

The remainder of the discussion is concerned with metabolic fate of nutrient particularly glucose metabolism, the biosynthetic pathway for lactose synthesis (Chapter VIII), the utilization of glucose in the whole body related to the utilization in the mammary gland in both control animals and rbST-treated animals (Chapter IX).

. It is clear that changes in milk yield during rbST administration were in part accounted for changes in intra-mammary factors (ChapterVIII). An increase in milk yield during bST administration is thought to be determined primarily by lactose secretion (Linzell and Peaker, 1971). Lactose is synthesized in the mammary secretory cell from glucose derived from the blood. The concentration of milk glucose significantly increased which coincided with an increase in milk yield during rbST administration in both early and mid-lactation (Chapter VIII). This would reflect to the intracellular glucose concentration (Kuhn and White,1975; Faulkner et al,1981), since glucose freely permeates across Golgi vesicles and apical membranes of the mammary secretory cells (Faulkner & Peaker, 1987). Mammary cell cannot synthesize free glucose because they lack glucose-6-phosphatase activity (Threadgold & Kuhn, 1979). It is likely that the high concentrations of milk glucose in rbST-treated animals are related to a high rate of glucose uptake by the mammary gland, consistent with the higher mammary blood flow to the mammary gland during rbST administration (Chaiyabutr et al., 2005). During early lactation, a large portion of the conversion of intracellular glucose to intermediary metabolites of rbST-treated animals, was mainly used in the lactose biosynthetic pathway, when compared with controls. Our results in Chapter VIII clearly indicate that rbST administration exerts its galactopoietic action, in part, through both intra-mammary and extra-mammary effects.

The experiment in Chapter IX showed that rbST treatment did not significantly affect the reversible turnover of [3-^{3}H]glucose throughout stages of lactation, while the irreversible turnover of [U-^{14}C]glucose was reduced during the early lactation but not for mid-and late lactation. Experiments in Chapter V showed that the insulin level increased during rbST treatment in different stages of lactation in crossbred HF animals (Chaiyabutr et al., 2005). It indicates that rbST administration during early lactation antagonizes whole body turnover of glucose stimulated by insulin. Growth hormone is thought to be antagonistic to the action of insulin in tissues that are sensitive to insulin (Rose and Obara,1995), preventing the uptake of glucose by peripheral tissue and thus sparing glucose to mammary gland , which are insensitive to insulin (McGuire et al., 1995). It also noted that glucose clearance which

stimulated by insulin was also reduced during early lactation. This speculative sparing glucose utilization in tissues sensitive to insulin would partially allow for increase in lactose synthesis and milk yield. As lactation advances, the irreversible turnover of [U-^{14}C]glucose of rbST treated animals was increased in mid lactation, which was significantly higher than that of control animals. The reversible turnover of [3-^{3}H]glucose may represent the total glucose turnover rate as the ^{3}H is not recycled from products of partial glucose degradation (Katz et al.,1965). There were no differences for an increased recycling of glucose-C between the controls and rbST treated animals during advanced lactation suggests that a constant level of tricarbon units originally derived from glucose being again reincorporated into glucose was not affected by rbST treatment .

In general an increasee in milk yield can be attributed to an increase in the rate of lactose synthesis (Linzell & Peaker, 1971). However, an increase in the lactose yield during rbST administration was not related to the lactose concentration in milk, which largely unchanged. These results can be attributed to a difference in the activity of the mammary epithelial cells between controls and rbST treated animals. The synthesis of lactose involves a combination of glucose and UDP-galactose. The UDP-galactose originates from glucose 6-phosphate(Ebner and Schanbacher,1974). In contrast to the control animals, an administration rbST showed increases in both milk yield and glucose uptake by mammary gland, which were accompanied by increases in the secretion of both milk glucose and milk glucose 6-phosphate (Chapter VIII). These results would coincide with the calculated of metabolism of glucose 6-phosphate to the galactose moiety of lactose in rbST treated animals which was higher than that of control animals in early lactation. The availability of cytosolic glucose 6-phosphate in the cells of rbST-treated animals in early lactation would be sufficient to account for the cytosolic lactose synthesis. Decreases in the metabolism of glucose 6-phosphate to the galactose moiety of lactose in mid and late lactation in both groups (Chapter VIII and IX), would affect the lactose synthesis and milk production. A low enzymatic activity for lactose synthesis might be expected to appear as lactation advances in the crossbred animal. However, lactose synthesis is a complex process (Kuhn et al.,1980). There is still a need for more information to elucidate the changes in enzymatic activity in this particular system. The quantitative utilization of the glucose taken up by the mammary gland is used directly in the synthesis of lactose, and in other portions is metabolized via the pentose phosphate pathway, Embden-Meyerhof pathway and the tricarboxylic acid cycle.

Glucose carbon was used by the mammary cell to produce lactose, citrate and triacylglycerol for milk secretion. The data obtained for the utilization of glucose carbon for the synthesis of lactose, triacylglycerol and citrate during mid and late lactation were higher in rbST treated animals as comparison with the control animals. The differences in these results between the controls and rbST treated animals without a reduction in feed intake may be explained by the difference of nutrient partition or utilization in the mammary gland. In addition to the use of glucose carbon for milk synthesis, the hydrogen from glucose has been shown to be incorporated into milk fat. Studies *in vitro* have shown that glucose metabolism via the pentose phosphate pathway may not be as important for NADPH production as in the rat. Fatty acid synthesis from acetate can occur in the absence of glucose in sheep mammary-tissue slices (Balmain et al., 1952) and the perfused goat udder (Hardwick et al., 1963). In the present studies, estimates of the contribution of the pentose phosphate pathway in providing NADPH for fatty acid synthesis *in vivo* have been based on the assumption that all the glucose that was oxidized to CO_2 was metabolized via the pentose phosphate pathway. The calculation of the metabolism of glucose 6-phosphate via the Embden-Meyerhof pathway or the pentose phosphate pathway has been estimated in the goat udder *in vivo* (Chaiyabutr et al., 1980). However, few data have been available from the *in vivo* study of crossbred lactating cows. In the present studies glucose 6-phosphate metabolized via the pentose phosphate pathway gave percentage values of 7% to18% for both groups. These estimations are in contrast to experiments in the isolated perfused cow udder by Wood and co-workers (1965), in which about 23% to 30% of the glucose was metabolized via the pentose phosphate pathway. The difference in estimation is probably due to no consideration of the recycling of glucose 6-phosphate which occurs when glucose ismetabolized via the pentose cycle in the udder with the consequent loss of ^3H from glucose 6-phosphate (Davis and Bauman, 1974). However, the net proportion of the metabolism of glucose 6-phosphate via the pentose cycle pathway during different stages of lactation in rbST treated animals was higher than those of control animals. Metabolism of glucose via the pentose phosphate pathway yields 2 molecules of NADPH per molecule of glucose, only one of which could be labelled with ^3H in the present experiments. The data presented here provided evidence that 24% to 34% of the NADPH was required during early lactation for fatty acid synthesis *de novo* from glucose metabolism in the udder of rbST treated animals, while 30% to 39 % was required in the control animals. If there is a common pool of glucose 6-phosphate which is

available for both lactose synthesis and pentose phosphate metabolism, then the recycling of glucose 6-phosphate within the udder would result in too low a value for NADPH production from glucose. The net metabolism of glucose in the pentose phosphate pathway can be calculated from the incorporation of ^{3}H from [3-^{3}H]glucose in fatty acids assuming that the NADPH formed is used exclusively for biosynthesis of fatty acids (Katz et al.,1974). This technique has been used to study the *in vitro* metabolism of rat mammary and adipose tissue (Katz and Wals 1970,1972; Katz et al., 1966) and it was also used for the study of the *in vivo* metabolism of goat mammary tissue (Chaiyabutr et al.,1980). Based on the techniques and calculations of Katz and co-workers (1974) and assuming that cytosolic NADPH is used only for fatty acid synthesis, it has been shown that the glucose phosphorylated by the udder of rbST treated animals was metabolized via the pentose phosphate pathway which was higher than those of control animals. In rbST treated animals, a high proportion of the glucose taken up by the udder which was oxidized in the tricarboxylic acid cycle would be apparent in mid- and late lactations. High values of both the proportion and absolute amount of glucose carbon incorporation to milk citrate and milk triacylglycerol of rbST treated animals during mid- and late lactation are evidences supporting an increased proportion of glucose 6-phosphate metabolized via the Embden-Meyerhof pathway. It has been shown that metabolism of glucose 6-phosphate by the Embden-Meyerhof pathway can result in ^{3}H being retained in glycerol if the triose phosphate isomerase reaction is not at equilibrium (Katz and Rognstad, 1976). Metabolism of glucose 6-phosphate by the pentose phosphate pathway usually results in the loss of all ^{3}H from [3-^{3}H]glucose in lactating cows. The high metabolism of glucose 6-phosphate in early lactation of rbST treated animals appeared to be due primarily to a high flux through the lactose synthesis and to pentose phosphate pathway, probably reflecting the high milk production during rbST treatment. Tritium and carbon-14 were also shown to be incorporated into milk citrate which showed increases during lactation advance in rbST treated animals whereas it remained the same levels as compared with pretreatment period for the carbon skeleton of citrate in control animals. It has been postulated that milk citrate could be synthesized from 2-oxoglutarate via the NADP-dependent isocitrate dehydrogenase reaction (Hardwick,1965). In addition ^{3}H is lost to NADPH or water in metabolism via the pentose phosphate pathway or glycolytic pathway, so it is likely that ^{3}H incorporation into milk citrate was also via NADP^{3}H. It is possible that the incorporation of ^{3}H into milk citrate may occur in different manners in the exchange

Figure 2 The metabolic pathway involved in the metabolism of the precursor of milk in the pretreatment period of initial lactation of control animals and rbST treated animals (The value shown are in micromole/min.)

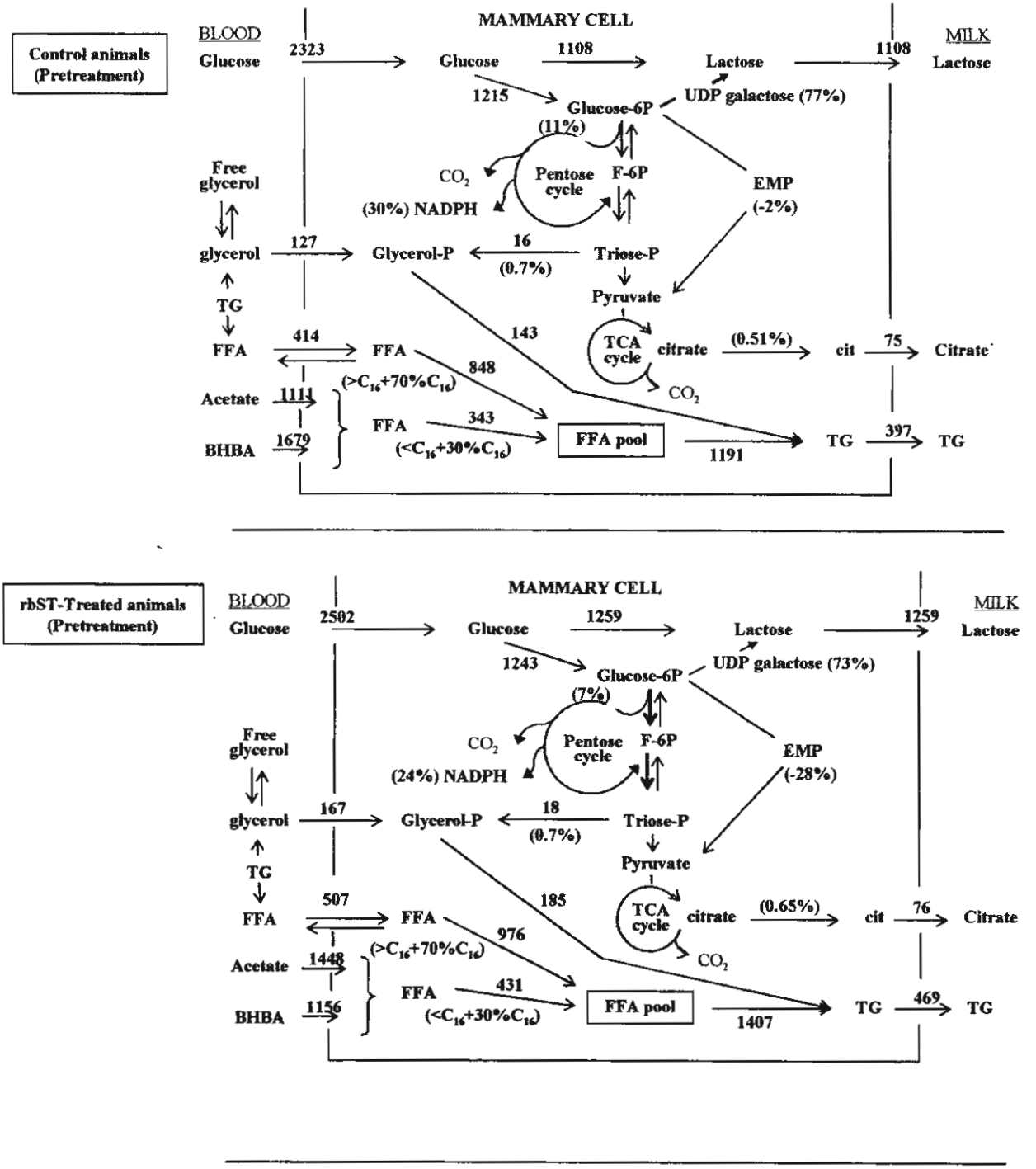
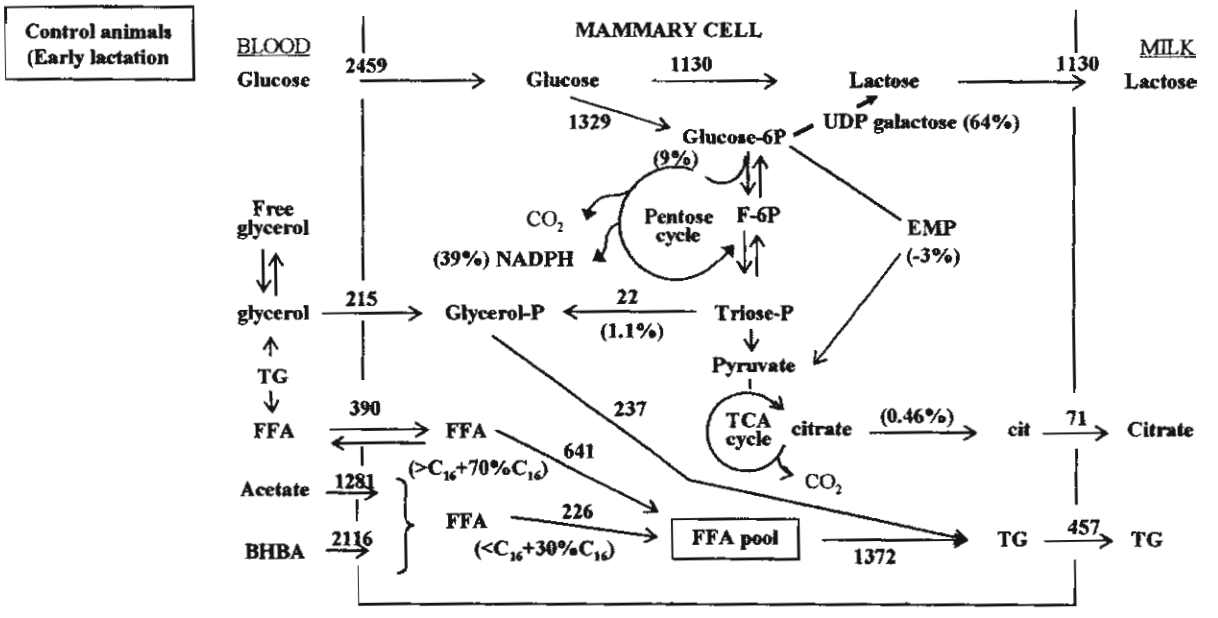


Figure 3. The metabolic pathway involved in the metabolism of the precursor of milk in the early lactation of control animals and rbST treated animals (The value shown are in micromole/min.)



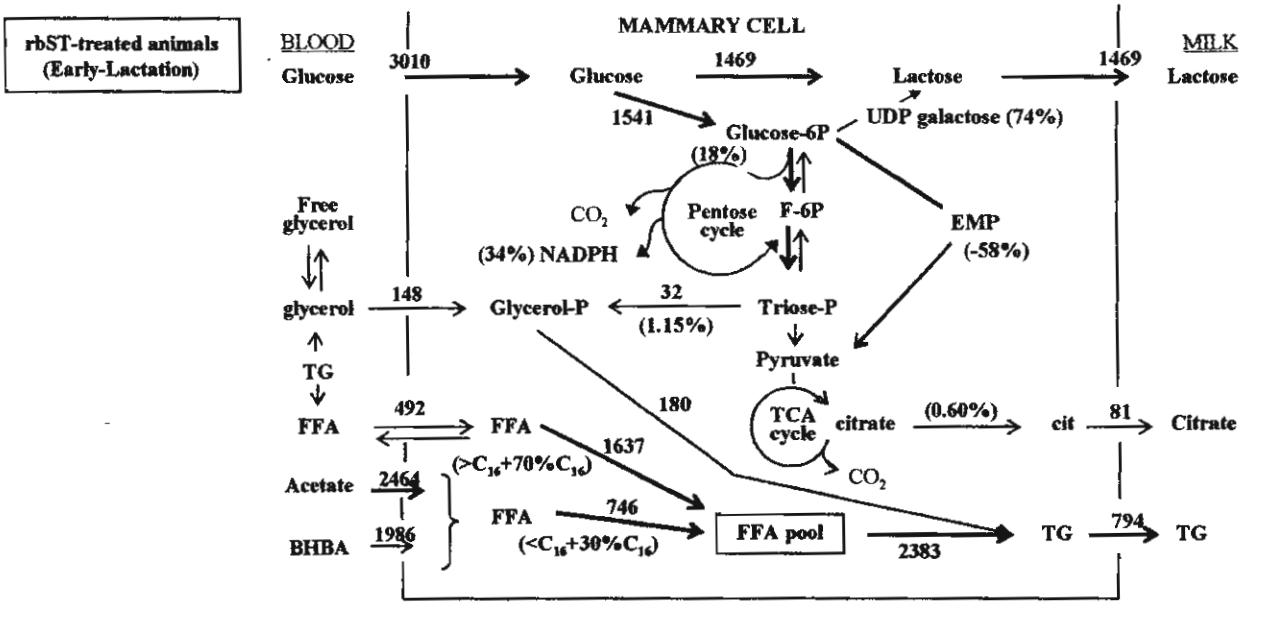
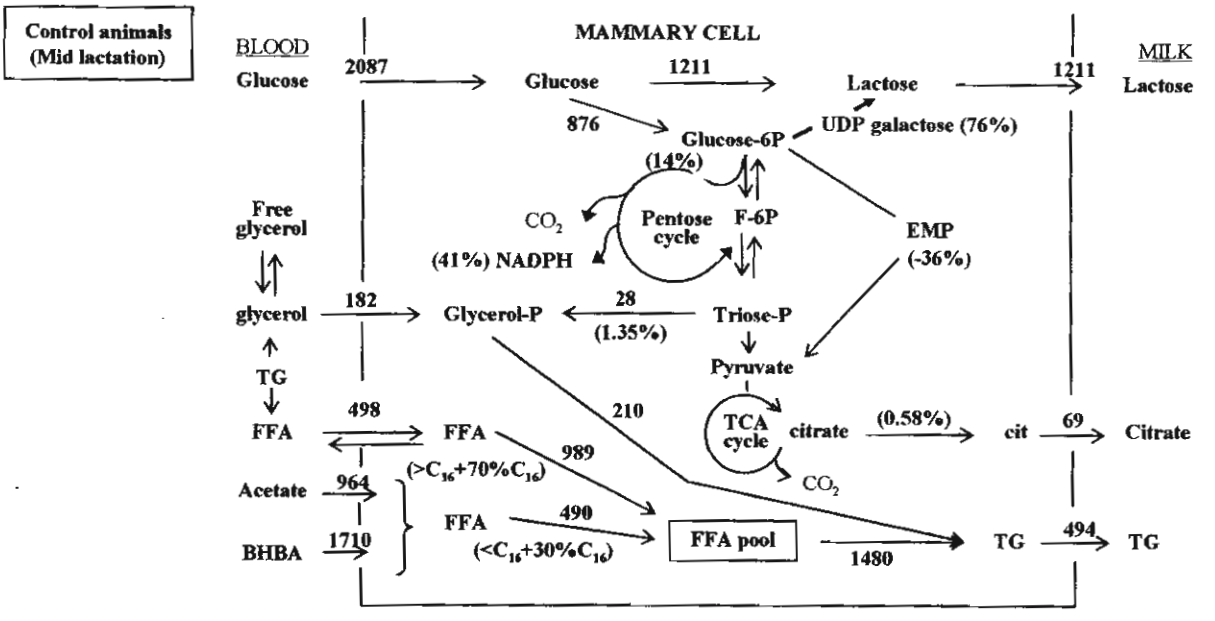


Figure 4. The metabolic pathway involved in the metabolism of the precursor of milk in the mid lactation of control animals and rbST treated animals (The value shown are in micromole/min.)



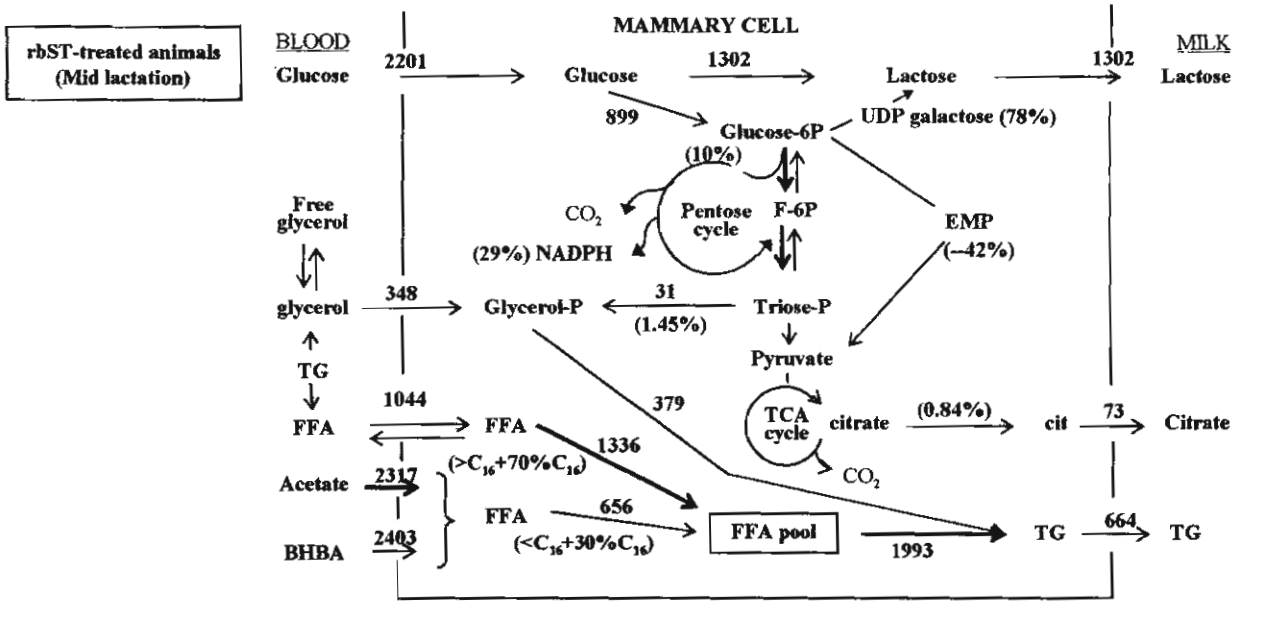
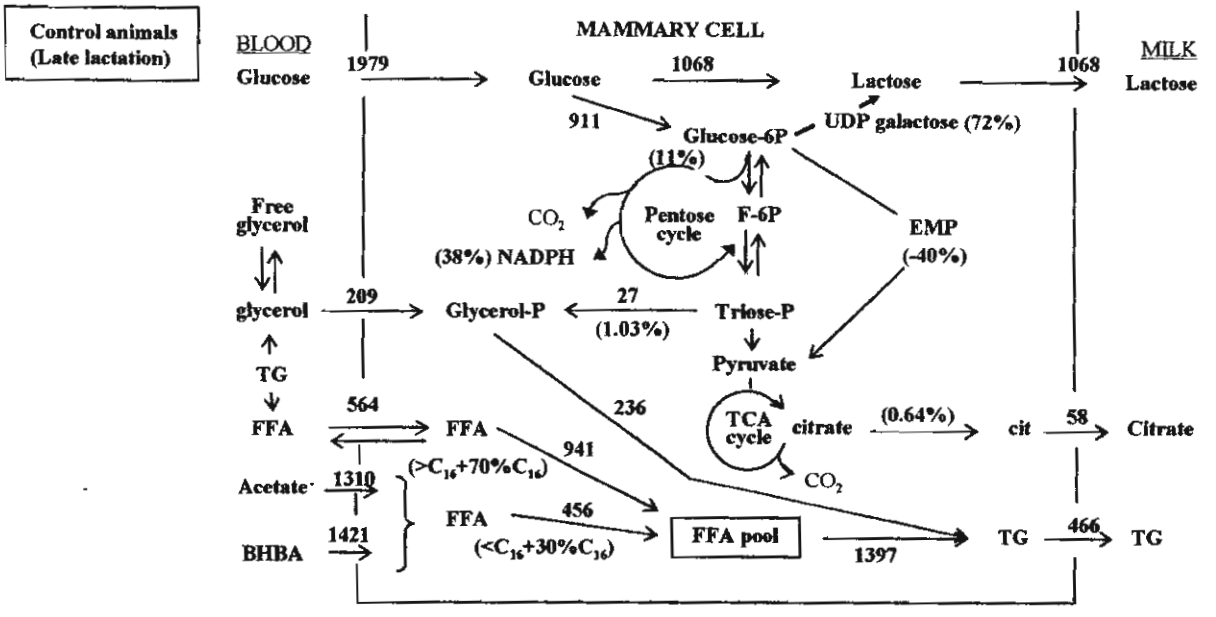
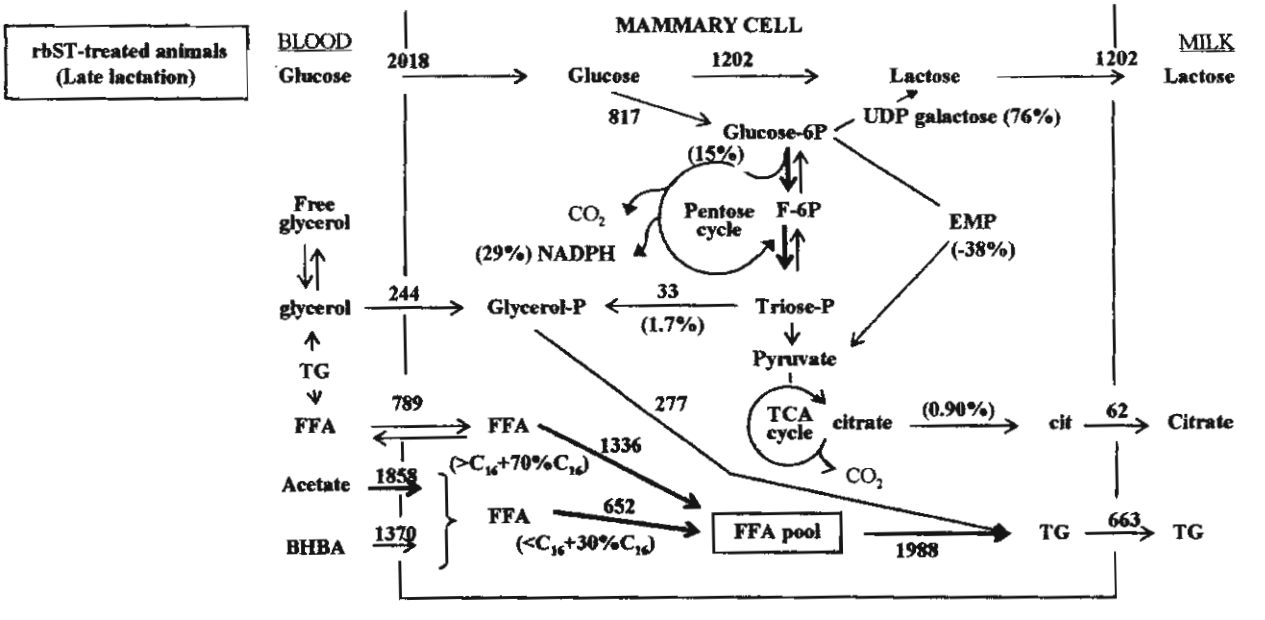


Figure 5 The metabolic pathway involved in the metabolism of the precursor of milk in the late lactation of control animals and rbST treated animals (The value shown are in micromole/min.)





reaction of the cytosolic NADP-dependent isocitrate dehydrogenase. Both fatty acid synthesis and the NADP-dependent isocitrate dehydrogenase reaction between control animals and rbST-treated animals may have different mechanisms with a common pool of cytosolic NADPH.

In conclusion, the data presented here represent the estimation *in vivo* of glucose metabolism in the udder and its distribution to lactose synthesis, the pentose phosphate pathway and the Embden-Meyerhof pathway during rbST administration in 87.5% HF animals. As shown in Fig 2, 3, 4 & 5 (summarized of Chapter X), the glucose taken up by the udder of rbST treated animals during early lactation, an average 18% and 34% were metabolized in the pentose phosphate pathway and contributed to NADPH production, respectively. The sufficient pool of intracellular glucose concentration during rbST administration, has effect on an increase in glucose 6-phosphate which increased flux through the lactose synthesis and pentose cycle pathway. Although we know a great deal of differences in regulating glucose metabolism that occur between the control animals and rbST treated animals, we do not know the different enzymatic activities during rbST administration in different stages of lactation which affect the rate of metabolic pathways. There is still a need for more information, for example,on whether the high enzymatic activity of fructose 1-6 diphosphatase or the lower enzymatic activity of pyruvate dehydrogenase occurs in rbST treated animals throughout the stages of lactation or occurs during early lactation which causes an increase in the metabolism of glucose 6-phosphate via the Embden-Meyerhof pathway and tricarboxylic acid cycle.

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