รายงานวิจัยฉบับสมบูรณ์

โครงการ

การตอบสนองทางสรีรวิทยาของโคนมลูกผสมโฮลสไตน์ต่อสภาพแวดล้อมอุณหภูมิสูงกับกลไกการควบคุมเพื่อลดผลกระทบต่อผลผลิตน้ำนม

Physiological responses of lactating crossbred Holstein cattle to high ambient temperature and control mechanisms to reduce its effect on milk production

โดย

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สัญญาเลขที่ BRG498004

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FINAL REPORT

PHYSIOLOGICAL RESPONSES OF LACTATING CROSSBRED HOLSTEIN CATTLE TO HIGH AMBIENT TEMPERATURE AND CONTROL MECHANISMS TO REDUCE ITS EFFECT ON MILK PRODUCTION

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PHYSIOLOGICAL RESPONSES OF LACTATING CROSSBRED HOLSTEIN CATTLE TO HIGH AMBIENT TEMPERATURE AND CONTROL MECHANISMS TO REDUCE ITS EFFECT ON MILK PRODUCTION

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Executive Summary

Project Title: Physiological responses of lactating crossbred Holstein cattle to high ambient temperature and control mechanisms to reduce its effect on milk production

Project Code: BRG498004

Project Objective: The overall objectives of the present study in terms of reference were to investigate effects of cooling and recombinant bovine somatotropin (rbST) supplementation on milk production of crossbred Holstein Friesians. The studies were carried out to clarify various physiological changes on the mechanism responsible for the control of the mammary function (extra-mammary functions) and the process of milk synthesis (intra-mammary functions) in different stages of lactation.

The experimental studies were carried out as following;

1. Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on water metabolism and mammary circulation in different stages of lactation in crossbred Holstein cattle.
2. Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on the plasma level of insulin like growth factor-1, insulin and plasma metabolites in different stages of lactation in crossbred Holstein cattle.
3. Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on the utilization of glucose in the mammary gland in different stages of lactation in crossbred Holstein cattle.
4. Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on cellular metabolites in milk secretion at different stages of lactation in crossbred Holstein cattle.
5. Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on plasminogen and plasmin system in the mammary gland in different stages of lactation in crossbred Holstein cattle.

6. Indicators of oxidative status in plasma of shaded cooled animals with recombinant bovine somatotropin (rbST) administration in different stages of lactation in crossbred Holstein cattle.
7. Changes in renal function and mammary circulation of shaded cooled animals and recombinant bovine somatotropin (rbST) administration in different stages of lactation in crossbred Holstein cattle.
8. Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) on milk yield, hematology and blood chemistry in different stages of lactation in crossbred Holstein cattle.
9. Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on changes in the rate of liquid flow from the rumen and milk production in different stages of lactation in crossbred Holstein cattle.

Materials and methods:

Primiparous crossbred cattle, containing 87.5 % Holstein (HF) genes, average body weight 358±32.5 kg, non pregnant and averaged 60 day postpartum at start of trial were used for the experiment. The animals were assigned randomly into two groups of five animals each. Animals in the first group were housed in open-sided barn with a tiled roof in normal shaded house (NS) as the non-cooled animals. Animals in the second group were housed in open-sided barn with a tiled roof (8 m long x 7 m wide x 3.5 m high) under misters and fans (MFC) as cooled animals. The open space cooling system consisted of two sets of misters and fans, which each system consisted of a 65 cm. diameter blade fan circulating 81 m^3/min of air, with oscillation coverage of 180º. The amount of water discharged from 4 splay heads was 7.5 L/h and size of mist droplet 0.01 mm. Animals were exposed to MFC for 45 minutes at 15-minute intervals from 06:00 h to 18:00 h. At night, animals were exposed to MFC for 15 minutes at 45-minute intervals from 18:00 h to 06:00 h. The ambient temperature and the relative humidity were read depending on wet and dry bulb temperature at cooled and non-cooled barns and a temperature-humidity index (THI) was calculated. from the average ambient temperature of dry and wet bulb temperatures. All cows were fed the same total mixed rations(TMR) twice daily and water was offered *ad libitum* throughout the experiments in both groups.

The experiment was divided into 3 phases, namely early- (approximately 2 months postpartum), mid- (approximately 4 months), and late lactation (approximately 6 months) periods. The pretreatment was conducted on the starting day of each phase. At the end of the pretreatment, within the same day, the subject was injected with the first dose/injection with 500 mg of recombinant bovine somatotropin (rbST) (POSILAC, Monsanto, USA). Subsequently, the subject was injected with two consecutive doses/injections of with 500 mg of recombinant bovine somatotropin (rbST) in every two weeks. Thereafter, within 2-5 days after the third injection, the treatment was conducted. The pretreatment, 3 doses of injections, and the treatment were performed during the first 30 days and the same procedures were followed for each phase. During the last 30 days of each phase, no experiments were conducted in order to allow the milk yield to return to control level. Thus, comparative studies of both groups in each stage of lactation, the four treatment combinations were normal shade without rbST injection (NS), normal shade plus rbST injection (NS + rbST), shade plus misty-fan cooling without injection (MFC), and shade plus misty-fan cooling with rbST injection (MFC + rbST).

Effects of cooling and recombinant bovine somatotropin (rbST) supplementation on mammary functions were carried out, where appropriate experiments were divided into different Chapters and series of studies to cover both intra- and extra-mammary functions.

Overview of results and conclusion:

During the experimental periods, values of ambient temperatures and temperature humidity index (THI) in the NS barn were significantly higher than in the MFC barn, while the relative humidity in the MFC barn was significantly higher than in the NS barn. The respiration rate and rectal temperature were significantly higher for non-cooled cows than for cooled cows during the daytime whether there was or was not rbST supplementation. Supplementation of rbST for either cooled or non-cooled cows significantly increased milk yields, dry matter intake and the efficiency of feed utilization. The initial experiment was designed to study the effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on regulation of body fluids and mammary circulation in different stages of lactation in crossbred Holstein cattle. It was shown that peak milk yield of both cooled and non-cooled cows supplemental rbST were significantly higher than those of the

pretreatment during early lactation; thereafter milk yields continued to decline in both groups as lactation advanced. Mammary blood flow (MBF) significantly increased during rbST supplementation in each stage of lactation in both cooled and non-cooled cows. The value of the water turnover rate (WTO) in cows supplemental rbST in each lactation tended to increase, while the biological half-life of tritiated water showed no significant differences in both cooled and non-cooled cows. The absolute values of both total body water space(TOH) and empty body water(EBW) of both cooled and non-cooled cows showed significant increases during supplemental rbST throughout lactation. Marked increases in extra cellular fluid (ECF), intra cellular fluid (ICF), blood volume (BV) and plasma volume (PV) in terms of absolute values were apparent in each stage of lactation in both cooled and non-cooled cows. The packed cell volume and plasma osmolality of both cooled and non-cooled cows supplemental rbST were unchanged throughout lactation. An increase in ECF by the effects of rbST in both cooled and non-cooled cows leads to an increase in MBF as secondary responses in facilitating increased milk production.

Further studies on the effects of supplemental rbST and cooling on alterations of plasma hormones and metabolites showed significant increase in the plasma IGF-I concentration in both cooled and non-cooled cows during rbST supplementation in each stage of lactation. The plasma concentrations of insulin had tendency to increase during supplemental rbST in early and mid-lactation, while the plasma thyroxine concentrations in both cooled and non-cooled cows were lower after rbST supplementation in early lactation. The plasma thyroxine concentration of cooled cows without rbST had tendency to be higher than those of non-cooled cows in each stage of lactation. No effects of cooling and supplemental rbST on the plasma cortisol concentration were apparent throughout periods of studies. There were no significant differences in plasma concentrations of glucose, acetate, β-hydroxybutyrate, triglyceride and protein in both cooled and non-cooled cows whether rbST supplementation or not in each stage of lactation. The lipolytic activity of rbST per se in adipose tissue was apparent with a marked increase in the plasma free fatty acid concentration in both cooled and non-cooled cows in each stage of lactation. The effect of exogenous rbST for increase in milk productions require IGF-I as a mediator to increase in mammary blood flow for increasing the availability of substrates to the mammary gland for milk synthesis.

As lactation advances, the concentration of plasmin-plasminogen activity and the milk plasminogen concentration increased in both groups. The supplementation of rbST showed an involvement of the activity of the plasmin–plasminogen system but not for maintaining tissue integrity in the mammary gland during high milk yield in early lactation. There were no significant differences of milk Na^+, K^+ and Cl^- concentrations in each stage of lactation. The milk sodium : potassium ratio of both cooled and non-cooled cows supplemental rbST was significantly increased than those of pretreatment period in the early lactation.

The further study was carried out to determine whether cows exposure to high temperatures and rbST supplementation would induce oxidative stress. The results of the study showed no changes of the oxidative stress markers in plasma for sulfhydryl (SH) residues and TBARS between non-cooled and cooled cows whether supplementation of rbST or not, while the plasma ascorbic acid concentration of non-cooled cows were lower than those of cooled cows. Their internal relationships, SH residues versus ascorbic acid concentration, SH residues versus TBARS and ascorbic acid versus TBARS, were not constant. The results indicated a different mechanism in each oxidative stress markers. The concentration of ascorbic acid in cow plasma was sensitive to high environmental temperature stress. In the present study, selected biochemical and haematological parameters remained within the normal physiological values and did not differ significantly between cooled and non-cooled cows before and after the supplementation of rbST.

The further experiment was carried out to study of the kidney function in regulation of volume and body compositions during exposure to high temperatures and rbST supplementation. Increases in MBF, TBW, ECF, BV and PV were apparent in both cooled and non-cooled cows receiving rbST without alterations of renal blood flow(RBF) and glomerular filtration rate (GFR) in each stage of lactation. Decreases in urinary excretion and fractional excretions of sodium, potassium and chloride ions appeared to correlate with reduction in the rate of urine flow and osmolar clearance during rbST administration. The effect of rbST supplementation to cows housing under either NS or MFC barn on body fluid volume expansion were attributed to changes in the rates of electrolytes excretion in the kidney. The increased availability of renal tubular reabsorption of sodium, potassium and chloride ions during rbST supplementation was a major factor consequence in retaining body water through its

colligative properties in exerting osmotic force mechanism formation. Lithium clearance study showed the site of response of sodium reabsorption in the proximal nephron segment which was mediated via increases in plasma levels of aldosterone and IGF-1 but not for vasopressin during rbST administration.

The study of diet digestibility, digestion kinetics and milk production were performed during supplementation of rbST in both cooled and non-cooled cows. Supplementation of rbST in either cooled or non-cooled cows significantly increased dry matter intake (DMI), the efficiency of feed utilization and milk yields. Digesta kinetics study using chromic oxide as an external marker showed a high digesta passage rate constant and low mean retention time of digesta in cows either by cooling or supplementation of rbST, whereas no changes were seen for the digestibility of dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF). The half-time of Cr_2O_3 in the whole digestive tract of cooled cows were lower than those of non-cooled cows and significantly decreased during rbST supplementation in both groups in all stages of lactation. The magnitude of responses for the digesta passage rate and efficiency of feed utilization were larger in animals supplemented with rbST than in animals under MFC cooling only. The main effect of cooling and supplemental rbST was to improve digestion by an increase in the rate of passage of digesta and in turn an increase in feed intake. Digestibility was not influenced by changes in passage rate of digesta either by cooling or rbST supplementation. The increased milk production induced by rbST supplementation was mediated by increased efficiency of feed utilization without changes in diet digestibility. The rbST-treated cows had higher total ruminal fermentation products as volatile fatty acid and ammonia nitrogen than the non-rbST treated cows and associated changes were greater in cooled animals in all stages of lactation. Exogenous rbST increased the concentrations of milk urea nitrogen in both groups. Changes in ruminal fermentation with greater production of total VFA and NH_3N in response to rbST in crossbred cows whether under misty-fan cooling or not, would be in part through an increase in feed intake, thereby making more substrate available to the mammary gland for milk synthesis.

The study on glucose metabolism and utilization of glucose in the mammary gland in vivo in both cooled and non-cooled cows were performed during supplementation with rbST. The glucose turnover rate was determined using a

continuous infusion of [U-^{14}C] and [3-^{3}H] glucose in each stage of lactation. Glucose turnover rates and plasma glucose concentrations were not significant different between cooled and non-cooled cows whether supplemental rbST or not. The glucose taken up by the mammary gland of both non-cooled and cooled cows increased flux through the lactose synthesis and the pentose cycle pathway with significant increases in NADPH formation for fatty acid synthesis during rbST supplementation. During early and mid lactation, the utilization of glucose carbon incorporation into milk appeared to increase in milk lactose and milk triacylglycerol but not for milk citrate of both non-cooled and cooled cows supplemental rbST. As advanced lactation, local changes for biosynthetic capacity within the mammary gland would be a factor in identification of the utilization of substrates in the rate of decline in milk yield. The proportion of glucose would be metabolized less for lactose synthesis, but metabolized more via the Embden-Meyerhof pathway and the tricarboxylic acid cycle as lactation advances to late lactation whether supplemental rbST or not. The uptake for glucose across the udder increased during supplementation of rbST in each stage of lactation in both cooled and non-cooled cows, which coincided with an increase in the milk glucose concentration in early and mid-lactation. The concentrations of milk Na^{+}, K^{+}, Cl^{-} and plasma osmolality of both cooled and non-cooled cows supplemental rbST were unchanged throughout lactation.

This report is based on the following publications, which will be referred to in the text by their roman numerals.

Abstract

The present study was to evaluate the the effects of misty-fan cooling and supplementation of rbST on milk production in relation to the mechanism responsible for the control of the mammary function in both intramammary factor and extramammary factors were performed in crossbred 87.5% Holstein cows. Primiparous cows were used for experiments. Cows in each experiment were divided into two groups and assigned under the normal shaded barn (NS) as non-cooled cows and shaded barn with misty-fan cooling (MF) as cooled cows. The NS barn was separated from MF barn by longed metal sheet wall from floor to roof. Each cow was injected subcutaneously with 500 mg of rbST in every 14 days for 3 consecutive doses in each stage of lactation (early, mid and late lactation). Cows were fed the same total mix ration *ad libitum* and water was freely offered. The experimental results demonstrated that an application of MF cooling could reduce ambient temperature (AT) and temperature humidity index (THI). A low respiratory rate (RR) and rectal temperature (RT) were occurred in cooled cows. Milk yield significantly increased in cows treated with rbST in each stage of lactation. Increases in mammary blood flow accompanied with increases in total body water (TBW), extracellular fluid (ECF), blood volume (BV) and plasma volume (PV) in both cooled and non-cooled cows receiving rbST in each stages of lactation. Cows were housed under MF cooling could reduce the negative effect of high temperatures on digestive function via an increase in the digesta passage rate resulting in an increase in feed intake. An increase in dry matter intake (DMI) in response to both cooling system and rbST supplementation would be partly attributed to an increase in rumen fermentation with increases in VFA, NH_3N and microbial protein. It was also found that an increase in water intake accompanying with an increase in DMI was apparent in rbST-supplemented cows under misty-fan cooling. An increase in gut water and liquid outflow rate from the rumen were apparent in rbST supplemented cows. The effect of MF cooling influenced to an increase in net water transfer through the ruminal wall. The rbST-supplemented cows under MF barn also showed a high level of water absorption through ruminal wall. These changes would be in part accounted for an increase in total body water (TBW). The present results indicate that the rbST exerts

its galactopoietic action, in part, through changes in body fluids associated with increased in gut water regulation and rumen function, which would be the consequence in distribution of nutrients to the mammary gland and for thermoregulatory mechanisms.

The study of renal function in both cooled and non-cooled cows whether supplemental rbST or not showed no significant changes in renal hemodynamics. There were decreases in the rate of urine flow, urinary electrolytes excretion and osmolar clearance in both cooled and non-cooled cows supplementation with rbST. During supplemental rbST in both cooled and non-cooled cows, a marked increase in plasma insulin like growth factor (IGF-I) coincided with an increase in the plasma aldosterone level, while there were no changes in plasma cortisol and vasopressin concentrations in all stages of lactation. The plasma thyroxine (T4) concentration was significantly decreased during early lactation. The lithium clearance study revealed the increases in sodium ion and water reabsorption in renal proximal tubule. The stimulatory effects of rbST on body fluid expansion could be in part stimulate sodium and water reabsorption in renal proximal tubule by mediated via increases in plasma levels of aldosterone and IGF-1 which may involve a stimulation of renin-angiotensin-aldosterone (RAAS) system, but not for vasopressin. The haematological and plasma biochemical values for systemic health monitoring during rbST supplementation in both cooled and non-cooled cows showed no significant changes and being within normal physiological limits. An antioxidative component, the SH residue concentration in the plasma, and the oxidation products of polyunsaturated lipid, (TBARS concentration) of both cooled and cooled cows showed no changes during periods of rbST supplementation. The ascorbic acid concentrations, antioxidative component in plasma, were not affected during rbST supplementation, but the ascorbic acid concentrations in plasma of cooled cows were significantly lower than those of non-cooled cows.

Milk yield of both cooled and non-cooled cows without rbST decreased as lactation advanced to late lactation. The mean arterial plasma concentrations for glucose, acetate, β-hydroxybutyrate and triacylglycerol were unchanged, while the mean arterial plasma concentrations of free fatty acid increased both cooled and non-cooled cows supplemental rbST. The net mammary glucose and triacylglycerol uptakes of cows in both groups markedly increased in mid and late stages of lactation,

while no significant changes of the arteriovenous differences (A-V differences) and mammary extraction across the mammary gland were apparent throughout lactation in both cooled and non-cooled cows supplemental rbST. No significant changes in the A-V differences, mammary extraction and mammary uptake for acetate, β-hydroxybutyrate were apparent during rbST supplementation in both cooled and non-cooled cows. Glucose turnover rates were not significant different between cooled and non-cooled cows whether supplemental rbST or not. The glucose taken up by the mammary gland of both non-cooled and cooled cows increased flux through the lactose synthesis and the pentose cycle pathway with significant increases in NADPH formation for fatty acid synthesis during rbST supplementation. The utilization of glucose carbon incorporation into milk appeared to increase in milk lactose and milk triacylglycerol of both cooled and non-cooled cows supplemental rbST during early and mid lactation but not for milk citrate as lactation advances. The proportion of glucose was metabolized less for lactose synthesis, but metabolized more via the Embden-Meyerhof pathway and the tricarboxylic acid cycle as lactation advances whether supplemental rbST or not.

These results can conclude that an increase in milk production by the effect of rbST supplementation to cows housed either in NS or MF barns is mediated primarily through body fluid volume expansion and secondary increased MBF to the mammary gland for distribution of nutrients for milk synthesis. Local changes for biosynthetic capacity within the mammary gland would be a factor in identification of the utilization of substrates in the rate of decline in milk yield with advancing lactation in both cooled and non-cooled cows whether supplemental rbST or not.

บทคัดย่อ

การศึกษาผลของการใช้ระบบพัดลมพ่นละอองน้ำทำความเย็นกับการการฉีดฮอร์โมนโบวายโซมาโตโทรปีน (rbST) ต่อการเพิ่มผลผลิตน้ำนมกับการเปลี่ยนแปลงกลไกที่มาควบคุมทั้งปัจจัยภายในและปัจจัยภายนอกต่อมน้ำนมในโคนมพันธุ์ผสมสายเลือด87.5%โฮลสไตน์ การศึกษาใช้โคนมท้องแรกในแต่ละการทดลอง โคถูกแบ่งออกเป็น 2 กลุ่ม จำนวนเท่าๆ กัน และจัดให้เลี้ยงในโรงเรือนแบบผูกยืนโรงที่ถูกกั้นแยกออกจากกันเป็นสองด้านด้วยแผ่นโลหะสูงจากพื้นจรดหลังคา กลุ่มแรกเลี้ยงอยู่ในด้านที่ไม่ติดตั้งระบบทำความเย็น (NS) กลุ่มที่สองเลี้ยงอยู่ในด้านที่ติดตั้งระบบทำความเย็นด้วยพัดลมพ่นละอองน้ำ(MF) โคนมทุกตัวได้รับการฉีดฮอร์โมนrbST ขนาด500มก เข้าใต้ผิวหนัง โดยให้ห่างกันทุกๆ 14 วันติดต่อกัน3 ครั้งในแต่ละระยะการให้นม (ระยะต้น กลาง และระยะท้ายของการให้นม) โคทั้งสองกลุ่มได้รับการจัดการ การให้อาหาร น้ำ และการจัดการอื่นๆเหมือนกันตลอดระยะการทดลอง ผลการทดลองพบว่าการใช้ระบบพัดลมพ่นละอองน้ำสามารถลด อุณหภูมิ และดัชนีอุณหภูมิความชื้นภายในโรงเรือน MF ลงได้ มีผลทำให้อัตราการหายใจและอุณหภูมิร่างกายวัดทางทวารหนักของแม่โคกลุ่มภายในโรงเรือน MF ต่ำลง อัตราการหลั่งน้ำนมจะเพิ่มขึ้นอย่างมีนัยสำคัญในกลุ่มโคที่ฉีด rbSTในทุกระยะของการให้นม การเพิ่มขึ้นของอัตราการไหลของเลือดสู่ต่อมน้ำนม ร่วมไปกับการเพิ่มขึ้นของปริมาณน้ำในร่างกาย ปริมาณน้ำนอกเซลล์ ปริมาณเลือดและปริมาณพลาสม่าในโคนมทั้ง 2 กลุ่มที่ได้รับ rbST ตลอดระยะการให้นม การเลี้ยงโคนมในโรงเรือน MF สามารถช่วยลดผลกระทบจากสภาพอากาศร้อนที่มีต่อระบบย่อยอาหารของโค โดยมีส่วนทำให้อัตราการไหลผ่านของอาหารเร็วขึ้น ทำให้โคสามารถกินอาหารได้เพิ่มขึ้น การตอบสนองของแม่โคต่อฮอร์โมน rbST และการปรับอุณหภูมิแวดล้อมด้วยระบบ MF ทำให้การกินอาหารเพิ่มขึ้นแล้ว ยังมีผลต่อการเพิ่มผลผลิตจากการหมักอาหารของจุรินทรีย์ในกระเพาะหมัก ได้แก่ กรดไขมันระเหยได้ แอมโมเนียไนโตรเจน รวมถึงการสังเคราะห์จุรินทรีย์โปรตีนเพิ่มขึ้นด้วย ในการทดลองครั้งนี้ยังพบว่าแม่โคที่ฉีดฮอร์โมนrbST และปรับอุณหภูมิแวดล้อมด้วยระบบ MF จะกินน้ำเพิ่มขึ้นสัมพันธ์กับการเพิ่มขึ้นของอาหารที่กินได้ ซึ่งเป็นสาเหตุหนึ่งของการเพิ่มน้ำในทางเดินอาหาร และมีการไหลผ่านของน้ำจากกระเพาะหมักไปยังกระเพาะส่วนอื่นและลำไส้เพิ่มขึ้น ส่วนอิทธิพลของโรงเรือนที่มีระบบMFพบว่ามีผลต่ออัตราการดูดซึมน้ำผ่านผนังกระเพาะหมักเพิ่มขึ้น จากผลดังกล่าวจึงเป็นสาเหตุสำคัญที่ทำให้มีการเพิ่มขึ้นของน้ำในร่างกาย และบางส่วนของน้ำในร่างกายยังถูกใช้ไปในกลไกควบคุมความร้อนในร่างกาย

การศึกษาการทำหน้าที่ของไตในการควบคุมของเหลวและส่วนประกอบในของเหลวในร่างกายใน โคที่เลี้ยงอยู่ในที่เย็น และไม่เลี้ยงอยู่ในที่เย็น ไม่ว่าจะให้ rbST เสริมหรือไม่ พบว่าไม่มีผลต่อการเปลี่ยนแปลงของ renal hemodynamics แต่มีผลต่อการลดลงของอัตราการขับปัสสาวะ

อัตราการขับทิ้งของอิเลคทรอไลท์ และ osmolar clearance ทั้งในโคที่อยู่ในที่เย็น และ โคไม่อยู่ในที่เย็นที่ได้รับ rbST การเสริม rbST ในโคที่ทั้งสองกลุ่ม พบการเพิ่มขึ้นของฮอร์โมนอินซุลินไลค์โกรท์แฟคเตอร์-I(IGF-I) ควบคู่ไปกับการเพิ่มขึ้นของ ฮอร์โมนอัลโดสเตอโรน แต่ไม่มีผลต่อการเปลี่ยนแปลงอย่างมีนัยสำคัญของ ฮอร์โมนคอร์ติซอล และ วาโสเพรสซิน ในทุกระยะของการให้นม นอกจากนี้ยังพบการลดลงอย่างมีนัยสำคัญทางสถิติของ ฮอร์โมนไทรอกซีนในระยะแรกของการให้นม จากการศึกษาโดยใช้ lithium clearance พบว่าการให้ rbST ทำให้มีการดูดกลับโซเดียมไอออนและน้ำเพิ่มขึ้นในบริเวณ proximal tubule ของไต การเพิ่มขึ้นของน้ำในร่างกายเป็นผลให้มีการเพิ่มขึ้นของปริมาณเลือดไปสู่ต่อมน้ำนมในการนำส่งสารอาหารในการสังเคราะห์น้ำนม ในการเพิ่มปริมาณน้ำนม ผลการกระตุ้นของ rbST ต่อการเพิ่มขึ้นของน้ำในร่างกายโดยผ่านการดูดกลับของ โซเดียมไอออนและน้ำที่บริเวณ proximal tubule จะผ่านการทำงานของฮอร์โมนอัลโดสเตอโรนและIGF-Iที่เพิ่มขึ้นซึ่งอาจจะกระตุ้นผ่านการทำงาน ของระบบเรนิน- แองจิโอเทนซิน-อัลโดสเตอโรน แต่ไม่เกี่ยวกับระดับ ฮอร์โมนวาโสเพรสซินที่ไม่เปลี่ยนแปลง การวัดค่าเลือดและค่าชีวเคมีในพลาสมาเพื่อบ่งถึงสุขภาพของโคนมระหว่างการให้ rbST ในโคที่เลี้ยงอยู่ในที่เย็น และ โคที่ไม่อยู่ในที่เย็นพบมีค่าอยู่เกณฑ์ปกติ การวัดค่าoxidative stressในพลาสมาของโคนมระหว่างการให้ rbSTในโคที่เลี้ยงอยู่ในที่เย็นและโคที่ไม่อยู่ในที่เย็น ไม่พบการเปลี่ยนแปลงของค่าความเข้มข้นของSH residue, และTBARSในพลาสมา แต่ความเข้มข้นของascorbic acid ในพลาสมาของโคนมที่เลี้ยงอยู่ในที่เย็นจะมีค่าต่ำกว่าของโคที่ไม่เลี้ยงอยู่ในที่เย็น

อัตราการหลั่งน้ำนมที่มากในระยะต้นของการให้นมในโคทั้ง 2 กลุ่มแม้ไม่ได้ให้rbST และจะลดลงเมื่อเข้าสู่ระยะท้ายของการให้นม การวัดระดับความเข้มข้นของกลูโคส อะซีเตท เบต้าไฮดรอกซี่บิวตาเรต และ ไตรกลีเซอไรด์ ในพลาสม่าของเลือดแดงไม่พบการเปลี่ยนแปลง แต่ความเข้มข้นของกรดไขมันอิสระจะเพิ่มขึ้นเมื่อให้rbSTในโคที่เลี้ยงทั้งในโรงเรือนปกติและโรงเรือนที่มีความเย็น อัตราการใช้กลูโคสและไตรกลีเซอไรด์ในต่อมน้ำนมจะเพิ่มขึ้นโดยเฉพาะในระยะกลางและระยะท้ายของการให้นม แต่ความแตกต่างของความเข้มข้นของสารอาหารระหว่างเลือดแดงและเลือดดำ (A-V difference)และสัดส่วนการใช้สารอาหารโดยต่อมน้ำนมในโคนมทั้งสองกลุ่มที่ให้rbSTไม่พบการเปลี่ยนแปลง ส่วนค่าความแตกต่างความเข้มข้นของอะซีเตทและเบต้าไฮดรอกซี่บิวตาเรตระหว่างเลือดแดงและเลือดดำ(A-V difference) และสัดส่วนการใช้และอัตราการใช้ในต่อมน้ำนมไม่พบการเปลี่ยนแปลงในช่วงที่มีการให้rbST ในโคนมทั้งสองกลุ่ม การศึกษาอัตราการหมุนเวียนของกลูโคสภายในร่างกาย ไม่พบการเปลี่ยนแปลงระหว่างโคนมทั้งสองกลุ่มที่ได้รับrbST หรือไม่ อัตราการใช้กลูโคสโดยต่อมน้ำนมถูกนำไปใช้ในวิถีของการสังเคราะห์แลคโตส และในวิถีเพนโตสในการเพิ่ม NADPH เพื่อการสังเคราะห์กรดไขมันในโคนมทั้งสองกลุ่มที่ให้rbST อัตราการใช้คาร์บอนอะตอมของกลูโคสเพิ่มขึ้นในน้ำนมแลคโตสและไขมันนมในโคนมที่เลี้ยงทั้งในที่เย็นและไม่ได้อยู่ในที่เย็นเมื่อได้รับrbSTในระยะต้นและระยะกลางของการให้นม แต่ไม่พบการ

เพิ่มขึ้นในน้ำนมซิเตรสเมื่อเข้าสู่ระยะท้ายๆของการให้นม สัดส่วนของการใช้กลูโคสในเซลล์ต่อมน้ำนมจะถูกเมแทบอไลซ์น้อยลงในกระบวนการสังเคราะห์แลคโตส แต่จะถูกเมแทบอไลซ์เพิ่มขึ้นโดยผ่านวิถี Embden-Meyerhof และวิถี Tricarboxylic acid มากขึ้นเมื่อเข้าสู่ระยะท้ายของการให้นม ไม่ว่าจะให้ rbST หรือไม่

จากผลการศึกษาดังกล่าว สามารถสรุปได้ว่าการเพิ่มผลผลิตน้ำนมจากการให้ rbST แก่โคนมที่เลี้ยงภายในโรงเรือนปกติและโรงเรือนเย็นที่ใช้ระบบพ่นด้วยละอองไอน้ำ เป็นผลจากการเพิ่มปริมาตรของเหลวภายในร่างกายในเบื้องต้นและจะเป็นผลตามมาในการเพิ่มอัตราการไหลของเลือดไปสู่ต่อมน้ำนมเพื่อนำสารอาหารไปในกระบวนการสังเคราะห์น้ำนม การเปลี่ยนแปลงความสามารถเฉพาะที่ภายในเซลล์ต่อมน้ำนมที่ลดลงจะเป็นปัจจัยหนึ่งที่บ่งชี้ถึงการลดการใช้สารอาหารในการสังเคราะห์น้ำนมลดลงเมื่อเข้าสู่ระยะท้ายๆของการให้นมทั้งในกลุ่มโคนมที่เลี้ยงในที่เย็นและไม่ได้เลี้ยงในที่เย็น ไม่ว่าจะให้ rbST หรือไม่

Chapter I

General Introduction and aim

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 rapid 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. The mechanism acting within the body to limit the rate of milk yield and shorter lactation persistency as lactation advances in crossbred dairy cattle are unknown. The problems of dairying in the tropics are multifaceted including nutrition, the hot climate, genetics, disease and management. The genetic 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 many tropical diseases and a high level of heat tolerance (Nakamura et al.1993). Crossbreeding has been exploited as an efficient tool for blending the adaptability of tropical cattle with the high milking potentials of exotic breeds for increase milk production. Several approaches have been attempted to try to improve dairy productivity. There is still a need to identify the type of crossbred cattle that are the most suitable for the tropics.

Our previous studies have been done to compare bodily function and mammary function between two different types of crossbred Holstein cattle (50% HF and 87.5%HF), feeding on two different types of roughage as diet (urea treated rice straw vs Pangola hay), during pregnancy and different stages of lactation. It has been revealed that either 50%HF or 87.5%HF animals showed no differences in physiological and biochemical responses both extra-mammary and intra-mammary parameters during feeding different forages between urea treated rice straw and Pangola hay. (Chaiyabutr**,** et al.,1997; 1998; 2000a; 2000b;2000c). However, 87.5%HF animals feeding either urea treated rice straw or Pangola hay showed differences in the distribution of their body fluids and mammary circulation from those of 50%HF animals during late pregnancy and different stages of lactation. The 87.5%HF animals showed lower efficiency in water retention mechanism and poor adaptation to tropical environment, in comparison to 50%HF animals (Chaiyabutr et al., 1997; 2000a). The decrease in blood flow to the mammary gland with a short persistent milk yield during the transition period from early to mid-lactation has been noted in the 87.5%HF animals (Chaiyabutr et al., 2000a). The control mechanism for mammary blood flow in different stages of lactation in crossbred dairy cattle has not been fully elucidated, although mammary blood flow (MBF) has been known to be a major determinant for the rate of substrates supply

for milk synthesis (Davis and Collier, 1985). 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 is known as a homeorrhetic hormone connected to both growth and lactation. BosTaurus animals normally have higher plasma bovine somatotropin (bST) during lactation. The importance of bST for maintaining milk output in ruminant is well established (see review Bauman, 1992). It has been reported by our studies that the concentration of plasma bovine somatotropin of 87.5% crossbred Holstein cattle decreased rapidly as lactation progressed to mid- and late lactation which coincided with the decrease in mammary blood flow. These decreases could contribute to a reduction in milk yield (Chaiyabutr et al. 2000a). 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). However, the high level of circulating bST involving in regulating mammary blood flow and milk yield might not be a major factor controlling milk production. Further study has been designed to clarify whether poorer lactation persistency in crossbred cattle containing 0.875Holstein genes was affected by a reduction in circulating growth hormone in association with changes of body fluid and mammary circulation. Long-term administration of exogenous recombinant bovine somatotropin in 87.5% HF animals, showed a marked increase in udder blood flow throughout lactation, but a short persistency of lactation in rbST treated animals was still similar to the control animals receiving placebo. The lack of effect of higher plasma IGF-I levels on persistency of lactation in rbST treated animals was also noted (Chaiyabutr et al., 2005). Increases in total body water, extracellular water and blood volume have been shown to coincide with an increase in mammary blood flow for milk production during rbST administration in all stages of lactation (Chaiyabutr et al., 2007a). During rbST administration in crossbred HF animals, no negative energy balance have been apparent during high peak yield in the early lactation, which differ from those of high yielding cows in temperate countries. 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 (Chaiyabutr 2005). Therefore, the interaction of genetics and environment (including nutrition) in the determination the response of lactating dairy cows to bST administration is not yet clear

One of the factors which limits milk production of tropical dairy cattle is high ambient temperature conditions (temperature and humidity). Prolonged exposure to high ambient temperature will develop heat stress in animal. Heat stress is a multifaceted adaptive response that occurs when an animal's capacity for heat dissipation is exceeded by the heat load acquired through excessive exposure to high environmental temperature and metabolic heat. The adaptive responses have important physiological consequences in terms of greatly increased body temperatures and impaired physiological functions including mammary function (Hahn and Becker, 1984).

A number of studies in other large ruminant e.g. buffalo, have been shown that an increase in intravascular volume compartment especially plasma volume was apparent during acute heat exposure (Chaiyabutr et al., 1987). An increase in plasma volume may come from the digestive tract, which increase in the rate of liquid passage from the rumen during acute heat exposure. This may be a way to transfer the water to the lower digestive tract when it is absorbed (Chaiyabutr et al., 1987). This positive water balance during acute heat exposure may relate to salt retention which would lead to a secondary water retention and thus to the expansion of the extracellular fluid pool (Macfarlane and Howard, 1970). From these results including our previous studies in crossbred dairy cattle, it was unclear from whether the appearance of a short persistency of lactation during longed-term rbST administration, was caused by disproportion between the utilization of body water retention for both heat dissipation mechanism and milk synthesis during exposure to high ambient temperature, or by the less stimulant effect of plasma somatotropin in late lactation or combination of both of these factors. 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).

Several approaches have been done in attempting to improve dairy productivity during heat exposure by management strategies. Management can minimize the adverse effects of heat stress in cattle. Environmental modification is one of management, which can alleviate severe heat stress in dairy cattle e.g. water splay with fans or evaporative cooling system. However, different types of environmental modifications and selecting the types of suitable crossbred cattle for the tropics are still further investigated. However, there is less information concerning the profitability of efficient utilization of the environmental modifications for dairy production in crossbred cattle, although the performance of crossbred animal has been found to differ from the pure breeds both in body composition and water turnover rate (Macfarlane 1968). Water turnover values in ruminants have been shown to be related to the

food and water intake and metabolism of the animal (Murphy 1992) including exposure to high ambient temperature (Chaiyabutr et al 1987).

During lactation, coordination between nutrient delivery and biosynthetic capacity are thought to be under endocrine control with homeorhetic mechanism. Previous study in 87.5%HF cows has been shown that the levels of plasma bovine somatotropin rose in early period of lactation and markedly reduced in mid and late lactation (Chaiyabutr et al., 2000b), which coincided with an appearance of a shorter lactation persistency during transition period from mid to late-lactation However, the mechanism of action for growth hormone on milk production in crossbred dairy cattle remains unclear. Studies on various physiological responses in both intra-mammmary and extra-mammary functions during long-term bovine somatotropin administration have been reported in crossbred Holstein cattle containing 87.5% Holstein genes in relation to the mechanism response for the control of milk secretion at different stages of lactation (Chaiyabutr et al.,2007a; 2007b; 2007c; 2008a; 2008b). However, few data are available for combined effects of reduction of environmental temperature and rbST supplementation on milk secretion in responsible for the short persistency of milk yield as lactation advance in crossbred Holstein cattle.

To provide this information, the present experiments were carried out to study thermoregulation and control mechanisms in crossbred dairy cattle containing 87.5%Holstein genes in maintaining milk yield during exposure to high environmental temperature and supplemental rbST. In appropriate management for reduction of heat stress may prove economically beneficial by increasing efficiency of milk production. An understand mechanisms in managing heat stress may help farmer mammary biologist and veterinarian attract new dairy business in the tropics.

Thus, the objective of the present study was to study effects of cooling and rbST supplementation on various physiological changes (extra-mammary function) and on intra-mammary functions during different stages of lactation in dairy crossbred Holstein Friesians.

To achieve the research objectives, the following experimental studies in both extra-mammary functions and intra-mammary functions were performed;

1. Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on water metabolism and mammary circulation in different stages of lactation in crossbred Holstein cattle.
2. Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on the plasma level of insulin like growth factor-1, insulin and plasma metabolites in different stages of lactation in crossbred Holstein cattle.

3. Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on the utilization of glucose in the mammary gland in different stages of lactation in crossbred Holstein cattle.
4. Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on cellular metabolites in milk secretion at different stages of lactation in crossbred Holstein cattle.
5. Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on plasminogen and plasmin system in the mammary gland in different stages of lactation in crossbred Holstein cattle.
6. Indicators of oxidative status in plasma of shaded cooled animals with recombinant bovine somatotropin (rbST) administration in different stages of lactation in crossbred Holstein cattle.
7. Changes in renal function and mammary circulation of shaded cooled animals and recombinant bovine somatotropin (rbST) administration in different stages of lactation in crossbred Holstein cattle.
8. Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) on milk yield, hematology and blood chemistry in different stages of lactation in crossbred Holstein cattle.
9. Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on changes in the rate of liquid flow from the rumen and milk production in different stages of lactation in crossbred Holstein cattle.

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Chapter II

Materials and Methods

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

Animals, housing and managements

Primiparous crossbred cattle, containing 87.5 % Holstein (HF) genes, average body weight 358±32.5 kg, non pregnant and averaged 60 day postpartum at start of trial were used for the experiment. The animals were assigned randomly into two groups of five animals each. Animals in the first group were housed in open-sided barn with a tiled roof in normal shaded house (NS) as the non-cooled animals. Animals in the second group were housed in open-sided barn with a tiled roof (8 m long x 7 m wide x 3.5 m high) under misters and fans (MFC) as cooled animals. The open space cooling system consisted of two sets of misters and fans, which each system consisted of a 65 cm. diameter blade fan circulating 81 m^3/min of air, with oscillation coverage of 180º. The amount of water discharged from 4 splay heads was 7.5 L/h and size of mist droplet 0.01 mm. Animals were exposed to MFC for 45 minutes at 15-minute intervals from 06:00 h to 18:00 h. At night, animals were exposed to MFC for 15 minutes at 45-minute intervals from 18:00 h to 06:00 h. The diet was fed twice a day for *ad lib* as the same ration of total mixed ration (TMR) throughout the experiments in both groups. The ambient temperature was recorded using a dry bulb thermometer. The relative humidity was read depending on wet and dry bulb temperature at cooled and non-cooled barns. A temperature-humidity index (THI) was calculated from the average ambient temperature of dry and wet bulb temperatures. A temperature-humidity index (THI) was calculated from the average ambient temperature of dry and wet bulb temperatures according to McDowell (1972), as follow:

$$THI = 0.72 (wb+db) + 40.6$$

Where; wb = wet bulb temperature and db = dry bulb temperature expressed in °C

All cows were fed the same total mixed rations (TMR) twice daily and water was offered *ad libitum* (Table 1).

Table 2.1. Feed ingredients and chemical compositions of the diet

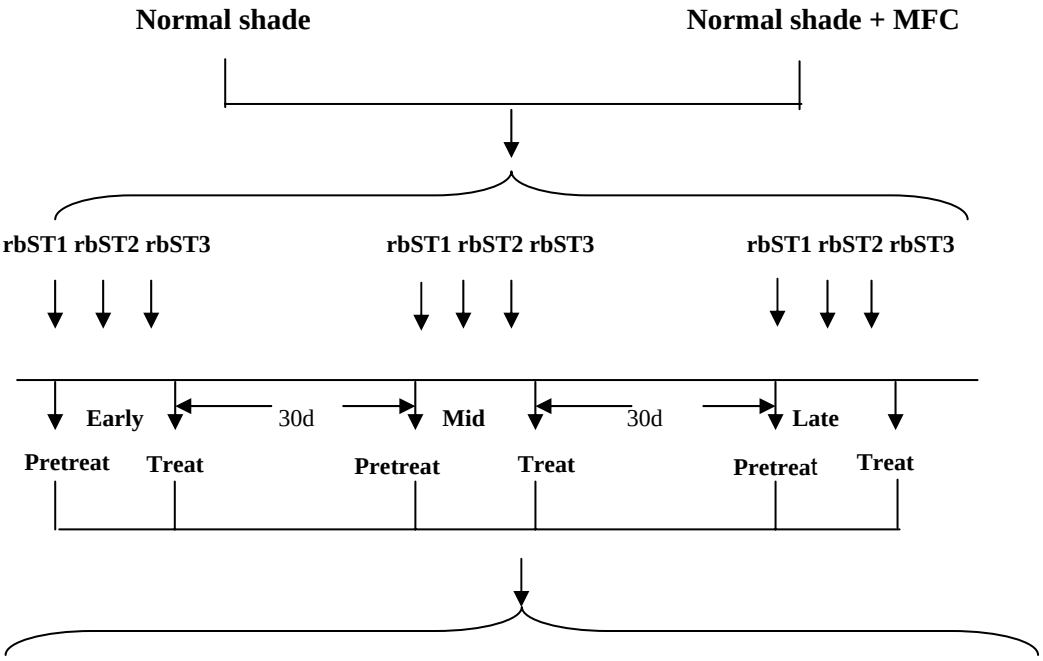
Ingredients	Kg (as fed basis)
Pine apple waste	50
Soybean meal	23
Rice bran	3.0
Cotton seed	20
Lime stone	1.4
Di-calcium phosphate	1.4
Sodium bicarbonate	0.3
Potassium chloride	0.1
Mineral and vitamin premix	0.8
Total	100
Chemical composition	
Dry matter (%)	39.1
Ash (% DM)	7.3
Organic matter (% DM)	92.7
Crude protein (% DM)	18.0
Acid detergent fiber (% DM)	20.1
Neutral detergent fiber (% DM)	33.9
Total digestible nutrients (% DM)	70.0
Metabolizable energy(Mcal/kg DM)	2.7

Experimental design

An overview of certain parts of the experimental designs in the experimental studies in Chapter III-XI is presented in Fig. 2.1. The procedures used in the present study were carried out in accordance with the principles and guidelines of the Faculty of Veterinary Science, Chulalongkorn University. These guidelines were formulated to comply with international standards and are in accordance with the principles and guidelines of the National Research Council of Thailand.

The experiment was divided into 3 phases, namely early- (approximately 2 months postpartum), mid- (approximately 4 months), and late lactation (approximately 6 months) periods. The pretreatment was conducted on the starting day of each phase. At the end of the pretreatment, within the same day, the subject was injected with the first dose/injection/cow of 500 mg of recombinant bovine somatotropin (rbST) (POSILAC, Monsanto, USA). Subsequently, the subject was injected with two consecutive doses/injections of with 500 mg of recombinant bovine somatotropin (rbST) in every two weeks. Thereafter, within 2-5 days after the third injection, the treatment was conducted. The pretreatment, 3 doses of injections, and the treatment were performed during the first 30 days and the same procedures were followed for each phase. During the last 30 days of each phase, no experiments were conducted in order to allow the milk yield to return to control level. Thus, comparative studies of both groups in each stage of lactation, the four treatment combinations were normal shade without rbST injection (NS), normal shade plus rbST injection (NS + rbST), shade plus misty-fan cooling without injection (MFC), and shade plus misty-fan cooling with rbST injection (MFC + rbST).

Effects of cooling and recombinant bovine somatotropin (rbST) supplementation on mammary functions were carried out, where appropriate experiments were divided into different Chapters and Series of studies to cover both intra- and extra-mammary functions.



Extra-mammary functions

- Body glucose and water metabolism
- Plasma hormones level
- Oxidative status in plasma
- Renal function and mammary circulation
- Hematology and blood chemistry
- Digestibility, digestion kinetics

Intra-mammary functions

- Mammary glucose utilization
- Cellular metabolites in milk secretion
- Plasminogen and plasmin system

Figure 2.1. Schematic diagrams illustrating the time course of the experiment in each cow supplemented with rbST at different stages of lactation. Pre-treat = timed study for pre-treatment; Treat = timed study for treatment

Statistical analysis

The statistic analyses were performed using General Linear Model procedures of statistical software package SPSS (SPSS for windows, V14.0; SPSS Inc., Chicago, IL, USA). The model used for each analysis was:

$$Y_{ijk} = \mu + A_l + H_i + A(H)_{il} + B_j + (HB)_{ij} + A(HB)_{ijl} + Cov_k + e_{ijkl}$$

Where Y_{ijk} = observation, μ = overall mean, A_l= Animal effect H_i= house effect as main plot (*i* = NS, MF), $A(H)_{il}$ = main plot error (animal *l* in house *i*), B_j = treatment effect (rbST) as a split plot (*j* = with and without rbST supplementation), $(HB)_{ij}$ = interaction effect between treatment and house , $A(HB)_{ijl}$ = split plot error (animal *l* in house *i* and treatment *j*), Cov_k = covariate effect and e_{ijk} = residual error.

A remain physiological parameters and environmental parameters were also analyzed by the similar model, but the covariate effect was excluded. Means values were used to evaluate the effect for all variables. Statistical significance was declared at $P<0.05$. and trends were declared at $0.05 < P \leq 0.10$. Duncan's new multiple range tests were used to detect the statistical significance between different treatment groups. In some cases a further comparison of consistent changes was made using either paired or unpaired t'test test.

Chapter III

Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on water metabolism and mammary circulation in different stages of lactation in crossbred Holstein cattle

Chapter III

Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on water metabolism and mammary circulation in different stages of lactation in crossbred Holstein cattle

INTRODUCTION

The low milk production of both exotic and crossbred cattle is still the main problem in dairy farming in the tropics. The regulation of milk secretion in crossbred cattle have shown to be inherited and being thought to be among the causes of differences in metabolic parameters (Chaiyabutr et al. 2000). It is known that an increase in milk production in dairy cow depends on the hormones especially bovine somatotropin (bST) mediating the interaction between genetic potential and nutrition for lactation performance (Bauman, 1992; Fike et al. 2002; West 2003; Settivari et al. 2007). The study in 87.5% crossbred Holstein cattle have been shown that the concentration of plasma bST decreased as lactation progressed to mid and late lactation. These reductions would concomitant to a reduction in both mammary blood flow and milk yield (Chaiyabutr et al. 2000). High environmental temperature would be another factor, which affect milk production in dairy cows in the tropic (Smith et al. 2006; Bohmanova et al. 2007). The high ambient heat load has been shown to reduce the response to bST treatment and low milk yields (Kronfeld 1988; Molett et al. 1986), while other studies showed no changes in milk yields (Cole and Hansen 1993).

In dairy cattle, body water is known to use for make up the largest portion of milk, for evaporative cooling during heat dissipation mechanism and for the vehicle in blood distribution to mammary glands. A few data are available for the interaction effects of combination of high ambient temperature and bST administration on body water regulation in crossbred dairy cows, although a number of studies 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). Somatotropin (ST) is well known to be responsible for galactopoiesis in ruminants, not prolactin or

adrenocorticotropin, while somatotropin administration has been shown to elevate total body water (TBW) and extracellular water (ECW) in both humans and crossbred dairy cattle (Janssen et al., 1997; Chaiyabutr et al. 2007). The stimulant effect for milk yield was less in late lactation even though a high level of body fluids and MBF during long-term administration of rbST (Chaiyabutr et al. 2007). These data substantiate the role of GH as a regulator of fluid homeostasis. Alleviation of heat stress during bST administration in heat-exposed animals would be in part through increases in body fluids, which is a matter of debate. Although, attempts have been made to minimize the impact of thermal stress on milk production in dairy cattle with different types of environmental modifications, such as water spray and fans, evaporative cooling system (Armstrong et al. 1985 ; Armstrong et al. 1993; Ryan et al. 1992 Chaiyabutr et al. 2008). Short persistency of lactation is occurred in 87.5% HF animals, whether by the effect of high ambient temperature or by the less stimulant effect of bovine somatotropin or combination of both of these factors during lactation advances. The effect of rbST administration on an increase in total body water of crossbred Holstein cattle has been noted (Chaiyabutr et al., 2007), which may involve in heat dissipation mechanism. A greater water reserve would not only provide a greater reservoir of soluble metabolites for biosynthesis for milk, but it is useful in slowing down the elevation in body temperature during heat stress. Few data are available for the study whether restoring body fluids during rbST administration in crossbred Holstein cattle could allow milk production at different stages of lactation to be maintained for a longer time during exposure to high environmental temperature. Therefore, the objectives of the present experiment were to 1) to evaluate the effects of providing cross-bred cattle with housing under shade with or without misters and fans and 2) demonstrating comparable galactopoietic activity of rbST supplementation in crossbred cattle housing under shade with or without misters and fans during three periods of the lactation (early, mid and late lactation). Measures to evaluate the effectiveness of these treatments were milk production and composition, body water regulation. This might lead to better understanding the adaptability in crossbred dairy cattle in body water regulation under a tropical condition.

MATERIAL AND METHODS

Animals, housing and managements

Ten primiparous crossbred cattle, containing 87.5 % Holstein (HF) genes, average body weight 358±32.5 kg, non pregnant and averaged 60 day postpartum at start of trial were used for the experiment. The animals were assigned randomly into two groups of five animals each. Animals in the first group were housed in open-sided barn with a tiled roof in normal shaded house (NS) as the non-cooled animals. Animals in the second group were housed in open-sided barn with a tiled roof (8 m long x 7 m wide x 3.5 m high) under misters and fans (MF) as cooled animals. The open space cooling system consisted of two sets of misters and fans, which each system consisted of a 65 cm. diameter blade fan circulating 81 m^3/min of air, with oscillation coverage of 180º. The amount of water discharged from 4 splay heads was 7.5 L/h and size of mist droplet 0.01 mm. Animals were exposed to MF for 45 minutes at 15-minute intervals from 06:00 h to 18:00 h. At night, animals were exposed to MF for 15 minutes at 45-minute intervals from 18:00 h to 06:00 h.

The diet was fed twice a day for *ad lib* as the same ration of total mixed ration (TMR) throughout the experiments in both groups. Ingredient and chemical compositions of feed are shown in Table 1. Animals are milked twice a day and milk yields are recorded at each milking. Each day, the food was given in equal portions at about 06:00 h and 17:00 h when the animals were milked using a milking machine and milk production was recorded daily. The dry matter intake of each animal was measured by weighing the TMR offered and refused each day. Samples of both feeds were collected and kept at -20 C for dry matter determination and chemical analysis. Samples of TMR were analyzed for dry matter, crude protein and ash using procedures described by AOAC (1990). ADF and NDF were analyzed according to Van Soest and Robertson (1991). The measurement of daily water consumption of each animal was measured by water meter of each animal. The daily water intake per animal in each period of lactation was recorded by averaging over seven days.

The ambient temperature was recorded using a dry bulb thermometer. The relative humidity was read depending on wet and dry bulb temperature at cooled and non-cooled barns. Ambient temperatures, humidity, rectal temperature and respiratory rate were measured weekly at, 14:00 h on specified day. Average values were considered to be the mean of all measurements taken for each date. A temperature-humidity index (THI)

was calculated from the average ambient temperature of dry and wet bulb temperatures according to McDowell (1972), as follow:

THI = 0.72 (wb+db) + 40.6

Where ; wb = wet bulb temperature and db = dry bulb temperature expressed in °C

Rectal temperature measurements were made with electronic thermometers. Respiratory rates were obtained by observing flank movements. All animals were weighed monthly throughout the experimental periods. Body weight was determined of each stage of lactation at the end of the pretreatment period and at the end of the treatment period.

Experimental design

The experiment was divided into 3 phases, namely early- (approximately 2 months postpartum or Day 60), mid- (approximately 4 months postpartum or Day 120), and late lactation (approximately 6 months postpartum or Day 180) periods. The pretreatment was conducted on the starting day of each phase. At the end of the pretreatment, within the same day, the subject was injected with the first dose/injection of with 500 mg of recombinant bovine somatotropin (rbST) (POSILAC, Monsanto, USA). Subsequently, the subject was injected with two consecutive doses/injections of with 500 mg of recombinant bovine somatotropin (rbST) every two weeks. Thereafter, within 2-5 days after the third injection, the treatment was conducted. The pretreatment, 3 doses of injections, and the treatment were performed during the first 30 days and the same procedures were followed for each phase. During the last 30 days of each phase, no experiments were conducted in order to allow the milk yield to return to control level. Thus, comparative studies of both groups in each stage of lactation, the four treatment combinations were normal shade without rbST injection (NS), normal shade plus rbST injection (NS + rbST), shade plus misty-fan cooling without injection (MFC), and shade plus misty-fan cooling with rbST injection (MFC + rbST)

Determinations of water metabolism and mammary blood flow

The water turnover rate, total body water space and empty body water were determined in each animal by tritiated water dilution techniques as previously described (Chaiyabutr et al., 2005). In brief, on the specified day around 0900h, the animal was injected intravenously via the ear vein with carrier free tritiated water in normal saline at

a single dose of 3,000 μCi per animal. The equilibration time was determined by taking blood samples for 3 days after the injection. Blood samples were collected at 20, 30, 40, 50, 60 min., 4, 8, 20, 26, 32, 44, 50, 56, 68 and 74 h subsequent to the injection. Preparation of samples for counting was achieved by the internal standardization technique as described by Vaughan and Boling (1961). The dilution curve of activity of tritiated water in plasma was described by an exponential equation using the compartment system model (Shipley and Clark, 1972) for determinations of the water turnover rate, total body water space (TOH). and empty body water (EBW). The exponential equation describing the one compartment model was calculated:

$Y_1 = Ae^{-k1t}$, where,Y is concentration of tritium in plasma at time t (nci/ml); A is plasma concentration intercept 1 in nci/ml. The extrapolated activity at theoretical zero time of complete mixing of radio-isotope was used to determine the TOH space. 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 / T1/2.

Empty body water (EBW) does not include water associated with gastrointestinal contents or the water in the fetus (Andrew et al., 1995), which was estimated from the disappearance curve of tritium in blood plasma for each animal. The two-compartment open system model (Shipley and Clark, 1972) was used to estimate the EBW. The exponential equation describing the two compartment model was calculated from the equation:

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

where, Y is the concentration of tritium in plasma at time t (nci/ml). A is plasma concentration intercept 1 of the fast phase of the plasma curve in nci/ml; B is plasma concentration intercept 2 of the in nci/ml; k1 and k2 are first order rate constant of the fast phase and the slow phase of each pool, respectively; and t is time in minutes. The sum of the two intercepts, A and B, equals the concentration of tritiated water in plasma at 0 time, and this concentration was used to estimate EBW in the equation:

EBW(ml) = [standard count (dis/min) x dose (ml)] / [radioactivity counts of A+B (dis/min)].

Blood flow 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).

Determinations of plasma volume, extracellular fluid and intracellular fluid

On the specified day, determinations of plasma volume (PV), extracellular fluid (ECF) and intracellular fluid (ICF) were performed coinciding with the measurement of body fluids. In each animal per measurement, 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) were given via an ear vein catheter to estimate ECF 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 ECF was modified from the method used by Medway and Kare (1959). Intracellular water (ICF) was calculated by subtracting ECF from EBW. Plasma osmolality was measured using the freezing point depression method (Advance Osmometer model 3, U.S.A.).

Statistical analysis

The statistic analyses were performed using General Linear Model procedures of statistical software package SPSS (SPSS for windows, V14.0; SPSS Inc., Chicago, IL, USA). The model used for each analysis was:

$$Y_{ijk} = \mu + A_l + H_i + A(H)_{il} + B_j + (HB)_{ij} + A(HB)_{ijl} + Cov_k + e_{ijkl}$$

Where Y_{ijk} = observation, μ = overall mean, A_l= Animal effect H_i= house effect as main plot (*i* = NS, MFC), $A(H)_{il}$ = main plot error (animal *l* in house *i*), B_j = treatment effect (rbST) as a split plot (*j* = with and without rbST supplementation), $(HB)_{ij}$ = interaction effect between treatment and house , $A(HB)_{ijl}$ = split plot error (animal *l* in house *i* and treatment *j*), Cov_k = covariate effect and e_{ijk} = residual error.

Means values were used to evaluate the effect for all variables. Statistical significance was declared at $P<0.05$. and trends were declared at $0.05 < P \leq 0.10$.

RESULTS

Ambient temperatures , relative humidity, temperature humidity index, rectal temperature and respiratory (Table 2)**.**

Data for ambient temperatures, relative humidity**,** rectal temperature and respiratory rate are shown in Table 3.2. Mean values of measurements at experimental site for ambient temperatures, relative humidity and temperature humidity index were highly significantly different between NS and MFC barn. The ambient temperatures and THI in the NS barn were significantly higher than the MFC barn but the relative humidity of MFC barn was significantly higher than the NS barn at all stages of lactation. The respiration rate and rectal temperature of cows under mister and fans were lower than non-cooled cows supplementatal rbST at all stages of lactation. During supplementation of rbST, respiration rate and rectal temperature were significantly higher than those of pretreatment periods in both cooled and non-cooled cows in all stages of lactation.

Changes in DM intake, water intake, mammary blood flow, milk yield and body weight (Table 3).

During supplemental rbST, the DM intake was higher than the pretreatment in both cooled and non-cooled cows throughout the lactation. The mean values of daily water intake in both cooled and non-cooled cows showed no significant increases during supplemental rbST when compared with the pre-treatment period. Milk yield of both cooled and non-cooled cows supplemental rbST were significantly higher than those of the pretreatment on each lactation. However, peak yields occurred during early lactation; thereafter yields continued to decline in both groups as lactation advances. Mammary blood flow (MBF) significantly increased during rbST supplementation in each stage of lactation in both cooled and non-cooled cows. No significant changes in MBF:milk yield ratio were apparent, which showed correlation of high blood flow and milk during rbST supplementation. Both cooled and non-cooled cows gained weight which were significantly above their initial weights for rbST-supplementation on early and mid lactation.

Changes in the water turnover rate (WTO), total body water space (TOH), empty boy water (EBW) (Table 4**).**

The absolute value of WTO and the value of WTO per fat free, wet, body weight ($kg^{0.82}$)(MacFarlane and Howard,1972) in cows supplemental rbST in each lactation tended to increase, while the biological half-life of tritiated water showed no significant differences in both cooled and non-cooled cows. The absolute values of both TOH and EBW of the both cooled and non-coooled cows showed significant increases during supplemental rbST throughout lactation, while the relative values of both TOH and EBW, as the percentage of body weight, showed no significant changes during supplemental rbST as compared with the pretreatment values

Changes in plasma volume, blood volume, extracellular water and intracellular water (Table 5).

Both cooled and non-cooled cows supplemental rbST showed marked increases in ECF, ICF, BV and PV in terms of absolute values in each stage of lactation, while there were no apparent for the relative values as a percentage of body weight in early and mid lactation. The packed cell volume and plasma osmolality of both cooled and non-cooled cows supplemental rbST were unchanged throughout lactation.

Table 1. Feed ingredients and chemical compositions of the diet

Ingredients:	Kg (as fed basis)
Pine apple waste	50
Soybean meal	23
Rice bran	3.0
Cotton seed	20
Lime stone	1.4
Di-calcium phosphate	1.4
Sodium bicarbonate	0.3
Potassium chloride	0.1
Mineral and vitamin premix	0.8
Total	100
Chemical composition:	
Dry matter (%)	39.1
Ash (% DM)	7.3
Organic matter (% DM)	92.7
Crude protein (% DM)	18.0
Acid detergent fiber (% DM)	20.1
Neutral detergent fiber (% DM)	33.9

Table 2. Ambient temperature, relative humidity, temperature humidity index, rectal temperature and respiration rate measurement at 1400h in cows treated with rbST housing under normal shade (NS) and cooling with misters and fans (MFC) at different stages of lactation

	Stages of	NS		MFC			[1]Effect		
Parameters	lactation	Pre	rbST	Pre	rbST	SEM	MFC	rbST	MFC*rbST
Ambient temperature (°C)	Early	33.9	35.1	31.6	31.8	0.74	0.001	0.386	0.473
	Mid	35.3	35.0	30.0	29.8	0.48	0.002	0.613	0.919
	Late	33.5	34.1	29.9	29.1	0.35	0.001	0.865	0.098
Relative humidity (%)	Early	49.5	52.8	66.0	68.8	3.28	0.001	0.396	0.942
	Mid	52.8	50.4	78.2	74.0	3.10	0.001	0.318	0.779
	Late	59.0	63.5	78.5	79.8	2.07	0.001	0.214	0.462
Temperature humidity index (THI)	Early	83.2	85.2	82.4	82.8	0.89	0.003	0.205	0.396
	Mid	85.5	84.8	81.5	80.8	0.39	0.019	0.116	0.928
	Late	83.9	85.2	81.4	80.7	0.29	0.004	0.316	0.011
Rectal temperature (°C)	Early	39.4	40.0	39.0	39.4	0.21	0.037	0.061	0.817
	Mid	39.7	40.1	38.6	39.5	0.13	0.002	0.002	0.090
	Late	39.2	39.9	38.4	38.8	0.16	0.015	0.016	0.309
Respiration rate (breath/min)	Early	73.0	82.3	55.5	68.0	4.13	0.023	0.039	0.708
	Mid	73.6	77.2	49.0	57.6	1.96	0.001	0.018	0.294
	Late	71.5	80.0	54.3	59.3	1.09	0.019	0.001	0.159

SEM = Standard error of the mean.

[1] P-values for the effects; MFC =Misty-fan cooling effect, rbST = rbST effect, MFC x rbST = interaction effect of MFC and rbST

Table 3. DMI, water intake, milk yield, mammary blood flow and body weight of crossbred Holstein cattle treated with rbST and housing under normal shade (NS) and shade plus misters and fans (MFC).

	Stages of	NS		MFC			[1]Effect		
Parameters	lactation	Pre	rbST	Pre	rbST	SEM	MFC	rbST	MFC *rbST
Milk yield	Early	10.65	11.95	12.23	12.79	0.378	0.477	0.039	0.390
(kg/day)	Mid	9.25	10.14	11.86	13.44	0.308	0.185	0.004	0.298
	Late	8.04	9.81	9.51	12.31	0.625	0.355	0.006	0.437
DMI	Early	6.14	7.05	7.22	8.49	0.312	0.049	0.088	0.575
(kg/day)	Mid	6.18	7.49	8.72	10.00	0.450	0.013	0.020	0.973
	Late	7.57	7.87	8.26	9.32	0.151	0.362	0.002	0.037
Water intake	Early	66.56	71.68	73.70	72.50	1.307	0.178	0.172	0.042
(kg/day)	Mid	68.68	69.96	73.10	75.60	1.198	0.138	0.153	0.624
	Late	69.20	67.94	70.40	74.5	1.153	0.251	0.247	0.047
MBF	Early	4969	5222	5241	6555	265.1	0.524	0.018	0.081
(ml /min)	Mid	4141	5053	4132	5434	388.1	0.821	0.021	0.629
	Late	3750	5096	4435	4968	248.6	0.735	0.005	0.141
MBF/milk yield	Early	485.92	454.64	434.18	511.42	23.27	0.685	0.723	0.172
(L/kg)	Mid	456.61	515.27	368.03	434.62	46.78	0.612	0.587	0.523
	Late	482.60	571.71	536.29	434.52	58.93	0.734	0.917	0.144
Body	Early	358.8	380.8	360.2	373.8	6.48	0.893	0.025	0.535
weight	Mid	382.4	383.2	381.8	411.4	4.17	0.586	0.007	0.009
(kg)	Late	398.2	393.0	425.0	423.0	4.89	0.268	0.483	0.752

SEM = Standard error of the mean.

[1] P-values for the effects; MFC =Misty-fan cooling effect, rbST = rbST effect, MFC x rbST = interaction effect of MFC and rbST

Table 4. Changes in the water turnover rate (WTO), total body water space (TOH) empty body water (EBW) and the biological half-life of tritiated water of crossbred Holstein cattle treated with rbST and housing under normal shade (NS) and misters and fans cooling (MFC) at different stages of lactation.

	Stages of	NS		MFC			[1]Effect		
Parameters	lactation	Pre	rbST	Pre	rbST	SEM	MFC	rbST	MFC *rbST
WTO (L/d)	Early	68.2	82.6	69.8	78.8	4.58	0.934	0.034	0.567
	Mid	59.4	72.4	71.8	78.5	6.28	0.398	0.156	0.625
	Late	77.9	94.8	53.6	73.1	9.13	0.048	0.082	0.885
WTO ($ml/kg^{0.82}/d$)	Early	554.7	638.1	561.5	613.8	31.98	0.937	0.067	0.639
	Mid	455.6	554.2	547.6	566.2	45.80	0.508	0.235	0.410
	Late	571.1	712.2	383.8	517.7	68.19	0.036	0.078	0.959
Biological half-Life (d)	Early	2.81	2.18	2.40	2.87	0.36	0.755	0.828	0.166
	Mid	2.74	3.19	2.42	2.77	0.38	0.382	0.316	0.894
	Late	2.49	2.32	3.70	3.18	0.31	0.062	0.287	0.581
TOH	Early	254.4	295.9	277.7	309.2	6.23	0.273	0.001	0.441
(L)	Mid	262.0	303.1	272.0	326.9	6.97	0.336	0.001	0.352
	Late	269.1	320.4	286.9	327.4	10.65	0.467	0.003	0.624
TOH	Early	71.6	78.1	77.0	82.8	1.75	0.259	0.008	0.853
(L/100kg)	Mid	68.6	79.4	71.2	79.9	2.19	0.624	0.002	0.657
	Late	67.3	81.7	67.8	77.8	2.66	0.563	0.002	0.429
EBW	Early	195.1	239.6	198.6	225.8	14.03	0.687	0.034	0.555
(L)	Mid	193.9	216.9	193.7	242.2	15.05	0.472	0.045	0.420
	Late	217.7	242.5	184.5	230.4	13.05	0.264	0.027	0.441
EBW	Early	54.8	61.9	55.2	60.6	3.97	0.910	0.155	0.852
(L/100 kg)	Mid	51.0	56.3	50.7	59.8	4.02	0.740	0.651	0.112
	Late	54.4	61.8	44.9	54.5	2.96	0.076	0.020	0.725

SEM = Standard error of the mean.

[1] P-values for the effects; MFC =Misty-fan cooling effect, rbST = rbST effect, MFC x rbST = interaction effect of MFC and rbST

Table 5. Extracellular fluid (ECF), plasma volume (PV), blood volume (BV) and packed cell volume (Hct) of crossbred Holstein cattle treated with rbST and housing under normal shade (NS) and misters and fans cooling (MFC) at different stages of lactation..

	Stages of	NS		MFC			[1]Effect		
Parameters	lactation	Pre	rbST	Pre	rbST	SEM	MFC	rbST	MFC *rbST
ECF (L)	Early	89.1	104.2	105.2	120.0	3.36	0.041	0.002	0.971
	Mid	99.9	111.3	116.0	123.3	4.54	0.081	0.073	0.666
	Late	99.7	111.2	115.5	128.7	3.68	0.086	0.010	0.826
ECF (L/100kg)	Early	25.2	27.6	28.3	32.1	0.97	0.114	0.014	0.489
	Mid	26.2	29.3	30.4	30.0	1.12	0.206	0.267	0.162
	Late	25.1	28.4	27.4	30.7	0.86	0.336	0.005	0.948
ICF (L)	Early	135.6	161.5	130.1	143.1	10.48	0.416	0.102	0.554
	Mid	128.1	148.7	113.3	156.5	10.18	0.820	0.014	0.299
	Late	143.7	170.3	113.8	142.2	8.31	0.069	0.011	0.917
ICF (L/100kg)	Early	38.0	42.4	36.0	38.4	2.78	0.411	0.261	0.718
	Mid	33.6	38.4	29.5	38.5	2.84	0.568	0.040	0.488
	Late	35.7	43.4	27.2	33.7	3.02	0.386	0.011	0.447
PV (L)	Early	18.31	21.04	17.00	19.36	1.07	0.244	0.045	0.865
	Mid	19.12	20.37	20.81	23.21	0.81	0.125	0.055	0.502
	Late	19.76	22.26	22.73	25.35	1.01	0.046	0.035	0.953
PV (L/100kg)	Early	5.15	5.53	4.74	5.20	0.29	0.339	0.183	0.894
	Mid	5.01	5.33	5.42	5.68	0.23	0.081	0.241	0.912
	Late	4.98	5.65	5.38	6.05	0.22	0.318	0.017	0.994
BV (L)	Early	24.72	27.03	22.61	25.19	1.46	0.304	0.132	0.931
	Mid	25.14	26.57	27.24	30.50	0.97	0.077	0.041	0.371
	Late	26.30	29.53	29.38	33.54	1.36	0.053	0.026	0.743
BV (L/100kg)	Early	6.92	7.04	6.09	6.78	0.40	0.275	0.337	0.494
	Mid	6.57	6.95	7.10	7.48	0.29	0.033	0.225	0.999
	Late	6.53	7.44	6.93	7.98	0.29	0.158	0.010	0.817
PCV (%)	Early	25.6	23.1	24.3	24.0	0.86	0.879	0.087	0.105
	Mid	23.9	23.3	23.9	24.2	0.54	0.728	0.774	0.419
	Late	24.8	24.4	23.9	23.9	0.32	0.754	0.488	0.565
Plasma osmolarity	Early	276.3	273.5	277.6	278.1	2.33	0.353	0.765	0.645
(mOSM/kg)	Mid	275.9	277.6	280.7	278.9	2.67	0.275	0.754	0.543
	Late	276.7	278.8	279.3	281.2	2.41	0.267	0.489	0.432

SEM = Standard error of the mean.

[1] P-values for the effects; MFC =Misty-fan cooling effect, rbST = rbST effect, MFC x rbST = interaction effect of MFC and rbST

DISCUSSION

The environmental temperatures measured in NS and MFC barn in the present study showed differences in ambient temperature and THI, especially in the afternoon throughout the experimental periods. However, MFC barn was not sufficient to completely eliminate heat stress in cows, because the range for THI measured at daytime under misters and fans throughout the experimental periods remained higher than the threshold level of comfortable zone, 72 for THI (Armstrong, 1994). The THI in both barns ranged from 80.7-85.5. Cows in both groups would be subjected to moderate heat stress (Fuquay, 1981). However, THI might not accurately reflect of heat stress in crossbred lactating cows under MFC cooling system that deliver a pressurized spray with considerable fan air movement in the barn, resulting in higher humidity but also causing a cooling effect. The cooling of cows under MFC was significantly lower in both respiratory rate and rectal temperature in comparison with those of non-cooled cows which indicate a partial alleviation of heat stress by MF system especially in the afternoon. The respiratory rate and rectal temperature were increased during rbST supplementation in both cooled and non-cooled cows. These results agree with previous reports (Sullivan et al., 1992; Tarazon et al., 1999) in cows treated with rbST. Although rbST-treated cows increases heat production associated with high milk yield, it also increases heat dissipation (Johnson et al., 1991; West, 1994). However, cows in both cooled and non-cooled cows gained weight throughout the experimental periods. A marked increase in milk yield with rbST supplementation without loss of body weight, especially during early lactation, may be due to the fact that cows were offered TMR diet to allow an adequate replacement of body reserves during lactations. Milk yield in the first lactating crossbred cows 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 gain of cows during their first lactation.

In the present results, the marked increases in blood flow to the mammary gland coincided with an increase in milk yield during rbST supplementation in both cooled and non-cooled cows. These results agree to previous studies by Chaiyabutr and co-worker (2005) that long-term administrations of rbST showed a marked increase in mammary blood flow throughout lactation. Factors that might affect to increase MBF during supplemental rbST could include an increasing relative mass of many organs and

tissue including mammary tissue (Moallem *et al.* 2004) and an increase in cardiac output (Soderholm et al., 1988) in bST treated cows. However, 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 mammary gland (Collier et al., 1984). An increase in MBF accompanying with an increase in circulating levels of IGF-I has been shown in either short-term or long-term rbST administration in different stages of lactation in crossbred HF animals (Chaiyabutr et al., 2005; Maksiri et al., 2005;Tanwattana et.al., 2003). In addition, study in vitro suggests that bST does not directly stimulate mammary secretory function (Gertler et al., 1983). The studies in goats and cows have shown that the effect of rbST on mammary circulation is indirect and mediated via IGF-I, although similar increases in milk secretion and mammary blood flow occurred during growth hormone treatment (Davis et al. 1988; Hart et al. 1980). It indicates that rbST plays a role for an increase in MBF requiring IGF-I as a mediator (Forsyth, 1996). However, the lack of effect of higher plasma IGF-I levels on persistency of lactation in rbST treated animals was also noted. (Chaiyabutr et al., 2005).

The supplementation of rbST markedly increased both the absolute values of PV BV, ECF and TOH in both cooled and non-cooled cows when compared with the pre-treatment period in each stage of lactation. An increase in ECF leads to an increase in MBF as secondary responses, thereby the increase in MBF drives nutrients supply per se to the mammary gland and increase in milk production in rbST treated cows. However, during lactation advanced to late lactation in both cooled and non-cooled cows, the decline in milk yields were still apparent, although MBF, ECF TOH, EBW were still in high levels during supplemental rbST. These results indicate that an increase in milk yield of dairy crossbred cattle in response to rbST administration will not be sustained for long and is influenced by the stage of lactation. These data suggest that changes in milk production during the progress of lactation in rbST treated animals might not be controlled systematically but also locally within the mammary gland (Chaiyabutr et al., 2005).

The high body water content of cows supplemental rbST 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, Tyrrell et al., 1988).

In the present study, the higher values of ECF and ICF were significantly apparent after supplemental rbST in each stage of lactation, These findings suggest that the expansion of body fluid in rbST-treated animals 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 NS and MFC barns in the similar moderate THI environment. Thus, the water turnover rate of both cooled and non-cooled cows in the present study would not be influenced by the effect of pregnancy (Chaiyabutr et al., 1997) or changes in environmental conditions (Ranjhan et al., 1982). However, the water turnover rate was slightly higher coinciding with higher milk yield in both groups of cows supplemental rbST throughout experiment. These findings would relate to the fact that lactation requires more water and more loss of water due to secretion in milk, which is generally known to be about 87% and would account for these phenomena. Water loss with the increase in milk yield of the rbST-treated animals might be compensated by a larger body water pool (TOH or EBW) which restores body fluids to equilibrium with no significant changes of water half-life. No obvious changes of WTO and half-life of tritiated water were apparent during periods of study in both controls and rbST-treated animals. During lactation advanced to late lactation, the marked reductions of relative values of both TOH and TBW of the control animals were apparent. Animals used in the present study being 0.875HF. They had genetic potential close to the exotic *bos taurus* breed which might be responsible to poor adjustment in a tropical environment (Chaiyabutr et al., 2000; Nakamura et al., 1993). An increase in ECF by the effects of rbST in both cooled and non-cooled cows leads to an increase in MBF as secondary responses, thereby facilitating increased milk production. Local changes for biosynthetic capacity within the mammary gland would be a factor in identification of the utilization of substrates in the rate of decline in milk yield with advancing lactation in both cooled and non-cooled cows whether supplemental rbST or not.

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Chapter IV

Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on the plasma level of insulin like growth factor-1, insulin and plasma metabolites in different stages of lactation in crossbred Holstein cattle

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INTRODUCTION

Dairy herds in tropical countries are of mixed exotic breeds and crossbreeds. The potential for milk production is governed by a variety of factors, for example, environmental temperature, stage of lactation and nutrition including functioning of the endocrine gland. Bovine somatotropin(bST) is a peptide hormone which is well known to be responsible for galactopoiesis in ruminants, not prolactin or adrenocorticotropin. It has been reported that the concentration of bST 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. 2000). 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). The bST is known as a homeorrhetic hormone concerned with both growth and lactation, but the mechanism of action of bST in crossbred dairy cattle on milk production is a controversial area. Receptors for growth hormone(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). An infusion of IGF-I into the pudic artery of lactating goats, which 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). It has been reported that GH can stimulate milk production under circumstances in which IGF-I does not (Prosser & Davis, 1992). Chaiyabutr et al. (2000) reported that the galactopoietic

effect of GH was 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 whole 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 (Tanwattana 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 whether it relate to the role of GH or a mechanism other than the circulating level of GH. High environmental temperature would be another factor which affects milk production in dairy cows in the tropic (Smith et al. 2006; Bohmanova et al. 2007). The interaction effects between thermal stress and the role of GH on lactation performance in crossbred lactating cattle is not yet clear. A number of studies in lactating cows showed that the high ambient temperatures would reduce the response to bST treatment and low milk yields (Molett et al. 1986), while other studies showed no changes in milk yields (Cole and Hansen 1993). Attempts have been made to minimize the impact of thermal stress on milk production in dairy cattle with different types of environmental modifications, such as water spray and fans, evaporative cooling system (Armstrong et al. 1993; Ryan et al. 1992 Chaiyabutr et al. 2008). During lactation, mammary gland function is dependent on hormonal stimuli and the provision of nutrients from the blood to sustain milk synthesis. To understand this apparent paradox, more data are required for the knowledge concerning link between bST supplementation in high ambient temperature exposure. The objective of the present study was to determine the relationship between housing under shade with or without misters and fans and supplementation with rbST or not during three period of lactation (early, mid and late lactation) of crossbred 87.5% HF animals. Measurements for evaluation the effectiveness of these treatments were circulating levels of IGF-I, insulin, thyroxine, cortisol, mammary blood flow and biological variables relevant to milk production. It might lead to better understanding adaptability in crossbred cattle and choosing suitable crossbred dairy cattle for increased milk production in the tropics.

MATERIALS AND METHOD

Animals, housing and managements

Ten primiparous, non pregnant crossbred cattle, containing 87.5 % Holstein (HF) genes, were used for the experiment. The animals with averaged 60 days postpartum at start of trial were assigned randomly into two groups of five animals each. Animals in the first group were housed in open-sided barn with a tiled roof in normal shaded house (NS) as the non-cooled animals. Animals in the second group were housed in open-sided barn with a tiled roof (8 m long x 7 m wide x 3.5 m high) under misty fan cooling (MFC) as cooled animals. The open space cooling system consisted of two sets of misty fan, which each system consisted of a 65 cm. diameter blade fan circulating 81 m^3/min of air, with oscillation coverage of 180º. The amount of water discharged from 4 splay heads was 7.5 L/h and size of mist droplet 0.01 mm. Animals were exposed to MCF for 45 minutes at 15-minute intervals from 06:00 h to 18:00 h. At night, animals were exposed to MCF for 15 minutes at 45-minute intervals from 18:00 h to 06:00 h.

The diet was fed twice a day for *ad lib* as the same ration of total mixed ration (TMR) throughout the experiments in both groups. Ingredient and chemical compositions of feed are shown in Table 1. Animals are milked twice a day using a milking machine and milk production was recorded daily. Each day, the food was given in equal portions at about 06:00 h and 17:00 h when the animals were milking. The ambient temperature was recorded using a dry bulb thermometer. The relative humidity at NS and MFC were read by psychrometric chart depending on wet and dry bulb temperature. Ambient temperatures, humidity, rectal temperature and respiratory rate were measured weekly at 09:00, 11:00, 13:00, 15:00 and 17:00 h on specified day. A temperature-humidity index (THI) was calculated from the average ambient temperature of dry and wet bulb temperatures according to McDowell (1972), as follow:

$$THI = 0.72 (wb+db) + 40.6$$

Where ; wb = wet bulb temperature and db = dry bulb temperature expressed in °C

Rectal temperature and respiratory rates were measured at different parts of the day with electronic thermometers and observing flank movements, respectively. Body weight (BW) of all animals were recorded by weighing monthly throughout the experimental periods.

Experimental design

The experiments were carried out throughout lactating period in each group. The experiment in each group was divided into 3 phases, namely early- (Day 60 postpartum), mid- (Day 120 postpartum), and late lactating periods (Day 180 postpartum). The pretreatment study was conducted on the starting day of each phase. At the end of the pretreatment, within the same day, the animal was injected with the first dose subcutaneous injection of 500 mg of recombinant bovine somatotropin (rbST) (POSILAC, Monsanto, USA). Subsequently, the animal was injected with two consecutive doses injections of rbST every 2 weeks. Thereafter, within 2 days after the third injection, the treatment study was conducted. The pretreatment, 3 doses of injections, and the treatment periods were performed during the first 30 days and the same procedures were followed for each phase. During the last 30 days of each phase, no experiments were conducted in order to allow the milk yield from the effect of rbST treatment to return to the control level (Etherton and Bauman 1998).

Sample collection and chemical analysis

Milk samples from morning milking were used to determine milk compositions concentrations using Milkoscan (Milko-Scan 133B, A/S N. Foss Electric, Hillerod, Denmark).

Mammary blood flow measurements

On specified days of the study 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 (Surflo® I.V. Catheter, Terumo Corporation, Philipines), under local anesthesia. This was done on the standing animal for the measurement of mammary blood flow. 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 via catheter, adapted from a method of measuring blood flow in the milk veins of cattle as previously described by Chaiyabutr et al. (1997).

Determination of plasma hormones and metabolite concentration

Plasma samples were collected from coccygeal vessel for determinations of hormonal concentrations of IGF-I by Chemiluminescence immunoassay using an IMMULITE® Analyzer (IMMULITE IGF-1, Diagnostic Products Corporation, Los Angeles, CA). The plasma bovine insulin concentration was quantified using ELISA

technique (Mercodia Bovine Insulin, Mercodia AB, Sylveninsgatan 8, Uppsala, Sweden). The plasma levels of thyroxine (T_4) were determined by Electrochemiluminescence immunoassay (ECLIA) (Roche Diagnostics GmbH, USA) by Elecsys 2010 analyzer. (Indianapolis, IN, USA). The plasma level of cortisol was analyzed by Chemiluminescence immunoassay using cortisol kit.

Plasma glucose concentrations were measured using enzymatic oxidation in the presence of glucose oxidase. The plasma concentration for acetate was assayed by acetic acid UV-method (R-Biopharm AG, Darmstadt, Germany), Plasma β–hydroxybutyrate concentrations were assayed using an enzymatic reaction in the presence of β-hydroxybutyrate dehydrogenase (Sigma Chemical Co.) and the plasma concentration for triglyceride was determined by enzymatic colorimetric test (Triglyceride liquicolor^{mono} Su-Trimr, Germany). Plasma free fatty acids were determined by colorimetry after plasma extraction with chloroform, heptane and methanol and TAN solution (Wang et al.,2004). Plasma protein concentrations were conducted with an automatic clinical chemistry analyzer (Operator Manual BT 2000 Plus, Biotecnica Instruments S.P.A Via Licenza, Rome, Italy)

Statistical analysis

The statistic analyses were performed using General Linear Model procedures of statistical software package SPSS (SPSS for windows, V14.0; SPSS Inc., Chicago, IL, USA). The model used for each analysis was:

$$Y_{ijk} = \mu + A_l + H_i + A(H)_{il} + B_j + (HB)_{ij} + A(HB)_{ijl} + Cov_k + e_{ijkl}$$

Where Y_{ijk} = observation, μ = overall mean, A_l= Animal effect H_i= house effect as main plot (*i* = NS, MFC), $A(H)_{il}$ = main plot error (animal *l* in house *i*), B_j = treatment effect (rbST) as a split plot (*j* = with and without rbST supplementation), $(HB)_{ij}$ = interaction effect between treatment and house , $A(HB)_{ijl}$ = split plot error (animal *l* in house *i* and treatment *j*), Cov_k = covariate effect and e_{ijk} = residual error.

Means values were used to evaluate the effect for all variables. Statistical significance was declared at $P<0.05$. and trends were declared at $0.05 < P \leq 0.10$.

RESULTS

Ambient temperature(AT), relative humidity (RH), temperature humidity index (THI), rectal temperature (RT) and respiration rate (RR)

An environmental parameters measurement and and physiological parameters at 1400h are shown in Table 2. Ambient temperature measured in NS barn was significantly higher than MFC barn throughout experimental period, while relative humidity of NS barn was lower than that of MFC barn. The significant differences of THI were apparent between MFC barn (range 80.7 to 82.8) in and NS barn (83.2to 85.5). The cows without rbST housed under MFC barn showed significantly lower RR and RT than those of cows housed under NS barn. After rbST supplementation, the cows showed significantly increases in RR and RT when compared with pre-treatment in each stage of lactation.

Milk yield, DMI, mammary blood flow and body weight

The body weight, dry matter intake (DMI), milk yield and mammary blood flow are shown in Table 3. It is obvious that both cooled and non-cooled cows supplemental rbST increased milk yield, which was significantly higher than that of the pretreatment period, but it decreased as lactation advances. It is obvious that both cooled and non-cooled cows supplemental rbST increased mammary blood flows, which were significantly higher than those of the pretreatment periods. The ratio of mammary blood flow to the rate of milk yield was not affected by the supplementation of rbST in both groups. The body weights of both cooled and non-cooled cows were increased stepwise as lactation advances whether supplemental rbST or not. Cooled cows showed lower DMI than those of non-cooled cows in all stages of lactation. Cows increased DMI after supplementation of rbST when compared with pretreatment in either cooled and non-cooled cows.

Arterial plasma concentrations of insulin, insulin like growth factor-I (IGF-I) thyroxine (T_4) and cortisol.

Effects of supplemental rbST and cooling on alterations of plasma hormones (IGF-I), insulin, cortisol and thyroxine are presented in Table 4. The mean values of the plasma IGF-I concentration were significantly increased in both cooled and non-cooled cows during rbST supplementation in each stage of lactation. The plasma

concentrations of insulin had tendency to increase during supplemental rbST in early and mid-lactation. No effects of cooling and supplemental rbST were apparent on the plasma cortisol concentration throughout periods of studies. The plasma thyroxine concentrations in both cooled and non-cooled cows were lower after rbST supplementation in early lactation but not in mid and late lactations. The plasma thyroxine concentration of cooled cows without rbST had tendency to be higher than those of non-cooled cows in each stage of lactation.

The concentration of plasma metabolites

Plasma concentrations of metabolites are shown in Table 5. There were no significant differences in plasma concentrations of glucose, acetate, β-hydroxybutyrate, triglyceride and protein in both cooled or non-cooled cows whether rbST supplementation or not in all stages of lactation. The plasma free fatty acid concentration was numerically increased during supplemental rbST in both cooled and non-cooled cows in each stage of lactation.

Table 1. Feed ingredients and chemical compositions of the diet

Ingredients:	Kg (as fed basis)
Pine apple waste	50
Soybean meal	23
Rice bran	3.0
Cotton seed	20
Lime stone	1.4
Di-calcium phosphate	1.4
Sodium bicarbonate	0.3
Potassium chloride	0.1
Mineral and vitamin premix	0.8
Total	100
Chemical composition:	
Dry matter (%)	39.1
Ash (% DM)	7.3
Organic matter (% DM)	92.7
Crude protein (% DM)	18.0
Acid detergent fiber (% DM)	20.1
Neutral detergent fiber (% DM)	33.9

Table 2. Ambient temperature, relative humidity, temperature humidity index (THI), rectal temperature and respiration rate measurement at 1400h in animals treated with rbST housing under normal shade (NS) and cooling with misters and fans (MFC) at different stages of lactation

.

	Stages of	NS		MFC			[1]Effect		
Parameters	lactation	Pre	rbST	Pre	rbST	SEM	house	rbST	house*rbST
Ambient temperature (°C)	Early	33.9	35.1	31.6	31.8	0.74	0.001	0.386	0.473
	Mid	35.3	35.0	30.0	29.8	0.48	0.002	0.613	0.919
	Late	33.5	34.1	29.9	29.1	0.35	0.001	0.865	0.098
Relative humidity (%)	Early	49.5	52.8	66.0	68.8	3.28	0.001	0.396	0.942
	Mid	52.8	50.4	78.2	74.0	3.10	0.001	0.318	0.779
	Late	59.0	63.5	78.5	79.8	2.07	0.001	0.214	0.462
Temperature humidity index	Early	83.2	85.2	82.4	82.8	0.89	0.003	0.205	0.396
	Mid	85.5	84.8	81.5	80.8	0.39	0.019	0.116	0.928
	Late	83.9	85.2	81.4	80.7	0.29	0.004	0.316	0.011
Rectal temperature (°C)	Early	39.4	40.0	39.0	39.4	0.21	0.037	0.061	0.817
	Mid	39.7	40.1	38.6	39.5	0.13	0.002	0.002	0.090
	Late	39.2	39.9	38.4	38.8	0.16	0.015	0.016	0.309
Respiration rate (breath/min)	Early	73.0	82.3	55.5	68.0	4.13	0.023	0.039	0.708
	Mid	73.6	77.2	49.0	57.6	1.96	0.001	0.018	0.294
	Late	71.5	80.0	54.3	59.3	1.09	0.019	0.001	0.159

SEM = Standard error of the mean

[1] P-values for the effects; MFC =Misty-fan cooling effect, rbST = rbST effect, MFC x rbST = interaction effect of MFC and rbST

Table 3**.** Milk yield, Dry matter intake (DMI), mammary blood flow (MBF), mammary plasma flow (MPF)and body weight in cows treated with rbST and housing under normal shade (NS) and misters and fans cooling (MFC) at different stages of lactation.

	Stages of	NS		MFC			[1]Effect		
Parameters	lactation	pre	rbST	Pre	rbST	SEM	house	rbST	house*rbST
Milk yield (kg/day)	Early	13.39	15.43	14.82	15.84	0.31	0.684	0.001	0.140
	Mid	11.13	13.10	13.79	15.73	0.54	0.269	0.003	0.549
	Late	10.31	11.77	11.29	15.00	0.61	0.372	0.003	0.101
DMI (kg/d)	Early	6.14	7.05	7.22	8.49	0.312	0.049	0.008	0.575
	Mid	6.18	7.49	8.72	10.00	0.450	0.013	0.020	0.973
	Late	7.57	7.88	8.26	9.32	0.151	0.326	0.002	0.037
MBF (ml/min)	Early	4969	5222	5241	6555	265.1	0.524	0.018	0.081
	Mid	4141	5053	4132	5434	388.1	0.821	0.021	0.629
	Late	3750	5096	4435	4968	248.6	0.735	0.005	0.141
MBF/milk yield (L/kg)	Early	535.0	491.6	554.5	583.0	19.29	0.685	0.701	0.100
	Mid	615.8	612.7	473.9	534.9	49.30	0.568	0.573	0.534
	Late	561.9	672.2	589.7	597.5	63.51	0.888	0.391	0.430
Body weight (kg)	Early	359	381	360	374	6.5	0.893	0.025	0.535
	Mid	383	383	382	411	4.2	0.586	0.007	0.009
	Late	398.	393	425	423	4.9	0.268	0.483	0.752

SEM = Standard error of the mean.
[1] P-values for the effects; MFC =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MFC and rbST

Table 4. Changes in arterial plasma concentrations of thyroxine(T_4), cortisol, insulin and insulin like growth factor-I (IGF-I) in cows treated with rbST and housing under normal shade (NS) and misters and fans cooling (MFC) at different stages of lactation.

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Parameter	Stages of lactation	NS		MFC		SEM	Effect		
		Pre	rbST	Pre	rbST		house	rbST	house*rbST
Insulin (µg /L)	Early	0.72	0.92	0.51	1.38	0.36	0.821	0.173	0.382
	Mid	1.19	1.01	0.97	1.23	0.27	0.996	0.887	0.436
	Late	0.57	1.24	1.76	1.21	0.29	0.102	0.830	0.071
IGF-I (ng/ml)	Early	118.2	196.0	87.4	114.8	18.8	0.301	0.023	0.216
	Mid	115.6	183.2	121.8	208.4	36.1	0.644	0.065	0.798
	Late	128.2	350.9	124.1	220.4	41.5	0.221	0.005	0.166
Cortisol (µg/dl)	Early	2.15	2.24	1.87	1.74	0.65	0.457	0.972	0.868
	Mid	3.20	1.37	2.56	2.01	0.73	0.998	0.139	0.404
	Late	1.54	2.22	2.14	1.63	0.68	0.989	0.907	0.405
T_4 (µg/dl)	Early	8.08	7.26	10.64	8.52	0.51	0.518	0.021	0.237
	Mid	9.96	10.82	13.87	13.04	1.90	0.337	0.993	0.673
	Late	7.95	7.99	10.89	12.15	2.04	0.276	0.760	0.774

SEM = Standard error of the mean.

[1] P-values for the effects; MFC =Misty-fan cooling effect, rbST = rbST effect, MFC x rbST = interaction effect of MFC and rbST

Table 5. The arterial plasma concentrations for glucose, acetate, β-hydroxybutyrate, free fatty acid and triacylglycerol in cows treated with rbST housing under normal shade (NS) and misters and fans cooling (MFC) at different stages of lactation.

	Stages of	NS		MFC			[1]Effect		
Parameters	lactation	Pre	rbST	Pre	rbST	SEM	house	rbST	house*rbST
Glucose:	Early	67.19	63.19	65.44	62.58	1.834	0.883	0.098	0.763
(mg/dl)	Mid	63.93	61.12	63.27	66.04	1.931	0.739	0.154	0.591
	Late	62.81	63.41	68.75	67.80	1.570	0.686	0.256	0.464
Acetate :	Early	3.90	3.25	2.62	2.81	0.28	0.111	0.919	0.463
(mg/dl)	Mid	2.78	3.09	3.89	3.24	0.22	0.276	0.554	0.453
	Late	4.05	3.58	3.58	2.39	0.42	0.193	0.338	0.825
β-OH-butyrate:	Early	8.85	9.08	6.78	7.26	0.66	0.143	0.610	0.860
(mg/dl)	Mid	7.83	8.47	9.19	7.72	0.56	0.831	0.480	0.093
	Late	9.09	9.70	7.81	7.74	1.17	0.332	0.823	0.783
Free fatty acids	Early	3.703	3.877	4.720	6.785	0.897	0.239	0.247	0.323
(mg/dl)	Mid	3.144	4.633	4.449	4.825	0.350	0.576	0.029	0.152
	Late	2.419	3.625	4.215	6.201	0.415	0.147	0.004	0.362
Triglyceride	Early	11.88	13.41	14.55	15.06	1.39	0.243	0.510	0.760
(mg/dl)	Mid	15.61	17.20	13.60	15.14	1.79	0.729	0.407	0.998
	Late	14.85	15.71	24.00	18.63	3.607	0.351	0.549	0..413
Total protein	Early	8.52	8.79	9.23	8.82	0.18	0.428	0.718	0.092
(g/dl)	Mid	9.11	8.49	8.57	8.97	0.29	0.956	0.706	0.114
	Late	8.70	8.13	8.98	8.88	0.22	0.460	0.179	0.327

SEM = Standard error of the mean.

[1] P-values for the effects; MFC =Misty-fan cooling effect, rbST = rbST effect, MFC x rbST = interaction effect of MFC and rbST

DISCUSSION

The application of misty-fans cooling could reduce ambient temperature and THI which were lower than those of normal shade barn in the present study. Although, relative humidity measured in MFC barn were relative high, but it could be partially mitigate heat load, which showing low RR and RT for lactating cows. This in agree with number of previous works (Fike et al., 2002; Gallardo et al., 2005). Cows housed in MFC barn significantly decreased both RR and RT as compared to those of cows housed in NS barn. These results are in agreement with the study of Smith et al. (2006) that using evaporative tunnel cooling can decrease peak daytime rectal temperatures by 0.6 to 1.0°C and respiration rates by 20 to 30 breaths/min when comparison with traditional cooling strategies. Although, supplementation of rbST increased body heat, but the extent of responses for increases in RR and RT were lower than those of effects of high temperatures. Thus, supplemental rbST would not be the main cause of heat stress in the present study. These findings might confirm the previous reports by Johnson et al. (1991) and West (1994) who proposed that rbST-treated cows increases heat production, it also increases heat dissipation.

The application of misty fans cooling system could reduce the effect of heat stress in lactating crossbred cows which showed greater milk yield than those of non cooled cows without rbST. Supplementation of rbST showed high milk yield in both cooled and non-cooled cows in each stage of lactation. These results suggest that the supplementation of rbST to lactating cows could improve milk production either under cooling or not. In the present results, increases in body weights during supplemental rbST in both cooled and non-cooled cows were apparent, but body weights of cows in both groups were increased when lactation progress. The primiparous cow using in this experiment would attribute to the growing effect as lactation advances.

High temperature has been known to influence milk yield and endocrine status which affect to the maintenance requirement (Collier and Beede, 1985). Changes of some blood metabolites, i.e. glucose, acetate, FFA and β-hydroxybutyrate could be used as indicator for energy status in lactating cows. In the present study, plasma metabolites for glucose, β-hydroxybutyrate, acetate, and triglyceride of cows showed no statistical differences in both cooled and non-cooled cows with or without

supplemental rbST. In the present study, the plasma FFA concentration was markedly increased after rbST supplementation in cooled cows. An increase in FFA during rbST administration would be the lipolytic activity of rbST per se in adipose tissue (Houseknecht et al., 2000).

In the present study the plasma IGF-1 level was increased during rbST supplementation in each stage of lactation in both groups. The synthesis and release of IGF-1 is mainly by the liver (Granner, 1996). Mechanisms for regulating the plasma IGF-1 level are known to be dependent on the availability in the liver of both bST and some nutritional factors (Clemmons and Underwood, 1991). From the present study, the increase in IGF-1 secretion would appear to be maintained by the availability of exogenous rbST in the liver. Exogenous of rbST in the present study was sufficient to achieve a satisfactory stimulation of IGF-1. Cows with a lower nutritional state having a lower basal level of IGF-1 (Hodgkinson, Bass & Gluckman, 1991) or a negative energy balance, have reduced hepatic IGF-1 production (Weller et al. 1994; Ketelsleger et al. 1995), would not be expected to occur in the present study, since no differences in the nutritional status among treatments. During early and mid lactation, plasma insulin levels had tendency to increase during rbST supplementation in both cooled and non-cooled cows. This suggests that the availability of both somatotropin and insulin to the liver would attribute to increase in IGF-I secretion (Luo & Murphy, 1991). The relationship between somatotropin and insulin was not apparent for rbST treated cows in late lactation. However, maintaining the plasma concentration of glucose with high concentrations of insulin during rbST supplementation in both cooled and non-cooled cows. This indicates that exogenous bovine somatotropin 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 yields were increased throughout lactation by the effect of rbST supplementation especially in early lactation. The milk yields of cow supplemental rbST in early lactation were highest when compared with other stages of lactation. The higher of milk production would be a consequence of increase in heat production (Manalu et al., 1988). Thyroid hormones are known to be a calorigenic hormone. Thus, increasing of heat production during supplementation of rbST may depress secretion of thyroid hormone which may assist the animal to regulate body temperature in maintaining homeothermy (Johnson et al., 1991). This result probably

accounted for a reduction of the plasma thyroxine (T_4) concentration in early lactation during rbST supplementation in the present study. It has been reported in patients with GH deficiency that rhGH therapy appears to increase serum T_3 level and decrease in mean of free T_4 level, resulting an increased conversion of thyroxine (T_4) to triiodothyronine (T_3) in peripheral tissues (Losa et al., 2008). The low level of plasma thyroxine remained within the normal range which does not induce hypothyroidism (Portes et al., 2000) and are not mediated via IGF-I (Hussain et al., 1996). However, the effect of rbST on the T_4 levels is varied. During hot and humid weather, the levels of triiodothyronine and thyroxine were not affected by bST administration in Holstein and Jersey cows (West et al., 1991). Johnson et al. (1991) founded in cows treated with rbST that plasma triiodothyronine were significantly decreased while it had no effect on the plasma T_4 level.

Cortisol is the major adrenal corticoid secreted in cattle which enable animals to tolerate stressful condition (Christison and Johnson, 1972). Plasma cortisol levels have been shown to reduce in prolonged heat exposure in cattle (Ingraham et al.,1979) and sheep (Tilton et al., 1975) including during rbST supplementation in both thermoneutral and hot condition (Johnson et al., 1991). However, plasma cortisol levels of dairy cattle were increased in response to exposure to cool environment (Guerrini and Bertchinger, 1982). The present results showed no changes in plasma cortisol concentrations in both cooled or non-cooled cows with or without rbST. These findings are in agreement with studies reported by Peel et al., (1982, 1983) and West et al.,(1991). However, increases in the level of adrenocorticortropin (ACTH) have been reported, although the level of cortisol did not change in rbST-treated cows (Adriaens et al., 1995), in high-producing cows (Shayanfar et al., 1990) and in early lactation (Koprowski and Tucker, 1973). The endogenous bST levels are naturally higher in early lactation and in high producing cows (Hart et al., 1980; Kazmer et al., 1986; Bonzek et al., 1988; Chaiyabutr et al., 1997). Therefore, rbST may influence in reduced adrenal ACTH responsiveness. It is suggested that this phenomenon is part of the coordinated metabolic adaptations required to support the increases in milk production (Adriaens et al., 1995).

In the present study, both cooled and non-cooled cows treated with rbST increased milk yields and the plasma IGF-I concentrations throughout lactation. These results supported the study of Bauman, (1992) that the effect of exogenous bST on increasing of milk productions requiring IGF-I as a mediator or nutrient partitioning.

IGF-I injection has been shown directly increase in mammary blood flow (Prosser et al., 1990; Etherton and Bauman, 1998). An increase in the mammary blood flow would be a factor for increasing the availability of substrates to the mammary gland for milk synthesis. The mechanism of regulation of secretion and synthesis IGF-I in the liver are known to dependent on action of GH and some nutritional factors (Clemmons and Underwood, 1991). The rbST supplemented cows would stimulate appetite in both cooled and non-cooled cows. An increase in dry matter intake would attribute to be a cause of increased nutrients for stimulating IGF-I synthesis. Thus, either the direct action of exogenous rbST or indirect action of an increase of nutrients by rbST administration would cause an increase in the plasma IGF-I concentration in present experiment. Moreover, It has been reported that IGF-I response to bST treatment would not take place during heat stress (McGuire et al., 1991; Settivari et al., 2007).

In the present study, THI values were averaged in moderate heat stress for lactating cows in both cooled and non-cooled cows. The effect of chronically heat stress cattle had tended to reduce metabolic hormone including thyroxine, growth hormone and glucocorticoid in *Bos taurus* (Collier et al., 1982a; Collier et al., 1982b; Alvarez and Johnson, 1973; Mitra et al., 1972; Niles et al., 1980). In the present experiment, the effect of environment did not affect on plasma levels of T_4, cortisol and IGF-I in either cooled or non-cooled cows alone. It is possible that 87.5% HF cows using in the present study had a low milk yield and heat tolerant which were a consequence of lower heat production as compared with exotic Holstein lactating cows.

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CHAPTER V

Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on the utilization of glucose in the mammary gland in different stages of lactation in crossbred Holstein cattle:

Series 5.1: The effects of supplemental bovine somatotropin and cooling on milk production relating to body glucose metabolism and the utilization of glucose by the mammary gland in crossbred Holstein cattle

Series 5.2: Effects of cooling and recombinant bovine somatotropin supplementation on body fluids, mammary blood flow, and nutrients uptake by the mammary gland in different stages of lactation of crossbred Holstein cattle

Chapter V (Series 5.1)

The effects of supplemental bovine somatotropin and cooling on milk production relating to body glucose metabolism and the utilization of glucose by the mammary gland in crossbred Holstein cattle

INTRODUCTION

It is known that low milk yield and short lactation period of either pure exotic or crossbred dairy cattle is still the major problem for the dairy practices in the tropics. The mechanisms that limit the rate of milk yield and shorter lactation persistency as lactation advances in crossbred dairy cattle in tropics are unknown. It is not only animal genetics that have to be considered but other factors, for example, high environmental temperatures and hormonal factors can influence milk production of cows (Collier et al., 1982). The study in 87.5% crossbred Holstein cattle (HF) have been shown that the concentration of plasma bovine somatotropin (bST) decreased rapidly as lactation progressed to mid and late lactation. This decrease would accompany with a reduction in both mammary blood flow and milk yield (Chaiyabutr et al., 2000a). Many studies have demonstrated the efficacy of bST for improvement in milk yield (Breier et al., 1991; Burton et al., 1994.). Long term exogenous recombinant bovine somatotropin (rbST) in 87.5% crossbred Holstein cattle increased in milk yield which accompanied with an increase in the rate of mammary blood flow, but the stimulant effect for milk yield was less in late lactation despite a high level of mammary blood flow (Chaiyabutr et al. 2007). It is not known which factors are the cause and which factors are the effects for such reduction.

Many technologies are required to improve milk production of dairy cattle in the tropics. It has well recognized that hot environments lowered milk production in dairy cattle both directly and indirectly. Environmental modification is the most common approach to increase milk production with alleviation of severe heat stress in dairy cattle, for example, fans and sprinklers (Fike *et al.* 2002), evaporative cooling system (Chan *et al.* 1997; Chaiyabutr et al., 2008a). In addition to environmental modification, the

application of exogenous bovine somatotropin has been reported to minimize the effects of heat stress and potentially increased milk yields (West *et al.* 1991; West 1994). Somatotropin is known to play a role in responsible for galactopoietic and contributing to homeostasis and homeorhesis in ruminants **(**Bauman and Currie 1980). However, few data are available on understanding the mechanisms of milk secretion involving extra-mammary factors and intra-mammary factors during the combined effects of high environmental temperatures and bST administration.

Glucose is known to be the principal precursor of lactose synthesis. Lactose is a highly osmotic component, which allows the drainage of water from blood to the alveolar compartment and its concentration in milk remains relatively stable (Linzell and Peaker, 1971). Regulation of the milk yield of animals is mainly based on the mechanisms governing the quantity of glucose extracted by the mammary gland and converted into lactose. The decrease in lactose biosynthetic pathways has been shown to account for a short persistency of lactation in 87.5% HF animals (Chaiyabutr et al., 2000b). These changes have been explained by a change of the mammary utilization of glucose. The estimation in vivo of glucose metabolism in the udder has been reported to be metabolized less for lactose synthesis and the pentose phosphate pathway but metabolized more via the Embden-Meyerhof pathway as lactation advances during long-term administration of rbST in 87.5% HF animals (Chaiyabutr et al., 2008b). 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. Although, a study the effect of an administration of bST can increase milk production in dairy cows, but it also increases heat production (West, 1994). Few data are available concerning intra-mammary factors for the utilization of glucose and glucose metabolism in the udders of crossbred Holstein dairy cattle keeping under high environmental temperature. More specifically, it is not known how much of the reduction in milk yield is due to high environmental temperatures alone and how much improved management could overcome reduced production. Therefore, the present study is designed to investigate the mechanisms of milk secretion involving extra-mammary factors and intra-mammary factors relating to body glucose metabolism and utilization of glucose by the mammary gland during rbST supplementation in 87.5% HF animals under misty-fan cooling system.

MATERIALS AND METHODS

Animals and managements

Ten pregnant heifer crossbred 87.5% Holstein cattle were selected for the experiment. Animals were randomly divided into two groups of five animals each. Animals in the control group were housed in the normal shade (NS) in individual stall, while animals in the experimental group were housed in shade with using mister and fans cooling to reduce the environmental temperature (MF). The MF barn had two sets of misters and fans cooling system, which each system consisted of a 26 inch diameter blade fan circulating 7,200 ft^3/min of air, with oscillation coverage of 180°. The amount of water discharged from 4 spray heads was 7.5L/hr and side of mist droplet 0.01 mm. Animal were exposed to MF for 45 minutes at 15-minute intervals from 0600h to 1800h. At night, animals were exposed to MFC for 15 minutes at 45-minute intervals from 1800h to 0600h. Animals in each group were fed with the same ration of TMR twice daily throughout the experiment. Each day, the diet was given in equal portion at about 0600h and 1700h when animal were milked. Water was available at all times. All animals were weighed monthly throughout the experiment.

The study was performed under a protocol approved by ethic committee of Faculty of Veterinary Science, Chulalongkorn University. The procedures used in the present study were formulated to comply with international standards and are in accordance with the principles and guidelines of the National Research Council of Thailand.

Experimental procedures

The experiments were carried out throughout lactating period in each group. The experiment in each group was divided into 3 stages, namely early- (Days 65-95 postpartum), mid- (Days 125-155 postpartum), and late lactating stages (Days 185-215 postpartum). The pretreatment study was conducted on the starting day of each lactating stage. At the end of the pretreatment, within the same day, the animal was injected with the first dose subcutaneous injection of 500 mg of recombinant bovine somatotropin (rbST) (POSILAC, Monsanto, USA). Subsequently, the animal was injected with two

consecutive doses injections of rbST every 2 weeks. Thereafter, within 2 days after the third injection, the treatment study was conducted. The pretreatment, 3 doses of injections, and the treatment periods were performed during the first 30 days and the same procedures were followed for each stage. During the last 30 days of each lactating stage, no experiments were conducted in order to allow the milk yield from the effect of rbST treatment to return to the control level (Kirchgessner et al., 1991).

Rectal temperature and respiration rate of individual animals were determined at the same time as recording ambient temperature. Ambient temperature and humidity were measured weekly throughout the experiment. The temperature humidity index (THI) was calculated according to West (1994) where: THI = db - (0.55-0.55RH) (db -58) with db = dry bulb temperature (°F), and RH = relative humidity. On each specified day of study, measurements of mammary blood flow, glucose metabolism and the utilization of glucose by the mammary gland were carried out. At around 10.00 h. both ear vein and milk vein were catheterized with the non-radiopaque intravenous catheter, gauge 18G (Surflo, Terumo Europe N.V., Belgium) under local anesthesia for infusion of solution. An arterial blood sample was collected from the coccygeal artery by venipuncture with a # 21 needle into heparinized tube. Blood samples from arterial and mammary venous blood in heparinized tube were kept in crushed ice and then centrifuge at 3000 rpm for 30 min at 4 ºC. Plasma samples were collected and frozen at -40°C in aliquots until time of assays for measurements the concentration of metabolites.

Glucose turnover measurements

The study on glucose kinetic and efficiency of glucose utilization by the mammary gland during pretreatment and treatment periods with rbST was performed at different stages of lactation: early, mid and late lactation by using both [U-^{14}C]-glucose and [3-^{3}H]-glucose infusion in animals, of both groups of animals. Glucose kinetic studies of each animal in each lactating stage were carried out as described previously by Chaiyabutr et al., (1998). Briefly, at about 10.00h of the specified day, a priming dose of radioactive glucose in 20 ml of sterile NSS containing 30 μCi [3-^{3}H]-glucose and 15 μCi[U-^{14}C]-glucose was administered intravenously via the ear vein catheter and followed by a continuous infusion of 1 ml/min of NSS (0.9%) containing 0.7 μCi/ml of

[U-^{14}C]-glucose and 1.5 μCi/ml of [3-^{3}H]-glucose for 3 h. (Peristatic pump; EYLA Model 3). During the last 1 hour (1200-1300h) of continuous 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 vessel by venipuncture with a # 21 needle. Blood samples in heparinized tubes were kept in crushed ice for chemical studies. Milk secretion was recorded for the last 1 hour of continuous infusion. Milk samples were used for measurement of radioactive glucose incorporation into other milk components. Milk yield was recorded by weight.

Mammary blood flow measurement

Measurements of the mammary blood flow through half of the udder were performed in duplicated by dye dilution technique using dye T-1824 (Evans blue) by a short term continuous infusion into the milk vein as described by Chaiyabutr et al. (1997). The rate of blood flow through half of the udder was calculated from plasma sample and the value of packed cell volume 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.

Chemical methods

Radiochemicals for [U-^{14}C]-glucose and [3-^{3}H]-glucose were obtained from the Radiochemical Center, Amersham Bucks, UK. The specific activity of labeled plasma glucose was determined by the method described by Chaiyabutr and Buranakarl (1989). The plasma glucose concentration was measured using enzymatic oxidation in the presence of glucose oxidase (Human GmBH, Germany). Plasma free fatty acid were determined by colorimetry after plasma extraction with chloroform, heptane and methanol and TAN solution (Wang et al.,2004). Plasma triacylglycerol concentration was determined by enzymatic colorimetric test (Triglyceride liquicolor, Wiesbaden, Germany).

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 fatty acids was extracted from 1 ml of an aliquot thawed milk in 2 ml of Dole's solution(Dole, 1965), (*iso*-propanol 40: *n*-heptane 10: 1N H_2SO_4 1, v/v) shaking in water bath for 30 min. After 1 ml hexane and 1ml H_2O was added to the vial and shaking, the upper layer containing fatty acids was transferred into two vials for radioactivity assay and determination of milk fatty acids concentration. Milk extraction solution in counting vial with a scintillation cocktail was measured radioactivity of ^{14}C and ^{3}H-fat by liquid scintillation counter (Liquid Scintillation Analyzer, Tricarb2300 TR, Packard Instrument Co. Inc. U.S.A.). Other portion of milk extraction was used to determine milk fatty acids concentration by colorimetry according to Wang et al.(2004) using chloroform, heptane and methanol and TAN solution. Milk fatty profiles was determined by gas chromatography (GC-2010 Gas Chromatograph, Shimazu) after extraction by chloroform and methanol(Christopherson & Glass 1969) in comparison with the appropriate internal standard of pentadecanoic acid (C: 15:0 fatty acid). The concentration of milk citrate was determined by spectophotometry 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}C]glucose or [3-^{3}H]glucose (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 glucose by the udder (U_G), expressed as μmol/min, was calculated from the equation:

$$U_G = MPFx (P_A - P_V)$$

where MPF = mammary plasma flow (mL/min), P_A = concentration of glucose in coccygeal arterial plasma (μmol/mL) and P_V = concentration of glucose of plasma from milk vein(μmol/mL).

Milk components output (MO), expressed as μmol/min, was calculated from the equation: MO = Ms x Cc/1000

where Ms = milk secretion rate (mL/min) and Cc = concentration of components in milk (μmol/L).

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

$$A = M_A/G_A \text{ x } t$$

where A = incorporation of radioactivity from glucose into milk components (μmol/min), M_A = total activity of ^{3}H or ^{14}C in the milk components (μCi),

G_A = specific activity of ^{14}C- or ^{3}H-glucose in arterial plasma at equilibrium (μCi/μ mol) 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:

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

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 & Linzell 1964).

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

$$G_6^P = U_G - L$$

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 & Wood 1963).

If the NADPH formed via PC were used exclusively for reductive biosynthesis of fatty acids, the ^{3}H-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:

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

The $^{3}H/^{14}C$ ratio in the plasma and related product was calculated from the equation:

$^{3}H/^{14}C$ glucose = $^{3}H/^{14}C$ in plasma glucose relative to $^{3}H/^{14}C$ ratio of 1 in the infusion,

$^{3}H/^{14}C$ lactose = $^{3}H/^{14}C$ in milk lactose relative to $^{3}H/^{14}C$ ratio of 1 in the infusion,

$^{3}H/^{14}C$ galactose = 2($^{3}H/^{14}C$ lactose) - ($^{3}H/^{14}C$ glucose),

$^{3}H/^{14}C$ citrate = $^{3}H/^{14}C$ in milk citrate relative to $^{3}H/^{14}C$ ratio of 1 in the infusion,

and $^{3}H/^{14}C$ triacyglycerol = $^{3}H/^{14}C$ in milk triacyglycerol relative to $^{3}H/^{14}C$ ratio of 1 in the infusion .

Statistical analysis

The statistic analyses were performed using General Linear Model procedure of statistical software package SPSS (SPSS for windows, V14.0; SPSS Inc., Chicago, IL, USA). The model used for each analysis was:

$$Y_{ijk} = \mu + A_l + H_i + A(H)_{il} + B_j + (HB)_{ij} + A(HB)_{ijl} + Cov_k + e_{ijkl}$$

Where Y_{ijk} = observation, μ = overall mean, A_l= Animal effect, H_i= house effect as main plot (*i* = NS, MF), $A(H)_{il}$ = main plot error (animal *l* in house *i*), B_j = treatment effect (rbST) as a split plot (*j* = with and without rbST supplementation), $(HB)_{ij}$ = interaction effect between treatment and house , $A(HB)_{ijl}$ = split plot error (animal *l* in house *i* and treatment *j*), Cov_k = covariate effect and e_{ijk} = residual error.

RESULTS

Ambient temperature, relative humidity, temperature humidity index (THI) respiratory rate and rectal temperature

Mean values of measurements at experimental site during periods of studies for daily temperatures, humidities, THI, the rectal temperature and respiratory rate are shown in Table1. Average values of ambient temperature in the barn during the daytime in the morning (0900 hours) between NS barn and MFC barn were not significantly different, while ambient temperatures during 1400 hours at NS were significantly higher than those of MFC. The high relative humidity were apparent at morning and it decreased onwards from morning to evening in both NS and MFC, whereas relative humidity in MFC was significantly higher than that of NS barn. THI values at the MFC barn in afternoon were significantly lower in comparison with NS barn. Cows in both groups exposed to high THI values (78.1 to 85.5) in both barns. Rectal temperature recording in the morning and afternoon (0900 to 1400 hours) of cooled and non-cooled cows were significant different whether rbST injection or not. The cooled cows showed lower rectal temperature than non-cooled cows during afternoon (1400 hours). There were significant increases in rectal temperature and respiration rate by the effect of supplemental rbST in different parts of the day. The cooled cow showed significantly lower respiratory rate than those of non-cooled cows throughout experimental periods.

Milk yield, milk compositions and its secretion

Milk yield, milk compositions and its secretion in cooled and non-cooled cows are shown in (Table 2.). It is obvious that both cooled and non-cooled cows supplemental rbST increased milk yield, which was significantly higher than that of the pretreatment period, but it decreased as lactation advances. The values of milk lactose concentration were unaltered during rbST supplementation as compared with pretreatment in both groups or among periods of lactation in the same group. The ratio of lactose output/glucose uptake were not different in comparison between cooled and non-cooled cows whether supplemental rbST or not in each stage of lactation, but it showed tendency

to decrease as lactation advances. In rbST-treated cows in both NS and MFC barns, the milk lactose secretion significantly increased as compared with pretreatment periods in all stages of lactation. The milk citrate concentration was significantly increased during supplemental rbST in early lactation, while its significantly decreased in late lactation in both cooled and non-cooled cows. However, during early and mid lactation, the secretions of milk citrate were significantly increased by the effect of supplemental rbST in both cooled and non-cooled cows. The concentration of milk triacylglycerol cows supplemental rbST had tendency to increase but a significant increases were apparent in early lactation in both groups. The secretions of milk triacylglycerol were significantly increased in both cooled and non-cooled cows during rbST supplementation in all stages of lactation.

Mammary plasma flow, plasma glucose concentration, mammary glucose uptake and percentage of glucose extraction.

The utilization of glucose across the mammary gland during rbST supplementation in both cooled and non-cooled cows are shown in Table 3**.** Mammary plasma flow of both cooled and non-cooled cows significantly increased after rbST administration in all stages of lactation. During rbST supplementation mammary glucose uptake increased in each stage of lactation in both cooled and non-cooled cows. The mammary glucose uptake of both non-cooled and cooled cows significantly increased during supplemental rbST in mid and late lactations by average 37% and 34%, respectively. Plasma glucose concentration remained in constant levels throughout lactation in both groups. There were no significant changes in A-V concentration differences for glucose across the mammary gland throughout the stage of lactation. The percentage of glucose extraction was not influenced by the supplementation of rbST in both groups.

Glucose turnover and related variables

The glucose turnover rate in both cooled and non-cooled cows supplemental rbST 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 are shown in Table 4. All absolute values of glucose turnover in both cooled and non-cooled cows

showed no significant changes in glucose entry and utilization rate throughout the stages of lactation. Plasma glucose concentration remained at the constant level at different stage of lactation in both cooled and non-cooled cows. Supplementation of rbST did not change in glucose entry and utilization rates in compared with the pretreatment. The recycling of glucose-C was estimated by simultaneous injection of [3-^{3}H] glucose and [U-^{14}C] glucose, which showed no differences between cooled and non-cooled cows whether supplemental rbST or not. Plasma glucose clearance remained unchanged during rbST administration in both cooled and non-cooled cows. Both absolute values and percentage of utilization of glucose by tissue other than the mammary gland were calculated from the total rate of glucose synthesis and the rate of glucose uptake by the mammary gland. The utilization of glucose of non-mammary tissues of both cooled and non-cooled cows increased as lactation advanced but it significantly decreased in cows supplemental rbST particular during mid lactation in comparison with the pretreatment periods. There were significant increases of body weight during the course of lactation in both groups. The body weights of both cooled and non-cooled cows whether supplemental rbST or not increased stepwise as lactation advances.

Utilization of glucose carbon in the mammary gland

Glucose uptake and incorporation into related products of lactose, citrate and triacylglycerol are shown in Table 5. A marked increase of the utilization of glucose carbon to milk lactose in absolute values were apparent in early and mid lactation of both cooled and non-cooled cows, while it decreased in late lactation. However, the percentage of utilization of glucose carbon for synthesis of milk lactose was not significantly different in early and mid lactation, but the significant decrease was apparent in late lactation of both cooled and non-cooled cows supplemental rbST. The absolute values and percentage of utilization of glucose carbon for synthesis of milk citrate of rbST-treated cows were significantly lower than those of the pretreatment period during mid and late lactation in both cooled and non-cooled cows. During supplementation of rbST, the utilizations of glucose carbon for synthesis of milk triacylglycerol were higher in both cooled and non-cooled cows in all stages of lactation.

Glucose metabolisms in different metabolic pathways in the udder

The effects of supplemental rbST and cooling on glucose metabolisms in different metabolic pathways in the udder are shown in Table 6. Data for glucose metabolism via pentose phosphate pathway have shown that the incorporation of ^{3}H from [3-^{3}H]glucose into fatty acids and the flux through the pentose phosphate pathway increased as lactation advances and during supplemental rbST in both cooled and non-cooled cows. Correction for the lower $^{3}H/^{14}C$ ratio likely to be present in intracellular glucose 6-phosphate still gave high flux values as lactation advances and during supplemental rbST in both cooled and non-cooled cows. The results of the net metabolism of glucose 6-phosphate via the pentose phosphate pathway has been defined as glucose 6-phosphate metabolized according to the equation:

glucose 6-phosphate $\longrightarrow$ glyceraldehyde 3-phosphate + $3CO_2$ [29] (Katz and Wood, 1963)

According to this equation, complete metabolism of one molecule of glucose 6-phosphate 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 present findings, during early and mid lactation, 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 in cows supplemental rbST, which were higher as compared with pretreatment period, but these values declined in late lactation. Values of metabolism of glucose 6-phosphate via the galactose moiety of lactose decreased as lactation advanced to late lactation in both groups. Metabolism of glucose 6-phosphate via the Embden-Meyerhof pathway was calculated in term of the proportion of metabolized glucose, which was considerable variation throughout stages of lactation in cooled and non-cooled cows. The absolute rate of metabolism of glucose via the Embden-Meyerhof pathway also appeared to increase on late lactation in both cooled and non-cooled cows whether supplemental rbST or not, but the increase was not statistical significant.

Milk fatty acid concentration (Table 7.)

The data in Table 7 showed the marked increases in the total milk fatty acids concentrations in all stages of lactation during supplemental rbST in both cooled and non-cooled cows. The statistical significant effects of rbST were apparent in early and mid lactation in both groups. The milk fatty acid concentrations, particularly with the long change length fatty acids (C_{16}-C_{18}) significantly increased in both cooled and non-cooled cows after supplemention of rbST in all stages of lactation.

NADPH production from glucose (Table 8.)

Data in Table 8 show the requirement of NADPH for fatty acid synthesis which was calculated from milk fatty compositions and output. The NADPH production for fatty acid synthesis significantly increased during supplementation with rbST in different stages of lactation in both cooled and non-cooled cows. The percentage of NADPH production from glucose via the pentose phosphate pathway was considerable variation throughout stages of lactation in cooled and non-cooled cows.

The 3H/14C ratio in glucose and related products (Table 9.)

$^{3}H/^{14}C$ ratios in plasma glucose and related products at different stages of lactation of cooled and non-cooled cows during supplementation with rbST are shown in Table 9. The $^{3}H/^{14}C$ ratio in arterial plasma glucose was lower than that of the infusion in both groups. These values were not different among cooled and non-cooled cows supplemental rbST in different stages of lactation, indicating some recycling of glucose-C in the whole animals during periods of study. A further decrease in the $^{3}H/^{14}C$ ratio was seen in milk lactose. As the glucose moiety of lactose arises directly from plasma glucose, this decrease in the ratio was due to metabolism of glucose 6-phosphate within the udder before incorporation into lactose as galactose. The $^{3}H/^{14}C$ ratio of milk triacylglycerol was slightly higher in both groups, indicating ^{3}H was removed and detected in milk triacylglycerol. 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.

Table 1. Ambient temperature, Relative humidity, temperature humidity index, mean values of rectal temperature and respiratory rate of crossbred Holstein cows housing in normal shade (NS) and shade plus misty-fan cooling (MFC).

Parameter	Stages of lactation		NS		MFC		SEM	[1]Effect		
			Pre	rbST	Pre	rbST		rbST	MFC	rbSTxMFC
Ambient temperature (°c)										
	Early	0900 h	28.00	27.60	27.10	27.50	0.30	1.000	0.276	0.261
		1400h	33.90	35.10	31.60	31.80	0.74	0.386	0.001	0.473
	Mid	0900 h	28.00	28.50	27.60	26.90	0.30	0.749	0.090	0.082
		1400h	35.30	35.00	30.00	29.80	0.48	0.613	0.002	0.919
	Late	0900h	28.30	28.40	27.30	27.40	0.49	0.903	0.040	0.903
		1400h	33.50	34.10	29.90	29.10	0.35	0.865	0.001	0.098
Relative humidity (%)										
	Early	0900 h	79.80	78.80	84.00	88.80	1.65	0.299	0.048	0.132
		1400h	49.50	52.80	66.00	68.80	3.28	0.396	0.001	0.942
	Mid	0900 h	78.60	78.80	85.20	83.60	1.27	0.595	0.009	0.497
		1400h	52.80	50.40	78.20	74.00	3.10	0.318	0.001	0.779
	Late	0900h	74.50	76.30	80.80	84.00	1.77	0.206	0.054	0.686
		1400h	59.00	63.50	78.50	79.80	2.07	0.214	0.001	0.462
Temperature humidity index										
	Early	0900 h	78.90	78.30	78.10	79.00	0.33	0.695	0.921	0.058
		1400h	83.20	85.20	82.40	82.80	0.89	0.205	0.003	0.396
	Mid	0900 h	79.00	79.60	78.90	77.80	0.40	0.495	0.216	0.064
		1400h	85.50	84.80	81.50	80.80	0.39	0.116	0.019	0.928
	Late	0900h	78.90	79.00	78.00	78.50	0.50	0.497	0.329	0.730
		1400h	83.90	85.20	81.40	80.70	0.29	0.316	0.004	0.011
Rectal temperature (°c)										
	Early	0900 h	38.50	38.90	38.10	38.50	0.08	0.003	0.032	1.000
		1400h	39.40	40.00	39.00	39.40	0.21	0.061	0.037	0.817
	Mid	0900 h	38.50	39.00	38.00	38.30	0.08	0.001	0.024	0.258
		1400h	39.70	40.10	38.60	39.50	0.13	0.002	0.002	0.090
	Late	0900h	38.50	38.80	38.00	38.30	0.10	0.015	0.007	0.723
		1400h	39.20	39.90	38.40	38.80	0.16	0.016	0.015	0.309
Respiratory rate (breaths/min)										
	Early	0900 h	40.00	42.50	35.00	38.00	0.56	0.003	0.003	0.670
		1400h	73.00	82.30	55.50	68.00	4.13	0.039	0.023	0.708
	Mid	0900 h	41.20	45.80	36.40	40.40	0.66	0.001	0.022	0.663
		1400h	73.60	77.20	49.00	57.60	1.96	0.018	0.001	0.294
	Late	0900h	40.50	44.50	37.00	41.50	1.44	0.025	0.101	0.868
		1400h	71.50	80.00	54.30	59.30	1.09	0.001	0.019	0.159

SEM = Standard error of the mean.

[1] P-values for the effects ; MFC =Misty-fan cooling effect, rbST = rbST effect, MFC x rbST = interaction effect of MFC and rbST

Table 2. Milk yield, milk lactose yield, lactose concentration and lactose output / glucose uptake during rbST administration at different stages of lactation of Holstein cows housing in normal shade (NS) and shade plus misty-fan cooling (MFC).

Parameter	Stages	NS		MFC			[1]Effect		
	of lactation	Pre	rbST	Pre	rbST	SEM	rbST	MFC	rbSTxMFC
Milk yield (kg/day) :									
	Early	13.39	15.43	14.82	15.84	0.31	0.001	0.684	0.140
	Mid	11.13	13.10	13.79	15.73	0.54	0.003	0.269	0.549
	Late	10.31	11.77	11.29	15.00	0.61	0.003	0.372	0.101
Lactose concentration (mmol/L):									
	Early	132.5	135.6	133.2	134.0	1.51	0.231	0.833	0.467
	Mid	129.3	130.6	130.5	131.2	1.29	0.658	0.536	0.195
	Late	130.5	129.7	131.7	133.6	1.68	0.769	0.338	0.448
Milk lactose secretion (µmol/min):									
	Early	1230.3	1458.4	1367.7	1471.3	36.12	0.002	0.716	0.123
	Mid	999.2	1188.0	1249.7	1497.5	48.38	0.003	0.225	0.100
	Late	936.6	1066.3	1028.6	1392.5	57.35	0.003	0.347	0.075
Lactose output / Glucose uptake (%) :									
	Early	65.1	70.4	60.1	57.7	5.6	0.799	0.537	0.510
	Mid	62.8	48.8	66.4	54.6	5.9	0.678	0.994	0.702
	Late	40.8	34.4	36.7	43.9	3.0	0.903	0.668	0.055
Milk citrate concentration (mmol/L):									
	Early	4.24	4.54	4.22	4.85	0.15	0.014	0.305	0.303
	Mid	4.70	4.71	5.67	5.78	0.11	0.575	0.016	0.645
	Late	4.74	4.14	5.24	4.38	0.15	0.001	0.042	0.404
Milk citrate secretion (µmol/min)									
	Early	39.51	48.81	43.81	52.95	1.74	0.001	0.578	0.965
	Mid	36.21	42.16	54.48	67.00	2.82	0.011	0.078	0.277
	Late	33.72	33.90	41.39	45.56	2.26	0.364	0.228	0.402
Milk triacylglycerol concentration (mmol/L):									
	Early	42.36	48.50	45.95	58.66	3.87	0.041	0.361	0.420
	Mid	58.77	56.92	57.53	64.99	3.12	0.395	0.699	0.174
	Late	61.13	69.36	54.67	66.41	5.61	0.113	0.503	0.762
Milk triacylglycerol secretion (µmol/min):									
	Early	374.05	491.30	483.14	632.77	40.09	0.010	0.105	0.697
	Mid	446.56	510.50	519.84	710.27	48.33	0.030	0.100	0.227
	Late	433.02	569.36	415.35	688.55	81.45	0.036	0.590	0.425

SEM = Standard error of the mean.

[1] P-values for the effects ; MFC =Misty-fan cooling effect, rbST = rbST effect, MFC x rbST = interaction effect of MFC and rbST

Table 3. Mammary plasma flow, arterial plasma glucose concentration, mammary glucose uptake and percentage of glucose extraction during rbST administration at different stages of lactation of Holstein cows housing in normal shade (NS) and shade plus misty-fan cooling (MFC).

Parameter	Stages of lactation	NS		MFC		SEM	[1]Effect		
		Pre	rbST	Pre	rbST		rbST	MFC	rbSTxMFC
Mammary plasma flow (ml/min):									
	Early	3748	4030	3923	5024	186	0.006	0.561	0.060
	Mid	3139	3871	3164	4141	303	0.023	0.822	0.696
	Late	2817	3843	3389	3792	185	0.005	0.676	0.131
Plasma glucose (µmol/ml) :									
	Early	3.73	3.51	3.64	3.48	0.10	0.098	0.883	0.763
	Mid	3.55	3.4	3.52	3.67	0.10	0.992	0.719	0.159
	Late	3.49	3.52	3.82	3.77	0.09	0.918	0.286	0.646
A-V (µmol/ml):									
	Early	0.66	0.67	0.76	0.61	0.08	0.485	0.858	0.261
	Mid	0.62	0.58	0.74	0.72	0.07	0.810	0.48	0.605
	Late	0.78	0.86	0.81	0.80	0.08	0.552	0.65	0.352
Mammary glucose uptake (µmol/min) :									
	Early	2299	2651	2438	2653	212	0.168	0.766	0.632
	Mid	1879	2437	1881	2745	355	0.042	0.624	0.982
	Late	2183	3235	2475	2936	253	0.051	0.53	0.203
Percentage of mammary glucose extraction (%):									
	Early	16.7	18.6	19.3	16.9	1.58	0.984	0.816	0.164
	Mid	17.1	16.7	19.6	18.8	1.57	0.696	0.461	0.398
	Late	22.2	24.4	21.5	21.5	1.62	0.530	0.901	0.373

SEM = Standard error of the mean.

[1] P-values for the effects ; MFC =Misty-fan cooling effect, rbST = rbST effect, MFC x rbST = interaction effect of MFC and rbST

Table 4. Glucose turnover rate, glucose-C-recycling, plasma glucose clearance, non-mammary glucose utilization and body weight during rbST administration at different stages of lactation of Holstein cows housing in normal shade (NS) and shade plus misty-fan cooling (MFC).

Parameter	Stages of lactation	NS		MFC		SEM	[1]Effect		
		Pre	rbST	Pre	rbST		rbST	MFC	rbSTxMFC
Glucose turnover :									
[U-14C] glucose (µmol/min):									
	Early	3377.4	3974.2	4547.8	4029.8	432.7	0.930	0.236	0.234
	Mid	4380.8	4388.2	5851.6	5144.4	510.4	0.512	0.135	0.504
	Late	4000.2	4302.8	5426.6	5428.8	414.9	0.723	0.110	0.727
[3-3H] glucose (µmol/min):									
	Early	4631.0	5064.6	5252.2	5032.4	539.8	0.848	0.703	0.562
	Mid	5493.4	5488.8	7926.6	6026.4	598.1	0.150	0.134	0.152
	Late	5309.2	5824.2	6707.0	8188.2	973.2	0.335	0.199	0.633
Glucose-C-recycling (%):									
	Early	24.9	22.1	21.0	19.3	5.71	0.698	0.347	0.927
	Mid	19.5	20.4	26.2	15.9	2.83	0.140	0.696	0.082
	Late	28.5	24.2	18.6	30.1	4.58	0.453	0.776	0.126
Plasma glucose clearance (ml/min):									
	Early	1403.9	1614.3	1391.9	1434.2	163.2	0.461	0.681	0.620
	Mid	1603.0	1588.3	2357.3	1643.3	182.5	0.081	0.192	0.092
	Late	1437.4	1737.8	1845.2	1881.2	283.4	0.252	0.264	0.866
Non mammary glucose utilization (µmol/min):									
	Early	2331.9	2413.6	2814.6	2379.1	659.4	0.754	0.898	0.746
	Mid	3614.1	3052.4	4965.4	3281.3	406.7	0.014	0.207	0.336
	Late	3126.2	2589.7	4231.6	3929.6	636.4	0.713	0.228	0.793
Non mammary glucose utilization (%):									
	Early	49.6	47.8	52.5	44.2	7.00	0.395	0.754	0.787
	Mid	65.4	59.1	74.3	53.8	3.32	0.001	0.903	0.207
	Late	58.6	44.6	58.9	55.9	4.49	0.223	0.538	0.301
Body weight (kg):									
	Early	358.8	380.8	363.8	380.2	6.54	0.019	0.908	0.680
	Mid	378.8	386.8	381.8	411.4	3.67	0.001	0.586	0.019
	Late	391.0	400.2	418.6	427.4	6.16	0.182	0.297	0.975

SEM = Standard error of the mean.

[1] P-values for the effects ; MFC =Misty-fan cooling effect, rbST = rbST effect, MFC x rbST = interaction effect of MFC and rbST

Table 5. Utilization of glucose carbon in the udder during rbST administration at different stages of lactation of Holstein cows housing in normal shade (NS) and shade plus misty-fan cooling (MFC).

Parameter	Stages of lactation	NS		MFC		SEM	[1]Effect		
		Pre	rbST	Pre	rbST		rbST	MFC	rbSTxMFC
[14C] Glucose incorporation (µmol/min) into:									
Milk lactose									
	Early	1102.9	1633.5	1809.7	2372.5	254.96	0.009	0.323	0.601
	Mid	1280.2	1405.7	1738.7	2113.4	237.83	0.856	0.602	0.452
	Late	1369.7	1034.0	1661.6	874.1	223.11	0.059	0.652	0.675
Milk triacylglycerol									
	Early	78.18	135.81	165.25	236.94	38.69	0.163	0.010	0.760
	Mid	126.85	217.62	197.00	197.66	29.82	0.244	0.660	0.251
	Late	154.49	205.94	118.87	231.53	51.25	0.226	0.930	0.638
Milk citrate									
	Early	25.45	21.19	23.78	16.41	4.58	0.793	0.822	0.597
	Mid	25.06	17.20	16.50	8.84	3.67	0.013	0.136	0.922
	Late	25.43	16.67	20.81	18.13	2.59	0.052	0.704	0.024
Percentage of glucose carbon appearing as:									
Milk lactose									
	Early	52.5	64.6	73.6	88.6	34.93	0.261	0.080	0.718
	Mid	81.3	58.5	91.9	77.9	19.45	0.628	0.377	0.831
	Late	58.6	32.4	57.8	28.3	11.34	0.012	0.199	0.429
Milk triacylglycerol									
	Early	3.7	7.6	8.2	11.4	1.98	0.227	0.011	0.577
	Mid	6. 9	10.3	11.6	7.6	1.74	0.424	0.354	0.649
	Late	6.7	6.4	4.9	8.4	2.28	0.632	0.655	0.590
Milk citrate									
	Early	1.46	1.09	0.98	0.75	0.38	0.556	0.714	0.853
	Mid	1.56	0.91	1.07	0.38	0.22	0.005	0.135	0.468
	Late	1.19	0.58	0.70	0.62	0.12	0.013	0.385	0.017

SEM = Standard error of the mean.

[1] P-values for the effects ; MFC =Misty-fan cooling effect, rbST = rbST effect, MFC x rbST = interaction effect of MFC and rbST

Table 6. Glucose metabolism in different metabolic pathway in the udder during rbST administration at different stages of lactation of Holstein cows housing in normal shade (NS) and shade plus misty-fan cooling (MFC).

Parameter	Stages of	NS		MFC			[1]Effect		
	lactation	Pre	rbST	Pre	rbST	SEM	rbST	MFC	rbSTxMFC
Flux through the pentose phosphate pathway calculated as ^{3}H incorporation into milk fatty acid (equivalent µmol of glucose/min):									
	Early	156.5	234.7	236.7	326.9	34.08	0.039	0.269	0.865
	Mid	212.7	294.2	300.0	344.1	64.36	0.358	0.25	0.779
	Late	421.3	412.9	376.0	282.9	68.33	0.479	0.415	0.552
Corrected ^{3}H incorporation into milk fatty acid (equivalent µmol of glucose/min):									
	Early	237.27	280.81	273.54	420.42	65.64	0.185	0.406	0.454
	Mid	280.22	352.92	406.02	551.58	77.09	0.195	0.096	0.649
	Late	537.57	572.53	472.91	420.03	103.24	0.933	0.501	0.682
Net metabolism of glucose 6-phosphate via the pentose phosphate pathway (µmol/min):									
	Early	70.1	102.68	97.13	143.49	18.04	0.060	0.336	0.713
	Mid	94.3	131.29	126.93	134.54	34.27	0.534	0.586	0.68
	Late	181.8	170.62	155.28	106.89	36.28	0.435	0.355	0.622
Net metabolism of glucose 6-phosphate via the pentose phosphate pathway (%):									
	Early	11.0	13.2	11.4	16.8	4.04	0.374	0.778	0.699
	Mid	13.1	14.2	16.5	14.9	4.24	0.544	0.347	0.406
	Late	12.5	8.7	9.7	6.2	2.60	0.195	0.409	0.952
Metabolism of glucose 6-phosphate via the galactose moiety of lactose (%):									
	Early	81.6	90.1	67.7	74.9	10.1	0.459	0.317	0.951
	Mid	87.1	87.4	97.2	78.3	16.3	0.583	0.972	0.571
	Late	56.0	42.7	58.9	58.3	11.2	0.553	0.515	0.588
Metabolism of glucose 6-phosphate via Embden-Meyerhof pathway (µmol/min):									
	Early	-115.8	-103.4	-197.5	-216.4	111.4	0.977	0.748	0.892
	Mid	-213.6	91.1	-345.9	-226.6	168.2	0.243	0.351	0.597
	Late	124.3	465.7	321.3	111.6	139.3	0.649	0.713	0.083
Metabolism of glucose 6-phosphate via Embden-Meyerhof pathway (%):									
	Early	-22.5	-42.1	-43.0	-25.9	11.4	0.914	0.949	0.146
	Mid	-25.6	-12.4	-61.1	-61.4	21.6	0.774	0.370	0.762
	Late	19.6	26.6	25.0	13.0	4.1	0.565	0.582	0.050

SEM = Standard error of the mean.

[1] P-values for the effects ; MFC =Misty-fan cooling effect, rbST = rbST effect, MFC x rbST = interaction effect of MFC and rbST

Table 7. Fatty acid composition of milk fat during rbST administration at different stages of lactation of Holstein cows housing in normal shade (NS) and shade plus misty-fan cooling (MFC).

Stages	Fatty acid	NS		MFC			[1]Effect		
of lactation	chain length	Pre	rbST	Pre	rbST	SEM	rbST	MFC	rbSTxMFC
Early lactation: (µmol/mL)									
	C6	0.97	1.30	0.53	1.79	0.20	0.004	0.937	0.051
	C8	0.37	0.68	0.95	0.84	0.11	0.373	0.060	0.080
	C10	0.81	1.40	2.06	1.67	0.19	0.582	0.041	0.030
	C12	0.88	1.47	2.20	1.75	0.15	0.670	0.021	0.009
	C14	4.16	5.59	6.74	6.47	0.45	0.231	0.040	0.094
	C16:0	16.67	21.64	22.38	25.35	1.30	0.016	0.131	0.464
	C16:1	0.17	0.67	0.66	0.82	0.08	0.004	0.065	0.079
	C18:0	5.72	4.92	4.00	5.68	0.40	0.304	0.586	0.014
	C18:1	11.94	12.05	8.91	16.89	1.58	0.037	0.605	0.033
	C18:2 trans	0.67	0.86	0.71	0.69	0.15	0.577	0.728	0.509
	C18:2 cis	1.64	0.91	0.80	0.86	0.38	0.408	0.325	0.327
	Total	45.25	50.26	49.93	62.81	2.93	0.016	0.195	0.217
Mid lactation: (µmol/mL)									
	C6	1.43	1.50	1.49	1.80	0.15	0.240	0.757	0.467
	C8	0.69	0.17	0.69	0.85	0.09	0.314	0.780	0.458
	C10	1.56	1.81	1.42	1.85	0.20	0.121	0.946	0.661
	C12	1.61	1.97	1.51	2.00	0.24	0.108	0.956	0.778
	C14	5.54	6.53	5.40	6.69	0.55	0.074	0.993	0.793
	C16:0	19.69	22.54	19.17	23.45	1.34	0.029	0.963	0.608
	C16:1	0.61	0.77	0.81	1.00	0.07	0.029	0.203	0.854
	C18:0	4.86	5.24	3.39	4.29	0.60	0.314	0.264	0.675
	C18:1	9.07	10.91	10.43	13.77	1.00	0.033	0.048	0.478
	C18:2 trans	0.16	0.14	0.13	0.20	0.02	0.195	0.679	0.016
	C18:2 cis	0.91	1.02	0.58	0.82	0.08	0.083	0.038	0.477
	Total	46.11	53.14	45.04	56.74	2.83	0.011	0.870	0.433
Late lactation: (µmol/mL)									
	C6	1.54	1.69	1.50	1.91	0.11	0.029	0.876	0.255
	C8	0.68	0.66	0.72	0.96	0.05	0.048	0.268	0.027
	C10	1.51	1.47	1.47	2.10	0.08	0.008	0.335	0.004
	C12	1.65	1.72	1.53	2.19	0.07	0.001	0.438	0.003
	C14	6.08	5.89	5.37	6.75	0.33	0.113	0.912	0.047
	C16:0	21.60	23.05	20.56	24.43	1.78	0.174	0.954	0.515
	C16:1	0.84	1.01	0.89	1.10	0.15	0.252	0.749	0.888
	C18:0	5.29	4.82	4.50	6.36	0.67	0.326	0.747	0.118
	C18:1	12.30	14.67	13.37	19.63	1.17	0.006	0.289	0.134
	C18:2 trans	0.21	0.20	0.23	0.27	0.02	0.478	0.371	0.135
	C18:2 cis	1.03	0.95	0.86	1.24	0.12	0.229	0.686	0.081
	Total	53.10	55.76	51.89	66.05	4.03	0.070	0.553	0.191

SEM = Standard error of the mean.

[1] P-values for the effects ; MF =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

Table 8. NADPH production for fatty acid synthesis in the udder during rbST administration at different stages of lactation of Holstein cows housing in normal shade (NS) and shade plus misty-fan cooling (MFC).

Parameter	Stages of lactation	NS		MFC		SEM	[1]Effect		
		Pre	rbST	Pre	rbST		rbST	MFC	rbSTxMFC
Requirement of all NADPH for fatty acid synthesis (µmol/min)	Early	1134	1761	1920	2067	134	0.020	0.203	0.112
	Mid	1235	1698	1428	2150	181	0.011	0.539	0.495
	Late	1240	1460	1154	2062	150	0.006	0.481	0.052
Requirement of all NADPH formation from glucose via the pentose phosphate pathway (%)	Early	16.7	18.5	19.5	24.9	3.14	0.286	0.482	0.594
	Mid	25.6	26.5	31.6	25.6	7.36	0.744	0.667	0.651
	Late	35.4	25.6	26.5	17.8	5.16	0.112	0.255	0.925

SEM = Standard error of the mean.

[1] P-values for the effects ; MFC =Misty-fan cooling effect, rbST = rbST effect, MFC x rbST = interaction effect of MFC and rbST

Table 9. 3H/14C ratios in plasma glucose and related products during rbST administration at different stages of lactation of Holstein cows housing in normal shade (NS) and shade plus misty-fan cooling (MFC).

Parameter	Stage of lactation	NS		MFC		SEM	[1]Effect		
		Pre	rbST	Pre	rbST		rbST	MFC	rbSTxMFC
Plasma glucose:									
	Early	0.76	0.81	0.86	0.82	0.08	0.973	0.393	0.589
	Mid	0.80	0.82	0.74	0.68	0.04	0.653	0.217	0.317
	Late	0.80	0.76	0.84	0.68	0.04	0.029	0.781	0.176
Milk lactose:									
	Early	0.83	0.73	0.65	0.74	0.06	0.930	0.104	0.173
	Mid	0.88	0.88	0.73	0.70	0.08	0.831	0.152	0.869
	Late	0.72	0.6	0.71	0.71	0.08	0.493	0.539	0.471
Milk galactose:									
	Early	0.86	0.87	0.60	0.66	0.07	0.576	0.047	0.715
	Mid	0.83	0.93	0.72	0.73	0.11	0.638	0.231	0.677
	Late	0.64	0.64	0.67	0.74	0.11	0.732	0.472	0.759
Milk triacylglycerol:									
	Early	1.43	2.14	2.74	1.64	0.60	0.852	0.176	0.076
	Mid	3.51	2.93	3.70	1.79	0.72	0.124	0.721	0.381
	Late	2.45	2.61	3.12	1.90	0.50	0.327	0.976	0.206
Milk citrate:									
	Early	0.86	0.74	0.78	0.81	0.05	0.410	0.964	0.189
	Mid	0.98	0.86	0.87	0.86	0.06	0.291	0.463	0.367
	Late	0.81	0.86	0.87	0.78	0.04	0.640	0.899	0.135

SEM = Standard error of the mean.

[1] P-values for the effects ; MFC =Misty-fan cooling effect, rbST = rbST effect, MFC x rbST = interaction effect of MFC and rbST

DISCUSSION

In the present study, the values of THI in NS and MF barns in either morning or afternoon, were always higher than critical value (THI 72) for lactating dairy cows in both barns (Smith *et al.* 2006). Animals were therefore always subjected to moderate heat stress throughout experimental periods (i.e. THI = 78.1 to 85.5). Thus the effect of misters and fans for cooling animals in the present study was not sufficient to completely eliminate heat stress. The values of THI might not accurately reflect heat stress when using a mister and fan system for evaporative cooling that result in higher humidity but also cause cooling. Although the cooling effect using the misty-fan system was not sufficient to adequately reduce THI in the barn, there is a beneficial effect as indicated by a lower RR and RT in cooled cows and also higher milk yield throughout lactation. These results support the study of Fike *et al.* (2002) that housing cows during the day with fans and sprinklers effectively reduced heat stress as indicated by lower body temperature and respiration rate. In the present study, an increase in milk yield of rbST-supplemented cows was accompanied by an increase in both RT and RR in comparison with cows without rbST supplementation in both cooled and non-cooled cows throughout the experimental periods. The observation for an increase in heat production during rbST supplementation agrees with the reports of West *et al.* (1991) and West (1994) that rbST-treated cows in a hot environment may increase heat production in high and lower milk producing cows.

It is known that dairy cattle adapt to high temperatures with variety of hormonal and metabolic responses, one of which may involve changes in the process of milk synthesis in the mammary gland. Milk yield, milk compositions and its secretion during rbST administration in the experiment is shown in Table 2. It is obvious that administration of rbST to cooled and non-cooled cows increased milk yield, which was significantly higher than that of the pretreatment period. Milk yield initially showed significant increases in early lactation of cooled and non-cooled cows either supplemental rbST or not and it decreased as lactation advances. These findings confirm that an increase in milk yield in response to rbST administration will not be sustained indefinitely (Bauman 1992), and 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 response to somatotropin for whole

lactation might be reduced if treatment begins very early in lactation (Bauman &Vernon 1993; Burton *et al*. 1994).

It is known that glucose is an important precursor of milk constituents and energy source for the lactating mammary gland. Milk production requires glucose for synthesis of lactose which is essential for milk secretion and glucose moiety of lactose arises directly from plasma glucose (Ebner & Schanbacher 1974). An increase in milk yield without an alteration of the plasma glucose concentration during supplemental rbST in both cooled and non-cooled cows indicates that this requires a substantial increase in supply of glucose to the mammary gland. An increase in mammary blood flow is a factor for glucose uptake by the mammary gland (Linzell 1973), which the rate of mammary plasma flow of cows supplemental rbST significantly increased ($P<0.05$) as compared with the pretreatment period. However, in the present study, an increase in mammary plasma flow during rbST supplementation in each stage of lactation would not be a major determinant in the mediation of nutrient delivery and uptake by the mammary glands for increase in milk production throughout lactation. Local changes for biosynthetic capacity within the mammary gland would be a factor in identification of the utilization of substrates in the rate of decline in milk yield with advancing lactation (Chaiyabutr *et al*. 2005).

Effects of supplemental rbST and cooling on glucose kinetics are shown in Table 3. Plasma glucose concentrations in both cooled and non-cooled cows maintained over a wide range at different stages of lactation. It indicates that steady state conditions between the rate of gluconeogenesis and the rate of utilization of glucose existed in the body pool of glucose in both groups. However, it has been reported that the plasma glucose concentration would increase during injection of bovine somatotropin in cows with low milk yield but not in cows with high milk yields (Bines et al, 1980). The difference in response in terms of changes in plasma glucose level may reflect the differences of utilizations for lactose synthesis between high yielding and low yielding cows.

The reversible turnover rate of [3-^{3}H]glucose (the total glucose entry rate) and the irreversible turnover rate of [U-^{14}C] glucose (the utilization rate of glucose) of cooled cows without rbST were slightly higher than those of non-cooled cows in all stages of lactation. It is probably that the turnover rate of glucose correlated positively with a higher milk yield in cool cows. However, administration of rbST showed non-

significant changes in both glucose entry and utilization rates in comparison with those of pretreatment periods in both cooled and non-cooled cows throughout lactation. During studies, both cooled and non-cooled cows with or without supplemental rbST were fed TMR diet to satisfy requirements for metabolizable energy and the body weights increased stepwise throughout lactating periods. It is possible that both cooled and non-cooled cows were in positive energy balance, since irreversible losses of glucose has been shown to increase in cows with negative energy balance (McDowell et al.,1987). The reversible turnover rate of [3-^{3}H]glucose represents the total glucose turnover rate as the ^{3}H is not recycled from products of partial glucose degradation (Katz *et al*.1965). Thus, recycling of glucose-C was estimated by simultaneous injection of [3-^{3}H]glucose and [U-^{14}C]glucose as in the present studies in cooled and non-cooled cows, which showed no differences between the pretreatment and rbST treated period in all stages of lactation. These findings suggest that a constant level of tricarbon units originally derived from glucose being again reincorporated into glucose, which was not affected by either cooling or the supplemental rbST.

The utilization of glucose across the mammary gland during supplemental rbST in both cooled and non-cooled cows at different stages of lactation are complex regulatory mechanisms, It would depend both on the partitioning of blood flow between extra-mammary tissues and local regulation. The present results for the mammary uptake of plasma glucose in both groups are not based on changes in A-V concentration differences and extraction ratio of glucose. An increase in the rate of blood flow to the mammary gland during supplemental rbST in both cooled and non-cooled cows, would be a major determinant of the rate of glucose uptake by the mammary gland. In all stages of lactation, the net mammary glucose uptake increased approximately 8-48% during supplemental rbST as compared with the pretreatment period in both groups. Glucose extracted by the mammary gland has several possible metabolic fates in mammary epithelial cells that may occur at another level than transmembrane transport (Xiao and Cant, 2003). The glucose uptake by the mammary gland during supplemental rbST and cooling was rate limiting for the transport of glucose to the mammary cell. The high blood flow to the mammary gland during supplemental rbST would decrease the transit time of glucose, thereby reduction for

prolonging the contact time between glucose in blood and glucose transporter in mammary epithelial cell (Chaiyabutr *et al*. 2007b).

It is known that glucose is an important intermediary of metabolism for the biosynthesis of lactose, triacylglycerol and citrate by the mammary gland. The bovine mammary gland cannot synthesize its own glucose because of lacking of glucose-6-phosphatase (Scott et al., 1976). Glucose plays a crucial role in their metabolism and lactose synthesis, which is formed in Golgi vesicles from a combination of glucose either directly or after phosphorylation to glucose 6-phosphate and conversion to UDP-galactose (Ebner & Schanbacher, 1974). The calculated amount of metabolism of glucose 6-phosphate to the galactose moiety of lactose during supplemental rbST in both cooled and non-cooled cows in different stages of lactation would be sufficient to account for the cytosolic lactose synthesis.The utilization of glucose carbon incorporation to lactose in the udder increased in both early and mid lactation but not for late lactation during supplemental rbST in both cooled and non-cooled cows. The decrease in the metabolism of glucose 6-phosphate to the galactose moiety of lactose as lactation advanced to late lactation in both cooled and non-cooled cows would affect to the lactose synthesis and milk production in both groups. A low enzymatic activity for lactose synthesis might be expected to appear as lactation advances. According to Davis and Bauman (Davis & Bauman 1974), 50 to 60% of the glucose in the glucose-6-phosphate pool is converted into galactose. Major part of the galactose has been shown to derive from mammary extracted glucose, as well as from glycerol and other metabolic pathways. However, glucose is not the sole carbon source for lactose synthesis but remains the main one. An increase in the glucose concentration in milk representing an increase in glucose concentration in the mammary epithelial cell during prolonged treatment of rbST has been noted (Chaiyabutr *et al*. 2008a).

It is known that 80–85% of lactose carbon atoms arise from glucose (Faulkner and Peaker, 1987; Bickerstaffe et al., 1974). The quantitative utilization of the glucose taken up by the mammary gland is used directly in the synthesis of lactose, while the remaining of extracted glucose can participate in the supply of ATP (Embden-Meyerhof pathway and the tricarboxylic acid cycle), other portions would be metabolized via the pentose phosphate pathway, In the present studies, glucose 6-phosphate metabolized via the pentose phosphate pathway by average 10-17% throughout lactation in both cooled and non-cooled cows without rbST, while it

increased from 13 % in pretreatment to 15 % on early and mid-lactation but it decreased in the late lactation after supplemental rbST (Table 6). These results also agree with prolonged treatment of rbST in crossbred HF cows showing that percentage values of glucose 6-phosphate metabolized via the pentose phosphate pathway were variable in different stages of lactation (Chaiyabutr et al., 2008b). These values are different in comparable to those obtained previously in the isolated perfused udder of cow by Wood and co-workers (1965), in which about 23- 30% of the glucose was metabolized via the pentose phosphate pathway. It is probable that no consideration of the recycling of glucose 6-phosphate metabolized via the pentose cycle in the udder with the consequent loss of ^{3}H from glucose 6-phosphate (Davis & Bauman 1974). However, the net proportion of the metabolism of glucose 6-phosphate via the pentose cycle pathway was increased during supplemental rbST at early stage of lactation of cooled and non-cooled cows. 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 ^{3}H in the present experiments. In the present studies, estimates of the contribution of the pentose phosphate pathway in providing NADPH for fatty acid synthesis *in vivo* have been estimated by based on the assumption that all the glucose that was oxidized to CO_2 was metabolized via the pentose phosphate pathway. High metabolism of glucose 6-phosphate in early lactation of rbST treated cows 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 supplementation.

The utilization of glucose carbon by the mammary epithelial cell for the synthesis of lactose, citrate and triacylglycerol are shown in Table 5. Absolute amount of glucose carbon incorporation to milk lactose was significantly higher during supplemental rbST in early and mid lactation in both cooled and non-cooled cows. As lactation advances of both cooled and non-cooled cows without rbST, high values of both the proportion and absolute amount of glucose carbon incorporation to milk citrate and milk triacylglycerol were apparent, which would be evidences supporting an increase in proportion of glucose 6-phosphate metabolized via the Embden-Meyerhof pathway and was oxidized in the tricarboxylic acid cycle. During supplemental rbST in each stage of lactation, both the proportion and absolute amount of glucose carbon incorporation to milk triacylglycerol were increased, while both the

proportion and absolute amount of glucose carbon incorporation to milk citrate were decreased. These changes can be interpreted in terms of metabolic shifts that are occurring within the mammary epithelial cell, and one might speculate that such changes reflect the high flux of the utilization of glucose carbon by the mammary epithelial cell through the rate of lactose synthesis and milk production during supplemental rbST. In addition to the use of glucose carbon for milk fat synthesis, the hydrogen from glucose has been shown to be incorporated into milk fat in early and mid lactation in both cooled and non-cooled cows supplemental rbST(Table5),although studies *in vitro* have shown that fatty acid synthesis could occur from the utilization of acetate in the perfused goat udder (Hardwick *et al.* 1963). It has been known that milk fat is synthesized from fatty acids of both blood lipids and from de novo synthesis within the mammary epithelial cells. However, an increase in milk fat after rbST supplementation was associated with the increased yield of long-chain fatty acids characteristic of plasma free fatty acids and body fat. Significant increases in plasma free fatty acids in rbST-treated cows have been published elsewhere (Chaiyabutr *et al*. 2007). Thus, the lipolytic activity would be a function of bST treatment per se in stead of the associated changes in energy balance.

Glucose can also participate in the milk fat formation, by supplying the glycerol (triose phosphate pathway) and the NADPH essential to elongating milk fatty acids (pentose phosphate and isocitrate dehydrogenase pathways). However,very marginally, less than 11% of glucose could supply carbon atoms for the synthesis of milk triacylglycerol in either supplemental rbST or cooling. Data findings in Table 8 provide evidence that 17% to 35 % of the NADPH required for fatty acid synthesis *de novo* in the udder of cooled and non-cooled cows without rbST arose from glucose metabolism, while 18% to 27% of the NADPH was required during rbST supplementation. 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 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 & 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).

. Metabolism of glucose 6-phosphate via the pentose phosphate pathway usually loss of all ^{3}H from [3-^{3}H]glucose in lactating cows. During lactation, a higher level of $^{3}H/^{14}C$ ratio in milk triacyglycerol (Table 9) was due to an increase in disequilibrium of the triose phosphate isomerase reaction occuring in the udder of crossbred animals ,which needs to be further investigated. Tritium and carbon-14 in glucose molecule were also shown to be incorporated into milk citrate which provided by averaged 22 μmol/min (16.5-.25.5) in cooled and non-cooled cows without rbST and provided by averaged 16 μmol/min(8.1-21.2) for the carbon skeleton of citrate during rbST supplementation in both groups. 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 may have different mechanisms with a common pool of cytosolic NADPH between cows without rbST and cows supplemental rbST. Significant increases in the concentration of FFA in milk were apparent in cooled and non-cooled cows supplemental rbST as compared with the pretreatment period in each stage of lactation (Table 7). A similar result for an increase in milk fat content due to prolonged administration of rbST has also been observed previously (West *et al*. 1991 Chaiyabutr et al., 2008b). It has been known that milk fat is synthesized from fatty acids of both blood lipids and from de novo synthesis within the mammary epithelial cells.

In conclusion, the data presented here represent the estimation *in vivo* of glucose metabolism in the mammary gland and its distribution to lactose synthesis, the pentose phosphate pathway and the Embden-Meyerhof pathway by the effects of supplemental rbST and cooling in 87.5% HF animals. The rbST exerts its galactopoietic action, in part, association with an increase in mammary blood flow, which partitions the distribution of glucose to the mammary gland. The stimulant effect for milk yield by supplemental rbST was transiently and the glucose turnover

rate was not significantly increased as compared with pre-treatment period in all stages of lactation. It indicates that rbST induced enhancement of milk yield in all stages of lactation, which would be compensated by mobilization of body energy reserves (i.e. plasma free fatty acids) to the extent of the elevated energy requirements for supporting the increased milk production. During early and mid lactation, the glucose taken up by the udder of both cooled and non-cooled cows without supplemental rbST, an average 13% and 23% were metabolized in the pentose phosphate pathway and contributed to NADPH production, respectively. During supplemental rbST, the glucose taken up by the udder of both cooled and non-cooled cows, an average 15% and 24% were metabolized in the pentose phosphate pathway and contributed to NADPH production, respectively. An increased flux of the sufficient pool of intracellular glucose 6-phosphate during early and mid lactation came across through the lactose synthesis and pentose cycle pathway during rbST supplementation. On late lactation of cooled and non-cooled cows, the glucose taken up by the udder were metabolized in the pentose phosphate pathway by averaged from 11% to 7.4% and contributed to NADPH production from 30% to 22% after supplemental rbST. In the present study, mammary plasma flow was significantly increased after rbST supplementation, while milk yield of rbST-treated cows was not significantly greater than that of pretreatment in late lactation. It would appear that a larger proportion of the glucose 6-phosphate is metabolized via Embden-Meyerhof pathway in late lactation[1] The present results indicate that the regulation of biosynthetic capacity within the mammary gland would be influenced more by local than by systemic factors in identification of the utilization of substrates in the rate of decline in milk yield with advanced lactation.

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Chapter V (Series 5.2)

Effects of cooling and recombinant bovine somatotropin supplementation on body fluids, mammary blood flow, and nutrients uptake by the mammary gland in different stages of lactation of crossbred Holstein

Effects of cooling and recombinant bovine somatotropin supplementation on body fluids, mammary blood flow, and nutrients uptake by the mammary gland in different stages of lactation of crossbred Holstein cattle

INTRODUCTION

Many factors affect milk production in dairy cattle in tropical areas, e.g. lower genetic potential for milk production in indigenous cattle including high environmental temperature and humidity. During exposure to high temperature, lactating cows require more free water than non-lactating cows, since milk production contains about 87% of water (Murphy, 1992). During lactation, cows increase in blood volume (Chaiyabutr et al., 1997) and cardiac output (Hanwell and Peaker, 1977). These changes would account for an increase in circulatory distribution including the blood supply to the mammary gland. The lactating mammary gland receives other signals from the rest of body in form of nutrient and hormones from blood during milk synthesis (Forsyth and Hayden, 1977). Higher milk production is believed to associate with changes in the uptake of substrates across the mammary gland, which is dependent on substrate concentrations in blood and mammary blood flow (Handerson and Peaker, 1983). The study in 87.5% HF animal has been shown that a shorter persistency of milk yield during the transition period from early to mid lactation accompanied with decreases in both blood flow to the mammary gland and the level of plasma bovine somatotropin (bST) (Chaiyabutr et al., 2000a). These decreases could contribute to a reduction in milk yield. It is not known which factors are the cause and which factors are the effects for such a reduction and whether a low level of bST of cows will decrease the metabolic rate and heat production during exposure to high temperatures (Tyrrell et al., 1988). Short persistency of lactation is occurred in 87.5% HF animals, whether by the effect of high ambient temperature or by the less stimulant effect of bovine somatotropin or combination of both of these factors during lactation advances. However, the control mechanism for milk production in different stages of lactation in crossbred dairy cattle has not been fully elucidated, although

mammary blood flow has been known to be a major determinant for the rate of substrate supply for milk synthesis (Davis and Collier, 1985).

Many studies have been done in attempting to improve dairy productivity by management strategies. The modification of surrounding environmental to reduce the impacts of high temperature has reported to increase milk production, for example water spray with fans (Fike et al., 2002), or evaporative cooling system (Chan et al., 1997; Chaiyabutr et al., 2008). However, there is less information concerning the profitability of efficient utilization of environment modification for dairy production in crossbred cattle. Body water is known to play a central role in the mechanism of heat dissipation including the process of lactation. Greater water retention during rbST administration would not only provide a greater reservoir of soluble metabolites for biosynthesis for milk, but it may be useful in slowing down the elevation in body temperature during heat exposure. It is known that the rate of milk production depends on function of number of mammary secretory cells and their metabolic activity. In view of an increase in total body water in recombinant bST-treated cows has been reported (Chaiyabutr et al. 2007a). It is necessary to study whether rbST supplementation in cows in high temperatures will minimize the effects of heat stress and whether increase in MBF will delivery of nutrients to the mammary gland to sustain the potentially increased milk yields. Bovine somatotropin is known as a homeorrhetic hormone connected with growth and lactation in ruminant is well established (Bauman, 1992). Few data are available for the additive effects of cooling and supplemental recombinant bovine somatotropin (rbST) in responsible for the short persistency of milk yield in crossbred Holstein cattle. Therefore, the aim of the present study was conducted to determine the differences of lactation physiology between cooled and non-cooled cows after rbST supplementation. The patterns of nutrients uptake by measuring body fluid, mammary blood flow and combining these with measurements plasma arterial concentrations of nutrients and arterial-venous concentration differences for the mammary uptake of nutrients during rbST supplementation in 87.5% HF cows under misty-fan cooling system were performed.

MATERIALS AND METHODS

Animals and management

Ten primiparous, crossbred 87.5% Holstein cows were selected for the experiment. Cows were randomly divided into 2 groups as control (n=5) and experimental groups (n=5). Animals in the control group were housed in the normal shade barn (NS), while cows in the experimental group were housed in normal shade plus misty-fan cooling system (MFC). The open space cooling system consisted of two sets of misty fan, which each system consisted of a 26 inch diameter blade fan circulating 7,200 ft^3/min of air, with oscillation coverange of 180°. The amount of water discharged from 4 spray heads was 7.5 L/hr and side of mist droplet 0.01 mm. Animals were exposed to MFC for 45 minutes at 15-minute intervals from 0600h to 1800h. At night, animals were exposed to MFC for 15 minutes at 45- minute intervals from 1800h to 0600h. Three consecutive periods of study were carried out in each group, consisting 60-95 days postpartum (early-lactation), 120-155 days postpartum (mid-lactation), 180-215 days postpartum (late-lactation). Cows in both groups were housed in tie stall barns and offered a total mixed ration (TMR) twice a day in equal portion, around 06.00 and 17.00. TMR samples were collected once a week throughout the whole experimental period and pooled. Feed values of TMR were calculated on the basis of the chemical composition of the ingredients, determined according to AOAC. The diets were fed as the same ration of TMR throughout the experiment. Each day, the diets were given when cows were milked. Water is available at all time. All animals were weighed monthly throughout the experiment.

The study was performed under a protocol approved by ethic committee of Faculty of Veterinary Science. The procedures used in the present study were carried in accordance with the principles and guidelines of Faculty of Veterinary Science, Chulalongkorn University, followed National Research Council of Thailand protocol.

Experimental protocol

Each cow in the control group was performed by the pretreatment study without rbST (NS), and the treatment study with rbST (NS + rbST). In the experimental group, cows in shade plus MFC without rbST injection (MFC) and treatment with rbST injection (MFC + rbST). The pretreatment periods of both groups were performed on days 60, 120, and 180 of early, mid, and late lactation, respectively. The studies at treatment periods were carried out at week 4 after the pretreatment study in each stage of lactation. Three consecutive subcutaneous

injections of 500 mg of rbST (The rbST was suspended in 792 mg of a prolonged-release formulation of sesame oil, POSILAC, Monsanto, USA) were performed in every 2 weeks interval after pretreatment). Thereafter, within 2 days after the third injection, the treatment study was conducted. The pretreatment, 3 doses of injections, and the treatment periods were performed during the first 30 days and the same procedures were followed for each stage. During the last 30 days of each lactating stage, no experiments were conducted in order to allow the milk yield from the effect of rbST treatment to return to the control level (Kirchgessner et al 1991). Rectal temperature and respiration rate of individual cow were determined at the same time as recording ambient temperature. Ambient temperature and humidity were measured weekly throughout the experiment. The temperature humidity index (THI) was calculated by THI = 0.72 (wb+db) + 40.6 where; wb = wet bulb temperature (ºC), db = dry bulb temperature (ºC) (McDowell, 1972).

On each specified day of each lactating period, at around 9.00 h, milk vein and ear vein were catheterized with non-radiopaque intravenous catheters, gauge 18G (Surflo, Terumo Europe N.V., Belgium) under local anesthesia for measurements of mammary blood flow through half of the udder and body fluids, respectively. Blood samples were collected from the coccygeal artery and milk vein by venipuncture with a # 21 needle into heparinized tubes. Blood samples were kept in crushed ice and then centrifuge at 3000 rpm for 30 min at 4 ºC. Arterial and venous plasma samples were collected and frozen at -20 ºC in aliquots until time of assays for measurements the level of metabolites.

Mammary blood flow measurement

The measurement of the mammary blood flow through half of the udder was performed at around 1100-1200h on the specified day. The duplicated measurements were done 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, the dye solution (100 mg/L of dye (T-1824) in sterile normal saline) was infused into the milk vein by a peristaltic pump (Gilson Medical Electronics) at a constant rate of 100 ml/min for 30 seconds. About 10 second after starting the infusion, 10 ml of adequate mixing of dye with 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 interval for calculation of blood flow of half of the udder. 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.

Body fluid measurements

At around 1300h on each specified day, intravenous injection with solutions containing 20 mL of sodium thiocyanate solution (10 % in normal saline), 20 mL of the 0.5 % Evans blue dye (T-1824, E. Merck, Darmstadt, Germany) and 1 mL of a single dose of 3000 μCi/ animal of carrier-free tritiated water were performed via an ear vein catheter for estimation of extra cellular fluid (ECF) volume, the plasma volume and total body water (TBW) respectively. Venous blood samples from the jugular vein were taken at 20, 30, 40 and 50 min after dye injection for ECF and plasma volume determination. Blood samples were subsequently collected at 1, 2, 3, 4, 5, 6, 7, 18, 24, 36, 48, 56 and 68 hour subsequent to the injection of tritiated water (^3H_2O) for determination of TBW. Total body water (TBW) was determined in each animal by dilution techniques using tritiated water as previously described (Chaiyabutr et al., 1997). TBW = (standard count (dis/min)×dose (ml))/(radio activity counts at zero time (dis/min)). The concentration of sodium thiocyanate in plasma was performed by the method of Medway and Kare (1959) for estimation of ECF volume. Blood volume was calculated from the plasma volume and packed cell volume (Chaiyabutr et al., 1980).

Metabolites determination

Both coccygeal arterial (A) and milk vein plasma (V) samples were determined for the plasma glucose concentration which was measured by using enzymatic oxidation in the presence of glucose oxidase (Human GmBH, Germany). The plasma concentration of acetate was assayed by the acetic acid UV-method (R-Biopharm, Darmstadt, Germany). Plasma β-hydroxybutyrate concentrations were determined by using an enzymatic reaction in the presence of β-hydroxybutyrate dehydrogenase (R-Biopharm, Darmstadt, Germany). Plasma free fatty acids were determined by colorimetry after plasma extraction with chloroform, heptane and methanol and TAN solution (Wang et al.,2004). Plasma triacylglycerol concentration was determined by

enzymatic colorimetric test (Triglyceride liquicolor, Wiesbaden, Germany). Mammary uptake of metabolites and extraction of metabolites by the mammary gland were calculated as follows; Mammary uptake = mammary plasma flow×arteriovenous differences (A-V); Mammary extraction = (A-V)/A.

Statistical analysis

Data were adjusted for covariate effects. The statistic analyses were performed using General Linear Model procedures of statistical software package SPSS (SPSS for windows, V14.0; SPSS Inc., Chicago, IL, USA). The model used for each analysis was:

$$Y_{ijk} = \mu + A_l + H_i + A(H)_{il} + B_j + (HB)_{ij} + A(HB)_{ijl} + Cov_k + e_{ijkl}$$

Where Y_{ijk} = observation, μ = overall mean, A_l= Animal effect H_i= house effect as main plot (i = NS, MF), $A(H)_{il}$ = main plot error (animal l in house i), B_j = treatment effect (rbST) as a split plot (j = with and without rbST supplementation), $(HB)_{ij}$ = interaction effect between treatment and house , $A(HB)_{ijl}$ = split plot error (animal l in house i and treatment j), Cov_k = covariate effect and e_{ijk} = residual error.

Means values were used to evaluate the effect for all variables. Statistical significances for respiratory rate and rectal temperature among treatments in each stage of lactation were also analyzed by a similar model, but the covariate effect was not included. Duncan's new multiple range tests were used to detect the statistical significance different among treatment groups. The statistical significant differences of environmental parameters between NS and MFC barn was determined by unpaired t-test.

Results

Ambient temperature, relative humidity, temperature humidity index (THI) respiratory rate and rectal temperature

Mean values of measurements at experimental site during periods of studies for daily temperatures, humidities, THI, the rectal temperature and respiratory rate are shown in Figure1 and Figure2. Average values of ambient temperature in the barn during the daytime (1400 hours) at NS were significantly higher than that of MFC. The relative humidity in MFC was significantly higher than that of NS barn. THI

values at the MFC barn were lower in comparison with NS barn. Cows in both groups exposed to high THI values (80.7 to 85.5) in both barns. Rectal temperature of cooled and non-cooled cows were significant different whether rbST injection or not. Cows housing under MFC barn showed lower rectal temperature than cows housing under NS barn during afternoon (1400 hours). There were increases in rectal temperature and respiration rate by the effect of supplemental rbST during the daytime. The cooled cow showed significantly lower respiratory rate than those of non-cooled cows throughout experimental periods.

Total body water (TBW), extracellular fluid (ECF), plasma volume (PV), blood volume (BV) and packed cell volume (Hct)

The supplementation of rbST markedly increased both the absolute values and relative values as percentage of body weight of TBW, ECF, PV and BV in each stage of lactation (Table 1). The cooling system did not affect TBW, ECF, PV and BV in absolute value or relative value as percentage of body weight. The packed cell volume was not affected by the supplementation of rbST in both cooled and non-cooled cows.

Change in milk yield, mammary blood flow and body weight

The milk yield, rate of mammary blood flow, plasma flow and body weight in cooled and non-cooled cows are shown in Table 2. It is obvious that both cooled and non-cooled cows supplemental rbST increased milk yield, which was significantly higher than that of the pretreatment period, but it decreased as lactation advances. It is obvious that both cooled and non-cooled cows supplemental rbST increased mammary plasma flow and mammary blood flow, which were significantly higher than those of the pretreatment periods. The ratio of mammary blood flow to the rate of milk yield was not affected by the supplementation of rbST in both groups. The body weights of both cooled and non-cooled cows were increased stepwise as lactation advances whether supplemental rbST or not.

Arterial plasma concentration, A-V concentration differences, mammary extraction and mammary uptake of glucose, acetate and β–hydroxybutyrate

The mean arterial plasma concentration for glucose were largely unchanged throughout periods of study in both cooled and non-cooled cows whether supplemental rbST or not (Table 3). There were no significant changes in A-V

concentration differences and mammary extractions for glucose across the mammary gland throughout the stage of lactation. During rbST supplementation mammary glucose uptake increased in each stage of lactation in both cooled and non-cooled cows. There were significant increases of mammary glucose uptake in rbST treated animals in mid and late lactation. The arterial plasma acetate concentration were unchanged throughout experimental periods in both groups of animals whether supplemental rbST or not. There were no significant changes in A-V concentration differences and mammary extraction for acetate across the mammary gland throughout the stage of lactation. During rbST supplementation, mammary acetate uptake was unaltered as compared with the pretreatment in each stage of lactation in both cooled and non-cooled cows. The means arterial plasma concentration for β–hydroxybutyrate were unchanged between cooled and non-cooled cows whether supplemental rbST or not in each stage of lactation. The A-V differences, mammary extraction and the mammary uptake for β–hydroxybutyrate were not influenced by the supplementation of rbST in both cooled and non-cooled cows.

Arterial plasma concentration, A-V concentration differences and mammary uptakes of free fatty acid and triacylglycerol

The arterial plasma fatty acid concentrations were increased significantly during mid and late lactation of cows supplementation with rbST in cooled and non-cooled cows (Table 4). There were no significant changes in A-V concentration differences, mammary extraction and the mammary uptake of fatty acid across the mammary gland in early and mid lactation, but there were significantly higher in late lactation after rbST supplementation. The mean arterial plasma concentration, A-V differences and mammary extraction for triacylglycerol showed no significant differences between cooled and non-cooled cows whether supplemental rbST or not in each stage of lactation. The mammary uptake of triacylglycerol in cows supplemental rbST had tendency to increase, but a significant increased were apparent in mid and late lactation in both cooled and non-cooled cows.

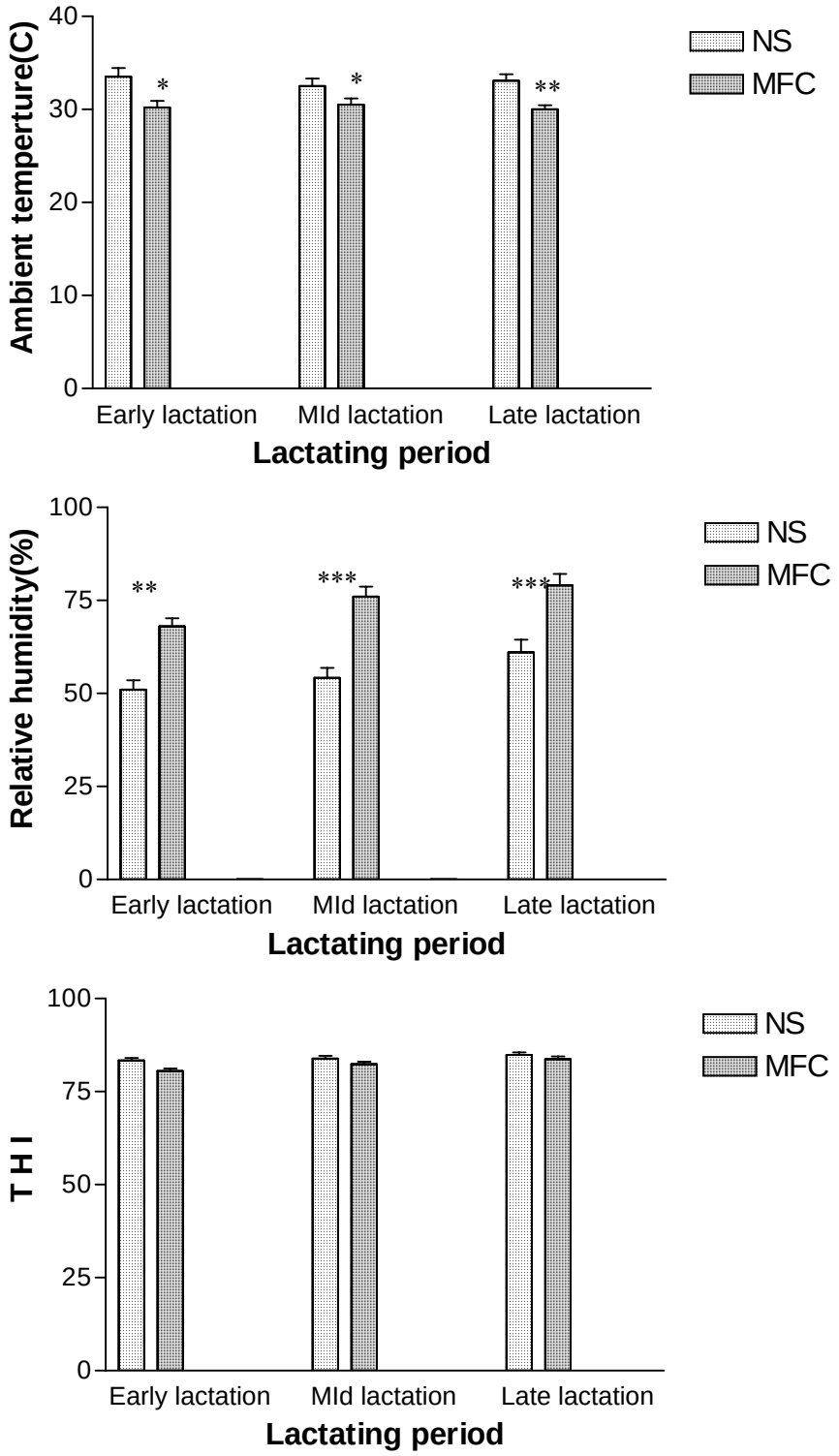

Figure1. Ambient temperature, relative humidity and temperature humidity index (THI), measuring at 1400h in normal shade (NS) barn and NS barn with misters and fans (MFC) at different stages of lactation**.** (Unpaired t-test, * $P<0.05$; ** $P<0.01$; *** $P<0.001$).

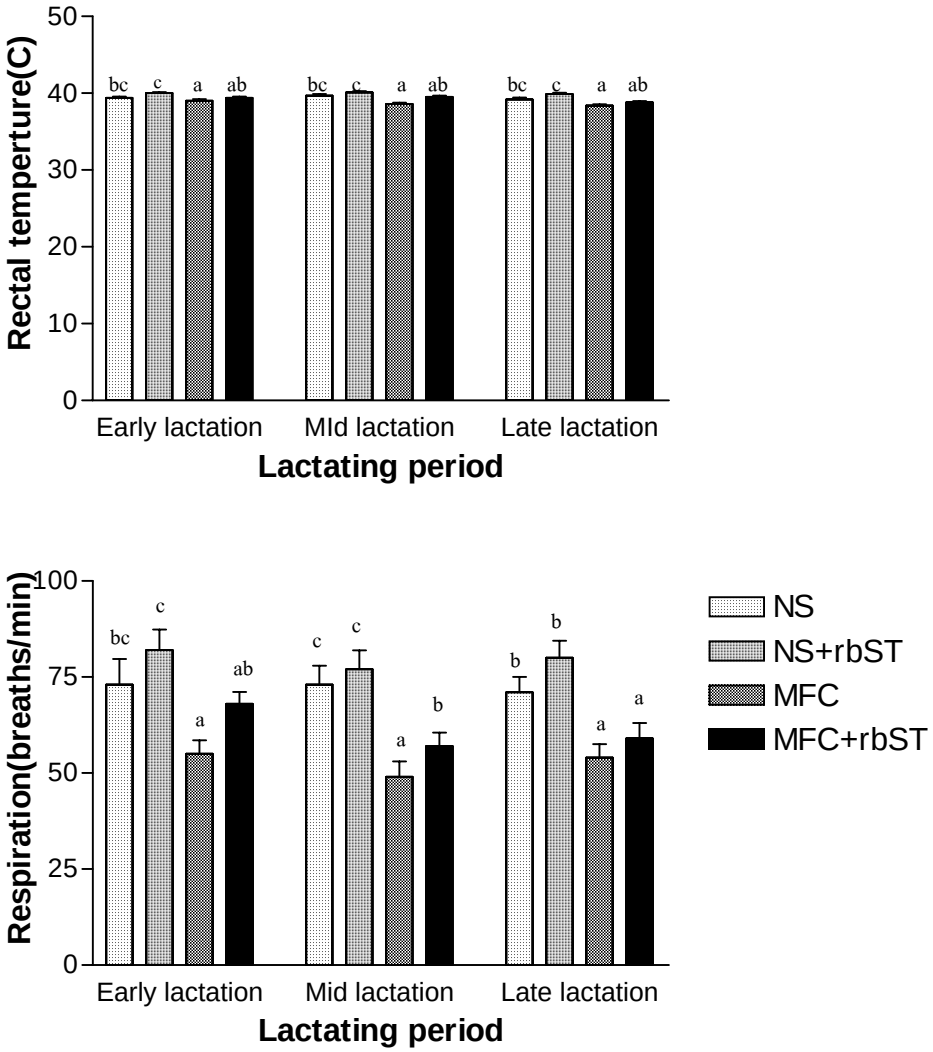

Figure 2. Rectal temperature and respiration rate measurement at 1400h in cows treated with rbST housing under NS and MFC barn at each stage of lactation**.** (Duncan'test; means in each stage of lactation with different superscripts (a.b,c) differ significantly, $P<0.05$)

Table 1. Total body water (TBW), extracellular fluid (ECF), Plasma volume (PV), blood volume (BV) and packed cell volume (PCV) in animals treated housingwith rbST under normal shade (NS) and misters and fans cooling (MFC) at different stages of lactation.

	Stages of	NS		MFC			[1]Effect		
Parameters	lactation	Pre	rbST	Pre	rbST	SEM	MFC	rbST	MFC*rbST
TBW	Early	254.4	295.9	277.7	309.2	6.23	0.273	0.001	0.441
(L)	Mid	262.0	303.1	272.0	326.9	6.97	0.336	0.001	0.352
	Late	269.1	320.4	286.9	327.4	10.65	0.467	0.003	0.624
TBW	Early	71.6	78.1	74.8	82.8	2.48	0.369	0.019	0.764
(L/100kg)	Mid	68.6	79.4	71.2	79.8	2.19	0.624	0.002	0.657
	Late	67.3	81.7	67.8	78.4	2.74	0.641	0.002	0.501
ECF	Early	92.87	106.4	108.1	123.2	3.00	0.029	0.001	0.805
(L)	Mid	103.1	114.5	119.1	126.6	4.52	0.050	0.068	0.668
	Late	102.3	112.7	118.6	131.9	3.58	0.034	0.011	0.693
ECF	Early	26.2	28.1	29.2	33.0	0.87	0.081	0.010	0.319
(L/100kg)	Mid	27.0	30.1	31.3	30.9	1.13	0.132	0.252	0.160
	Late	25.8	28.7	28.2	31.5	0.85	0.208	0.006	0.842
PV	Early	18.8	20.6	17.8	19.5	0.95	0.364	0.104	0.937
(L)	Mid	18.6	20.1	21.3	24.0	0.79	0.017	0.028	0.430
	Late	19.8	21.7	23.3	26.0	0.96	0.007	0.042	0.671
PV	Early	5.3	5.4	4.8	5.2	0.25	0.378	0.274	0.618
(L/100kg)	Mid	4.9	5.2	5.6	5.9	0.23	0.037	0.154	0.992
	Late	5.0	5.5	5.5	6.2	0.23	0.152	0.022	0.637
BV	Early	24.3	26.4	23.5	25.0	1.32	0.548	0.205	0.819
(L)	Mid	24.2	26.1	27.5	30.7	1.00	0.016	0.034	0.541
	Late	25.9	28.4	30.0	34.0	1.20	0.006	0.027	0.535
BV	Early	6.8	6.9	6.4	6.7	0.35	0.532	0.477	0.758
(L/100kg)	Mid	6.4	6.8	7.2	7.6	0.30	0.039	0.192	0.860
	Late	6.5	7.2	7.1	8.2	0.27	0.092	0.012	0.506
PCV	Early	22.3	21.8	24.1	22.3	0.52	0.423	0.057	0.261
(%)	Mid	23.0	23.1	22.8	21.9	0.56	0.290	0.506	0.407
	Late	23.8	23.6	22.5	23.7	0.60	0.768	0.438	0.300

SEM = Standard error of the mean.

[1] P-values for the effects; MFC =Misty-fan cooling effect, rbST = rbST effect, MFC x rbST = interaction effect of MFC and rbST

Table 2. Milk yield, mammary blood flow (MBF), mammary plasma flow (MPF)and body weight in cows treated with rbST housing under normal shade (NS) and misters and fans cooling (MFC) at different stages of lactation.

	Stages of	NS		MFC			[1]Effect		
Parameters	lactation	Pre	rbST	Pre	rbST	SEM	MFC	rbST	MFC*rbST
Milk yield	Early	13.39	15.43	14.82	15.84	0.31	0.684	0.001	0.140
(kg/day)	Mid	11.13	13.10	13.79	15.73	0.54	0.269	0.003	0.549
	Late	10.31	11.77	11.29	15.00	0.61	0.372	0.003	0.101
MBF	Early	4969	5222	5241	6555	265.1	0.524	0.018	0.081
(ml /min)	Mid	4141	5053	4132	5434	388.1	0.821	0.021	0.629
	Late	3750	5096	4435	4968	248.6	0.735	0.005	0.141
MPF	Early	3748	4030	3923	5024	186	0.561	0.006	0.060
(ml/min)	Mid	3139	3871	3164	4141	303	0.822	0.023	0.696
	Late	2817	3843	3389	3792	185	0.676	0.005	0.131
MBF/milk yield	Early	535.0	491.6	554.5	583.0	19.29	0.685	0.701	0.100
(L/kg)	Mid	615.8	612.7	473.9	534.9	49.30	0.568	0.573	0.534
	Late	561.9	672.2	589.7	597.5	63.51	0.888	0.391	0.430
Body	Early	358.8	380.8	360.2	373.8	6.48	0.893	0.025	0.535
weight	Mid	382.4	383.2	381.8	411.4	4.17	0.586	0.007	0.009
(kg)	Late	398.2	393.0	425.0	423.0	4.89	0.268	0.483	0.752

SEM = Standard error of the mean.
[1] P-values for the effects; MFC =Misty-fan cooling effect, rbST = rbST effect, MFC x rbST = interaction effect of MFC and rbST

Table 3. The arterial plasma concentrations, arteriovenous differences (A-V), mammary extraction and mammary uptake for glucose, acetate and β-hydroxybutyrate in cows treated with rbST housing under normal shade (NS) and misters and fans cooling (MFC) at different stages of lactation.

	Stages of	NS		MFC			[1]Effect		
Parameters	lactation	Pre	rbST	Pre	rbST	SEM	MFC	rbST	MFC*rbST
Glucose:									
Plasma conc.:	Early	3.73	3.51	3.64	3.48	0.10	0.883	0.098	0.763
(µmol/ml)	Mid	3.55	3.40	3.52	3.67	0.10	0.719	0.992	0.159
	Late	3.49	3.52	3.82	3.77	0.09	0.286	0.918	0.646
A-V difference:	Early	0.66	0.67	0.76	0.61	0.08	0.858	0.485	0.261
(µmol/ml)	Mid	0.62	0.58	0.74	0.72	0.07	0.480	0.81	0.605
	Late	0.78	0.86	0.81	0.80	0.08	0.650	0.552	0.352
Extraction (%):	Early	16.7	18.6	19.3	16.9	1.58	0.816	0.984	0.164
	Mid	17.1	16.7	19.6	18.8	1.57	0.461	0.696	0.398
	Late	22.2	24.4	21.5	21.5	1.62	0.901	0.530	0.373
Udder uptake:	Early	2299	2651	2438	2653	212	0.766	0.168	0.632
(µmol/min)	Mid	1879	2437	1881	2745	355	0.624	0.042	0.982
	Late	2183	3235	2475	2936	253	0.530	0.051	0.203
Acetate :									
Plasma conc.:	Early	650.47	541.77	437.13	468.93	54.27	0.215	0.499	0.232
(µmol /L)	Mid	462.43	514.47	648.60	540.13	60.66	0.287	0.654	0.222
	Late	668.57	602.77	555.00	439.43	71.09	0.181	0.238	0.735
A-V difference:	Early	363.67	305.43	273.07	293.57	48.20	0.661	0.706	0.438
(µmol /L)	Mid	248.23	316.73	450.87	343.80	64.94	0.239	0.774	0.213
	Late	424.93	408.27	409.10	249.53	52.43	0.384	0.131	0.21
Extraction (%):	Early	52.8	48.9	62.7	57.1	4.99	0.548	0.364	0.874
	Mid	51.4	54.2	68.1	60.9	5.87	0.281	0.722	0.419
	Late	58.4	64.9	66.8	63.3	5.10	0.763	0.772	0.352
Udder uptake:	Early	1534.4	1358.5	1212.0	1663.5	247.8	0.99	0.593	0.241
(µmol /min)	Mid	831.2	1165.5	1512.5	1473.0	260.0	0.234	0.586	0.493
	Late	1377.1	1612.0	1519.3	1169.3	231.3	0.925	0.304	0.076
β-OH-butyrate:									
Plasma conc.:	Early	850	872	652	697	63.1	0.143	0.610	0.860
(µmol/L)	Mid	752	814	883	742	53.3	0.831	0.480	0.093
	Late	874	932	750	744	112.4	0.332	0.823	0.783
A-V difference :	Early	312	266	224	269	49.53	0.268	0.992	0.385
(µmol/L)	Mid	240	252	333	282	26.67	0.366	0.486	0.271
	Late	302	270	220	326	70.52	0.859	0.614	0.356
Extraction (%):	Early	36.9	29.4	34.8	40.4	5.35	0.277	0.855	0.253
	Mid	30.6	29.8	39.5	37.4	4.11	0.105	0.733	0.885
	Late	34.7	26.6	28.5	43.1	5.72	0.346	0.585	0.082
Udder uptake:	Early	1262.4	1083.9	912.9	1253.8	178.6	0.781	0.661	0.184
(µmol /min)	Mid	726.6	882.8	1059.8	1140.1	129.0	0.157	0.386	0.776
	Late	881.5	1043.0	784.3	1137.8	255.8	0.997	0.344	0.717

SEM = Standard error of the mean.

[1] P-values for the effects; MFC =Misty-fan cooling effect, rbST = rbST effect, MFC x rbST = interaction effect of MFC and rbST

Table 4. The arterial plasma concentrations, arteriovenous differences (A-V), mammary extraction and mammary uptake for free fatty acid and triacylglycerol in cows treated with rbST housing under normal shade (NS) and misters and fans cooling (MFC) at different stages of lactation.

	Stages of	NS		MFC			[1]Effect		
Parameters	lactation	Pre	rbST	Pre	rbST	SEM	MFC	rbST	MFC*rbST
Free faty acids :									
Plasma conc.:	Early	156.89	164.27	199.99	287.49	38.02	0.239	0.247	0.323
(µmol/L)	Mid	133.24	196.31	188.53	204.45	14.82	0.576	0.029	0.150
	Late	102.52	153.59	178.58	262.77	17.14	0.147	0.004	0.362
A-V difference									
(µmol/L)	Early	-4.85	-18.32	17.03	90.5	29.6	0.158	0.341	0.180
	Mid	-31.11	11.86	5.10	-11.18	18.76	0.727	0.497	0.153
	Late	-32.47	29.68	-14.86	25.09	16.89	0.539	0.017	0.530
Extraction (%)	Early	-20.2	-12.2	10.1	21.5	11.22	0.063	0.414	0.882
	Mid	-23.5	6.2	2.9	-4.7	13.05	0.531	0.421	0.191
	Late	-30.2	16.3	-11.8	9.2	8.20	0.266	0.003	0.158
Udder uptake	Early	-59.65	-122.58	73.11	462.00	155.5	0.105	0.325	0.184
(µmol/min)	Mid	-71.17	34.63	27.57	-13.93	59.63	0.643	0.604	0.252
	Late	-100.38	121.07	-57.31	98.1	67.38	0.821	0.023	0.637
Triacylglycerol :									
Plasma conc.:	Early	159.25	179.71	195.07	201.93	17.29	0.702	0.452	0.704
(µmol/L)	Mid	209.2	230.62	182.25	202.92	24.03	0.729	0.407	0.988
	Late	199.09	210.64	321.77	249.76	48.36	0.351	0.549	0.413
A-V difference	Early	42.92	32.33	58.39	70.21	6.48	0.209	0.927	0.122
(µmol/L)	Mid	44.96	69.89	54.99	84.37	23.95	0.508	0.29	0.928
	Late	58.23	87.99	52.71	90.80	21.22	0.941	0.149	0.849
Extraction (%)	Early	36.4	23.6	32.9	37.3	4.06	0.660	0.330	0.066
	Mid	25.5	36.3	28.7	41.2	7.33	0.475	0.151	0.909
	Late	33.9	43.9	23.5	39.5	6.56	0.426	0.084	0.655
Udder uptake	Early	167.65	136.88	238.33	388.11	44.04	0.162	0.214	0.075
(µmol/min)	Mid	146.31	260.41	167.29	336.48	68.98	0.543	0.074	0.700
	Late	160.18	333.33	193.37	358.83	72.19	0.696	0.047	0.959

SEM = Standard error of the mean.

[1] P-values for the effects; MF =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

DISCUSSION

The environmental temperatures measured in NS and MFC barn in the present study showed differences in ambient temperature and THI, especially in the afternoon throughout the experimental periods. However, MFC barn was not sufficient to completely eliminate heat stress in cows, because the range for THI measured at daytime under misters and fans throughout the experimental periods remained higher than the threshold level of comfortable zone, 72 for THI (Armstrong, 1994). The THI in both barns ranged from 80.7-85.5. Cows in both groups would be subjected to moderate heat stress (Fuquay, 1981). However, THI might not accurately reflect of heat stress in crossbred lactating cows under MFC cooling system that deliver a pressurized spray with considerable fan air movement in the barn, resulting both high humidity and a cooling effect. The cooling of cows under MFC was significantly lower in both respiratory rate and rectal temperature in comparison with those of non-cooled cows which indicate a partial alleviation of heat stress by MF system especially in the afternoon. The respiratory rate and rectal temperature were increased during rbST supplementation in both cooled and non-cooled cows. These results agree with previous reports (Sullivan et al., 1992; Tarazon et al., 1999) in cows treated with rbST. Although rbST-treated cows increases heat production associated with high milk yield, it also increases heat dissipation (Johnson et al., 1991; West, 1994). However, cows in both groups gained in weight as lactation progress.

It is known that milk production is the result of coordination between nutrient delivery to and biosynthetic capacity of the mammary glands (Linzell and Mepham, 1974). The arterial plasma concentration of nutrients including mammary gland biosynthetic capacity and mammary blood flow would be factors affect to the mode of nutrient uptake by the gland. In the present results, the marked increases in blood flow to the mammary gland coincided with an increase in milk yield during rbST supplementation in both cooled and non-cooled cows. These results agree to previous studies by Chaiyabutr and co-worker (2005) that long-term administrations of rbST showed a marked increase in mammary blood flow throughout lactation. Factors that might affect to increase MBF during supplemental rbST could include an increasing relative mass of many organs and tissue including mammary tissue (Moallem *et al.* 2004) and an increase in cardiac output (Soderholm et al., 1988) in bST treated cows. However, the supplementation of rbST markedly increased both the absolute values of

plasma volume and blood volume, ECF and TBW in both cooled and non-cooled cows when compared with the pre-treatment period in each stage of lactation. An increase in ECW leads to an increase in MBF as secondary responses, thereby the increase in MBF drives nutrients supply per se to the mammary gland and increase in milk production in rbST treated cows. However, during lactation advanced to late lactation in both cooled and non-cooled cows, the decline in milk yields were still apparent, although MBF, ECF TBW were still in high levels during supplemental rbST. These results indicate that an increase in milk yield in response to rbST administration will not be sustained for long, which is influenced by the stage of lactation. These data suggest that changes in milk production during lactation advances might not be controlled systematically but also locally within the mammary gland (Chaiyabutr et al., 2005).

It is believed that the effect of somatotropin on MBF occurs by a mechanism which does not involve the direct action of somatotropin on the mammary gland (Collier et al., 1984). An increase in MBF accompanying with an increase in circulating levels of IGF-I has been shown in either short-term or long-term rbST administration in different stages of lactation in crossbred HF animals (Chaiyabutr et al., 2005; Maksiri et al., 2005;Tanwattana et.al., 2003). In addition, no direct effect of bST on mammary secretory function has been noted (Gertler et al., 1983). The studies in goats and cows have shown that the effect of rbST on mammary circulation is indirect and mediated via IGF-I, although similar increases in milk secretion and mammary blood flow occurred during growth hormone treatment (Davis et al. 1988; Hart et al. 1980). It indicates that bST plays a role for an increase in MBF requiring IGF-I as a mediator (Forsyth, 1996). However, the lack of effect of higher plasma IGF-I levels on persistency of lactation in rbST treated animals was also reported (Chaiyabutr et al., 2005).

The present results for the effect of supplemental rbST in both cooled and non-cooled cows on the mammary uptake of plasma substrates were not based on changes in mammary extraction and A-V concentration differences of substrates across the mammary gland. Glucose is known to be the major precursor for lactose synthesis. The supply of glucose to the mammary gland is an important factor in the control of milk yield. In the present study an increase in MBF would be a major determinant of an increase in the mammary glucose uptake in both cooled and non-cooled cows. No

alterations in arterial plasma glucose concentrations, A-V concentration differences and mammary extraction of glucose were apparent as lactation advances in either cooled or non-cooled cows supplemental rbST. In contary to other investigations that mammary glucose uptake was depended on an increase in the arterial plasma glucose concentration during bST administration (Sandles et al., 1988; Fullerton et al., 1989), whereas other works have demonstrated no differences (McDowell et al., 1987; Mepham, 1993). The present results support the latter observations during rbST supplementation. However, no changes in both A-V concentration differences and the mammary extraction of glucose during supplemental rbST were apparent. It indicates that the contact time between glucose in blood and mammary epithelial cell did not affect to transit time of glucose during high blood flow to the mammary gland. The local factor may also influence in the control of glucose uptake. It is possible that during rbST supplementation, an increase in body protein synthesis including a number of specific glucose transporters at the mammary cell membrane might be proportionate to an increase in the delivery of glucose by high MBF (Prosser, 1988; Madon et al., 1990). Therefore, the limited transport of glucose into mammary cell would not apparent by these means.

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, mammary extraction and mammary uptake of acetate were not affected during rbST supplementation in different stages of lactation in both cooled and non-cooled cows. Acetate uptake was not dependent upon the rate of mammary blood flow. It is known that 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 fatty acids in milk fat was not altered by rbST supplementation (Chaiyabutr et al., 2000b), indicating that acetate was partially redirected from oxidation to de novo fatty acid synthesis. In the present results, levels of A-V concentration differences and mammary extraction of β-hydroxybutyrate across the mammary gland including the arterial plasma concentration were not affected during rbST supplementation. It indicates that the utilization of β-hydroxybutyrate by the mammary tissue was not obvious during rbST administration in 87.5% HF cows. It is known that the circulating β-hydroxybutyrate arise mainly from rumen butyrate in the fed animal

(Leng and West, 1969), and the principal effect of bST has been shown to increase oxidation of free fatty acids during negative energy balance in high yield lactating cows. An increase in the concentration of plasma β-hydroxybutyrate would be consistent with an increase in oxidation of free fatty acids (Bauman et al., 1988). The present study, the greater energy requirement resulting in increased hepatic ketogenesis due to greater mobilization of fat reserves (Schultz, 1974) were not apparent during rbST-supplementation in both cooled and non-cooled cows.

In the present study, the mean values for the arterial plasma concentration of free fatty acids but not for triacylglycerol increased during rbST supplementation which was more sensitive to alteration than other blood substrates. This phenomenon has been proposed as an indication of under-nutrition (Reid and Hinks, 1962). However, cows in both cooled and non-cooled cows gained weight throughout the experimental periods. A marked increase in milk yield with rbST supplementation without loss of body weight, especially during early lactation, may be due to the fact that cows were offered TMR diet with an adequate replacement of body reserves during lactations. Milk yield in the primiparous lactating crossbred cows 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 gain of cows during their first lactation. During early lactation, the metabolic demands of lactation during supplemental rbST in both cooled and non-cooled cows were met by dietary intake, thus not causing mobilization of body tissues as indicated by no alteration of the levels of plasma triglyceride. The marked increases in the plasma concentrations of FFA were apparent in cows supplemental rbST in both cooled and non-cooled cows especially in mid and late stages of lactation. Thus, the lipolytic activity would be a function of rbST treatment per se in stead of the associated changes in energy balance.

The measurement of A-V 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. The high uptake of triacylglycerol by the mammary gland especially significant increase in the late lactation in cows supplemental rbST, which is agree with the results reported by Miller et al. (1991). It is possible that the negative mammary uptakes of free fatty acids may reflect hydrolysis of triacylglycerol, 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 releasing

of FFA would be as a result of enzymatic activity of lipoprotein lipase in the mammary tissue which has been reported to be higher in the mammary tissue relative to other tissue (Shirley et al., 1973; Bauman and Griinari, 2003).

In conclusion, the present study demonstrates that an increase in MBF during rbST supplementation would be a major determinant in the mediation of nutrient delivery and uptake by the mammary glands for increase in milk production. Local changes for biosynthetic capacity within the mammary gland would be a factor in identification of the utilization of substrates in the rate of decline in milk yield with advancing lactation in both cooled and non-cooled cows whether supplemental rbST or not.

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CHAPTER VI

Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on cellular metabolites in milk secretion at different stages of lactation in crossbred Holstein cattle

Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on cellular metabolites in milk secretion at different stages of lactation in crossbred Holstein cattle

INTRODUCTION

Exogenouse bovine somatotropin to dairy ruminants is known to stimulate milk secretion (Bauman and Vernon, 1993 Chaiyabutr et al 2007). A previous study in lactating crossbred animals containing 87.5% Holstein genes has shown the decline in the concentration of bovine somatotropin in plasma and a rapid decrease in the peak rate of milk yield as lactation progressed to mid- and late lactation (Chaiyabutr *et al*. 2000a). Alterations of the level of plasma bovine somatotropin are among the causes of persistent lactation performance (Knight *et al*. 1990). This shorter lactation persistency in 87.5% crossbred Holsteins might be explained by both the reduction of nutrient uptake by the mammary gland with the decline in mammary blood flow (MBF) (Chaiyabutr *et al*. 2000b). However, this does not appear to be the only factor responsible for increase in milk yield as direct effects on the metabolism in the mammary gland have also been observed (Chaiyabutr et al 2008). High environmental temperature would be another factor, which affect milk production in dairy cows in the tropic (Smith et al. 2006; Bohmanova et al. 2007). The high ambient heat load has been shown to reduce the response to bST treatment and low milk yields (Kronfeld 1989; Molett et al. 1986), while other studies showed no changes in milk yields (Cole and Hansen 1993).

A number of studies of mammary gland metabolism in cows, goats and rats under different experimental and physiological conditions have measured changes in the concentrations of cellular metabolites present in the milk (Kuhn & White 1975; Chaiyabutr *et al*. 1981; Faulkner et al.,1982; Faulkner & Pollock 1989). However, detailed information on changes in the concentrations of metabolites in the mammary gland corresponding to the short persistency of lactation by the effect of high environmental temperatures and bovine somatotropin is not available in the crossbred dairy cattle. Measurement of milk metabolites reflecting intracellular constituents such

as glucose has been proved a useful, non invasive, method of following changes in mammary metabolism in goat (Chaiyabutr et al., 1981). Changes in the concentrations of the other minor constituents have been found to correlate significantly with changes in milk production under a variety of situations such as starvation in goat (Chaiyabutr et al., 1981) and prolonged exogenous bovine somatotropin in crossbred dairy cattle (Chaiyabutr et al.,2008). The volume of milk secreted is known to closely relate to the rate of lactose synthesis. Lactose is synthesized within the mammary secretory cell from glucose in the lumen of the Golgi apparatus by an enzyme lactose synthase. If changes in the availability of glucose within the mammary gland are important for of milk synthesis. A study on the concentration of glucose and relating metabolites in milk can be interpreted in terms of changes in the 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 & Peaker, 1982). Therefore, the purpose of this study was to obtain a more complete picture of the effect of cooling and bovine somatotropin supplementation on a possible mechanisms that could regulate the persistency of lactation in 87.5% crossbred Holstein. Measurement for changes in the concentrations of metabolites in milk could be interpreted as biochemical changes of the rate of lactose synthesis occurring in the mammary gland in crossbred dairy cattle.

MATERIAL AND METHODS

Animals, housing and managements

Ten primiparous crossbred cattle, containing 87.5 % Holstein (HF) genes, average body weight 358±32.5 kg, non pregnant and averaged 60 day postpartum at start of trial were used for the experiment. The animals were assigned randomly into two groups of five animals each. Animals in the first group were housed in open-sided barn with a tiled roof in normal shaded house (NS) as the non-cooled animals. Animals in the second group were housed in open-sided barn with a tiled roof (8 m long x 7 m wide x 3.5 m high) under misters and fans (MF) as cooled animals. The open space cooling system consisted of two sets of misters and fans, which each system consisted of a 65 cm. diameter blade fan circulating 81 m^3/min of air, with oscillation coverage of 180º. The amount of water discharged from 4 splay heads was 7.5 L/h and size of mist droplet 0.01 mm. Animals were exposed to MF for 45 minutes at 15-minute intervals from

06:00 h to 18:00 h. At night, animals were exposed to MF for 15 minutes at 45-minute intervals from 18:00 h to 06:00 h.

The ambient temperature was recorded using a dry bulb thermometer. The relative humidity was read depending on wet and dry bulb temperature at cooled and non-cooled barns. Ambient temperatures, humidity, rectal temperature and respiratory rate were measured weekly at, 14:00 h on specified day. Average values were considered to be the mean of all measurements taken for each date. A temperature-humidity index (THI) was calculated from the average ambient temperature of dry and wet bulb temperatures according to McDowell (1972), as follow:

$$THI = 0.72 (wb+db) + 40.6$$

Where; wb = wet bulb temperature and db = dry bulb temperature expressed in °C

Rectal temperature measurements were made with electronic thermometers. Respiratory rates were obtained by observing flank movements. All animals were weighed monthly throughout the experimental periods. Body weight was determined of each stage of lactation at the end of the pretreatment period and at the end of the treatment period. The diet was fed twice a day for *ad lib* as the same ration of total mixed ration (TMR) throughout the experiments in both groups. Ingredient and chemical compositions of feed are shown in Table 1. Animals are milked twice a day and milk yields are recorded at each milking. Each day, the food was given in equal portions at about 06:00 h and 17:00 h when the animals were milked using a milking machine and milk production was recorded daily.

Experimental design

The experiment was divided into 3 phases, early (2 months postpartum), mid- (4 months postpartum), and late lactation (6 months postpartum) periods. The pretreatment was conducted on the starting day of each phase. At the end of the pretreatment, within the same day, the subject was injected with the first dose/injection of with 500 mg of recombinant bovine somatotropin (rbST) (POSILAC, Monsanto, USA). Subsequently, the subject was injected with two consecutive doses/injections of with 500 mg of recombinant bovine somatotropin (rbST) every two weeks. Thereafter, within 2-5 days after the third injection, the treatment was conducted. The pretreatment, 3 doses of injections, and the treatment were performed during the first 30 days and the same procedures were followed for each phase. During the last 30 days of each phase,

no experiments were conducted in order to allow the milk yield to return to control level. Thus, comparative studies of both groups in each stage of lactation, the four treatment combinations were normal shade without rbST injection (NS), normal shade plus rbST injection (NS + rbST), shade plus misty-fan cooling without injection (MFC), and shade plus misty-fan cooling with rbST injection (MFC + rbST)

Mammary blood flow measurement

On the specify day, during the 1000-1100h, measurements of the mammary blood flow through half of the udder were performed in duplicated by dye dilution technique using dye T-1824 (Evans blue) by a short term continuous infusion into the milk vein as described by Chaiyabutr et al.(1997). A venous blood sample was collected from the milk vein via a catheter while an arterial blood sample was collected from the coccygeal vessel by venipuncture with a # 21 needle. Blood samples in heparinized tubes were kept in crushed ice for glucose studies.

Collection of milk sample and estimation of milk metabolites

On each specified day of study, the milk sample was collected from each animal at the evening milking on the day of study of each period, which was kept at 4°C until next day of preparation for metabolite analysis. The samples of milk were defatted, deproteinized and analyzed for metabolites. Briefly, the milk was centrifuged at 12,000 rpm for 15 min at 4°C. The aqueous phase below the solidified fat layer was removed. The supernatant were performed for milk glucose which 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 electrolytes in milk were estimated for sodium (Na) and Potassium (K) using Flame photometer. The chloride in milk was measured by Chloridometer (Chloride analyzer 925, Ciba Corning Inc., USA). The osmolarity of milk was determined by osmometer (Osmometer 3D3, Advance Instrument Inc., USA).

Statistical analysis

The statistic analyses were performed using General Linear Model procedures of statistical software package SPSS (SPSS for windows, V14.0; SPSS Inc., Chicago, IL, USA). The model used for each analysis was:

$$Y_{ijk} = \mu + A_l + H_i + A(H)_{il} + B_j + (HB)_{ij} + A(HB)_{ijl} + Cov_k + e_{ijkl}$$

Where Y_{ijk} = observation, μ = overall mean, A_l= Animal effect H_i= house effect as main plot (*i* = NS, MFC), $A(H)_{il}$ = main plot error (animal *l* in house *i*), B_j = treatment effect (rbST) as a split plot (*j* = with and without rbST supplementation), $(HB)_{ij}$ = interaction effect between treatment and house , $A(HB)_{ijl}$ = split plot error (animal *l* in house *i* and treatment *j*), Cov_k = covariate effect and e_{ijk} = residual error.

Means values were used to evaluate the effect for all variables. Statistical significance was declared at $P<0.05$. and trends were declared at $0.05 < P \leq 0.10$.

RESULTS

Ambient temperatures , relative humidity, temperature humidity index, rectal temperature and respiratory (Table 2)

The ambient temperatures and THI in the NS barn were significantly higher than the MFC barn but the relative humidity of MFC barn was significantly higher than the NS barn at all stages of lactation. The respiration rate and rectal temperature of cows under mister and fans were lower than non-cooled cows supplemental rbST at all stages of lactation. During supplementation of rbST, respiration rate and rectal temperature were significantly higher than those of pretreatment periods in both cooled and non-cooled cows in all stages of lactation.

Mammary plasma flow, plasma glucose concentration, mammary glucose uptake, lactose concentration and lactose secretion, milk yield (Table 3).

During supplemental rbST, mammary plasma flow was higher than the pretreatment in both cooled and non-cooled cows in each stage of lactation. The mean values of plasma glucose concentration in both cooled and non-cooled cows showed no significant changes during supplemental rbST when compared with the pre-treatment period. The uptake for glucose across the udder increased in each stages of lactation in both cooled and non-cooled cows supplemental rbST. Milk yield of both cooled and non-cooled cows supplemental rbST were significantly higher than those of the pretreatment in each stage lactation. Lactose secretion in both cooled and non-cooled cows significantly increased during supplementation of rbST, while no alterations of the milk lactose concentrations were apparent.

The concentrations of milk glucose, Na^+, K^+, Cl^-, milk osmolarity and citrate (Table 4).

Both cooled and non-cooled cows supplemental rbST had tendency to increase in the milk glucose concentration in early and mid-lactation, while milk glucose concentration was not increased in the late lactation in cows under normal shade. There were no apparent for the relative values as a percentage of body weight in early and mid lactation. The concentrations of milk Na^+, K^+, Cl^- and plasma osmolality of both cooled and non-cooled cows supplemental rbST were unchanged throughout lactation. The milk citrate concentration significantly increased after rbST supplementation in early lactation of both cooled and non-cooled cows. In mid lactation, the milk citrate concentration significantly increased by the effect of cooling, while in late lactation the milk citrate concentration significantly increased by the effect of both cooling and rbST supplementation.

Table 1 Feed ingredients and chemical compositions of the diet

Ingredients:	Kg (as fed basis)
Pine apple waste	50
Soybean meal	23
Rice bran	3.0
Cotton seed	20
Lime stone	1.4
Di-calcium phosphate	1.4
Sodium bicarbonate	0.3
Potassium chloride	0.1
Mineral and vitamin premix	0.8
Total	100
Chemical composition:	
Dry matter (%)	39.1
Ash (% DM)	7.3
Organic matter (% DM)	92.7
Crude protein (% DM)	18.0
Acid detergent fiber (% DM)	20.1
Neutral detergent fiber (% DM)	33.9

Table 2. Ambient temperature, relative humidity, temperature humidity index at 1400h, rectal temperature, respiration rate measurement and milk yield in cows treated with rbST and housing under normal shade (NS) and cooling with misters and fans (MFC) at different stages of lactation.

Parameters	Stages of	NS		MFC			[1]Effect		
	lactation	Pre	rbST	Pre	rbST	SEM	MFC	rbST	MFC*rbST
Ambient temperature (°C)	Early	33.9	35.1	31.6	31.8	0.74	0.001	0.386	0.473
	Mid	35.3	35.0	30.0	29.8	0.48	0.002	0.613	0.919
	Late	33.5	34.1	29.9	29.1	0.35	0.001	0.865	0.098
Relative humidity (%)	Early	49.5	52.8	66.0	68.8	3.28	0.001	0.396	0.942
	Mid	52.8	50.4	78.2	74.0	3.10	0.001	0.318	0.779
	Late	59.0	63.5	78.5	79.8	2.07	0.001	0.214	0.462
Temperature humidity index (THI)	Early	83.2	85.2	82.4	82.8	0.89	0.003	0.205	0.396
	Mid	85.5	84.8	81.5	80.8	0.39	0.019	0.116	0.928
	Late	83.9	85.2	81.4	80.7	0.29	0.004	0.316	0.011
Rectal temperature (°C)	Early	39.4	40.0	39.0	39.4	0.21	0.037	0.061	0.817
	Mid	39.7	40.1	38.6	39.5	0.13	0.002	0.002	0.090
	Late	39.2	39.9	38.4	38.8	0.16	0.015	0.016	0.309
Respiration rate (breath/min)	Early	73.0	82.3	55.5	68.0	4.13	0.023	0.039	0.708
	Mid	73.6	77.2	49.0	57.6	1.96	0.001	0.018	0.294
	Late	71.5	80.0	54.3	59.3	1.09	0.019	0.001	0.159

SEM = Standard error of the mean.

[1] P-values for the effects; MFC =Misty-fan cooling effect, rbST = rbST effect, MFC x rbST = interaction effect of MFC and rbST

Table 3. Mammary plasma flow, arterial plasma glucose concentration, mammary glucose uptake, lactose concentration and lactose secretion during rbST administration at different stages of lactation of Holstein cows and housing in normal shade (NS) and shade plus misty-fan cooling (MFC).

Parameter	Stages of lactation	NS		MFC		SEM	[1]Effect		
		Pre	rbST	Pre	rbST		MFC	rbST	rbSTxMFC
Mammary plasma flow (ml/min)	Early	3748	4030	3923	5024	186	0.561	0.006	0.060
	Mid	3139	3871	3164	4141	303	0.822	0.023	0.696
	Late	2817	3843	3389	3792	185	0.676	0.005	0.131
Plasma glucose: (µmol/ml)	Early	3.73	3.51	3.64	3.48	0.10	0.098	0.883	0.763
	Mid	3.55	3.4	3.52	3.67	0.10	0.992	0.719	0.159
	Late	3.49	3.52	3.82	3.77	0.09	0.918	0.286	0.646
Mammary glucose uptake (µmol/min)	Early	2299	2651	2438	2653	212	0.766	0.168	0.632
	Mid	1879	2437	1881	2745	355	0.624	0.042	0.982
	Late	2183	3235	2475	2936	253	0.530	0.051	0.203
Lactose concentration (mmol/L)	Early	131.6	141.3	138.8	135.8	3.87	0.846	0.411	0.140
	Mid	134.7	133.8	133.9	136.5	2.14	0.820	0.687	0.452
	Late	132.4	132.8	126.8	131.1	2.41	0.542	0.372	0.443
Milk lactose secretion (µmol/min)	Early	1230.3	1458.4	1367.7	1471.3	36.12	0.716	0.002	0.123
	Mid	999.2	1188.0	1249.7	1497.5	48.38	0.225	0.003	0.100
	Late	936.6	1066.3	1028.6	1392.5	57.35	0.347	0.003	0.075
Milk yield (kg/day)	Early	10.65	11.95	12.23	12.79	0.378	0.477	0.039	0.390
	Mid	9.25	10.14	11.86	13.44	0.308	0.185	0.004	0.298
	Late	8.04	9.81	9.51	12.31	0.625	0.355	0.006	0.437

SEM = Standard error of the mean.

[1] P-values for the effects ; MFC =Misty-fan cooling effect, rbST = rbST effect, MFC x rbST = interaction effect of MFC and rbST

Table 4. The concentrations of metabolites in milk for glucose, Na^+, K^+, Cl^-, milk osmolarity and citrate in cows treated with rbST and housing under normal shade (NS) and cooling with misters and fans (MFC) at different stages of lactation.

Parameter	Stages of	NS		MFC			[1]Effect		
	lactation	Pre	rbST	Pre	rbST	SEM	MFC	rbST	MFC*rbST
Milk glucose	Early	288.9	333.3	222.2	397.8	77.9	0.989	0.196	0.425
(µmol/L)	Mid	246.7	340.0	355.6	426.7	79.4	0.252	0.331	0.892
	Late	380.0	288.9	211.1	288.9	77.0	0.448	0.933	0.305
Na+	Early	29.80	31.20	27.20	27.60	1.31	0.250	0.449	0.670
(mmol/L)	Mid	28.80	29.20	27.80	28.00	0.79	0.651	0.714	0.902
	Late	32.60	32.20	29.00	32.00	2.05	0.700	0.544	0.432
K+	Early	38.70	36.88	36.80	36.04	1.27	0.434	0.341	0.688
(mmol/L)	Mid	37.06	36.16	36.32	35.08	0.90	0.582	0.267	0.854
	Late	35.16	34.74	33.84	34.50	1.07	0.609	0.914	0.628
Cl^-	Early	31.00	33.00	36.40	35.80	0.78	0.277	0.398	0.136
(mmol/L)	Mid	33.20	32.80	29.80	27.60	0.78	0.255	0.132	0.279
	Late	33.40	32.60	33.80	41.60	3.75	0.541	0.378	0.284
Osmolarity	Early	277.6	277.8	279.6	279.0	3.37	0.710	0.754	0.908
(mOsm/kg)	Mid	276.6	272.6	279.8	277.2	1.66	0.289	0.081	0.684
	Late	269.8	275.0	302.2	283.4	9.60	0.265	0.499	0.246
Citrate	Early	4.24	4.54	4.22	4.85	0.15	0.301	0.014	0.298
(mM)	Mid	4.70	4.71	5.67	5.78	0.11	0.016	0.575	0.645
	Late	4.74	4.14	5.24	4.38	0.15	0.042	0.001	0.398

SEM = Standard error of the mean.
[1] P-values for the effects ; MFC =Misty-fan cooling effect, rbST = rbST effect, MFC x rbST = interaction effect of MFC and rbST

DISCUSSION

The environmental temperatures measured in NS and MFC barn in the present study showed differences in ambient temperature and THI, especially in the afternoon throughout the experimental periods. However, MFC barn was not sufficient to completely eliminate heat stress in cows, because the range for THI measured at daytime under misters and fans throughout the experimental periods remained higher than the threshold level of comfortable zone, 72 for THI (Armstrong, 1994). The THI in both barns ranged from 80.7-85.5. Cows in both groups would be subjected to moderate heat stress (Fuquay, 1981). However, THI might not accurately reflect of heat stress in

crossbred lactating cows under MFC cooling system that deliver a pressurized spray with considerable fan air movement in the barn, resulting in higher humidity but also causing a cooling effect. The cooling of cows under MFC was significantly lower in both respiratory rate and rectal temperature in comparison with those of non-cooled cows which indicate a partial alleviation of heat stress by MF system especially in the afternoon. The respiratory rate and rectal temperature were increased during rbST supplementation in both cooled and non-cooled cows. These results agree with previous reports (Sullivan et al., 1992; Tarazon et al., 1999) in cows supplemental rbST. Although rbST-treated cows increases heat production associated with high milk yield, it also increases heat dissipation (Johnson et al., 1991; West, 1994).

Increased milk yield in response to rbST supplementation is well known, but the mechanisms have not been completely elucidated in crossbred dairy cattle. The present results show that the concentration of glucose in the aqueous phase of milk increased in response to rbST supplementation. The changes in glucose concentrations in milk would reflect intracellular glucose concentrations within the mammary epithelial cell (Faulkner,1980), since it equilibrates rapidly across the apical membrane of lactating cell (Neville *et al*. 1990). An increase in intracellular glucose concentrations would act to stimulate lactose production and an increase in lactose secretion would coincide with an increase in milk yield during rbST supplementation. This is believed to be the process of milk secretion in the mammary glands by which water is drawn osmotically from the inside of the cell into the Golgi vesicle during lactose formation and increasing milk yield by bulk water movement into milk (Linzell & Peaker 1971).

It is known that the rate of blood flow to the mammary gland determines the rate of nutrient uptake by the mammary gland. During supplementation of rbST in crossbred dairy cattle, it has been noted that milk yield increased with an increase in MBF, but rbST has a less stimulating effect for milk yield as lactation advances to late lactation, despite a high level of MBF (Chaiyabutr *et al*. 2007). These results indicate that an increase in the milk yield of dairy crossbred cattle, in response to rbST supplementation, is not sustained for long and is influenced by the stage of lactation. Our previous studies showed that an increase in MBF in rbST-treated cows coincided with an increase in the circulating levels of insulin-like growth factor I (IGF-I) throughout lactation (Chaiyabutr *et al*. 2005). It is suggested that bST plays a role in MBF, requiring IGF-I as a mediator for increasing MBF directly (Forsyth 1996). However,

several investigations for mammary nutrients uptake like glucose uptake have been shown to depend on an increase in the plasma glucose concentration during bST administration (Sandles *et al*. 1988; Fullerton *et al*. 1989), whereas other works have found no differences (McDowell *et al*. 1987). The present study supports the latter observations that there are no changes in the plasma glucose concentration in rbST-treated cows. The intracellular glucose concentration is mainly derived from the blood for the process of milk synthesis (Linzell & Peaker 1971), since the mammary cell cannot synthesize free glucose because of the lack of glucose-6-phosphatase activity (Threadgold & Kuhn 1979). The intracellular glucose freely permeates across the Golgi vesicles of the mammary secretory cells (Faulkner & Peaker 1987). An increase in the concentration of milk glucose would reflect the increase in intracellular glucose concentration (Kuhn& White 1975; Faulkner *et al*. 1981). In the present results, milk glucose concentration was not increased in the late lactation in cows supplemental rbST under normal shade. Thus, changes in the availability of intracellular glucose would be one of the intramammary factors limiting lactose production and hence milk yields during advanced lactation. The low number of glucose transporters (Prosser, 1988) or an elevation of glucose utilization by the mammary epithelial cell as lactation advanced to late lactation could be attributed to the reduction in the milk yield. An increase in milk glucose concentrations would accompany with both increases glucose uptake by the mammary glands and milk yield during rbST supplementation. It has been reported that glucose and electrolytes secrete across the mammary epithelium from the blood side to milk via membrane route (Linzell et al., 1976), while lactose and citrate in the Golgi vesicle are secreted into the alveolar lumen by exocytosis (Kuhn *et al*. 1980), because Golgi and apical membranes of mammary secretory cells are impermeable to lactose but freely permeable to water. Glucose must cross the Golgi membrane to reach the site of lactose synthesis. The levels of milk citrate were varied by the effects of cooling and rbST supplementation in different stages of lactation. It is probable that citrate exists in the aqueous phase of milk in a variety of chemical forms such as calcium citrate^{-}, citrate^{2-}, and citrate^{3-}. The relative proportion of each chemical form depends on factors such as H^{+}, Ca^{2+}, and Mg^{2+} and thus citrate can be considered as an important milk buffer. In the present study the concentrations of milk Na^{+} K^{+} and Cl^{-} were not change during supplementation of rbST in both cooled and non-cooled cows. It indicates that the mammary gland is able to generate and maintain large Na^{+} K^{+} and

Cl^- gradient between milk and plasma. These ions make a substantial contribution to maintain the osmolality of milk.

In conclusion, a marked response with increases in milk yield, lactose output and milk glucose concentration during early lactation in rbST-treated animals would depend on the rate of entry of glucose into mammary cell. An increase in MBF would not be responsible for milk synthesis throughout lactation. Milk glucose concentrations, reflecting intracellular glucose concentrations during rbST administration, would be one of the factors regulating the rate of lactose production. During early lactation, a larger portion of the conversion of intracellular glucose to intermediary metabolites of rbST-treated animals was used mainly in the lactose biosynthetic pathway when compared with controls. These findings illustrate that rbST administration exerts its galactopoietic action more by intramammary than by extra-mammary factors.

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CHAPTER VII

Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on plasminogen and plasmin system in the mammary gland in different stages of lactation in crossbred Holstein cattle

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INTRODUCTION

The low milk production of both exotic and crossbred cattle is still the main problem in dairy farming in the tropics. Many factors can affect milk production in dairy cattle in the hot, humid tropics, Several approaches have been used to try to improve dairy productivity in the tropics. It has been known that mammary gland is dependent on hormonal stimuli and the provision of nutrients to sustain milk synthesis. The concentration of plasma bovine somatotropin (bST) of lactating crossbred cattle containing 87.5% Holstein genes markedly decreased as lactation advances to mid- and late lactation. This decrease could be attributed to decreases in milk yield and mammary blood flow (Chaiyabutr et al 2000). However, the stimulant effect for milk yield was less in late lactation even though a high level of MBF during long-term administration of rbST (Chaiyabutr et al. 2007). Little information is available for the role of bST in the short persistency of lactation in crossbred dairy cattle in the tropics, although the importance of bST for enhancing and maintaining milk production in dairy ruminants is well established (Bauman 1999). A number of studies have examined the importance of the plasmin-plasminogen system in the bovine mammary gland. Plasmin would be a marker for gradual involution. The declining phase of lactation has been shown to correlate with a marked conversion of plasminogen (PG) to plasmin (PL). The PG:PL ratio, an index independent of changes in milk volume, declines as lactation advances to late lactation (Politis et al. 1989). The study in crossbred Holstein cattle by Chaiyabutr *et al*. (2007) showed that the concentration of plasminogen : plasmin ratio decreased in the control animals while it increased in the rbST-treated animals as lactation advanced. These findings demonstrate that rbST is involved the activity of the plasmin–plasminogen system but it is not involved in maintaining tissue integrity in the mammary gland during late

lactation in crossbred dairy cattle, although a number of studies indicate that bST can delay the 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 (Politis *et al*. 1990; Baldi *et al*. 1997). There is evidence that the progressive loss of the capacity of mammary epithelial cells to synthesize milk occurs during mammary involution. Cows exposed to high environmental temperatures usually respond with reduction in milk yield and milk quality (Collier et al., 1982; Johnson, 1987; Huber et al.1994). During thermal stress, whether plasmin productions in milk increase in determining milk production and initiate the onset of involution within the mammary gland (Ossowski *et al*. 1979). Little is known about what is responsible for this proteolysis relating to the role of growth hormone and thermal stress on the persistency of lactation in crossbred cattle. Extending lactation may be a strategy worth considering for improving the longevity of dairy cows. Longer lactations are more profitable when they are combined with a strategy that reduces the rate of decline in yield after peak production. Thus, the objective of the present study was to determine the relationship between milk plasmin–plasminogen and milk yield, including milk compositions, during animal cooling under misters and fans and supplementation with rbST in different stages of lactation in 87.5% Holstein Friesian (HF) animals. Relationships between stage of lactation, and thermal stress for activities of milk PL, PG, and PA were evaluated.

MATERIALS AND METHOD

Animals and managements

Ten primiparous, non pregnant crossbred cattle, containing 87.5 % Holstein (HF) genes, were used for the experiment. The animals with averaged 60 days postpartum at start of trial were assigned randomly into two groups of five animals each. Animals in the first group were housed in open-sided barn with a tiled roof in normal shaded house (NS) as the non-cooled animals. Animals in the second group were housed in open-sided barn with a tiled roof (8 m long x 7 m wide x 3.5 m high) under misty fan cooling (MFC) as cooled animals. The open space cooling system consisted of two sets of misty fan, which each system consisted of a 65 cm. diameter blade fan circulating 81 m^3/min of air, with oscillation coverage of 180º. The amount of water

discharged from 4 splay heads was 7.5 L/h and size of mist droplet 0.01 mm. Animals were exposed to MCF for 45 minutes at 15-minute intervals from 06:00 h to 18:00 h. At night, animals were exposed to MCF for 15 minutes at 45-minute intervals from 18:00 h to 06:00 h.

The diet was fed twice a day for *ad lib* as the same ration of total mixed ration (TMR) throughout the experiments in both groups. Ingredient and chemical compositions of feed are shown in Table 1. Animals are milked twice a day using a milking machine and milk production was recorded daily. Each day, the food was given in equal portions at about 06:00 h and 17:00 h when the animals were milking. The ambient temperature was recorded using a dry bulb thermometer. The relative humidity at NS and MFC were read by psychrometric chart depending on wet and dry bulb temperature. Ambient temperatures, humidity, rectal temperature and respiratory rate were measured weekly at 09:00, 11:00, 13:00, 15:00 and 17:00 h on specified day. A temperature-humidity index (THI) was calculated from the average ambient temperature of dry and wet bulb temperatures according to McDowell (1972), as follow:

$$THI = 0.72 (wb+db) + 40.6$$

Where ; wb = wet bulb temperature and db = dry bulb temperature expressed in °C

Experimental design

The experiments were carried out throughout lactating period in each group. The experiment in each group was divided into 3 phases, namely early- (Day 60 postpartum), mid- (Day 120 postpartum), and late lactating periods (Day 180 postpartum). The pretreatment study was conducted on the starting day of each phase. At the end of the pretreatment, within the same day, the animal was injected with the first dose subcutaneous injection of 500 mg of recombinant bovine somatotropin (rbST) (POSILAC, Monsanto, USA). Subsequently, the animal was injected with two consecutive doses injections of rbST every 2 weeks. Thereafter, within 2 days after the third injection, the treatment study was conducted. The pretreatment, 3 doses of injections, and the treatment periods were performed during the first 30 days and the same procedures were followed for each phase. During the last 30 days of each phase, no experiments were conducted in order to allow the milk yield from the effect of rbST treatment to return to the control level.

Milk sampling and determination of milk composition

The animals were normally milked at around 06.00 hours and 17.00 hours by a milking machine. The daily milk yield (kg/day) was recorded and the weekly average of each animal was calculated. Milk was collected in the afternoon of a specified day and divided to two portions. One was kept fresh to determine the plasmin–plasminogen concentration and other portion of 60 ml of milk was kept using 0.1ml of bronopol (2-Bromo-2-nitropropane-1,3 diol) (0.02w/w). Milk was kept at 4°C for determinations of lactose by the colorimetric method (Tele *et al*. 1978), fat by the Gerber method and protein concentrations by infrared method using Milkoscan, respectively. The concentrations of electrolytes in the aqueous phase of milk were estimated for sodium (Na^+) and potassium (K^+) using Flame photometry and chloride (Cl^-) concentration by Chloridometer (Chloride Analyzer, 925).

Plasmin and plasminogen determination

The concentrations of plasmin and plasminogen in milk or casein fractions were determined by 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 405 nm. One 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.

RESULTS

The milk plasminogen and plasmin activity and milk electrolyte concentrations (Na^+, K^+, Cl^-) in cows supplemental with rbST and housing under normal shade (NS) and misters fans cooling (MFC) at different stages of lactation (Table 1).

The milk plasminogen concentrations were not significantly different between the cooled cows and the non-cooled cows as lactation advances. The total plasminogen and plasmin activities tended to increase during lactation advances in both cooled and non-cooled cows supplemental rbST. Milk plasmin activities were not affected by the effect of rbST supplementation. The plasminogen slightly increased in the rbST-treated animals in each stage of lactation. The plasminogen: plasmin ratio was not affected in either cooled or non-cooled cows supplemental rbST in different stages of lactation. The milk Na^+, K^+ and Cl^- concentrations showed no significant changes between cooled and non-cooled cows whether supplemental rbST or not in each stage of lactation. The Na^+/K^+ ratio significantly increased in early lactation during rbST supplementation of both cooled and non-cooled cows.

Milk yield and milk compositions in animals in cows treated with rbST and housing under normal shade (NS) and misters and fans cooling (MFC) at different stages of lactation (Table 2).

Milk yield significantly increased after supplemental rbST as compared with the pretreatment period in each stage of lactation in both cooled and non-cooled cows. The low responses in milk yield during rbST supplementation as lactation advanced. The significant increases in the milk fat concentrations after supplemental rbST in both cooled and non-cooled cows. Milk from both cooled and non-cooled cows supplemental rbST showed no significant differences of milk lactose, solid not fat and total solid in each stage of lactation. During supplementation of rbST, the concentration of milk protein increased in the early and mid-lactation of both cooled and non-cooled cows.

Table 1. Effects of the application of misters and fans and supplementation with rbST on the milk plasminogen, plasmin activity and milk electrolyte concentrations (Na^+, K^+, Cl^-) in cows treated with rbST and housing under normal shade (NS) and misters and fans cooling (MFC) at different stages of lactation.

	Stages of	NS		MFC			[1]Effect		
Parameter	lactation	Pre	rbST	Pre	rbST	SEM	house	rbST	house*rbST
Native plasmin activity (unit/ml)	Early	2.38	6.14	5.22	4.70	1.74	0.798	0.380	0.255
	Mid	5.38	9.94	4.84	7.94	2.36	0.823	0.143	0.765
	Late	8.64	8.72	5.94	4.44	2.12	0.496	0.747	0.719
Plasmin & plasminogen activity (units/ml)	Early	148.54	152.28	139.60	147.86	17.79	0.745	0.745	0.902
	Mid	125.08	179.22	158.66	174.40	35.12	0.684	0.349	0.599
	Late	176.14	183.20	185.48	188.94	25.83	0.890	0.844	0.946
Plasminogen activity (units/ml)	Early	146.12	146.12	134.38	143.18	18.29	0.729	0.816	0.816
	Mid	119.72	165.28	153.82	166.48	35.66	0.642	0.438	0.657
	Late	167.50	174.46	179.52	184.50	26.23	0.844	0.826	0.971
Plasminogen / Plasmin	Early	66.8	77.1	72.0	71.3	33.0	0.995	0.888	0.872
	Mid	55.6	80.5	53.2	38.7	14.5	0.579	0.680	0.193
	Late	79.7	68.4	92.9	124.8	25.8	0.608	0.702	0.427
Sodium (mM)	Early	29.80	31.20	27.20	27.60	1.31	0.250	0.449	0.670
	Mid	28.80	29.20	27.80	28.00	0.79	0.651	0.714	0.902
	Late	32.60	32.20	29.00	32.00	2.05	0.700	0.544	0.432
Potassium (mM)	Early	38.70	36.88	36.80	36.04	1.27	0.434	0.341	0.688
	Mid	37.06	36.16	36.32	35.08	0.90	0.582	0.267	0.854
	Late	35.16	34.74	33.84	34.50	1.07	0.609	0.914	0.628
Na^+/K^+ ratio	Early	0.78	0.85	0.73	0.77	0.022	0.392	0.038	0.464
	Mid	0.78	0.82	0.77	0.80	0.025	0.868	0.180	0.984
	Late	0.94	0.93	0.87	0.94	0.083	0.858	0.727	0.639
Chloride (mM)	Early	31.00	33.00	36.40	35.80	0.78	0.277	0.398	0.136
	Mid	33.20	32.80	29.80	27.60	0.78	0.255	0.132	0.279
	Late	33.40	32.60	33.80	41.60	3.75	0.541	0.378	0.284

SEM = Standard error of the mean.

[1] P-values for the effects; MFC =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MFC and rbST

Table 2. Milk yield and milk compositions in animals in cows treated with rbST and housing under normal shade (NS) and misters and fans cooling (MFC) at different stages of lactation.

	Lactating	NS		MFC			Effects[1]		
	period	Pre	rbST	Pre	rbST	SEM	MFC	rbST	MFCx rbST
Milk yield	Early	10.81	12.30	12.19	12.82	0.25	0.580	0.002	0.146
	Mid	9.19	10.44	11.58	12.70	0.36	0.222	0.002	0.413
	Late	8.24	9.73	9.38	12.30	0.54	0.362	0.003	0.217
Protein	Early	3.37	3.61	3.48	3.63	0.15	0.788	0.227	0.790
(gm%)	Mid	3.79	3.84	4.09	4.26	0.15	0.104	0.466	0.695
	Late	4.25	4.03	4.30	4.32	0.17	0.518	0.586	0.499
Fat	Early	3.27	4.29	3.89	4.76	0.24	0.325	0.004	0.757
(gm%)	Mid	3.53	4.25	3.87	4.44	0.21	0.593	0.013	0.732
	Late	4.27	4.58	4.11	5.15	0.33	0.732	0.075	0.301
Lastose	Early	4.74	5.09	5.00	4.89	0.14	0.846	0.411	0.140
(gm%)	Mid	4.85	4.82	4.82	4.91	0.08	0.820	0.698	0.452
	Late	4.77	4.78	4.41	4.72	0.11	0.358	0.186	0.217
SNF	Early	8.61	9.39	9.18	9.22	0.21	0.614	0.079	0.110
(gm%)	Mid	9.34	9.37	9.61	9.87	0.16	0.071	0.385	0.474
	Late	9.72	9.37	9.41	9.74	0.23	0.906	0.953	0.179
TS	Early	13.24	12.79	13.42	14.54	0.85	0.401	0.703	0.381
(gm%)	Mid	14.87	13.42	14.92	15.05	0.49	0.590	0.215	0.147
	Late	14.68	14.40	15.22	16.09	0.80	0.447	0.722	0.494

SEM = Standard error of the mean.
[1] P-values for the effects; MFC =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MFC and rbST

DISCUSSION

The previous study showed a rapid decrease in the peak rate of milk yield concomitant as lactation advances with decreases in both mammary blood flow and the concentration of plasma growth hormone in crossbred cattle containing 87.5% Holstein genes(Chaiyabutr *et al*. 2000). In the present study, the milk yield significantly increased after supplemental rbST as compared with the pretreatment period in each stage of lactation in both cooled and non-cooled cows. However, the low responses in milk yield during rbST supplementation as lactation advanced are similar to previous reports in dairy crossbred cattle (Phipps *et al*. 1991). These results indicate that an increase in the milk yield of crossbred dairy cattle in response to rbST administration is not sustained for long, and is influenced by the stage of lactation.

This indicates that exogenous rbST cannot exert its effects in limiting the natural loss of secretory cell activity during the involution of the mammary tissue as lactation advances in crossbred dairy cattle.

The significant increases in the milk fat concentrations after supplemental rbST in both cooled and non-cooled cows and the control animals found in this study indicate an increase in fatty acid synthesis de novo in the mammary gland during rbST treatments. 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). The administration of exogenous bST has been known to reduce lipid accretion and fatty acids made available for oxidation and are precisely coordinated with an increase in milk fat yields (McCutcheon & Bauman 1986). However, it has been noted that concentrations of fat, including protein in milk, may or may not change in animals treated with bovine somatotropin (Baldi *et al*. 1997). Thus, the total yield of fat and protein of cows supplemental rbST would be higher through the effect of increased milk yield during the treatment period.

The plasminogen and plasmin activities tended to increase during lactation advances in both cooled and non-cooled cows supplemental rbST. The plasminogen–plasmin system has been known to be involved in the tissue remodeling associated with the declining phase of lactation and mammary gland involution in dairy ruminants. 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). An administration of bST in cows has been shown to prevent an increase in milk plasmin activity during late lactation suggesting bST delaying mammary gland involution (Politis *et al*. 1990). However, in the present results, the effect of rbST on preventing an increase in milk plasmin activities was not apparent. These results are in agreement with the previous results during long-term administration of rbST (Chaiyabutr et al., 2007). In both cooled and non-cooled cows there was a tendency increase in milk plasminogen concentrations during supplementation of rbST. A different pattern of this enzymatic system in crossbred dairy cattle would be expected. During supplementation of rbST, the concentration of milk protein increased in the early and mid-lactation of both cooled and non-cooled cows. These results might be ascribed to an increase in milk plasminogen

concentrations. The effect of growth hormone on the synthesis and secretion of α-casein in the bovine mammary epithelial cell line has been reported (Sakamoto *et al*. 2005). An increase in the plasmin activity in milk coinciding with an increase in the concentration of casein in milk has also been noted (Dulley 1972). Milk plasmin is known to be influenced by the availability of plasminogen and the plasminogen activators. As plasminogen is ubiquitous in the body, the plasminogen concentration in milk in cooled and non-cooled cows supplemental rbST would not be expected to be limiting in the present study. The milk plasminogen concentrations were not significantly different between the cooled cows and the non-cooled cows as lactation advances, which was similar to that of findings in cows by Politis *et al*. (1990). However, the plasminogen slightly increased in the rbST-treated animals in each stage of lactation. The plasminogen: plasmin ratio is a useful index of plasminogen activation, which is independent of milk volume. The data for the PG:PL ratio in both cooled and non-cooled cows are similar to those of studies in New Zealand cows, which reported that the PG:PL ratio in bovine milk ranged between 31.3 and 81.2 (Stelwagen et al., 1994; Lacy-Hulbert et al., 1999), suggesting that PL was extremely low in both crossbred Holstein and New Zealand milk. It is not similar to the reports of Politis et al. (1990) that the PG:PL ratio ranged between 6.3 (early lactation milk) and 3.6 (late lactation milk) and Baldi et al. (1996), who investigated the PG:PL ratio in bovine milk of Italian dairy herds between 2.4 and 6. The present data concerning the PG:PL ratio obtained herein were not affected in either cooled or non-cooled cows supplemental rbST in different stages of lactation, which indicate that PG is not converted to PL more efficiently in crossbred cow milk during rbST administration. It is probably that rbST supplementation delayed massive activation of plasminogen and production of plasmin whether cows housing under misty fan or not. Therefore, it does not exclude the possibility in the present results that rbST was involved in maintenance of the tissue function.

The mechanism of ion transport in the mammary cell has been proposed as occurring by either a transcellular route or a paracellular route (Linzell and Peaker 1971). In the present study, milk from both cooled and non-cooled cows supplemental rbST showed no significant differences of milk Na^+, K^+ and Cl^- concentrations in each stage of lactation. However, the effect of supplementation of rbST on Na^+: K^+ ratio in milk of both cooled and non-cooled cows were significantly high in early

lactation. These results indicate that during high milk yield in early lactation, the mammary gland tissue permeability is markedly increased and the breakdown of junctions between adjacent epithelial cells is thought to be a cause of this result. Therefore, changes in the profile of milk ion concentrations indicate that the role of rbST could not maintain tissue integrity in the mammary gland during early lactation.

These findings suggest that the decrease in milk secretion during lactation advances might not be controlled by changes in extra-mammary factors but, may in part, occur through changes within the mammary gland relating to the activity of the plasmin–plasminogen system.

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CHAPTER VIII

Indicator of oxidative status in plasma of shaded cooled animals with recombinant bovine somatotropin (rbST) administration in different stages of lactation in crossbred Holstein cattle

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Indicator of oxidative status in plasma of shaded cooled animals with recombinant bovine somatotropin (rbST) administration in different stages of lactation in crossbred Holstein cattle

INTRODUCTION

One of the most problems for dairy animal production in the tropics is high environmental temperature and it is often considered as one of the main causes of stress. Heat stress affects the performance of dairy cows by reducing their dry matter intake, feed efficiency and milk production (Fuquay, 1981; Shibata & Mukai 1979). The occurring during heat stress of dairy cattle disrupt their homeostasis and metabolism, including changes in the activity of enzymes and hormones. It is known that the heat stress-induced production of oxygen-derived free radicals consequently shows up in some of the deleterious effects of heat stress (Mitchell & Russo 1983; Loven 1988). Oxidative stress, which stimulated the production of free radicals and reactive oxygen species, is believed to be involved in the etiology of many diseases (Halliwell & Gutteridge 1990), and to reduce the metabolic activity of several tissues, consequently decreasing the performance of domestic animals. A number of studies on oxidative stress in domestic animals under hot condition have been reported, especially on poultry (Donkoh 1989; Mujahid *et al*. 2005). A few data are available for the effects of heat stress on the oxidative stress index in the plasma of crossbred dairy cows, compared with other domestic animals. Lakritz *et al*. (2002) reported that heat stress decreased reduced glutathione contents and increased the oxidized glutathione contents in the whole blood of adult cows. Although the performance of exotic Holstein dairy cows was affected by hot conditions and oxidative stress, the changes of some oxidative stress markers in the plasma of crossbred Holstein cattle was obscure. The low milk production is still the main problem in dairy farming in the tropics. Our previous report (Chaiyabutr et al., 2007) indicated that growth hormone act as a regulator of fluid homeostasis, administration of bST increased in total body

water of crossbred Holstein cattle. Whether exogenous rbST would help to improve negative responses in lactating cows during heat stress,which is a matter of debate. Despite those studies for the regulation of milk secretion in crossbred cattle have shown to be inherited and being thought to be among the causes of differences in metabolic parameters (Chaiyabutr et al. 2000). Few data are available for the study whether restoring body fluids during rbST administration in crossbred Holstein cattle could reduce oxidative stress allowing milk production at different stages of lactation to be maintained for a longer time during exposure to high environmental temperature. Environmental modifications have been known to alleviate severe heat stress in dairy cattle. Management strategies are needed to minimize the effects of reactive oxygen species (ROS) generated during thermal stress, especially in the tropic. It was reported that heat stress influenced the plasma concentration of antioxidative vitamins such as ascorbic acid and tocopherol ~~and minerals such as zinc and chromium (Halliwell & Gutteridge 1989; Iwagami 1996~~; Sahin *et al*.2002), and increased lipid peroxidation in_tissue(Altan et al.,2003). Therefore, to clarify the oxidative stress of dairy cows under hot conditions, alterations of oxidative stress markers of sulfhydryl (SH) residue, ascorbic acid and thiobarbituric acid reactive substance (TBARS) concentration in the plasma of crossbred Holstein dairy cows at high environmental temperatures and rbST supplementation were investigated.

MATERIALS AND METHOD

Animals and managements

Ten primiparous, non pregnant crossbred cattle, containing 87.5 % Holstein (HF) genes, were used for the experiment. The animals with averaged 60 days postpartum at start of trial were assigned randomly into two groups of five animals each. Animals in the first group were housed in open-sided barn with a tiled roof in normal shaded house (NS) as the non-cooled animals. Animals in the second group were housed in open-sided barn with a tiled roof (8 m long x 7 m wide x 3.5 m high) under misty fan cooling (MFC) as cooled animals. The open space cooling system consisted of two sets of misty fan, which each system consisted of a 65 cm. diameter blade fan circulating 81 m^3/min of air, with oscillation coverage of 180º. The amount of water discharged from 4 splay heads was 7.5 L/h and size of mist droplet 0.01 mm. Animals were exposed to MCF for 45 minutes at 15-minute intervals from 06:00 h to

18:00 h. At night, animals were exposed to MCF for 15 minutes at 45-minute intervals from 18:00 h to 06:00 h.

The diet was fed twice a day for *ad lib* as the same ration of total mixed ration (TMR) throughout the experiments in both groups. Ingredient and chemical compositions of feed are shown in Table 1. Animals are milked twice a day using a milking machine and milk production was recorded daily. Each day, the food was given in equal portions at about 06:00 h and 17:00 h when the animals were milking. The ambient temperature was recorded using a dry bulb thermometer. The relative humidity at NS and MFC were read by psychrometric chart depending on wet and dry bulb temperature. Ambient temperatures, humidity, rectal temperature and respiratory rate were measured weekly at 09:00, 11:00, 13:00, 15:00 and 17:00 h on specified day. A temperature-humidity index (THI) was calculated from the average ambient temperature of dry and wet bulb temperatures according to McDowell (1972), as follow:

$$THI = 0.72 (wb+db) + 40.6$$

Where ; wb = wet bulb temperature and db = dry bulb temperature expressed in °C

Rectal temperature and respiratory rates were measured at different parts of the day with electronic thermometers and observing flank movements, respectively.

Experimental design

The experiments were carried out throughout lactating period in each group. The experiment in each group was divided into 3 phases, namely early- (Day 60 postpartum), mid- (Day 120 postpartum), and late lactating periods (Day 180 postpartum). The pretreatment study was conducted on the starting day of each phase. At the end of the pretreatment, within the same day, the animal was injected with the first dose subcutaneous injection of 500 mg of recombinant bovine somatotropin (rbST) (POSILAC, Monsanto, USA). Subsequently, the animal was injected with two consecutive doses injections of rbST every 2 weeks. Thereafter, within 2 days after the third injection, the treatment study was conducted. The pretreatment, 3 doses of injections, and the treatment periods were performed during the first 30 days and the same procedures were followed for each phase. During the last 30 days of each phase, no experiments were conducted in order to allow the milk yield from the effect of rbST treatment to return to the control level.

Measurements of SH residue, TBARS and ascorbic acid concentration in plasma

On each specified day, blood sample was obtained from each cross-bred Holstein cattle. Blood was collected from the coccygeal vessel by venopuncture immediately. Heparin was used as an anticoagulant in all samples. The concentration of Sulfhydryl residue(SH), the TBARS concentration and the total ascorbate concentration in the plasma were measured by spectrophotometric methods. The concentration of plasma Sulfhydryl (SH) residue in the plasma was measured by the methods of Motchnik *et al*. (1994) using dithionitrobenzene (DTNB) in the reaction. The TBARS concentration in the plasma, a breakdown product of lipid peroxidation, was determined by methods according to previous reports (Ohkawa *et al*.1979; Chitra *et al*. 20a03) with an extraction solution contained pyridine and 1-buthanol using the concentration of malondialdehyde as the standard. The total ascorbate concentration in the plasma was measured according to the method described by Omaye *et al*. (1979). The ascorbic acid was oxidized by copper ions to form dehydroascorbic acid and diketogulonic acid, which react with 2,4- dinitrophenylhydrazine to form the derivative bis-2,4- dinitrophenylhydrazone. A standard curve was made using several concentrations of sodium ascorbate. The total protein and albumin concentrations in the plasma were determined by an Automatic analyzer (Operator Manual BT 2000 Plus, Biotecnica Instruments S.P.A Via Licenza, Rome, Italy.

Statistical analysis

The statistic analyses were performed using General Linear Model procedures of statistical software package SPSS (SPSS for windows, V14.0; SPSS Inc., Chicago, IL, USA). The model used for each analysis was:

$$Y_{ijk} = \mu + A_l + H_i + A(H)_{il} + B_j + (HB)_{ij} + A(HB)_{ijl} + Cov_k + e_{ijkl}$$

Where Y_{ijk} = observation, μ = overall mean, A_l= Animal effect H_i= house effect as main plot (*i* = NS, MFC), $A(H)_{il}$ = main plot error (animal *l* in house *i*), B_j = treatment effect (rbST) as a split plot (*j* = with and without rbST supplementation), $(HB)_{ij}$ = interaction effect between treatment and house , $A(HB)_{ijl}$ = split plot error (animal *l* in house *i* and treatment *j*), Cov_k = covariate effect and e_{ijk} = residual error.

Means values were used to evaluate the effect for all variables. Statistical significance was declared at $P<0.05$. and trends were declared at $0.05 < P \leq 0.10$.

RESULTS

Ambient temperature, relative humidity, temperature humidity index, rectal temperature and respiration rate in animals treated with rbST under normal shade (NS) and misters and fans cooling (MF) at different stages of lactation (Table 2).

An environmental parameters measurement at 1400h are shown in Table2. Ambient temperature measured in NS barn was significantly higher than MFC barn throughout experimental period, while relative humidity of NS barn was lower than that of MFC barn. The significant differences of THI were apparent between MFC barn and NS barn. Cows without rbST housed under MFC barn showed significantly lower RR and RT than those of cows housed under NS barn. After rbST supplementation in both cooled and non-cooled cows showed significantly increases in RR and RT when compared with pre-treatment in each stage of lactation.

Effects of the application of misters and fans and supplementation with rbST on the concentrations of malondialdehyde (MDA) TBARS, ascorbate, Sulfhydryl residue and plasma albumin of crossbred Holstein cows (Table 3).

One antioxidative component, the SH residue concentration in the plasma, and the oxidation products of polyunsaturated lipid, (TBARS concentration) of both cooled and non-cooled cows showed no changes during periods of rbST supplementation. Another antioxidative component of ascorbic acid concentration in plasma were not affected during rbST supplementation, but the ascorbic acid concentrations in plasma of cooled cows were significantly lower than those of non-cooled cows in early and mid-lactation. The albumin concentration in the plasma of both cooled and non-cooled cows was not affected by the high temperatures and rbST supplementation.

Table 1. Feed ingredients and chemical compositions of the diet

Ingredients:	Kg (as fed basis)
Pine apple waste	50
Soybean meal	23
Rice bran	3.0
Cotton seed	20
Lime stone	1.4
Di-calcium phosphate	1.4
Sodium bicarbonate	0.3
Potassium chloride	0.1
Mineral and vitamin premix	0.8
Total	100
Chemical composition:	
Dry matter (%)	39.1
Ash (% DM)	7.3
Organic matter (% DM)	92.7
Crude protein (% DM)	18.0
Acid detergent fiber (% DM)	20.1
Neutral detergent fiber (% DM)	33.9

Table 2. Ambient temperature, relative humidity, temperature humidity index, rectal temperature and respiration rate in animals treated with rbST under normal shade (NS) and misters and fans cooling (MF) at different stages of lactation.

		Treatments					Effects[1]		
	Lactating period	NS	NS+rbST	MF	MF+rbST	SEM	MF	rbST	MFxrbST
Ambient temperature (°C)	Early	34.6	35.3	31.6	29.8	0.95	0.001	0.578	0.224
	Mid	33.4	33.1	30.7	30.0	0.61	0.004	0.439	0.753
	Late	32.5	32.3	27.8	30.1	0.81	0.001	0.229	0.16
Relative humidity (%)	Early	56.0	51.0	60.2	75.5	3.31	0.006	0.159	0.015
	Mid	57.6	60.0	71.5	75.4	4.48	0.017	0.503	0.871
	Late	60.0	61.8	79.2	64.4	4.35	0.054	0.173	0.093
Temperature humidity index (THI)	Early	85.5	85.3	82.2	81.8	1.13	0.002	0.816	0.924
	Mid	83.9	84.1	82.7	82.1	0.75	0.043	0.778	0.671
	Late	83.3	83.2	79.2	80.6	0.72	0.001	0.369	0.354
Rectal temperature (°C)	Early	38.8	39.0	38.0	38.2	0.07	0.001	0.023	0.886
	Mid	39.4	39.9	38.6	38.9	0.13	0.005	0.011	0.245
	Late	39.1	39.3	38.5	38.8	0.10	0.057	0.023	0.565
Respiration Rate (breath/min)	Early	72.0	78.0	54.0	63.2	2.52	0.001	0.017	0.544
	Mid	70.4	73.4	52.8	55.0	1.22	0.001	0.065	0.751
	Late	71.6	78.4	52.2	57.6	1.16	0.001	0.004	0.657

SEM = Standard error of the mean.
[1] P-values for the effects ; MF =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

Table 3. Effects of the application of misters and fans and supplementation with rbST on the concentrations of malondialdehyde (MDA) TBARS, ascorbate, Sulfhydryl residue and plasma albumin of crossbred Holstein cows

Parameter	Stages of lactation	NS		MFC		SEM	[1]Effect		
		Pre	rbST	Pre	rbST		MCF	rbST	MCF*rbST
Ascorbic acid (µmole/l)	Early	22.26	22.20	32.10	30.62	1.98	0.023	0.708	0.730
	Mid	20.91	19.29	25.48	28.79	1.61	0.085	0.612	0.164
	Late	22.21	25.01	25.25	26.62	1.73	0.440	0.262	0.691
Sulfhydryl residue (µmole/l)	Early	393.21	363.23	365.67	361.59	17.01	0.763	0.346	0.468
	Mid	379.95	363.12	378.93	389.64	14.89	0.742	0.842	0.382
	Late	388.62	419.26	375.87	418.22	19.94	0.872	0.105	0.777
Malondialdehyde,TBARS (nmole/ml)	Early	1.69	2.11	2.61	2.06	0.11	0.243	0.590	0.003
	Mid	1.72	2.29	1.79	1.92	0.24	0.539	0.181	0.400
	Late	1.89	2.16	1.89	2.28	0.16	0.814	0.075	0.729
Albumin (µmole/l)	Early	636.6	635.1	630.0	634.6	10.33	0.901	0.890	0.779
	Mid	630.3	614.9	616.9	627.1	10.72	0.755	0.765	0.294
	Late	642.9	648.7	625.9	621.4	13.54	0.604	0.715	0.831
SH-residue/Albumin	Early	0.62	0.58	0.59	0.57	0.027	0.818	0.308	0.496
	Mid	0.60	0.59	0.61	0.61	0.031	0.765	0.465	0.344
	Late	0.61	0.65	0.60	0.66	0.026	0.804	0.543	0.431

SEM = Standard error of the mean.

[1] P-values for the effects ; MF =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

DISCUSSION

It is known that the effects of hot environment on lactation performance are dependent on the breed, age stage of lactation, degree of acclimatization and the level of milk production itself. The reduction in milk production in a hot environment is usually attributed to a fall in feed intake. This shows direct effect on the mechanism of lactation, since the reduction in milk production occurred under high temperatures even cows fed fixed level of feed intake (Wayman et al., 1962). However, the study in 87.5% crossbred Holstein cattle in the tropic have been shown that the concentration of plasma bST decreased as lactation progressed to mid and late lactation. These reductions would concomitant to a reduction in both mammary blood flow and milk yield (Chaiyabutr et al. 2000). The environmental temperatures measured in MFC barn in the present study showed low ambient temperature and THI as compared with in NS barn, especially in the afternoon throughout the experimental periods. However, the THI in both barns ranged from 80.7-85.5 remained higher than the threshold level of comfortable zone, 72 for THI (Armstrong, 1994). Cows in both groups would be subjected to moderate heat stress (Fuquay, 1981). The cooling of cows under MFC was significantly lower in both respiratory rate and rectal temperature in comparison with those of non-cooled cows which indicate a partial alleviation of heat stress by MF system especially in the afternoon.

High environmental temperatures affect to bodily functions including a number of performance cells in dairy cow. High ambient temperatures influencing oxidative stress in cultured tissue has been noted (Skibba *et al*. 1989, 1990, 1991). The free radicals detected by radical electron paramagnetic resonance spin signals in heat-stressed cells had increased (Flanagan *et al*. 1998). An increase in oxidative stress derived from radical molecules in the body may decrease in dairy performance with a decrease in milk production under heat-stressed conditions. In the present study, clarifications of the oxidative stress under high ambient temperatures with determination of the alterations of plasma oxidative stress markers were performed. Ascorbic acid is known to be the most important water soluble antioxidative vitamin (Frei *et al*. 1989). Bovines have not been considered to need dietary ascorbic acid because they are able to synthesize a high enough level of ascorbic acid in themselves. The reduction of ascorbic acid concentrations in the plasma of cows was apparent under NS barn independent on the effect of rbST. There are many factors

can affect the level of plasma ascorbic acids. The reduction of the concentration of ascorbic acid in the plasma has been shown in calves under stressful conditions of cowshed environments (Cummins & Brunner 1991) and in lactating cows with mastitis (Weiss *et al*. 2004). Ascorbic acids as an antioxidant is known to donate a free molecule of hydrogen that detoxifies the harmful ROS generated by the body during heat stress. Thus, hot conditions under moderate heat stress (high THI) in NS barn seemed to promote oxidative stress with the reduction of plasma ascorbic acid concentration. However, the components of SH residue, TBARS and the plasma albumin concentration in cows under NS were not affected as compared with the values of cows in MFC barn whether supplemental rbST or not. Albumin was thought to be one of resources of SH residue in the plasma. It is known that reversible oxidations of sulfur residues are common and fundamentally important in the control of cell functions (Moran *et al*. 2001). SH residues are abundant both inside and outside the cell as non-protein and protein SH groups which predominate over the oxidized form. The concentration of SH residues in plasma of both cooled and non-cooled cows supplemental rbST were closed to the plasma albumin concentrations indicating that most protein SH groups are found on plasma albumin (Radi *et al*. 1991). Albumin possess only one SH residue in a molecule (Carter & Ho 1994). It was observed that no changes in of the ratio of SH residues to albumin concentrations were apparent in plasma in both cooled and non-cooled cows supplemental rbST. It has been reported that oxidized ascorbic acid by oxidative stress was re-reduced using the reducing equivalent of SH residues in rat plasma (Vethanayagam *et al*. 1999). Ascorbic acid concentrations in the plasma of dairy cows seem to be important in reducing its equivalent in body fluid and is sensitive to oxidative stress. These results suggest that the ascorbic acid concentrations in cow plasma are sensitive to environmental. However, the SH concentration did not show pattern as ascorbic acid in the plasma. It is possible that the oxidation of the SH residues by oxidative stress did not utilize the same mechanism as that of ascorbic acid oxidation. The concentration of a breakdown product of lipid peroxidation, TBARS, known to be one of the oxidative stress markers in plasma (Nielsen *et al*. 1997). The TBARS concentration in plasma of both cooled and non-cooled crossbred cows were not derived from oxidative stress under moderately heat stress. It has been reported that oxidative stress increased TBARS (Halliwell & Chirico 1993), and the cytotoxic

effects of TBARS is well known (Zollner *et al*. 1991). The results of the present study showed that the profiles of oxidative stress markers in plasma of non-cooled cows were not different from those of cooled cows. Their internal relationships, namely, SH versus ascorbic acid concentration, SH versus TBARS and ascorbic acid versus TBARS, were not constant, which suggested there are different mechanisms in each oxidative stress markers' response to oxidative stress.

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CHAPTER IX

Changes in renal function and mammary circulation of shaded cooled animals and recombinant bovine somatotropin (rbST) administration in different stages of lactation in crossbred Holstein cattle:

Series 9.1 Effects of supplemental recombinant bovine somatotropin (rbST) and misters and fans cooling on renal function relation to body fluids regulation in different stages of lactation in crossbred Holstein cattle

Series 9.2 Effects of supplemental recombinant bovine somatotropin and mist-fan cooling on renal tubular handling of sodium in different stages of lactation in crossbred Holstein cattle

CHAPTER IX (Series 9.1)

Effects of supplemental recombinant bovine somatotropin (rbST) and misters and fans cooling on renal function relation to body fluids regulation in different stages of lactation in crossbred Holstein cattle

INTRODUCTION

The low milk production of both exotic and crossbred cattle is still the main problem in dairy farming in the tropics. The regulation of milk secretion in different types of crossbred cattle have shown to be inherited and being thought to be among the causes of differences in bodily functions. The lower efficiency in water retention and poor adaptation in tropical environment have been reported in 87.5%HF animals in comparison with those of 50%HF (Chaiyabutr et al., 1997; 2000). There is a rapid reduction of milk yield as lactation advanced to mid and late lactation in 87.5% HF animal. The reduction of milk yield is attributed the decrease in mammary blood flow (MBF) coinciding with the decline of the plasma bovine somatotropin (bST) concentration. These changes would account for the short lactation persistency (Chaiyabutr et al., 2000). In addition to animal genetic, other factors may affect milk production in dairy cattle in the tropics, such as high environmental temperature. Animals in high ambient temperatures will cause excessive heat load and impairment of physiological function including body fluids (Hahn et al., 1999).

Many technologies are required to improve milk production of dairy cattle in the tropics. Environmental modification is the most common approach to increase milk production with alleviation of severe heat stress in dairy cattle, for example, fans and sprinklers (Fike et al., 2002), evaporative cooling system (Chan et al., 1997 Chaiyabutr et al., 2008). Other technologies can increase milk production in dairy cattle in hot weather, for example the application of exogenous bovine somatotropin (West, 1994). There are inconsistent results as regards the relationship between effects of high environmental temperature and the application of exogenous bST on milk production. Some reports showed that bST-treated lactating cows increased heat production (Tyrrell

et al., 1988; Elvinger et al., 1992; Cole and Hansen, 1993) and caused a decrease in milk production during thermal imbalance. Some showed dairy cows treated with exogenous bST increase in milk production in both normal thermal zone and hot environment (Johnson et al., 1991; Staples et al., 1988). The increase in heat production in bST treated cows in high temperature may not be great enough to alter the cow's ability to maintain homeothermy; since it has been reported that exogenouse rbST to crossbred dairy cows increased milk yields accompanying with increases in total body water (TBW) and extra cellular water (ECW) (Maksiri et al., 2005 ; Chaiyabutr et al., 2007). With these facts, the higher total body water may be useful in slowing down the elevation in body temperature in hot conditions through evaporative cooling during heat dissipation. These parameters are considered to be factors that may be involved in shorter persistency of lactation if animals can not maintain their body fluids.

The kidney has known to be an important organ in the regulation of body fluids and compositions. Fluid volume and body compositions also depend on the coordinated action of multiple mechanisms regulating water intake and excretion. However, there is still no physiological evidence to interpret the influence of bST on controlling the kidney function in regulation of body fluids in dairy cows. Growth hormone (GH) administration has been reported to cause sodium retention and volume expansion in humans with severe growth hormone deficiency (Johannsson et al., 2002). In view of the study for an increase in total body water in rbST-treated cows (Chaiyabutr et al., 2007), we hypothesized that rbST supplementation would involve in the regulation of body fluids by accompanying with changes in the kidney function. In an attempt to explain the mechanism which is responsible for body fluid expansion after rbST supplementation, the study would focus exclusively on the renal events of crossbred dairy cows during rbST supplementation under high ambient temperature with or without misty cooling. Both assumptions, however, remain hypothetical to date because no experiments on dairy cows have been published investigating this mutual influence. Therefore, the objective of this studies were 1) to evaluate the effect of providing crossbred cattle with housing under shade with or without misters and fans and 2) supplementation of cows with rbST or not during period of lactation (early, mid and late lactation). Measures to evaluate the effectiveness of these treatments relative to renal function in regulation of body fluids were performed. This information might contribute to better insight into the physiological basis for the regulation in milk secretion under high temperature and supplemental rbST.

MATERIALS AND METHODS

Animal managements

Ten, first lactation, non pregnant, 87.5% lactating crossbred Holstein cattle were randomly selected and divide into two groups of five animals each. Animals in both groups were housed in open-sided barn with a tiled-roof. Animals in group 1 were housed in normal shaded barn (NS) and animals in group 2 were housed in shaded barn with misters and fans cooling (MFC). The barn (16 m long x 7 m wide x 3.5 m high) was separated into two parts. The first part (8 m long x 7 m wide x 3.5 m high) was arranged for animals in normal shade and the second part of barn equipped with two misters and fans system for cooled animal. Each system consisted of a 65 cm. diameter blade fan circulating 81 m^3/min of air, with oscillation coverage of 180º. The amount of water discharged from 4 mister spray heads (mounted relative to the fan) was 7.5 L/h and size of mist droplet 0.01 mm. Animals were exposed to MFC for 45 minutes at 15-minute intervals from 06:00 h to 18:00 h. At night, animals were exposed to MCF for 15 minutes at 45-minute intervals from 18:00 h to 06:00 h. All animals were fed with total mixed ration (TMR) throughout the experiments. Samples of TMR were analyzed for dry matter (DM) and chemical analysis (Table 1). Individual feed intake was recorded daily and analyzed for dry matter intake (DMI) Samples of TMR was determined for crude protein and ash using procedures described by AOAC (1990). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed according to Van Soest et al. (1991).

The ambient temperature was recorded by a dry bulb thermometer. The relative humidity was calculated from the reading of dry and wet bulb thermometer. Ambient temperature and humidity were measured once weekly during the period of hottest daily temperature (13.00 to 14.00 h). The temperature humidity index (THI) was calculated according to West (1994) where: THI = td-(0.55-0.55RH) (td-58) with td = dry bulb temperature (°F), and RH = relative humidity. Animal was normally milked at around 0600 h and 1700 h using a milking machine and milk production was recorded daily. Before treatment and at weekly intervals during treatment, cows were weighed.

Experimental procedures

The experiment in each group was divided into 3 phases, namely early- (Day 75 postpartum), mid- (Day 135 postpartum), and late lactating periods (Day 195 postpartum). The pretreatment study was conducted on the starting day of each phase. At the end of the pretreatment, within the same day, the animal was injected with the first dose subcutaneous injection of 500 mg of recombinant bovine somatotropin (rbST) (POSILAC, Monsanto, USA). Subsequently, the animal was injected with two consecutive doses injections of rbST every 2 weeks. Thereafter, within 2 days after the third injection, the treatment study was conducted. The pretreatment, 3 doses of injections, and the treatment periods were performed during the first 30 days and the same procedures were followed for each phase. During the last 30 days of each phase, no experiments were conducted in order to allow the milk yield from the effect of rbST treatment to return to the control level (Kirchgessner et al., 1991). Animals were normally milked at around 0600 h and 1700 h using a milking machine and milk production was recorded daily.

On each specified day of both pretreatment and treatment period, measurements of mammary blood flow, renal function and total body fluids were performed. The measurement of mammary blood flow (MBF) was performed in the morning (9.00-10.00 h). 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 for determination of MBF as previously described (Chaiyabutr et al., 2007).

After the study of MBF, the study of renal function and body fluids were performed subsequently. The catheter for isotope injection, dye injection and para-aminohippurate (PAH) solution infusion was inserted into an ear vein, under local anesthesia. The catheter was flushed with heparinized, normal saline (heparin 25 i.u./ml normal saline) and was left in place during the experiment. The urinary bladder had been fitted with an indwelling catheter (Foley catheter, no 22) in the bladder for urine collection. The free end balloon catheter was introduced into the bladder and secured with the inflated retaining cuff (60 ml. bulb capacity) during urine collection.

The procedures used in the present study were carried in accordance with the principles and guidelines of the Faculty of Veterinary Science, Chulalongkorn University. These guidelines were formulated to comply with international standards and

are in accordance with the principles and guidelines of the National Research Council of Thailand

Determinations of renal hemodynamics and electrolytes excretion

Measurement of renal hemodynamics was started by injection of 20 ml priming dose solution (2.5% PAH solution) via ear vein and followed immediately by a sustaining infusion of 0.5% PAH in normal saline at the rate of 2 ml/min. The solution was infused at a constant rate throughout the experimental study using a peristaltic pump (Eyela, MP-3, Tokyo Rikakikai, Japan). After equilibration period of a 2 h. of infusion, the experiments were carried out in duplicate of urine sample collection over an accurately timed period about 15-20 min. To ensure each accurate collection, the urine sample was started after voidness the bladder. The coccygeal blood sampling at midpoint of urine collection was performed. Plasma and urine samples were kept at -20 C for determinations of endogenous creatinine, PAH, electrolytes and osmolarity.

The clearance (C) of endogenous creatinine and PAH were used to measured GFR and ERPF respectively, based on the Fick Principle as previously described by Chaiyabutr et al., (1992). The effective renal plasma flow (ERPF) was measured by PAH clearances using standard techniques (Smith, 1962). Renal blood flow (RBF) was obtained by dividing ERPF by 1-packed cell volume. Filtration fraction (FF) was obtained by dividing GFR by ERPF. Plasma and urine samples were analyzed for concentrations of sodium and potassium ions by flame photometer (Flame photometer 410C, Ciba Corning Inc., USA), chloride ion by Chloridometer (Chloride analyzer 925, Ciba Corning Inc., USA) and osmolality by osmometer (Osmometer 3D3, Advance Instrument Inc., USA). Fractional excretion of electrolyte (%FE) was obtained by dividing clearance of electrolyte by GFR. Tubular solute-free water clearance (C_{H2O}) was calculated by substraction the rate of urine flow (V) from osmolar clearance (C_{osm}).

Determinations of water intake, total body water, extracellular fluid, plasma volume and blood volume

Estimation of the rate of water intake of each animal in each period of experimental was recorded by an average over three days from weighing daily water consumption by water meter. On each specified day, in the afternoon (13.00-14.00h), the measurements of total body water (TBW), extracellular fluid (ECF) and plasma volume (PV) were performed. The injection of 1ml of tritiated water (2,500 μci per animal), 20 ml of

sodium thiocyanate solution (10 g/100 ml normal saline) and 20 ml the Evans blue dye (T-1824) (0.5 g/100 ml normal saline) were performed via an ear vein catheter for estimation of TBW, ECF and PV, respectively. After dye injection, blood samples from the jugular vein were taken at 20, 30, 40, 50 and 60 min for ECF and PV determinations. Plasma samples were collected at 4, 8, 20, 26, 32, 44, 50, 56, 68 and 72 hr subsequent to the injection for determination of TBW.

Total body water (TBW) was determined in each animal by dilution techniques using tritiated water as previously described (Chaiyabutr et al., 1997). TBW = [standard count (dis/min) × dose (ml)] / [radio activity counts at zero time (dis/min)].

The concentration of plasma sodium thiocyanate solution was performed by method of Medway and Kare (1959) for estimation of ECF volume. Blood volume was calculated from the plasma volume and packed cell volume (Chaiyabutr et al., 1980).

Determination of mammary blood flow

Blood flow through half of the udder for MBF was determined by measuring in the dilution of dye T-1824 (Evan blue) by short term continuous infusion as previously described (Chaiyabutr et al., 1997).

Statistical analysis

Data for milk yield, DMI and water intake, in each lactating period were adjusted for covariate effects using mean value of 14 d before the pretreatment study. The stages of lactation (early, mid and late) were separated analysis. The statistic analyses were performed using general linear models procedure of statistical software package SPSS (SPSS for windows, V13.0; SPSS Inc., Chicago, IL, USA). The model used for each analysis was:

$$Y_{ijk} = \mu + A_l + H_i + A(H)_{il} + B_j + (HB)_{ij} + A(HB)_{ijl} + Cov_k + e_{ijkl}$$

Where Y_{ijk} = observation, μ = overall mean, A_l= Animal effect H_i= house effect as main plot (*i* = NS, MF), $A(H)_{il}$ = main plot error (animal *l* in house *i*), B_j = treatment effect (rbST) as a split plot (*j* = with and without rbST administration), $(HB)_{ij}$ = interaction effect between treatment and house , $A(HB)_{ijl}$ = split plot error (animal *l* in house *i* and treatment *j*), Cov_k = covariate effect and e_{ijk} = residual error.

RESULTS

Ambient temperature, relative humidity, temperature humidity index, rectal temperature and respiration rate. (Table 2)

The mean values of ambient temperature in NS and MF barns, respiratory rate and the rectal temperature of animals are shown in Table 3. At the period of hottest daily temperature (13.00 to 14.00 h), ambient temperature and temperature humidity index (THI) of the MF barn were significantly lower ($P<0.05$), while relative humidity in MF barn was significantly higher than that of NS barn throughout periods of study. The mean values of both respiratory rate and the rectal temperature of cows under MF were significantly lower than those of cows under NS with or without treatment of rbST. Both cooled and non-cooled cows showed significant increases in RR and RT after rbST treatment in all stage of lactation.

Dietary dry matter intake, water intake, milk yield and mammary blood flow (Table 3).

Effects of supplemental rbST and misters and fans cooling on dietary dry matter intake (DMI), water intake, milk yield and mammary blood flow (MBF) are shown in Table 3. The DMI of cows housing in MF barn with or without treatment of rbST were significantly higher than those of cows housing in NS barn in all stages of lactation. Cows treated with rbST under either MF or NS showed significantly increase in DMI than pre-treatment period. The highest values of DMI were apparent in cooled cow treated with rbST. Water intake was not significantly different among cows; however, the mean values of water intake of cooled cows tended to be higher than those of non-cooled cows. Cows treated with rbST under either MF or NS showed significantly increase in water intake than pre-treatment period. There was no evidence of interaction of cooling system and rbST treatment on water intake. Milk yield was significantly increased ($P<0.01$) during rbST administration. The milk yields of cooled cows were slightly higher than those of non-cooled cows. The milk yield showed significantly higher in cows treated with rbST in all stages of lactation. Cows treated with rbST under either MF or NS showed significantly increase in mammary blood flow than pre-treatment period. There was no evidence of interaction of cooling system and rbST treatment on mammary blood flow.

Total body water, extracellular fluid, Plasma volume, blood volume and hematocrit (Table 4).

Effects of supplemental rbST and misters and fans cooling on total body water (TBW), extracellular fluid (ECF), plasma volume (PV), blood volume (BV) and hematocrit are shown in Table 4. The absolute value of TBW and as a percentage of body weight were significantly increased ($P<0.01$) by rbST treatment in all stages of lactation. The application of misters and fans did not affect to the level of TBW. Mean values either the absolute values or as a percentage of body weight of ECF, PV and BV in cows without rbST showed no significant differences between cows in NS and MF barn in all stages of lactation except cows in MF barn showed higher absolute values of PV during late lactation. The absolute values of ECF, PV and BV markedly increased during rbST treatment in all stages of lactation. The value of ECF, PV and BV as a percentage of body weight of cows with rbST treatment tended to increase but no significant differences as compared with pretreatment period. Neither rbST treatment nor the misters and fans cooling affected the hematocrit values.

Renal hemodynamics of crossbred Holstein cows supplemented with rbST (Table5)

Effects of supplemental rbST and misters and fans cooling on renal hemodynamics are shown in Table 5. No significant changes of renal hemodynamic (GFR, ERPF, ERBF and FF) were apparent in cows during rbST treatment or under the application of misters and fans cooling in all stages of lactation. The rate of urine flow tended to decrease in cows treated with rbST by averaged 14.9%.in compared with pre-treatment period in cows housing in either MF or NS barn.

Urinary and fractional electrolytes excretion, osmolar clearance and free water clearance of crossbred Holstein cows (Table6)

Effects of supplemental rbST and misters and fans cooling on urinary and fractional (FE) electrolytes excretion, osmolar clearance and free water clearance are shown in Table 6. The urinary excretion and fractional excretion of sodium tended to decrease during rbST administration in cows housing either NS or MF barns. The significant effect of rbST on the decreases in both urinary excretion and fractional excretion of sodium was apparent ($P<0.01$) in mid lactation. The potassium excretion was decreased ($P<0.05$) during rbST treatment in early and mid lactation. The potassium excretion tended to decrease in cows treated with rbST administration under misters and fans cooling in both early and mid lactation. The effect of cooling system and rbST treatment on changes in fractional excretion of potassium was

significantly apparent (P<0.05) in early lactation. Chloride excretion and fractional excretion of chloride tended to decrease during rbST administration, but no statistically difference (P>0.05) in all stages of lactation. Osmolar clearance decreased significantly (P<0.05) during rbST treatment in both early and mid lactation, while free water clearance was not significantly affected (P>0.05) by rbST treatment or the application of misters and fans throughout lactation.

The plasma electrolytes concentration and plasma osmolarity (Table7)

Effects of supplemental rbST and misters and fans cooling on the plasma electrolytes concentration and plasm osmolarity are shown in Table 7. No changes in the concentrations of plasma Na^+, K^+, Cl^- and osmolarity in cows treated with rbST or housing MF barn.

Table1. Feed ingredients and chemical compositions of the TMR diet

Ingredients	Kg
Pine apple waste	50
Soybean meal	23
Rice bran	3.0
Cotton seed	20
Lime stone	1.4
Di-calcium phosphate	1.4
Sodium bicarbonate	0.3
Potassium chloride	0.1
Mineral and vitamin premix	0.8
total	100
Chemical composition	%
Dry matter (DM)	39.1
	---%DM---
Organic matter	92.7
Crude protein	18.0
Acid detergent fiber	20.1
Neutral detergent fiber	33.9

Table 2. Ambient temperature, relative humidity, temperature humidity index, rectal temperature and respiration rate in animals treated with rbST under normal shade (NS) and misters and fans cooling (MF) at different stages of lactation.

	Lactating period	Treatments				SEM	Effects[1]		
		NS	NS+rbST	MF	MF+rbST		MF	rbST	MFxrbST
Ambient temperature (°C)	Early	34.6	35.3	31.6	29.8	0.95	0.001	0.578	0.224
	Mid	33.4	33.1	30.7	30.0	0.61	0.004	0.439	0.753
	Late	32.5	32.3	27.8	30.1	0.81	0.001	0.229	0.16
Relative humidity (%)	Early	56.0	51.0	60.2	75.5	3.31	0.006	0.159	0.015
	Mid	57.6	60.0	71.5	75.4	4.48	0.017	0.503	0.871
	Late	60.0	61.8	79.2	64.4	4.35	0.054	0.173	0.093
Temperature humidity index (THI)	Early	85.5	85.3	82.2	81.8	1.13	0.002	0.816	0.924
	Mid	83.9	84.1	82.7	82.1	0.75	0.043	0.778	0.671
	Late	83.3	83.2	79.2	80.6	0.72	0.001	0.369	0.354
Rectal temperature (°C)	Early	38.8	39.0	38.0	38.2	0.07	0.001	0.023	0.886
	Mid	39.4	39.9	38.6	38.9	0.13	0.005	0.011	0.245
	Late	39.1	39.3	38.5	38.8	0.10	0.057	0.023	0.565
Respiration Rate (breath/min)	Early	72.0	78.0	54.0	63.2	2.52	0.001	0.017	0.544
	Mid	70.4	73.4	52.8	55.0	1.22	0.001	0.065	0.751
	Late	71.6	78.4	52.2	57.6	1.16	0.001	0.004	0.657

SEM = Standard error of the mean.

[1] P-values for the effects ; MF =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

Table 3. Dietary dry matter intake (DMI), water intake, milk yield and mammary blood flow (MBF) in animals treated with rbST under normal shade (NS) and misters and fans cooling (MF) at different stages of lactation.

	Lactating period	Treatments				SEM	Effects[1]		
		NS	NS+rbST	MF	MF+rbST		MF	rbST	MFxrbST
DMI (kg/d)	Early	6.12	7.04	7.20	8.22	0.32	0.043	0.016	0.879
	Mid	6.16	7.62	8.92	9.98	0.52	0.001	0.042	0.709
	Late	7.36	7.76	8.48	9.16	0.31	0.010	0.122	0.666
Water intake (kg/d)	Early	10.70	11.28	12.26	12.76	0.96	0.163	0.590	0.967
	Mid	9.28	10.10	11.18	12.32	1.18	0.134	0.430	0.895
	Late	8.10	9.68	8.88	11.04	1.39	0.295	0.216	0.840
Milk yield (kg/d)	Early	10.81	12.30	12.19	12.88	0.25	0.580	0.002	0.146
	Mid	9.19	10.44	11.58	13.46	0.36	0.222	0.002	0.413
	Late	8.24	9.73	9.38	12.30	0.54	0.362	0.003	0.217
MBF (ml/min)	Early	2,551	2,904	3,158	4,129	206	0.180	0.018	0.184
	Mid	2,204	2,636	2,333	2,914	236	0.617	0.076	0.766
	Late	1,984	3,361	2,569	2,843	98	0.929	0.001	0.001

SEM = Standard error of the mean.

[1] P-values for the effects ; MF =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

Table 4. Total body water (TBW), extracellular fluid (ECF), Plasma volume (PV), blood volume (BV) and packed cell volume (Hct) in animals treated with rbST under normal shade (NS) and misters and fans cooling (MF) at different stages of lactation.

	Lactating period	Treatments				SEM	Effects[1]		
		NS	NS+rbST	MF	MF+rbST		MF	rbST	MFxrbST
TBW (L)	Early	262.7	312.2	280.6	323.9	7.92	0.426	0.001	0.709
	Mid	263.9	312.6	272.9	336.5	7.74	0.512	0.001	0.375
	Late	267.3	321.7	275.9	330.3	13.15	0.712	0.006	0.999
TBW (L/100kg)	Early	74.5	83.5	72.1	76.6	2.81	0.520	0.026	0.800
	Mid	71.7	84.6	66.2	76.5	1.78	0.374	0.001	0.503
	Late	68.6	85.1	65.0	74.8	3.80	0.349	0.090	0.410
ECF (L)	Early	97.9	104.3	111.1	123.1	3.79	0.171	0.010	0.086
	Mid	97.3	116.7	108.6	123.6	6.97	0.114	0.049	0.759
	Late	106.3	115.3	103.3	130.4	6.84	0.467	0.040	0.238
ECF (L/100kg)	Early	27.67	27.83	26.02	30.26	0.64	0.852	0.013	0.018
	Mid	26.17	31.94	26.36	28.12	2.20	0.330	0.138	0.396
	Late	24.97	30.42	24.37	29.48	1.16	0.409	0.004	0.885
PV (L)	Early	18.85	20.88	17.00	19.55	1.11	0.185	0.047	0.627
	Mid	19.08	19.72	20.45	23.79	0.66	0.100	0.018	0.079
	Late	19.16	21.94	22.07	26.14	1.07	0.023	0.035	0.918
PV (L/100kg)	Early	5.36	5.48	4.44	5.07	0.28	0.211	0.226	0.401
	Mid	4.98	5.14	5.17	6.19	0.28	0.253	0.068	0.170
	Late	4.83	5.62	5.30	5.97	0.35	0.077	0.069	0.862
BV (L)	Early	25.36	26.8	23.20	25.82	1.33	0.437	0.166	0.668
	Mid	24.96	25.54	25.45	31.83	1.30	0.096	0.028	0.056
	Late	25.34	29.09	28.62	31.96	1.79	0.076	0.044	0.892
BV (L/100kg)	Early	7.18	7.03	6.27	6.82	0.36	0.385	0.594	0.356
	Mid	6.50	6.65	6.82	7.91	0.29	0.342	0.064	0.142
	Late	6.38	7.45	6.68	7.93	0.25	0.093	0.002	0.727
Hct (%)	Early	25.69	22.06	22.17	22.42	1.17	0.502	0.187	0.137
	Mid	23.48	22.70	22.59	22.41	0.66	0.696	0.489	0.662
	Late	24.34	24.40	23.08	22.80	0.95	0.424	0.607	0.564

SEM = Standard error of the mean.

[1] P-values for the effects ; MF =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

Table 5. Glomerular filtration rate (GFR), effective renal plasma flow(ERPF), effective renal blood flow (ERBF), filtration fraction (FF) and urine flow rate in animals treated with rbST under normal shade (NS) and misters and fans cooling (MF) at different stages of lactation.

	Lactating	Treatments					Effects[1]		
	period	NS	NS+rbST	MF	MF+rbST	SEM	MF	rbST	MFx rbST
GFR	Early	2.03	1.95	1.63	1.45	0.09	0.076	0.188	0.599
(ml/min/kg)	Mid	2.12	2.07	1.51	1.44	0.07	0.065	0.204	0.773
	Late	1.96	2.01	1.51	1.50	0.06	0.172	0.847	0.671
ERPF	Early	6.01	5.83	5.43	5.44	0.23	0.592	0.715	0.682
(ml/min/kg)	Mid	6.57	6.56	5.82	5.56	0.33	0.074	0.692	0.708
	Late	6.28	6.43	4.87	4.75	0.40	0.092	0.977	0.746
ERBF	Early	8.14	7.45	6.94	6.98	0.37	0.481	0.400	0.349
(ml/min/kg)	Mid	8.62	8.49	7.51	7.17	0.42	0.077	0.597	0.806
	Late	8.38	8.57	6.29	6.06	0.48	0.081	0.972	0.672
FF	Early	34.7	33.6	30.7	27.3	1.3	0.092	0.112	0.387
(%)	Mid	32.2	30.5	26.5	25.8	1.6	0.192	0.456	0.748
	Late	31.0	31.5	30.7	31.3	1.0	0.917	0.598	0.944
Urine flow	Early	17.65	13.34	16.08	13.96	2.52	0.916	0.238	0.675
rate	Mid	14.94	13.88	16.29	13.78	2.12	0.927	0.425	0.742
(ml/min)	Late	17.61	15.88	10.52	8.48	4.55	0.146	0.748	0.979

SEM = Standard error of the mean.

[1] P-values for the effects ; MF =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

Table 6. Urinary electrolytes excretion, Fractional excretion of electrolytes, Osmolar clearance and Free water clearance in animals treated with rbST under normal shade (NS) and misters and fans cooling (MF) at different stages of lactation.

	Lactating period	Treatments				SEM	Effects[1]		
		NS	NS+rbST	MF	MF+rbST		MF	rbST	MFxrbST
Na^+ excretion	Early	485	384	614	465	90	0.344	0.215	0.801
(µmol/min)	Mid	613	514	613	411	43	0.713	0.008	0.272
	Late	653	515	442	393	202	0.226	0.288	0.176
Fractional Na^+	Early	0.52	0.37	0. 73	0.57	0.06	0.154	0.044	0.911
excretion	Mid	0.49	0. 41	0. 66	0. 48	0.04	0.318	0.007	0.177
(%)	Late	0. 95	0.52	0.56	0.46	0.19	0.396	0.249	0.425
K^+ excretion	Early	1,611	1,471	1,500	1,010	80	0.087	0.004	0.060
(µmol/min)	Mid	1,612	1,457	2,066	1,281	143	0.624	0.011	0.052
	Late	1,620	1,309	1,547	1,476	199	0.799	0.349	0.587
Fractional K^+	Early	45.6	53.7	55.6	45.2	3.58	0.941	0.750	0.032
excretion	Mid	43.2	36.3	60.4	47.3	5.45	0.129	0.103	0.587
(%)	Late	54.2	40.9	42.7	50.6	5.50	0.440	0.658	0.087
Cl^- excretion	Early	487	300	360	258	114	0.484	0.241	0.717
(µmol/min)	Mid	366	375	639	439	108	0.372	0.399	0.362
	Late	722	324	437	409	121	0.666	0.115	0.165
Fractional Cl^-	Early	0. 63	0. 47	5.80	0.46	0.13	0.874	0.284	0.867
Excretion	Mid	0. 42	0. 42	0.86	0.64	0.14	0.171	0.456	0.464
(%)	Late	0. 91	0. 40	0.86	0.86	0.13	0.663	0.097	0.097
Osmolar	Early	23.50	15.89	17.39	14.95	1.69	0.283	0.018	0.166
clearance	Mid	22.20	19.59	22.99	16.16	1.48	0.624	0.013	0.191
(ml/min)	Late	22.68	19.59	20.96	18.88	1.83	0.637	0.194	0.788
Free water	Early	-5.84	-2.55	-1.31	-0.99	3.10	0.463	0.576	0.645
clearance	Mid	-7.26	-5.72	-6.70	-2.38	3.26	0.766	0.395	0.682
(ml/min)	Late	-5.09	-3.71	-9.60	-7.43	3.95	0.419	0.665	0.923

SEM = Standard error of the mean.

[1] P-values for the effects ; MF =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

Table 7. Plasma sodium, plasma chloride, plasma potassium, plasma osmolarity in animals treated with rbST under normal shade (NS) and misters and fans cooling (MF) at different stages of lactation.

	Lactating period	Treatments				SEM	Effects[1]		
		NS	NS+rbST	MF	MF+rbST		MF	rbST	MFx rbST
Plasma Na^+	Early	139.4	139.6	138.0	139.2	0.79	0.570	0.400	0.543
(mEq/l)	Mid	140.0	140.6	139.2	139.0	0.59	0.426	0.744	0.518
	Late	139.4	139.2	140.4	139.8	0.50	0.582	0.447	0.700
Plasma K^+	Early	4.76	4.56	4.44	4.54	0.09	0.345	0.602	0.142
(mEq/l)	Mid	4.64	4.86	4.58	4.64	0.07	0.580	0.077	0.279
	Late	4.52	4.40	4.64	4.64	0.08	0.444	0.473	0.473
Plasma Cl^-	Early	101.4	100.2	97.6	100.0	1.23	0.380	0.637	0.180
(mEq/l)	Mid	100.0	99.0	101.0	101.0	0.35	0.431	0.195	0.195
	Late	100.6	101.0	101.6	100.4	1.12	0.889	0.730	0.495
Plasma	Early	275.0	272.4	275.8	277.0	2.53	0.333	0.789	0.473
osmolarity	Mid	275.8	276.4	281.0	279.2	1.57	0.176	0.713	0.467
(mOsm/kg)	Late	276.2	279.4	281.2	280.0	2.11	0.374	0.648	0.327

SEM = Standard error of the mean.
[1] P-values for the effects ; MF =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

DISCUSSION

In the present study, the temperature-humidity index (THI) was derived from ambient temperature and humidity taken at both barns ranging 79-85 throughout stages of lactation. Cows in both groups would be subjected to moderate heat stress (Fuquay, 1981), since the onset of heat stress is about 72 THI (Amstrong, 1994). It indicates that application of misters and fans in the present study was not sufficient to completely eliminate heat stress in cows. However, THI might not accurately reflect heat stress in misty fan evaporative cooling systems that deliver a pressurized spray with considerable air movement above the cow's back, resulting in higher humidity but also causing the cooling effect. The significant lower in rectal temperatures and respiratory rates of cooled cows at the period of hottest daily temperatures (1300 to 1400 h) showed that a partial alleviation of heat stress resulted from misters and fans cooling. The partial alleviation of heat stress by the cooling effect in cooled animals was also existed which was confirmed by a response of an increase in milk production than those of non-cooled

animals throughout stages of lactation. Thermal stress alone affecting high body temperatures or high respiratory rates was not an extra-mammary factors causing a reduction in milk production in crossbred HF animals. Other factors might involve, since crossbred HF animals treated with bovine somatotropin under high environment temperature could increase in milk yield (Chaiyabutr et al., 2007).

Crossbred Holstein cows treated with rbST increased milk yield accompanying with increases in TBW, ECF, blood volume and PV under with or without misty fan cooling in all stages of lactation. These results confirmed the previous reports of Maksiri et al., (2005) and Chaiyabutr et al., (2007) that exogenous rbST exerts the galactopoietic action in part through increases in body fluids and mammary blood flow (MBF) in distribution nutrients to the mammary gland for milk synthesis. The studies for increases in both MBF and milk secretion in animals given exogenous growth hormone were also noted in goats (Hart et al. 1980) and *Bos Taurus* cows (Davis et al., 1988). The marked increase in the MBF has been shown to asssociate with an increase in the level of plasma insulin like growth factor-I (IGF-I) during prolonged treatment with rbST in crossbred cows (Chaiyabutr et al., 2005). The effect of rbST on MBF and milk production is thought to be indirectly mediated via IGF-I (Bauman, 1992), since infusion of IGF-I into the pudic artery of lactating goat has been shown to increase in MBF and milk yield on the infused side (Prosser et al., 1990; 1994). Mammary blood flow is known to be a major determining factor for supply of nutrients for milk synthesis. However, in the present study, the pattern of progressive decline in milk yield as lactation advances of rbST-treated cows under with or without misters and fans were apparent even though a higher level of MBF. The decline in milk yield during rbST treatment without facilitating of MBF would attribute to a local change within the mammary gland as lactation advances. The action of rbST can affect higher blood flow to the mammary gland, but it seem unlikely for blood flow to the kidney, despite a high level of TBW and ECF during rbST administration. No alterations of the glomerular filtration rate (GFR), effective renal plasma flow (ERPF), effective renal blood flow (ERBF) and filtration fraction (FF) were apparent in rbST-treated cows housing under NS or MF barns in all stages of lactation. The action of rbST on renal hemodynamics in different manner from the mammary gland indicates that the kidneys are able to perform their normal function in the mechanism of autoregulation to regulate RBF and GFR constantly during experimental periods. It is probable that the kidneys of ruminant respond differently from the mammary gland to the high level of endogenous IGF-I, which could be inferred to

secrete during rbST administration in crossbred cows (Chaiyabutr et al., 2005). The action of IGF-I may appear in the blood vessel directly, but better in the mammary gland in ruminant. The present findings seemingly contradict to those of other studies in among different species for the effect of IGF-I on the hyperfiltration in the kidney (Jaffa et al., 1994). An infusion of IGF-I or recombinant human IGF-I has been shown to decrease renal vascular resistance, increased GFR and RBF both in man (Giordano and Defronzo, 1995; Guler et al., 1989; Hirschberg et al., 1993; Jaffa et al., 1994) and rat model (Inishi et al., 1997). The different response in ruminant is an interesting finding that deserves further investigation.

The kidneys are known to be responsible for retaining as much water as possible. From the present results, the effect of rbST on the kidney function in the regulation of body fluids would influence more on renal tubular function than renal hemodynamics. The GFR and filtered load for Na^+, K^+ and Cl^- ions (GFR x plasma concentration) of rbST-treated cows remained constant, while the absolute values of urinary excretion of these ions including FE_{Na}, FE_K and FE_{Cl} were decreased below the pre-treatment values whether under either NS or MF barn. Thus, on the other hand, these results obviously indicate an elevation of renal tubular ions reabsorption. These results also agree with findings in rat model showing that the urinary excretion of sodium, potassium, and chloride and urinary volume were decreased after treatment with growth hormone (Dimke et al., 2007), or in term of an increase in renal tubular reabsorption of sodium after somatotropin administration was also reported (Wyse et al., 1993). Physiologically, the renal tubular reabsorption of ions is generally known to be under hormonal functions, which may involve a stimulation of the renin-angiotensin-aldosterone system (Moller et al., 1995; Ho and Weissberger, 1990). Several studies demonstrated that growth hormone increased sodium and water reabsorption via stimulation of the renin-angiotensis-aldosterone system (Cuneo et al., 1991; Herlitz et al., 1994; Moller et al., 1997). Growth hormone also activates an increase in the plasma aldosterone concentration coinciding with increases in IGF-I and renin-angiotensin (Hanukoglu et al., 2001). However, the mediator of the effect of these hormones on the kidney function is still unsettled. The mechanism of action of growth hormone on sodium and fluid retention has been reported not to be primarily due to enhanced aldosterone secretion, but more likely to a direct renal tubular effect (Hoffman et al., 1996). Growth hormone has been shown to exert its acute antinatriuretic and antidiuretic effects through indirect activation of Na^+, K^+, $2Cl^-$ co-transporter in the medulla thick ascending limbs (mTAL)

(Dimke et al., 2007). The interaction of these hormones on kidney functions in ruminant is still speculative.

Body fluid volume and compositions depend on the coordinated action of multiple mechanisms regulating water intake and excretion. The increase in body fluids and thereby restoring body fluids in rbST treated animals might not be the result of water consumption, although the water intakes were higher in rbST treated cows as compared with non-treated cows, which the correlation of DM intake to water consumption has been noted (MacFarlane et al., 1959). According to the classical view, an increased plasma sodium concentration stimulates vasopressin secretion and thirst which leads to enlarged plasma volume. However, in the present results, both the plasma sodium concentration and Posm of cows were maintained constantly during rbST treatment in NS or MF barns. Thus, the secretion of vasopressin acting on water reabsorption from the distal tubules and the collecting ducts of the kidneys might not expect to occur to save water and thereby increasing the ECF in rbST treated cows, since no significant changes of the C_{H2O} values showed independent of any direct effect of the rbST on free-water formation. However, it has been shown that vasopressin administration increased potassium excretion and diuresis in sheep (Beal, 1976). In the present results, the observed decrease in the rate of urine flow during supplemental rbST was related to the decrease in electrolytes excretion, which would create lower osmotic diuretic effect resulting in the decline in the rate of urine flow. This is also evident from the observed decrease in the Cosm. It is known that sodium ion is the main cation in the ECF and it plays the dominant role for the regulation of body fluid homeostasis by its osmotic action. It can be postulated from the present findings that, an increase in the renal tubular reabsorption of electrolytes (Na^+, K^+, Cl^-) during rbST administration will increase the number of electrolytes in the body. This is a part of the effects of enlarge body fluid volume that would be responsible for increase body fluid volume from its colligative properties with exerting osmotic forces for retaining body water, which would be an explanation for an increase in body fluid volume during rbST administration. The Posm was not affected by rbST, probably because Na^+ is the osmotic factor of ECF and water is required in proportion to the amount of body fluids produced

In conclusion, cows supplemented with rbST supplemental and housing under misters and fan could increase milk yield in all stages of lactation. Cows housing either NS or MF barn without rbST did not affect the kidney function for both renal haemodynamics and electrolyte excretion. An increase in body fluid volume during rbST

supplementation appears partly due to changes in renal tubular functions by stimulating tubular electrolytes reabsorption without changes in renal hemodynamics. Further studies are required for a better understanding the mechanisms about exogenous bovine somatotropin on segmental tubular sodium handling in the kidney relation to aldosterone and vasopressin activation in the regulation body fluid volume in crossbred cattle.

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CHAPTER IX (Series 9.2)

Effects of supplemental recombinant bovine somatotropin and mist-fan cooling on renal tubular handling of sodium in different stages of lactation in crossbred Holstein cattle

INTRODUCTION

In the tropical countries, crossbreeding 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. However, crossbred cattle, containing 87.5 % Holstein (HF) genes have lower efficiency in water retention than 50%HF (Chaiyabutr et al., 1997; 2000). There is a rapid reduction of milk yield as lactation advanced to mid and late lactation in 87.5% HF cow. The reduction of milk yield is attributed the decrease in mammary blood flow (MBF) coinciding with the decline of the plasma bovine somatotropin (bST) concentration. These changes would account for the short lactation persistency (Chaiyabutr et al., 2000). The genetic is not only a cause of the problem. High environmental temperature is a factor, which involve milk production in dairy cattle in the tropics. In lactating animal, body water is known to play a central role in the mechanism of heat dissipation and the process of lactation. The effect of excessive heat load leads to increase in heat dissipation (Hahn et al., 1999). Such changes may lead to a loss body water and decrease in milk yield in lactating cattle. Environmental modifications can reduce heat stress and increase milk production in dairy cattle, for example, fans and sprinklers (Fike et al., 2002), evaporative cooling system (Chan et al., 1997; Chaiyabutr et al., 2008). Some studies have reported that cows treated with rbST under environmental modification would give highest milk yield in hot weather (Tarazo'N-Herrera et al., 1999). Although administration of bST can increase milk production in dairy cows, it also increases heat production (West, 1994). However, exogenous somatotropin has been shown to play an important role in water regulation, in part, through increases in total body water (TBW) and extracellular fluid (ECF), which are the consequence in distribution of nutrients to the mammary gland. (Maksiri et al., 2005; Chaiyabutr et al., 2007).

The kidney is known as an important organ in the regulation of body fluids and compositions. The mechanisms of rbST involving renal function for fluid retention are not fully elucidated in dairy cattle. In man and rat model, growth hormone administration causes increased body fluid and changes in renal function especially hyperfiltration, antinatriuretic and antidiuretic (Dimke et al., 2007; Hirschberq and Kopple, 1992; Ritz et al., 1991). The increases in glomerular filtration rate (GFR) and renal blood flow(RBF) have been shown in animals given exogenous growth hormone ~~(GH)~~ and insulin-like growth factor 1(IGF-1) (Ikkos et al., 1956, Falkheden and Sjogren, 1964; Jaffa et al., 1994). Our previous study in 87.5% HF under misters and fans in all stages of lactation has shown that rbST did not affect RBF and GFR, it appeared only decreases in the urinary excretions of sodium, potassium and chloride ions (Boonsanit et al., 2010). The renal tubular reabsorption of ions is generally known to be under hormonal functions. Several studies demonstrated that growth hormone increased sodium and water reabsorption via stimulation of the renin-angiotensin-aldosterone system (Cuneo et al., 1991; Herlitz et al., 1994; Moller et al., 1997). Growth hormone also activates an increase in the plasma aldosterone concentration coinciding with increases in IGF-1 and renin-angiotensin (Hanukoglu et al., 2001). However, the mediator of the effect of these hormones on the kidney function is still unsettled in dairy cattle. In particular, changes in segmental tubular handling in proximal and distal tubular sodium reabsorption after rbST administration in crossbred dairy cattle have not yet been reported. No data have been reported in providing a direct method for measurement the proximal fluid uptake and changes in transport in these segments in conscious dairy cattle.

The present study was therefore designed to investigate effects of supplementation of rbST in crossbred cows housing in normal shade (NS) and shade plus mist-fan cooling (MF) on the control mechanism for body fluid expansion; the study would focus exclusively on the renal events for renal hemodynamics, renal tubular handling of electrolytes and water, including plasma aldosterone and vasopressin levels. The method, namely lithium clearance technique (C_{Li}) was chosen to estimate the rate of renal proximal and distal tubular reabsorption of sodium and water in the present study. C_{Li} has been shown to be useful to estimate end-proximal fluid delivery including cortical and juxtamedullary nephons as a single population (Thomsen 1984; Koomans et al., 1989). An information may contribute to better understanding the

mechanisms involved body fluids regulation and milk production in rbST-treated cows.

MATERIALS AND METHODS

Animal managements

Ten, first lactation, non pregnant, 87.5% lactating crossbred Holstein cattle were used in the experiment. They were divided into two groups of five animals each. All animals were housed in open-sided barn with a tiled-roof. The barn (16 m long x 7 m wide x 3.5 m high) was separated into two parts by a metal sheet wall (3.5 m high). The first part (8 m long x 7 m wide x 3.5 m high) was arranged for non-cooled cows in group 1 in normal shade (NS) and the second part of barn for cooled cows under shade plus two misters and fans systems (MF). Each system consisted of a 65 cm. diameter blade fan circulating 81 m^3/min of air, with oscillation coverage of 180º. The amount of water discharged from 4 mister spray heads (mounted relative to the fan) was 7.5 L/h and size of mist droplet 0.01 mm. Animals were exposed to MF for 45 minutes at 15-minute intervals from 06:00 h to 18:00 h. At night, animals were exposed to MF for 15 minutes at 45-minute intervals from 18:00 h to 06:00 h. Animal in each group were fed with total mixed ration (TMR) throughout the experiments. The chemical compositions of feeds are presented in Table 1. Samples of TMR were analyzed for dry matter (DM), crude protein and ash using procedures described by AOAC (1990). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed according to Van Soest et al. (1991). Individual feed intake was recorded daily and analyzed for dry matter intake (DMI). Ambient temperature and humidity were measured once weekly during the period of hottest daily temperature (13.00 to 14.00 h). The ambient temperature was recorded by a dry bulb thermometer. The relative humidity was calculated from the reading of dry and wet bulb thermometer. The temperature humidity index (THI) was calculated according to West (1994) where: THI = td-(0.55-0.55RH) (td-58) with td = dry bulb temperature (°F), and RH = relative humidity. Animal was normally milked at around 0600 h and 1700 h using a milking machine and milk production was recorded daily. Body weights of the cows were estimated at weekly intervals during treatment and before treatment.

All experiments were approved by Animal Ethics Committee in accordance with the principles and guidelines of the Faculty of Veterinary Science, Chulalongkorn

University. These guidelines were formulated to comply with international standards and are in accordance with the principles and guidelines of the National Research Council of Thailand.

Experimental procedures

The experiment in each group was studied during early, mid and late lactation. The pretreatment study was started on each specified day (day 70 post-partum of early lactation, day 130 post-partum of mid lactation and day 190 post-partum of late lactation). At the end of the pretreatment, within the same day, the animal was injected with the first dose subcutaneous injection of 500 mg of recombinant bovine somatotropin (rbST) (POSILAC, Monsanto, USA). Subsequently, the animal was injected with three consecutive doses injections of rbST in every 2 weeks. Thereafter, within 2 days after the third injection, the treatment study was conducted. The pretreatment, 3 doses of injections, and the treatment periods were performed during the first 30 days and the same procedures were followed for each stage of lactation. During the last 30 days of each stage, no experiments were conducted in order to allow the milk yield from the effect of rbST treatment to return to the control level (Kirchgessner et al., 1991). On each specified day of each stage of lactation, both pretreatment and treatment period, measurements of the renal function and lithium clearance were performed in the morning (9.00-12.00 h). At the end of lithium clearance study, measurements of total body water (TBW), extracellular fluid (ECF) and plasma volume (PV) were carried out.

Animal preparation

On the day specified day of both pretreatment and treatment period, the non-radiopaque intravenous catheter, gauge 18G (Surflo, Terumo Europe N.V., Belgium), was inserted into an ear vein under local anesthesia for infusion of both para-aminohippurate (PAH) and lithium chloride solution. The catheter was flushed with heparinized normal saline (heparin 25 i.u./ml normal saline) and was left in place during the experiment. The urinary bladder was inserted by Foley catheter (no 22) for urine collection. The free end balloon catheter was introduced into the bladder and secured with the inflated retaining cuff (60 ml. bulb capacity) during urine collection.

Determinations of renal hemodynamics, lithium clearance and electrolytes excretion

Renal hemodynamics, lithium clearance (C_{Li}) and electrolytes excretion were performed by infusion of priming dose with 20 ml of 5% of lithium chloride solution (5.0g/100 ml of normal saline) and 20 ml of 2.5% PAH solution (2.5g/100ml of normal saline) via ear vein and followed immediately by a sustaining infusion infusion of solution containing 1.25% lithium chloride and 0.5% PAH in normal saline at the rate of 2 ml/min. The solution was infused at a constant rate by a peristaltic pump (Eyela, MP-3, Tokyo, Rikakikai, Japan) throughout the experimental study. After equilibration period of 90 minutes, the experiments were carried out in duplicate of urine sample collection over an accurately timed period about 15-20 min. To ensure each accurate collection, the urine sample was started after voidness the bladder. The coccygeal blood sampling at midpoint of urine collection was performed. Plasma and urine samples were kept at -20ºC for determinations of the plasma concentrations of endogenous creatinine, PAH, osmolarity, lithium, sodium, potassium and chloride ions.

The clearance (C) of endogenous creatinine, lithium and PAH were used to measure GFR, C_{Li} and effective renal plasma flow (ERPF), respectively. The ERPF was measured by PAH clearances using standard techniques (Smith, 1962). Plasma and urine samples were analyzed for concentrations of sodium, and potassium ions by flame photometer (Flame photometer 410C, Ciba Corning Inc., USA), chloride ion by chloridometer (Chloride analyzer 925, Ciba Corning Inc., USA), lithium ion by using Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES model PLASMA-1000) and osmolality by osmometer (Osmometer 3D3, Advance Instrument Inc., USA).

The renal sodium handing were evaluated by determining of proximal absolute reabsorption of sodium (PAR_{Na}), proximal fractional reabsorption of sodium (PFR_{Na}), distal absolute reabsorption of sodium (DAR_{Na}), distal fractional reabsorption of sodium (DFR_{Na}), distal absolute reabsorption of water (DAR_{H2O}) and distal fractional reabsorption of water (DFR_{H2O}).

Determinations of water intake, total body water, extracellular fluid, plasma volume and blood volume

Estimation of the rate of water intake of each animal in each period of experimental was recorded by an average over three days from weighing daily water consumption by water meter. On each specified day, at the end of lithium clearance study, The injection of 1ml of tritiated water (2,500 μci per animal), 20 ml of sodium thiocyanate solution (10 g/100 ml normal saline) and 20 ml the Evans blue dye (T-1824) (0.5 g/100 ml normal saline) were performed via an ear vein catheter for estimation of total body water (TBW), extracellular fluid (ECF) and plasma volume (PV), respectively. After dye injection, blood samples from the jugular vein were taken at 20, 30, 40, 50 and 60 min for determinations of ECF and PV and other blood samples were collected at 4, 8, 20, 26, 32, 44, 50, 56, 68 and 72 hr subsequent to the tritiated water injection for determination of total body water (TBW) as previously described (Chaiyabutr et al., 1997). The concentration of plasma sodium thiocyanate was performed by method of Medway and Kare (1959) for estimation of ECF. Blood volume was calculated from the plasma volume and packed cell volume (Chaiyabutr et al., 1980).

Determination of plasma hormone concentrations

On each specified day of each stage of lactation, blood sample was collected via venipuncture of the jugular vein with the 18 gauge needle into heparinzed tube after the first meal of the day. Blood was centrifuged at 2200g for 20 min at 4ºC and plasma sample was separated and kept at -20 °C for determinations of hormone concentrations. The plasma aldosterone concentration was determined by radioimmunoassay (Cayman's Aldosterone EIA kit, Michigan, U.S.A.). Plasma vasopressin concentration was determined by radioimmunoassay (Assay Designs' Vasopressin Enzyme Immunoassay (EIA) kit, Michigan, U.S.A.). The concentration of plasma insulin like growth factor 1(IGF-1) was measured by using a chemiluminescence immunoassay in an immulite analyzer (DPC, Los Angeles, CA).

Calculation for renal hemodynamics and electrolytes excretion

The renal hemodynamics and electrolytes excretion were calculated from the equation:

Glomerular filtration rate (GFR) $= U_{cr} \times V/P_{cr}$

Effective renal plasma flow (ERPF) $= U_{PAH} \times V/P_{PAH}$

Effective renal blood flow (ERBF) = (ERPF×100)/(100-Hct)

Filtration fraction (FF) = (GFR×100)/ERPF

Osmolar clearance (C_{osm}) = U_{osm}×V/P_{osm}

Free water clearance (C_{H2O}) = V- C_{osm}

Fractional excretion of electrolyte (FE) = {(U_e×V) ×100}/(P_e×GFR)

Urinary electrolyte excretion (U_eV) = U_e×V

2.8. Calculation for renal tubular handling of sodium

On the basis of assumptions that lithium is reabsorbed only in the proximal tubules in the same proportion as sodium and water and that lithium is not reabsorbed in the distal tubules, C_{Li} represents the delivery of isotonic fluid at the end of the proximal tubules (Thomsen and Leyssac, 1987). The estimation of segmental tubular handling of sodium and water could be calculated using C_{Li} as follow:

Lithium clearance (C_{Li}) = U_{Li}×V/P_{Li}

Proximal absolute reabsorption of sodium (PAR_{Na}) = (GFR- C_{Li}) ×P_{Na}

Proximal fractional reabsorption of sodium (PFR_{Na}) = (1- C_{Li}/GFR) ×100%

Distal absolute reabsorption of sodium (DAR_{Na}) = (C_{Li}- C_{Na}) × P_{Na}

Distal fractional reabsorption of sodium (DFR_{Na}) = (1- C_{Na}/C_{Li}) ×100%

Distal absolute reabsorption of water (DAR_{H2O}) = C_{Li}-V

Distal fractional reabsorption of water (DFR_{H2O}) = (1-V/C_{Li}) ×100%

Statistical analysis

The stages of lactation (early, mid and late) were separated analysis. The statistic analyses were performed using general linear models procedure of statistical software package SPSS (SPSS for windows, V13.0; SPSS Inc., Chicago, IL, USA). The model used for each analysis was: $Y_{ijk} = \mu + A_l + H_i + A(H)_{il} + B_j + (HB)_{ij} + A(HB)_{ijl} + Cov_k + e_{ijkl}$ Where Y_{ijk} = observation, μ = overall mean, A_l= Animal effect H_i= house effect as main plot (*i* = NS, MF), $A(H)_{il}$ = main plot error (animal *l* in house *i*), B_j = treatment effect (rbST) as a split plot (*j* = with and without rbST administration), $(HB)_{ij}$ = interaction effect between treatment and house , $A(HB)_{ijl}$ = split plot error (animal *l* in house *i* and treatment *j*), Cov_k = covariate effect and e_{ijk} = residual error. The significance was set as $p<0.05$. Ambient temperature, relative humidity, temperature humidity index, rectal temperature and respiration rate were presented as

the mean ± SE. Statistical significant difference between groups was determined by the unpaired t-test.

RESULTS

Ambient temperature, relative humidity, temperature humidity index, rectal temperature and respiration rate

The average of ambient temperature, relative humidity, temperature humidity index, rectal temperature and respiration rate of animals in NS and MF barns are presented in Table 2. Ambient temperature and humidity were recorded during the period of hottest daily temperatures (from 13.00 to 14.00 h). Ambient temperatures in MF barn were significantly lower but the values of relative humidity were higher when compared with NS barn throughout periods of study. Ambient temperature and humidity were created an average THI above 80 in both barns. THI in MF barn tended to decrease but no significant differences as compared with NS barn. The rectal temperature and respiration rate of cooled cows in MF barn significantly decreased ($P<0.005$) when compared with non-cooled cows in NS barn.

Dietary dry matter intake, water intake, milk yield and Body weight

Effects of supplemental rbST and mist-fan cooling on dietary dry matter intake (DMI), water intake and milk yield are presented in Table 3. The DMI of cooled cows with or without supplemental rbST were slightly higher than those of non-cooled cows in all stages of lactation. The values of DMI and water intake in cows treated with rbST showed significant increases ($P<0.05$) in cooled and non- cooled cows in all stages of lactation, which coincided with an increase in milk yield. The values of DMI and milk yield were highest in cooled cow treated with rbST. The mean value of body weight of cows treated with rbST tended to be higher than those of cows without rbST.

Total body water, extracellular fluid, plasma volume, blood volume and hematocrit

Effects of supplemental rbST and mist-fan cooling on total body water (TBW), extracellular fluid (ECF), plasma volume (PV), blood volume (BV) and hematocrit are presented in Table 4. The absolute values of TBW and relative values as a percentage of body weight significantly increased ($P<0.05$) in rbST-treated cows in

early and mid of lactation but not for late lactation. The absolute values and relative values of ECF in early and late lactation were increased in cows treated rbST in NS and MF barns but not for mid lactation. Mean values either the absolute values or relative values of PV and BV significantly increased ($P<0.05$) during rbST treatment in all stages of lactation. The absolute values and relative values of TBW, ECF, PV and BV were not different between cooled and non-cooled cows alone. There were no effects of supplemental rbST and mist-fan cooling on hematocrit values throughout experimental periods.

Renal hemodynamics, osmolar clearance, plasma osmolarity and free water clearance

Effects of supplemental rbST and mist-fan cooling on renal hemodynamics osmolar clearance, plasma osmolarity and free water clearance are presented in Table 5. There were no effects of supplemental rbST and mist-fan cooling on renal hemodynamic (GFR, ERPF, ERBF and FF) throughout experimental periods. Administration of rbST showed significant reduction of the rate of urine flow in both cooled and non-cooled cows particularly in early and late lactation. Osmolar clearance significantly ($P<0.05$) decreased during rbST treatment in all stages of lactation, while free water clearance and plasma osmolarity showed no significant differences in both cooled and non-cooled cows treated with rbST throughout lactation.

Urinary and fractional electrolytes excretion and plasma electrolytes concentration

Effects of supplemental rbST and mist-fan cooling on urinary and fractional (FE) electrolytes excretion and plasma electrolytes concentration are presented in Table 6. The factional and urinary excretion of sodium ion in cooled and non-cooled cows tended to be decreased during rbST supplementation but no statistical differences ($P>0.05$) in all stages of lactation. Urinary potassium excretion tended to decrease in rbST treated cows in both groups, while the significant reduction ($P<0.05$) of FE_K in rbST treated cows were apparent during early and late lactation. No changes in the fractional and urinary chloride excretion including the concentration of plasma electrolytes (Na^+, K^+ and CL^-) were apparent in both cooled and non-cooled cows treated with rbST in all stages of lactation.

Lithium clearance, renal proximal and distal tubular reabsorption of sodium and water, plama levels of aldosterone, arginine vasopressin and IGF-1

Effects of supplemental rbST and mist-fan cooling on lithium clearance, renal proximal and distal tubular reabsorption of sodium and water, plama levels of aldosterone, arginine vasopressin and IGF-1 were presented in Table7. C_{Li} study showed no significant differences in both cooled and non-cooled cows during rbST supplementation. At the proximal tubule, PAR_{Na} in rbST-treated cows significantly increased ($P<0.05$) especially in early and mid lactation when compared with pretreatment period. The PFR_{Na} of cooled and non-cooled cows treated with rbST tended to increase in early and mid lactation, while the significant increase ($P<0.05$) was apparent in late lactation. At the distal tubule, DAR_{Na} tended to decrease coinciding with DAR_{H2O}, while DFR_{Na} tended to increase during rbST supplementation in cooled and non-cooled cows in all stages of lactation. DFR_{H2O} was not affected by rbST administration in cooled and non-cooled cows in all stages of lactation. The proportion of the filtered load of sodium ($GFRxP_{Na}$) reabsorbed in the proximal and distal tubules during rbST supplementation are demonstrated in Fig.1. The significant increase in reabsorption of sodium was apparent in the proximal tubules from 81.58±1.77% in pretreatment to 87.05±1.16% ($P<0.01$) during rbST supplementation. In contrast to proximal tubule, the reabsorption of sodium in the distal tubule during rbST supplementation (12.46±1.13%) significantly decreased ($P<0.01$) when compared with pretreatment (17.34±1.71%). The effects of mist-fan cooling alone had no influence on changes of proximal and distal tubular reabsorption of sodium and water. The plasma IGF-I concentration was significantly increased ($P<0.05$) during rbST supplementation in both cooled and non-cooled cows in all stages of lactation. The plasma aldosterone levels tended to increase during rbST supplementation in cooled and non-cooled cows in all stages of lactation especially in early lactation ($P<0.05$). There were no effects of supplemental rbST and mist-fan cooling on plasma vasopressin concentration in all stages of lactation.

Table 1
Feed ingredients and chemical compositions of the TMR diet

Ingredients	Kg
Pine apple waste	50
Soybean meal	23
Rice bran	3.0
Cotton seed	20
Lime stone	1.4
Di-calcium phosphate	1.4
Sodium bicarbonate	0.3
Potassium chloride	0.1
Mineral and vitamin premix	0.8
total	100
Chemical composition	%
Dry matter (DM)	39.1
	---%DM---
Organic matter	92.7
Crude protein	18.0
Acid detergent fiber	20.1
Neutral detergent fiber	33.9

Table 2
Ambient temperature (AT), relative humidity (RH), temperature humidity index (THI), rectal temperature (RT) and respiration rate (RR) in cows treated with rbST under normal shade (NS) and shade plus mist-fan cooling (MF) at different stages of lactation.

		Treatments		P-value
		NS	MF	
AT (°C)	Early	30.88±0.48	28.94±0.59	P < 0.023
	Mid	31.06±0.37	29.19±0.16	P < 0.001
	Late	30.94±0.50	29.44±0.27	P < 0.020
RH (%)	Early	66.81±4.21	79.88±2.54	P < 0.019
	Mid	74.88±1.62	80.19±1.69	P < 0.039
	Late	68.50±2.59	75.25±2.31	P < 0.072
THI	Early	82.05±0.28	81.12±0.70	P < 0.237
	Mid	83.77±0.60	81.64±0.32	P < 0.007
	Late	82.46±0.38	81.29±0.39	P < 0.051
RR (breath/min)	Early	72.19±2.61	69.47±1.75	P < 0.034
	Mid	69.60±1.06	51.50±1.46	P < 0.001
	Late	68.00± 1.64	51.60±1.87	P < 0.001
RT (ºC)	Early	39.37±0.13	39.21±0.11	P < 0.005
	Mid	39.55±0.06	38.83±0.13	P < 0.001
	Late	39.30 ±0.11	38.66 ±0.10	P < 0.001

Mean±S.E.
Comparison of P-values of NS vs MS using unpaired t-test

Table 3
Dietary dry matter intake (DMI), water intake, milk yield and body weight in cows treated with rbST under normal shade (NS) and shade plus mist-fan cooling (MF) at different stages of lactation.

		Treatments				SEM	Effects[1]		
		NS	NS+rbST	MF	MF+rbST		MF	rbST	MFxrbST
DMI (kg/d)	Early	8.92	10.98	9.67	10.44	0.30	0.919	0.003	0.075
	Mid	9.45	10.74	10.65	11.78	0.12	0.117	0.027	0.860
	Late	7.56	8.78	9.78	10.65	0.15	0.147	0.024	0.637
Water intake (kg/d)	Early	15.75	20.30	17.41	19.11	0.66	0.919	0.003	0.075
	Mid	16.93	19.77	19.58	22.09	0.92	0.117	0.027	0.860
	Late	12.75	15.44	17.64	19.57	0.77	0.147	0.024	0.637
Milk yield (kg/d)	Early	12.77	15.52	13.64	16.32	0.45	0.784	0.001	0.944
	Mid	9.94	11.67	13.38	16.40	0.21	0.069	0.001	0.021
	Late	8.42	8.77	12.36	15.40	0.57	0.058	0.026	0.059
Body weight (kg)	Early	382.5	395.0	372.5	386.5	1.89	0.578	0.006	0.705
	Mid	400.0	402.5	393.0	400.5	3.23	0.745	0.175	0.469
	Late	412.0	419.0	405.0	411.5	3.00	0.147	0.065	0.936

SEM = Standard error of the mean.
[1] P-values for the effects; MF =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

Table 4
Total body water (TBW), extracellular fluid (ECF), Plasma volume (PV), blood volume (BV) and packed cell volume (Hct) in cows treated with rbST under normal shade (NS) and shade plus mist-fan cooling (MF) at different stages of lactation.

	Lactating	Treatments					Effects[1]		
	period	NS	NS+rbST	MF	MF+rbST	SEM	MF	rbST	MFxrbST
TBW	Early	292.8	334.1	277.4	329.8	13.35	0.583	0.013	0.693
(L)	Mid	300.5	317.1	254.0	294.2	8.45	0.135	0.015	0.214
	Late	285.9	313.6	266.6	288.7	23.46	0.305	0.329	0.908
TBW	Early	75.15	84.77	74.61	85.26	3.12	0.994	0.018	0.873
(L/100kg)	Mid	75.20	79.01	64.67	73.74	2.26	0.222	0.029	0.288
	Late	69.38	74.78	65.84	70.21	5.23	0.457	0.386	0.925
ECF	Early	88.60	108.2	96.84	118.7	5.63	0.376	0.010	0.846
(L)	Mid	113.3	123.5	109.9	123.0	5.73	0.881	0.088	0.805
	Late	99.6	111.1	115.5	128.6	3.68	0.086	0.010	0.823
ECF	Early	23.15	27.39	26.29	31.10	1.44	0.357	0.020	0.852
(L/100kg)	Mid	28.36	30.72	28.20	30.92	1.39	0.997	0.116	0.901
	Late	25.14	28.36	27.36	30.68	0.87	0.337	0.005	0.955
PV	Early	19.44	21.74	16.38	21.80	0.87	0.487	0.004	0.122
(L)	Mid	21.04	22.90	18.45	22.39	0.86	0.466	0.015	0.274
	Late	18.86	22.75	20.19	22.57	0.45	0.827	0.001	0.144
PV	Early	4.32	5.27	4.40	5.64	0.18	0.651	0.001	0.437
(L/100kg)	Mid	5.26	5.70	4.71	5.58	0.23	0.498	0.029	0.381
	Late	4.57	5.43	4.99	5.49	0.112	0.713	0.001	0.153
BV	Early	27.43	30.40	22.72	30.54	1.29	0.479	0.006	0.108
(L)	Mid	29.21	31.88	25.76	31.04	1.01	0.534	0.006	0.188
	Late	26.84	31.50	28.36	31.12	0.71	0.880	0.002	0.230
BV	Early	6.051	7.332	6.090	7.897	0.25	0.693	0.001	0.343
(L/100kg)	Mid	7.299	7.925	6.570	7.834	0.27	0.564	0.013	0.285
	Late	6.506	7.518	7.011	7.566	0.16	0.764	0.003	0.199
Hct	Early	28.94	28.44	27.84	28.46	0.98	0.660	0.953	0.589
(%)	Mid	27.94	28.21	28.19	28.75	0.50	0.731	0.442	0.782
	Late	29.63	27.86	28.69	27.39	0.78	0.567	0.098	0.774

SEM = Standard error of the mean.
[1] P-values for the effects; MF =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

Table 5.
Glomerular filtration rate (GFR), effective renal plasma flow (ERPF), effective renal blood flow (ERBF), filtration fraction (FF), urine flow rate, osmolar clearance, free water clearance and plasma osmolarity in cows treated with rbST under normal shade (NS) and shade plus mist-fan cooling (MF) at different stages of lactation.

		Treatments				SEM	Effects[1]		
		NS	NS+rbST	MF	MF+rbST		MF	rbST	MFxrbST
GFR	Early	189.1	202.8	202.6	233.0	10.1	0.660	0.072	0.442
(ml/min/100kg)	Mid	196.2	215.6	187.4	187.5	5.5	0.722	0.125	0.129
	Late	172.7	198.6	196.9	201.2	15.1	0.641	0.355	0.501
ERPF	Early	603.0	610.3	623.0	701.8	25.2	0.671	0.138	0.205
(ml/min/100kg)	Mid	641.5	624.3	632.8	618.8	11.7	0.930	0.229	0.894
	Late	517.0	568.8	524.3	470.8	43.9	0.626	0.985	0.276
ERBF	Early	846.3	854.3	863.6	976.1	27.0	0.705	0.067	0.101
(ml/min/100kg)	Mid	891.4	870.0	880.2	868.7	14.8	0.956	0.309	0.749
	Late	735.2	785.9	733.5	648.8	54.7	0.588	0.766	0.262
FF	Early	31.81	33.95	32.43	33.07	0.98	0.983	0.207	0.473
(%)	Mid	29.11	33.28	29.86	30.48	1.15	0.844	0.081	0.173
	Late	34.91	36.35	38.58	42.94	1.91	0.384	0.181	0.475
Urine flow	Early	6.65	3.98	6.33	3.4	1.05	0.427	0.037	0.909
rate	Mid	5.66	3.49	3.74	2.56	1.22	0.065	0.221	0.700
(ml/min/100kg)	Late	4.57	3.40	3.53	2.17	0.39	0.528	0.017	0.816
Osmolar	Early	7.54	5.62	7.48	4.74	0.47	0.700	0.003	0.423
clearance	Mid	7.31	4.17	6.02	4.61	0.69	0.732	0.016	0.255
(ml/min/100kg)	Late	5.53	4.48	5.57	3.83	0.39	0.779	0.012	0.418
Free water	Early	-1.05	-1.64	-1.30	-1.00	0.69	0.802	0.504	0.790
clearance	Mid	-1.65	-0.68	-2.28	-2.04	0.87	0.345	0.511	0.687
(ml/min/100kg)	Late	-0.97	-1.08	-2.04	-1.66	0.45	0.599	0.778	0.597
Plasma	Early	274.8	274.0	272.3	273.5	1.85	0.705	0.897	0.607
osmolarity	Mid	276.0	270.8	274.5	271.5	1.78	0.938	0.060	0.552
(mOsm/kg)	Late	278.5	275.3	276.3	276.0	1.91	0.859	0.395	0.463

SEM = Standard error of the mean.
[1] P-values for the effects; MF =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

Table 6
Electrolytes excretion, Fractional excretion and plasma of electrolytes concentration (Na^+, K^+ and Cl^-) in cows treated with rbST under normal shade (NS) and shade plus mist-fan cooling (MF) at different stages of lactation.

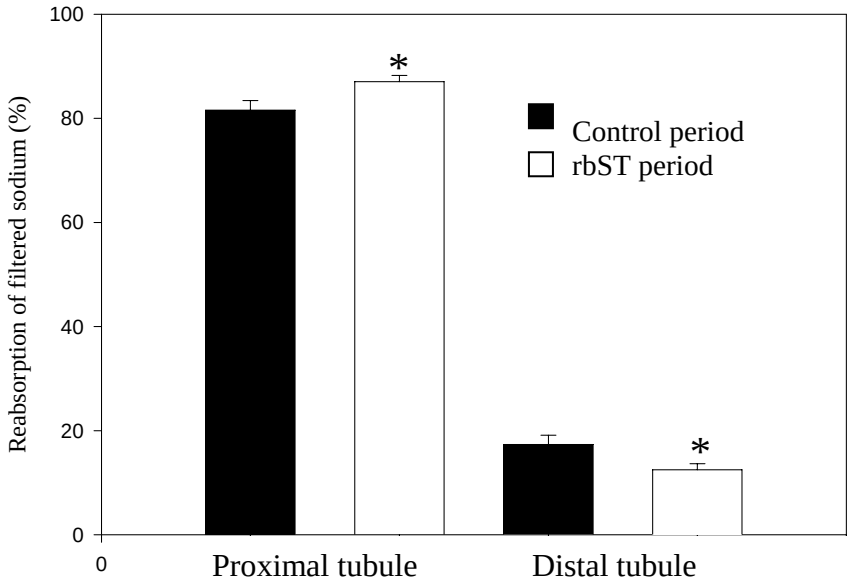
		Treatments				SEM	Effects[1]		
		NS	NS+rbST	MF	MF+rbST		MF	rbST	MFxrbST
Na^+ excretion	Early	198.9	155.0	205.1	121.5	27.1	0.688	0.057	0.491
(µmol/min/100kg)	Mid	307.3	209.1	262.7	156.0	43.1	0.660	0.055	0.925
	Late	186.5	133.0	189.4	118.2	26.9	0.895	0.060	0.753
fractional	Early	0.98	0.68	0.80	0.41	0.17	0.431	0.081	0.809
excretion Na^+	Mid	1.38	0.85	1.11	0.66	0.20	0.662	0.052	0.850
(%)	Late	0.85	0.54	0.81	0.47	0.15	0.811	0.074	0.921
K^+ excretion	Early	434.2	346.7	586.8	324.2	78.1	0.343	0.066	0.305
(µmol/min/100kg)	Mid	572.0	406.5	344.0	335.1	81.7	0.191	0.327	0.375
	Late	338.1	293.1	374.5	369.5	16.6	0.605	0.181	0.272
fractional	Early	59.52	48.13	62.98	32.20	6.18	0.548	0.014	0.168
excretion K^+	Mid	73.61	50.98	43.75	45.98	8.11	0.169	0.255	0.176
(%)	Late	52.76	38.92	49.65	46.34	2.33	0.903	0.010	0.065
Cl^- excretion	Early	86.2	110.7	84.5	80.2	13.8	0.637	0.493	0.337
(µmol/min/100kg)	Mid	214.8	90.3	176.1	58.9	63.0	0.676	0.104	0.956
	Late	149.2	113.1	119.3	47.3	48.4	0.467	0.306	0.724
fractional	Early	0.49	0.50	0.50	0.37	0.08	0.693	0.480	0.381
excretion Cl^-	Mid	1.00	0.41	0.99	0.34	0.30	0.910	0.085	0.907
(%)	Late	0.78	0.56	0.73	0.26	0.25	0.621	0.223	0.638
Plasma Na^+	Early	126.8	129.5	130.8	129.8	1.1	0.058	0.473	0.152
(mEq/l)	Mid	130.0	129.3	131.8	131.0	1.5	0.291	0.631	1.000
	Late	130.8	132.5	129.0	131.0	1.8	0.143	0.342	0.947
Plasma K^+	Early	4.10	3.95	4.15	4.08	0.15	0.395	0.492	0.816
(mEq/l)	Mid	4.15	4.13	4.15	4.08	0.05	0.889	0.382	0.654
	Late	3.93	4.03	4.05	4.25	0.21	0.427	0.502	0.820
Plasma Cl^-	Early	98.0	101.8	93.8	99.0	2.4	0.194	0.116	0.770
(mEq/l)	Mid	101.5	100.8	99.5	99.5	0.6	0.064	0.524	0.524
	Late	102.0	100.0	98.8	100.0	0.9	0.205	0.675	0.105

SEM = Standard error of the mean.
[1] P-values for the effects; MF =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

Table 7
Lithium clearance, aldosterone, vasopressin and insulin like growth factor 1 (IGF-1) in cows treated with rbST under normal shade (NS) and shade plus mist-fan cooling (MF) at different stages of lactation.

	Lactating	Treatments					Effects1		
	period	NS	NS+rbST	MF	MF+rbST	SEM	MF	rbST	MFxrbST
CLi	Early	33.90	25.95	35.17	25.47	5.75	0.953	0.175	0.884
(ml/min/100kg)	Mid	26.52	20.57	30.54	26.29	3.31	0.249	0.175	0.806
	Late	41.65	35.22	29.82	18.34	4.43	0.179	0.090	0.589
PARNa	Early	19.58	22.88	21.97	26.92	1.28	0.610	0.018	0.543
(mmol/min/100kg)	Mid	22.22	25.12	20.67	21.18	0.62	0.670	0.034	0.104
	Late	17.12	21.69	21.51	23.88	1.95	0.369	0.125	0.593
PFRNa	Early	78.26	85.42	82.88	88.56	3.49	0.538	0.116	0.839
(%)	Mid	84.92	90.03	83.35	85.99	1.81	0.193	0.076	0.522
	Late	75.05	81.74	85.02	90.54	2.37	0.130	0.042	0.814
DARNa	Early	4.08	3.23	4.34	3.22	0.73	0.885	0.226	0.860
(mmol/min/100kg)	Mid	3.08	2.52	3.74	3.32	0.36	0.159	0.222	0.858
	Late	5.26	4.51	3.63	2.30	0.56	0.156	0.115	0.624
DFR Na	Early	94.95	95.97	92.77	97.30	1.64	0.773	0.142	0.327
(%)	Mid	90.04	94.60	93.35	96.13	1.79	0.169	0.085	0.636
	Late	96.66	97.00	94.42	94.73	0.75	0.069	0.676	0.983
DAR H2O	Early	27.41	20.70	28.86	21.74	6.09	0.861	0.299	0.974
(ml/min/100kg)	Mid	20.86	17.08	26.80	23.73	3.29	0.139	0.339	0.918
	Late	37.08	31.82	26.29	16.17	4.14	0.163	0.112	0.578
DFRH2O	Early	78.90	78.07	72.00	85.36	7.44	0.981	0.432	0.377
(%)	Mid	80.14	76.52	87.69	89.73	5.78	0.073	0.896	0.642
	Late	89.58	90.60	88.29	85.39	1.74	0.531	0.611	0.303
aldosterone	Early	470.3	580.6	447.7	705.7	70.4	0.562	0.040	0.334
(pg/ml)	Mid	488.2	756.4	461.8	556.7	80.4	0.218	0.065	0.323
	Late	553.0	576.8	423.8	481.5	46.9	0.513	0.419	0.731
vasopressin	Early	227.8	227.2	219.9	188.0	26.3	0.477	0.553	0.569
(pg/ml)	Mid	227.1	178.6	185.9	241.1	38.5	0.716	0.933	0.215
	Late	221.3	259.5	195.4	217.9	33.7	0.262	0.394	0.822
IGF-1	Early	118.2	196.0	87.4	114.8	18.8	0.301	0.023	0.216
(ng/ml)	Mid	115.6	183.2	112.2	218.0	32.7	0.644	0.029	0.575
	Late	128.2	350.9	124.1	220.4	41.5	0.221	0.005	0.166

SEM = Standard error of the mean.
[1] P-values for the effects; MF =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST



P-values of control period vs. rbST-treated period using paired t-test, * P <0.01 (n=24)

Figure 1. Comparison of the reabsorption of filtered sodium at the proximal and distal tubules between the control period and the period of supplemental rbST.

DISCUSSION

It has been found that the application of misters and fans caused a decrease ambient temperature in the barn about 1-2°C throughout the experiment. The present results are similar with our previous report that the temperature-humidity index (THI) in both barns had values ranging 80-84 (Boonsanit et al 2010). It might be considered that animals in both groups exposed to moderate heat stress (Fuquay, 1981), which THI exceeded 72 of threshold (Amstrong, 1994). The THI value might not accurately indicate heat stress by using misters and fans cooling systems. The misters and fans cooling systems would create a highly humidity surrounding the animals by delivering a pressurized spray with air movement above the cow's back. However, alleviation of heat stress in cows by mist-fan cooling in the present study was confirmed by reductions in RT and RR including an increase in milk yield of cooled cows throughout stages of lactation.

The rbST-treated animals increased dry matter intake (DMI) and milk yields in cows in both cooled and non-cooled cows. These results are agreed to those reports that the DMI was positively correlated with milk yield during rbST treatment (Murphy, 1992). DMI is known to be a major influence on water intake in ruminant. An increase in dry matter intake will correlate with an increase in water consumption (MacFarlane et al. 1959). An increase in water consumption would be a rout in affecting an increase in body fluids by an influx of water via the GI tract. It has been reported previously that exogenous rbST exerts the galactopoietic action in part through increases in body fluids and mammary blood flow which are the consequence in distribution of nutrients to the mammary gland for milk synthesis (Maksiri et al., 2005; Chaiyabutr et al., 2007). The elevation of body fluids (TBW, ECF, BV and PV) in cooled and non-cooled cows supplementation with rbST confirmed our previous studies (Boonsanit et al. 2010).

Since the kidney is known to play the major role in maintaining salt and water balance. The effect of rbST on expansion of extracellular volume in cows would be attributable to changes in the renal tubular reabsorption of electrolytes rather than a direct effect on the renal hemodynamics. These findings confirmed our previous report (Boonsanit et al 2010) that the kidneys are able to maintain their normal function in the mechanism of autoregulation to regulate RBF and GFR constantly during experimental periods. In contrast to what has been observed subjected to growth hormone (GH) administration which showed increases in GFR and RPF (Tönshoff et al., 1993). However, changes in GFR and RPF in human did not correlate with increased levels of circulating GH, but rather with elevations of circulating IGF-I (Hirschberg and Kopple, 1993: Hirschberg et al., 1989). Infusion of IGF-1 has shown to increase GFR and RBF (Giordano and Defronzo, 1995; Guler et al., 1989) with a decrease in renal vascular resistance (Hirschberg and Kopple, 1992, Hirschberg et al., 1993). IGF-1 causing renal vasodilatation and being mediated by nitric oxide secretion (Haylor et al., 1991; Hoogenberg et al., 1994) or kinin (Jaffa et al., 1994) was also noted. However, no alteration of renal hemodynamics and filtration fraction in rbST-treated cows in the present study, despite a marked increase in the plasma levels of IGF-1, gives evidence that more complex processes of regulation exist via both vasodilating and vasoconstricting mediators.

The decreases in urinary and fractional excretions of electrolytes during supplemental rbST were not due in part to changes in filtered load of electrolytes

which GFR remained constant in both cooled and non-cooled cows. These findings are similar to our studies reported previously (Boonsanit et al. 2010). A net increase in the renal tubular reabsorption of electrolytes (Na^+, K^+, Cl^-) of rbST-treated cows would create a low osmotic diuretic effect resulting in the decline in osmolar clearance (Cosm) and led to decrease in the rate of urine flow. Increases in the renal tubular reabsorption of electrolytes during rbST supplementation would increase the number of electrolytes in the ECF. Posm was not affected by rbST, probably because sodium is an osmotic skeletal in ECF and water is required for equilibrium of sodium with progressive body fluid retention.

The proximal absolute reabsorption of sodium (PAR_{Na}) and fractional reabsorption of sodium (PFR_{Na}), as assessed by lithium clearance, increased in both cooled and non-cooled cows during rbST supplementation. The proximal tubule is known to be a site of action of angiotensin II (AII) on sodium reabsorption (Harris and Navar, 1985). In the present study reabsorption of sodium in the proximal tubules may have resulted from an increase AII although no measurements of the plasma AII were performed during rbST supplementation. An increase in the plasma aldosterone concentration in rbST treated-cows would be attributable to involve the action of the renin-angiotensin system (RAS). The effect of growth hormone on the regulation of renin-angiotensin-alsosterone system (RAAS) in human and rat model were also noted (Lampit et al., 1998; Wyse et al., 1993). Therefore, an increase in sodium reabsorption in the proximal nephron is most likely explained by the indirect effect of rbST.

Although it is known that aldosterone plays a role increase in the renal tubular reabsorption of sodium. Several mechanisms have been proposed that an increase in sodium reabsorption in the proximal tubule is likely to involve a direct action of IGF-1 (Guler et al. 1989). The study in fetal sheep has been shown that IGF-1 enhanced proximal tubular reabsorption of sodium and stimulated the renin-angiotensin system (Marsh et al., 2001). The present study showed increases in the level of plasma IGF-1 after rbST supplementation in both cooled and non-cooled cows. Thus, the effects of rbST on proximal tubular reabsorption of sodium are partly mediated by both AII and IGF-1. In contrast to the study in human, a direct action of GH and IGF-I took place mainly in the distal nephron (Johannsson et al. 2002). An increase in distal absolute reabsorption of sodium has also been reported in human treated with GH (Hansen et al., 2001). However, in the present study there was less extent for a stimulatory action

of aldosterone on distal absolute reabsorption of sodium reabsorption sodium (DAR_{Na}) although the plasma concentration of aldosterone markedly increased during rbST supplementation. DAR_{Na} had tendency to decrease during rbST supplementation by an average 19.7% in non-cooled cows and 46.8% in cooled cows. It is possible for the present findings that the low reabsorption of sodium in distal tubule was only partially counterbalanced by increased reabsorption in the proximal tubule. However, the slight increase in distal fractional reabsorption of sodium (DFR_{Na}) in rbST-treated cows, would be partly mediated by an increase in aldosterone levels.

It is possible that an increase in proximal absolute reabsorption of sodium (PAR_{Na}) during rbST supplementation may lead to decreased proximal tubular fluid output. A reduction in the delivery of sodium via the tubular fluid to macula densa cells may stimulate the release of renin, which will stimulate angiotensin and aldosterone production (Guyton, 1991). It is known that aldosterone plays an important role in regulating sodium reabsorption in distal tubule. Wyse et al. (1993) reported that aldosterone was secreted by stimulation of GH and AII, via the action of the RAS. Conversely, in GH-deficient children treated with GH increased sodium reabsorption in distal tubule without changes in plasma aldosterone (Moller et al., 1991). Thus, a stimulatory action of GH may act through other mechanism such as IGF-1, since IGF-1 receptors has been shown in the site of apical and basolateral membrane of either proximal and distal tubules or collecting ducts (Feld and Hirschberg, 1996).

The continuing decreases in the rate of urine flow were apparent in rbST-treated cows in all stages of lactation. It indicates that more proximal reabsorption of electrolytes particularly sodium during rbST supplementation, and thereby decrease the delivery of filtrate to the diluting segment. Filtrate sodium delivered out of the proximal nephron to the ascending limb and were reabsorbed there but low amounts of sodium escaped into the urine (as indicated by the decreased C_{OSM}). Additionally, the stimulatory action of GH on reabsorption of renal electrolytes (Na^+, K^+, Cl^-) and water has been shown to occur in the medulla thick ascending limbs (mTAL) with activation of Na^+, K^+, $2Cl^-$ co-transporter (Dimke,et al., 2007). The present studies found that no significant changes in DAR_{H2O}, and DFR_{H2O} were obtained which coincided with no significant changes in the plasma vasopressin concentration in cooled and non-cooled cows treated with rbST in all stages of lactation. It indicates that action of vasopressin on water reabsorption at the distal tubules and the collecting ducts might not occur to save water and thereby increasing the ECF in rbST treated

cows. No significant changes of the C_{H2O} values also showed independent of any direct effect of the rbST on free-water excretion.

It is concluded that cows supplemented with rbST under misters and fans were increased milk yields of in all stages of lactation. The action of rbST plays a role in expansion of extracellular volume in 87.5% lactating crossbred Holstein cattle via changes in the kidney function. The increased renal tubular sodium and water reabsorption in response to rbST supplementation took place mainly in the proximal nephron segment, most likely due to a stimulatory action of endogenous aldosterone ,AII and IGF-1 but not for vasopressin.

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CHAPTER X

Effects of exogenous bovine somatotropin and cooling on hematological and biochemical parameters in different stages of lactation of crossbred Holstein cattle in the tropic

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INTRODUCTION

Low milk yield and short lactation period of dairy cattle in the tropics is still the major problem for the dairy practices. Low productivity of crossbred dairy cattle is influenced by a large number of factors. A high environmental temperature is known to be one of the problems for animal production in tropical area. Heat stress affects the performance of dairy cows by reducing their dry matter intake, feed efficiency and milk production (Fuquay, 1981; Shibata & Mukai 1979; Collier et al., 1982; Johnson, 1987; Huber et al.1994). Environmental modifications have been performed in attempt to alleviate severe heat stress in dairy cattle for example, water spray and fans (Armstrong et al., 1985; Armstrong et al. 1993), evaporative cooling, (Armstrong et al., 1993; Armstrong et al., 1985; Armstrong et al., 1988; Ryan et al., 1992; Chaiyabutr et al., 2008). In dairy cows, milk synthesis is depended on hormonal stimuli and the provisions of nutrients from the blood to the mammary gland for sustain milk production. It has been reported from the previous study in crossbred cattle containing 87.5 % Holstein (HF) genes that a short persistency of lactation was found with decreases in the plasma bovine somatotropin level (bST) and blood flow to the mammary gland during the transition period from early to mid-lactation (Chaiyabutr et al. 2000). However, cows treated with rbST exhibited increase in milk yield which coincided with increases in both total body water and mammary blood flow (Chaiyabutr et al 2007). Administration of bST has been shown to be one of technology in management strategies that are needed to minimize the effects of heat stress and that will maintain sufficient DMI to sustain the potentially increased milk yields (West et al., 1994). An increase in total body water in bST-treated cow may play a central role both for process of lactation and heat dissipation. However, controversial results for

the use of bST are regarded due to its effects on animal health. A number of studies have been noted for the effect of bST on bovine health in the risk of clinical mastitis, reduction in fertility and risk of developing clinical signs of lameness (Dohoo et al 2003). The adverse effects of exogenous bST have also been reported in other species animal (Scarda et al 1991). The relative mass of many organs and tissue growth by the bST administration have also been reported (Moallem**,** et al, 2004), which may reflect changes in bodily functions. According to this information, prevention the metabolic disease or other disorders will be the priority for intensive milk production. Blood analysis is an useful tool for the diagnosis and health monitoring of animals in veterinary medicine (Payne and Pyne, 1987). Few data are available for the additive effects of supplemental rbST and cooling on both health and productivity of crossbred dairy cattle. Therefore, the aim of this study was to determine the hematological values and the pattern of variability of metabolic parameters and production performance of crossbred dairy cows during supplementation with rbST and providing cows with housing under shade with or without misters and fans in different stages of lactation. The results of the study will provide values of hematology and blood metabolites for systematic health monitoring and nutritional status and to suggest possible preventive measures to reduce the risk of adverse effects during rbST supplementation in the crossbred dairy cattle under high environmental temperatures.

MATERIALS AND METHOD

Animals and managements

The experiment was carried out in ten primiparous, non-pregnant crossbred cattle, containing 87.5 % Holstein (HF) genes. The animals with averaged 60 days postpartum at start of trial were assigned randomly into two groups of five animals each. Animals in the first group were housed in open-sided barn with a tiled roof in normal shaded house (NS) as the non-cooled animals. Animals in the second group were housed in open-sided barn with a tiled roof (8 m long x 7 m wide x 3.5 m high) under misty fan cooling (MFC) as cooled animals. The open space cooling system consisted of two sets of misty fan, which each system consisted of a 65 cm. diameter blade fan circulating 81 m^3/min of air, with oscillation coverage of 180º. The amount of water discharged from 4 splay heads was 7.5 L/h and size of mist droplet 0.01 mm.

Animals were exposed to MFC for 45 minutes at 15-minute intervals from 06:00 h to 18:00 h. At night, animals were exposed to MFC for 15 minutes at 45-minute intervals from 18:00 h to 06:00 h.

The diet was fed twice a day for *ad lib* as the same ration of total mixed ration (TMR) in equal portions at about 06:00 h and 17:00 h when the animals were milking throughout the experiments in both groups. Ingredient and chemical compositions of diet are shown in Table 1. Animals are milked twice a day using a milking machine and milk production was recorded daily. On the same day as blood sampling, the breathing rate (RR) of cows was recorded by counting flank movements during two 30-s cycles (between 1300 and 1400 hours) and rectal temperature (RT) was recorded using a digital thermometer (between 1400 and 1500 hours). The ambient temperature and humidity were recorded inside the barn, using a dry bulb thermometer and wet bulb thermometer placed above the feeding lane. The relative humidity at NS and MFC were read by psychrometric chart depending on wet and dry bulb temperature. A temperature-humidity index (THI) was calculated from the average ambient temperature of dry and wet bulb temperatures according to McDowell (1972), as follow: THI = 0.72 (wb+db) + 40.6. Where ; wb = wet bulb temperature and db = dry bulb temperature expressed in °C.

Experimental design

The experiments were carried out throughout lactating period in each group. The experiment in each group was divided into 3 phases, namely early- (Day 60 postpartum), mid- (Day 120 postpartum), and late lactating periods (Day 180 postpartum). The pretreatment study was conducted on the starting day of each phase. At the end of the pretreatment, within the same day, the animal was injected with the first dose subcutaneous injection of 500 mg of recombinant bovine somatotropin (rbST) (Posilac, Monsanto, USA). Subsequently, the animal was injected with two consecutive doses injections of rbST every 2 weeks. Thereafter, within 2 days after the third injection, the treatment study was conducted. The pretreatment, 3 doses of injections, and the treatment periods were performed during the first 30 days and the same procedures were followed for each phase. During the last 30 days of each phase, no experiments were conducted in order to allow the milk yield from the effect of rbST treatment to return to the control level (Kirchgessner et al., 1991).

Sample collection and chemical analysis

On specified days of the study, blood samples were taken at around 1100 h in order to avoid daily variations in activities of each stage of lactation at the end of the pretreatment period and at the end of the treatment period. Two blood samples (one for whole blood, one for plasma) were collected from the coccygeal vessel by venipuncture with a 21-gauge needle into heparinized tubes as an anticoagulant, and the samples were immediately placed in an ice bath until plasma separation. Just before blood centrifugation, blood samples were determined for hemoglobin, erythrocytes count(RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), leukocytes count(WBC), leukocyte differential counts for lymphocyte, monocyte and granulocyte and platelets by Automated, an in vitro Diagnostic medical Equipment (Model Mythic18 (IVD) C2 Diagnostics; Montrellier, France). Blood sample in a microhematocrit tube was centrifuged at 12,000 rpm for 5 min, and the spun packed cell volume (PCV) was measured. The remaining blood was centrifuged at 3,500 g for 10 min to separate plasma, which was stored at −20°C for subsequent analysis.

The frozen plasma samples were used to determine the following parameters; urea nitrogen (BUN), creatinine, inorganic phosphorus, total protein, albumin, glucose, total cholesterol, triglycerides. Enzyme activities were determined for alanine aminotransferase(ALT), aspartate aminotransferase(AST), alkaline phosphatase. All determinations of blood chemistry were conducted on plasma at 37°C, with an automatic clinical chemistry analyzer (Operator Manual BT 2000 Plus, Biotecnica Instruments S.P.A Via Licenza, Rome, Italy). Plasma free fatty acids were determined by colorimetry after plasma extraction with chloroform, heptane and methanol and TAN solution (Wang et al.,2004). The plasma sodium (Na^+) and potassium (K^+) concentrations were determined by Flame photometer (Clinical Flame Photometer 410C, Ciba-Corning Diagnostic, Halstead Essex, England). The plasma chloride(Cl^-) concentration was measured by Chlorimeter (Chloride analyzer 925, Ciba-Corning Inc., USA). Milk samples from morning milking were used to determine milk compositions using Milkoscan (Milko-Scan 133B, A/S N. Foss Electric, Hillerod, Denmark).

Statistical analysis

Data for BW, milk yield and DMI were adjusted for covariate effects using data from the 14 d pretreatment period and analyzed by the general linear models procedure of SPSS for windows (version 14.0) using the following statistical model:

$$Y_{ijk} = \mu + A_l + H_i + A(H)_{il} + B_j + (HB)_{ij} + A(HB)_{ijl} + Cov_k + e_{ijkl}$$

Where Y_{ijk} = observation, μ = overall mean, A_l= Animal effect H_i= house effect as main plot (*i* = NS, MFC), $A(H)_{il}$ = main plot error (animal *l* in house *i*), B_j = treatment effect (rbST) as a split plot (*j* = with and without rbST administration), $(HB)_{ij}$ = interaction effect between treatment and house , $A(HB)_{ijl}$ = split plot error (animal *l* in house *i* and treatment *j*), Cov_k = covariate effect and e_{ijk} = residual error.

Other data were also analyzed by the general linear models procedure of SPSS using a similar model, but the covariate effect was excluded. Least square means were used to evaluate the housing x treatment effect for all variables. Differences between means at a probability of <0.05 was considered significant. Values were compared between normal shade (NS) and shade plus misty-fan cooling (MFC), mean differences were examined statistically by using Student's unpaired *t*-test.

RESULTS

Ambient temperature, relative humidity and temperature humidity index

The mean values of ambient temperature in NS and MF barns are shown in Table 1 At the period of hottest daily temperature (13.00 to 14.00 h), ambient temperature and temperature humidity index (THI) of the MF barn were significantly lower, while relative humidity in the MF barn was significantly higher than in the NS barn throughout the periods of study.

Rectal temperature, respiration rate, milk yield and milk compositions

Effects of supplemental rbST and misty-fan cooling on milk yield and milk composition are shown in Table 2. Milk yield was significantly increased during rbST supplementation in both cooled and non-cooled cows. The milk yields of cooled cows were slightly higher than those of non-cooled cows. Milk protein, lactose, total solid (TS) and solid not fat (SNF) concentrations were not significantly different between cows treated with rbST and housing either NS or MF barns in each stage of lactation. The concentration of milk fat of cooled and non-cooled cows significantly increased

during supplementation of rbST in early and mid lactation, while it had a tendency to increase in late lactation.

Plasma electrolyte concentrations and plasma osmolarity

Effects of supplemental rbST and MF cooling on the concentrations of plasma electrolytes and on plasma osmolarity are shown in Table 5. There were no changes in the concentrations of plasma Na^+, K^+, Cl^- and osmolarity in both cooled and non-cooled cows treated with rbST or when housed in the MF barn.

Effects of cooling and rbST supplementation on hematological and biochemical parameters of crossbred Holstein cattle in normal shade and shade plus misty-fan cooling (Table 3 and 4)

Administration of rbST had no general effects on hematology or serum chemistry variables. The values of haematological parameters obtained after rbST supplementation in cooled and non-cooled cows were not significantly different. In the present results, significant increases in values of MCV were apparent in early lactation in both cooled and non-cooled cows supplemental rbST, whereas the values of packed cell volume were not altered**.** Platelets were not affected by the cooling system but were decreased by rbST supplementation.

The rbST supplementation in both cooled and non-cooled cows had no effect on plasma levels of triglyceride, glucose and protein throughout lactation. The concentrations of FFA in plasma were not affected by the cooling but were increased by rbST supplementation. The activities of plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (AlP) did not change. In late lactation, both the plasma cholesterol levels and plasma alkaline phosphatase (ALP) activity in both cooled and non-cooled cows supplemental rbST were significantly increased. The concentration of plasma urea were not affected by cooling but had tendency to decrease by rbST supplementation.

Plasma electrolyte concentrations and plasma osmolarity

Effects of supplemental rbST and MF cooling on the concentrations of plasma electrolytes and on plasma osmolarity are shown in Table 5. There were no changes in the concentrations of plasma Na^+, K^+, Cl^- and osmolarity in both cooled and non-

cooled cows treated with rbST or when housed in the MF barn.

Table 1. Ambient temperature, relative humidity and temperature humidity index in animals treated with rbST under normal shade (NS) and misty-fans cooling (MF) at different stages of lactation.

		Treatments		P-value
		NS	MF	
Ambient temperature (°C)	Early	34.95±0.58	30.70±0.62	P=0.003
	Mid	33.25±0.51	30.35±0.40	P=0.001
	Late	32.40±0.58	28.95±0.49	P=0.003
Relative humidity (%)	Early	53.50±2.52	67.85±3.55	P=0.004
	Mid	58.80±3.62	73.45±2.64	P=0.015
	Late	60.90±3.01	71.80±3.98	P=0.109
Temperature humidity index (THI)	Early	85.37±0.50	81.96±0.76	P=0.001
	Mid	84.04±0.44	82.40±0.53	P=0.036
	Late	83.24±0.44	79.92±0.46	P=0.001

Mean±S.E.

Table 2. Rectal temperature, respiration rate, milk yield and milk compositions of cows treated with rbST under normal shade (NS) and misty-fans cooling (MF) at different stages of lactation.

Parameter	Stages of lactation	NS		MF		SEM	[1]Effect		
		pre	rbST	Pre	rbST		MF	rbST	MFx rbST
Rectal : temperature (°C)	Early	38.8	39.0	38.0	38.2	0.07	0.001	0.023	0.886
	Mid	39.4	39.9	38.6	38.9	0.13	0.005	0.011	0.245
	Late	39.1	39.3	38.5	38.8	0.10	0.057	0.023	0.565
Respiration: Rate (breaths/min)	Early	72	78	54	63	2.5	0.001	0.017	0.544
	Mid	70	73	52	55	1.2	0.001	0.065	0.751
	Late	71	78	52	57	1.2	0.001	0.004	0.657
Milk yield: (kg/day/cow)	Early	10.81	12.30	12.19	12.82	0.25	0.580	0.002	0.146
	Mid	9.19	10.44	11.58	12.70	0.36	0.222	0.002	0.413
	Late	8.24	9.73	9.38	12.30	0.54	0.362	0.003	0.217
Protein: (gm%)	Early	3.37	3.61	3.48	3.63	0.15	0.788	0.227	0.790
	Mid	3.79	3.84	4.09	4.26	0.15	0.104	0.466	0.695
	Late	4.25	4.03	4.30	4.32	0.17	0.518	0.586	0.499
Fat: (gm%)	Early	3.27	4.29	3.89	4.76	0.325	0.757	0.004	0.325
	Mid	3.53	4.25	3.87	4.44	0.593	0.732	0.013	0.593
	Late	4.27	4.58	4.11	5.15	0.732	0.301	0.075	0.732
Lastose: (gm%)	Early	4.74	5.09	5.00	4.89	0.846	0.411	0.140	0.846
	Mid	4.85	4.82	4.82	4.91	0.820	0.698	0.452	0.820
	Late	4.77	4.78	4.41	4.72	0.358	0.186	0.217	0.358
SNF: (gm%)	Early	8.61	9.39	9.18	9.22	0.614	0.079	0.110	0.614
	Mid	9.34	9.37	9.61	9.87	0.071	0.385	0.474	0.071
	Late	9.72	9.37	9.41	9.74	0.906	0.953	0.179	0.906
TS: (gm%)	Early	13.24	12.79	13.42	14.54	0.401	0.703	0.381	0.401
	Mid	14.87	13.42	14.92	15.05	0.590	0.215	0.147	0.590
	Late	14.68	14.40	15.22	16.09	0.447	0.722	0.494	0.447

SEM = Standard error of the mean.
[1] P-values for the effects; MF =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

Table 3. Effects of cooling and rbST supplementation on hematological measurements of crossbred Holstein cattle in normal shade (NS) and shade plus misty-fan cooling (MF)

	Stages of	NS		MF			[1]Effect		
Parameter	lactation	pre	rbST	Pre	rbST	SEM	MF	rbST	MFx rbST
RBC :	Early	5.07	4.39	4.76	4.31	0.37	0.648	0.170	0.757
($x10^6$/µl)	Mid	4.71	4.44	4.87	4.41	0.37	0.801	0.351	0.791
	Late	4.65	4.39	4.34	4.90	0.23	0.858	0.533	0.109
Hb:	Early	7.72	7.51	7.58	7.88	0.32	0.751	0.885	0.445
(g/dl)	Mid	7.66	8.24	8.16	7.38	0.55	0.741	0.861	0.253
	Late	8.04	7.71	7.48	8.00	0.13	0.862	0.508	0.013
Hct:	Early	23.4	22.5	23.2	24.7	0.95	0.427	0.753	0.225
(%)	Mid	22.9	25.3	24.9	22.2	1.20	0.748	0.865	0.062
	Late	23.6	22.7	22.5	23.6	0.35	0.801	0.119	0.002
MCV:	Early	46.72	53.04	43.40	52.10	2.38	0.528	0.013	0.630
	Mid	49.02	54.80	45.70	49.44	3.06	0.298	0.158	0.747
	Late	49.22	49.32	45.73	50.48	1.70	0.811	0.191	0.208
MCH:	Early	15.84	17.38	14.46	17.10	1.16	0.452	0.110	0.649
	Mid	16.32	17.96	16.20	16.50	0.88	0.526	0.301	0.467
	Late	17.44	18.88	16.66	17.62	1.50	0.457	0.447	0.877
MCHC:	Early	33.72	33.03	33.40	32.86	1.03	0.845	0.567	0.942
	Mid	33.48	33.00	35.82	35.72	1.31	0.097	0.831	0.888
	Late	35.42	38.70	33.26	34.14	2.17	0.098	0.366	0.595
Platelets:	Early	413.60	383.20	512.20	401.80	70.67	0.587	0.348	0.587
(10^3/µl)	Mid	699.40	287.60	697.80	279.80	174.68	0.983	0.045	0.986
	Late	321.00	478.00	588.40	279.40	125.23	0.853	0.561	0.100
WBC:	Early	15.67	9.34	9.34	11.98	2.68	0.612	0.511	0.132
($x10^3$/µl)	Mid	8.16	13.80	9.58	7.55	2.12	0.309	0.419	0.108
	Late	9.43	12.42	13.56	17.84	1.09	0.253	0.010	0.569
Lymphocyte:	Early	27.76	16.78	18.30	19.32	3.79	0.565	0.226	0.152
(%)	Mid	15.03	18.23	15.70	18.45	4.53	0.944	0.536	0.962
	Late	19.13	20.23	25.85	25.18	3.05	0.256	0.947	0.781
Monocyte:	Early	6.90	3.88	5.38	6.33	1.57	0.846	0.534	0.254
(%)	Mid	5.63	5.58	5.80	5.33	1.84	0.986	0.889	0.913
	Late	2.08	3.23	4.03	4.33	0.63	0.125	0.292	0.524
Granulocyte:	Early	68.18	79.48	75.10	71.48	3.07	0.927	0.258	0.051
(%)	Mid	75.08	79.58	78.50	76.15	6.49	1.000	0.874	0.616
	Late	90.10	72.05	69.83	68.88	5.32	0.150	0.124	0.159

SEM = Standard error of the mean.

[1] P-values for the effects; MF =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

Table 4. Effects of cooling and rbST supplementation on blood chemistry measurements of crossbred Holstein cattle in normal shade (NS) and shade plus misty-fan cooling (MF)

	Stages of	NS		MF			[1]Effect		
Parameter	lactation	pre	rbST	Pre	rbST	SEM	MF	rbST	MFx rbST
SGPT:	Early	15.02	14.58	15.06	12.50	1.73	0.717	0.410	0.556
(I.U./l)	Mid	16.50	14.77	15.80	17.42	2.64	0.685	0.984	0.544
	Late	15.60	17.20	16.80	17.08	3.41	0.880	0.790	0.851
SGOT:	Early	58.74	60.92	71.28	58.06	3.53	0.538	0.157	0.061
(I.U./l)	Mid	63.54	49.60	68.06	70.98	5.22	0.035	0.322	0.145
	Late	62.28	57.70	60.00	78.18	4.25	0.358	0.148	0.028
Alkaline :	Early	103.98	122.60	88.90	92.38	8.55	0.375	0.233	0.402
phosphatase	Mid	132.04	92.38	102.72	122.60	19.49	0.985	0.625	0.165
(I.U./l)	Late	77.00	118.60	115.58	146.20	9.69	0.110	0.006	0.587
BUN:	Early	9.64	8.36	7.04	9.48	1.60	0.709	0.726	0.277
(mg/dl)	Mid	12.38	10.20	10.35	7.38	1.65	0.188	0.158	0.818
	Late	11.20	10.00	9.00	8.47	1.19	0.385	0.488	0.783
Creatinine:	Early	1.46	1.54	1.41	1.49	0.05	0.759	0.141	1.000
(mg/dl)	Mid	1.41	1.68	1.52	1.61	0.07	0.912	0.037	0.255
	Late	1.59	1.71	1.48	1.47	0.09	0.327	0.548	0.482
Total									
protein:	Early	8.52	8.79	9.23	8.82	0.18	0.428	0.718	0.092
(g/dl)	Mid	9.11	8.49	8.57	8.97	0.29	0.956	0.706	0.114
	Late	8.70	8.13	8.98	8.88	0.22	0.460	0.179	0.327
Albumin:	Early	4.27	4.22	4.18	4.21	0.06	0.820	0.868	0.513
(g/dl)	Mid	4.18	4.08	4.14	4.20	0.07	0.755	0.765	0.294
	Late	4.27	4.31	4.18	4.19	0.06	0.604	0.715	0.831
Cholesterol:	Early	183.00	169.60	128.10	136.14	5.40	0.092	0.633	0.083
(g%)	Mid	153.80	143.50	150.60	154.96	11.33	0.811	0.800	0.536
	Late	162.60	169.60	141.32	190.80	9.45	0.999	0.017	0.055
Triglyceride:	Early	14.24	13.73	13.36	12.72	0.84	0.755	0.516	0.939
(mg%)	Mid	14.72	13.80	12.82	13.50	1.60	0.671	0.942	0.631
	Late	11.84	12.54	14.84	18.68	1.68	0.140	0.245	0.408
Free fatty									
acid:	Early	3.70	3.88	4.72	6.78	0.90	0.239	0.247	0.323
(mg%)	Mid	3.14	4.63	4.45	4.82	0.35	0.576	0.029	0.150
	Late	2.42	3.62	4.21	6.20	0.40	0.147	0.004	0.362
Glucose:	Early	90.18	84.62	88.36	85.80	3.35	0.904	0.260	0.666
(mg%)	Mid	91.44	78.56	84.58	94.86	6.24	0.308	0.840	0.100
	Late	79.90	84.66	96.58	98.18	2.18	0.123	0.183	0.489
Inorganic :	Early	6.38	6.34	5.75	7.09	0.40	0.916	0.141	0.123
phosphate	Mid	7.10	6.77	6.48	6.70	0.47	0.644	0.906	0.572
(mg%)	Late	6.44	7.40	6.53	6.84	0.45	0.640	0.197	0.493

SEM = Standard error of the mean.

[1] P-values for the effects; MF =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

Table 5. Plasma electrolytes (Na^+, K^+ Cl^-) concentration and plasma osmolarity of cows treated with rbST under normal shade (NS) and misty-fans cooling (MF) at different stages of lactation.

	Lactating	Treatments					Effects[1]		
	period	NS	rbST	MF	rbST	SEM	MF	rbST	MFxrbST
Plasma Na+:	Early	139.4	139.6	138.0	139.2	0.79	0.570	0.400	0.543
(mEq/l)	Mid	140.0	140.6	139.2	139.0	0.59	0.426	0.744	0.518
	Late	139.4	139.2	140.4	139.8	0.50	0.582	0.447	0.700
Plasma K+:	Early	4.76	4.56	4.44	4.54	0.09	0.345	0.602	0.142
(mEq/l)	Mid	4.64	4.86	4.58	4.64	0.07	0.580	0.077	0.279
	Late	4.52	4.40	4.64	4.64	0.08	0.444	0.473	0.473
Plasma Cl-:	Early	101.4	100.2	97.6	100.0	1.23	0.380	0.637	0.180
(mEq/l)	Mid	100.0	99.0	101.0	101.0	0.35	0.431	0.195	0.195
	Late	100.6	101.0	101.6	100.4	1.12	0.889	0.730	0.495
Osmolarity:	Early	275.0	272.4	275.8	277.0	2.53	0.333	0.789	0.473
(mOsm/kg)	Mid	275.8	276.4	281.0	279.2	1.57	0.176	0.713	0.467
	Late	276.2	279.4	281.2	280.0	2.11	0.374	0.648	0.327

SEM = Standard error of the mean.

[1] P-values for the effects; MF =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

DISCUSSION

The objective of the present study was to evaluate the effect of rbST administration and cooling with misters and fans on the haematological and plasma biochemical values of crossbred Holstein cattle in different stages of lactation. The results obtained in the present study show that cows were housed at both NS and MF barns which had the THI ranging 79-85 throughout periods of study. The THI was more than 72 which was considered to moderate heat stress (Fuquay, 1981; Amstrong, 1994). It indicates that the effect of misters and fans was not sufficient to completely eliminate heat stress in cows. However, THI might not accurately reflect heat stress in misty fan evaporative cooling systems that deliver a pressurized spray above the cow's back, resulting in higher humidity but also causing the cooling effect. A partial alleviation of heat stress from misters and fans cooling showed the significant lower in rectal temperatures and respiratory rates of cooled cows at the period of hottest daily temperatures (1300 to 1400 h). A response of an increase in milk production in cooled cows than those of non-cooled cows in each stage of lactation was noted.

It is known that the blood chemistry and hematological parameters remained the most relevant and accurate on the spot diagnostic parameters for the determination of health, metabolic and nutritional status. The present data shows that both the haematological and plasma biochemical values of the crossbred Holstein cows were within normal physiological limits in comparison with those of the clinical healthy cows (Kaneko et al., 1997; Schalm et al., 1975). The results for haematological values did not show specific disorder during rbST supplementation in both cooled and non-cooled cows. An increase in ECF and plasma volume during rbST administration in crossbred Holstein cattle (Chaiyabutr et al. 2007), might not cause of variations in the haematological and plasma biochemical values during hemodilution in each stage of lactation. However, increases in values of MCV of red blood cells were apparent in both cooled and non-cooled cows supplemental rbST whereas the values of packed cell volume were not altered. It indicates that rbST supplementation would increase body fluid in both ECF and ICF.

The rbST supplementation in crossbred Holstein cows had no effect on plasma levels of triglyceride in each stage of lactation. However, the concentrations of FFA in plasma were significantly increased by the effect of rbST supplementation in both cooled and non-cooled cows. These results were in accordance with the results

reported in high producing lactating cows that marked increases in plasma FFA levels were observed following administration of large doses of growth hormone (Kronfeld,1965). These results indicate the greater mobilization of adipose tissue to supply the extra nutrients for the increased milk production. An elevation of the plasma FFA concentration did not depend on the stage of lactation, although cows in early lactation generally have a negative energy balance in stimulating mobilization of adipose tissue for an increase in the concentrations of plasma FFA. Cows in both groups in the present study were fed with balanced diets, which would imply no mobilize of fat tissue. As lactation advances, the milk production was declined and thus cows would gain body fat in preparation for the coming lactation. However, it has been shown that bovine somatotropin does not exert directly in acute lipolytic effects on adipose tissue. An alteration of lipogenesis or lipolysis by the effect of bST may occur indirectly via its anti-insulin effects (McDowell,1991).

Supplementation of rbST in both cooled and non-cooled cows showed no effect on plasma protein levels in each stage of lactation. The concentrations of total plasma proteins of both cooled and non-cooled cows either with or without rbST had average 8.13-9.11 g%. The present results did not agree to the other reports that the concentration of protein in blood during bST administration will be influenced by changes in relative body mass of many organs and tissue growth for the role in protein metabolism (Moallem**,** et al, 2004). Among proteins, the plasma albumin concentration had an average 4.14-4.31 g% of total protein which are similar to those found in literature (Kaneko, 1997). In the present study, no effects of rbST and misty fan cooling on the plasma albumin concentration were apparent. Plasma albumin concentration in cows is influenced by their physiological stage and it is closely connected with nutrition and the amount of nitrogen taken (Roil et al., 1974). Animals in both groups were equally well-fed throughout periods of study, which indicate that dietary protein was not limiting and that liver function was likely to be normal. In the present study, no differences of the plasma BUN concentrations between non-cooled cows and cooled cows without rbST, although several reports have shown that animals exposure to high temperatures increased in the plasma urea (Ronchi et al. , 1999) and plasma creatinine levels (Broucek et al. 1986 Chaiyabutr and Johnson, 1991). However, the marked decrease in BUN of either cooled or non-cooled cows supplemented with rbST, while no significant changes in the creatinine concentrations were apparent. The reduction of the plasma urea concentration might not reflect the dry matter crude

protein intake which showed higher in cows supplemental rbST. The decreased plasma concentrations of urea during supplementation of rbST may be due to effects of the hormone bST, via N- actyl glutamate, on carbamyl phophate synthetase, a key enzyme in the urea cycle (Oddy and Linsay, 1986). In the present study, cows exposed to moderate heat stress during experimental periods might not expect to cause a higher utilization of amino acids as energy source for increase in the urea level or mobilization of protein from muscle mass with subsequent creatinine delivery in the plasma (Fekry et al. 1989). It indicates that the kidneys are able to perform their normal function.

It is known that the activity of aminotransferases in the blood is important in acting as a catalyst in connecting the metabolism of amino acids and carbohydrates. Changes in their activities in the blood can be a consequence of their activities in liver cells and also reflection of cell structure damage. High activities of AST and ALT are most often found in acute and chronic liver disorder, e.g. fatty liver syndrome in dairy cows (Cebra et al., 1997), starvation and the appearance of ketosis during early lactation (Steen, 2001), even in a subclinical disorder (Meyer and Harvey, 1998). However, the liver is known to be the source of synthesis and secretion of IGF-I in ruminant, which are dependent on the availability of both plasma growth hormone level and some nutritional factors (Clemmons and Underwood, 1991). Animals with a lower nutritional state (Hodgkinson et al., 1991) or a negative energy balance, have reduced hepatic production IGF-I (Weller et al. 1994; Ketelsleger et al. 1995), which would not be expected to occur in the present study. The present results did not show any changes of the liver function for the activity of the plasma GPT (12.5-17.4 I.U/L) and GOT(49.6-78.2 I.U/L) during rbST supplementation in both cooled and non-cooled cows, although an increase in plasma IGF-I concentration originates primarily from liver has been shown to be affected by rbST administration in cows (Chaiyabutr et al., 2007). Variations of the plasma cholesterol levels and plasma alkaline phosphatase (ALP) activity in both cooled and non-cooled cows were in normal levels. It did not confirm the hypothesis that a reduction of plasma cholesterol concentration and plasma ALP activity would coincide with a reduction in liver activity in cows exposed to high temperatures (Abeni et al. 2007). Enzyme ALP activity has been indicated to be a quick and reliable blood-marker for heat stress (Abeni et al. 2007). It is probable that the crossbred cows in both groups experienced to moderate heat stress and those cows were on the borderline between moderate and high heat stress in the

hotter periods. In late lactation, both the plasma cholesterol levels and plasma alkaline phosphatase (ALP) activity in both cooled and non-cooled cows supplemental rbST were significantly increased. These findings could be due to an increase in lipids mobilization and lowutilization by peripheral tissues during rbST supplementation.

It is known that Na^+ and Cl^- are the major contributors to the anion-cation balance and pH of extracellular fluid, and K^+ is the major cation in ruminant sweat (Maltz et al., 1994). Maltz et al., (1994) has shown that during heat stress, K^+ loses causing negative electrolyte balance as sweating with impairing the absorption of Na^+ and Cl^- from the rumen coinciding with the depression of the rumen fermentation. However, in the present study, plasma concentrations of K^+, Na^+, and Cl^- in both cooled and non-cooled cows with or without rbST were not significantly different. Therefore, it is suggested that the supplemental rbST would not harmful changes in electrolytes in cooled and non-cooled cows.

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CHAPTER XI

Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on changes in the rate of liquid flow from the rumen and milk production in different stages of lactation in crossbred Holstein cattle

Series 11.1: Effects of using misters and fans and supplemental bST on milk production and rumen function of cross-bred Holstein cattle during early, mid and later lactation in a tropical environment

Series 11.2: Effects of mist-fan cooling and supplemental recombinant bovine somatotropin on diet digestibility, digestion kinetics and milk production of crossbred Holstein cattle in the tropics

CHAPTER XI(Series 11.1)

Effects of using misters and fans and supplemental bST on milk production and rumen function of cross-bred Holstein cattle during early, mid and later lactation in a tropical environment

INTRODUCTION

In tropical countries dairy herds are mixed exotic breeds and/or cross-bred animals. It is known that crossbreeding of *Bos taurus* and *Bos indicus* has been an efficient tool for blending the adaptability of endogenous tropical cattle with the high milk potential of exotic breeds. However, low level milk production of exotic breeds and cross-bred animals is still the main problem in dairy farming in the tropics. Regulation of milk secretion in dairy cattle, including exotic and cross-bred animals, as well as pure-bred cows, is inherited and mediated by hormone and growth factors, among others, and differences in milk producing ability likely is due to differences in these mediators on the mammary system and metabolism. Important among the hormone is somatotropin, which is know to be a mediator of the interaction between genetic potential and nutrition and milk production (Fike et al. 2002; Settivari et al. 2007). The study in 87.5% crossbred Holstein cattle have been shown that the concentration of plasma bST decreased as lactation progressed to mid and late lactation. This decrease would contribute to a reduction in mammary blood flow (Chaiyabutr et al. 2000). Long-term supplementation of recombinant bovine somatotropin (rbST) in cross-bred dairy cattle (87.5% crossbred Holsteins) increased in milk yield which coincided with an increase in the rate of mammary blood flow, but the stimulatory effect of bST on milk production was less in late lactation despite a high level of mammary blood flow (Chaiyabutr et al. 2007a). These changes were not apparent in crossbred dairy cattle containing 50%Holstein genes (Chaiyabutr et al. 2000). It is not known which factors are the cause and which factors are the effects for such reduction. High environmental temperature and humidity are other factors that are associated with reduced milk production in tropical environments (Bohmanova et al. 2007). The interaction effects between thermal stress and the role of bST on lactation performance in crossbred lactating cattle is not yet

clear. It may not be entirely clear but there is a substantial literature published that addresses the effects of temperature, humidity, housing, management on physiology and nutritional effects. An increase in milk yields of bST-treated cows has been shown to remain greater than that of the control cow even in heat stress condition (Johnson et al. 1991). An occurrence of greater heat stress in bST treated cows has been suggested to be due to an increase in metabolic activity and heat production associated with higher milk yield (West 1994; Settivari et al. 2007). It has been suggested that even though bST-treated cows increase in heat production; it also increases heat dissipation (Johnson et al. 1991; West 1994). An increase in milk production of the bST-treated cows exposure to hot environment would attribute to either an increase in DMI or no changing in feed intake (Staples et al. 1988). However, studies are few on the effects of combination of high ambient temperature and bST supplementation on bodily function particularly digestive system of crossbred dairy cows. There are inconsistent results for the relationship between environmental temperature and digestive system especially the rumen function in ruminant. A number of experiments showed an increase in total volatile fatty acids (VFA) under high environmental temperature (Martz et al. 1971), while another study showed decrease in VFA concentrations (Olbrich et al. 1972). The changes in ruminal VFA during heat exposure is not only due to a decline in feed intake but also the direct effect of heat stress (Olbrich et al. 1972). During heat stress, rumen fermentation would be affected by depression of rumination, reticular motility and blood flow to rumen epithelium including the decrease in microbial synthesis

Attempts have been made to minimize the impact of thermal stress on milk production of cows by using passive and active environmental modifications, such as shade, shade plus water spray with or without fans, or evaporative cooling (Chaiyabutr et al. 2008). Management strategies to minimize the effects of heat stress in lactating cows has been suggested to maintain sufficient DMI for the potential increase in milk yields by technology of bST supplementation (West 1994). More data are required for the knowledge of ruminal characteristics concerning link between bST supplementation and high ambient temperature exposure. The objective of the current research were to 1) to evaluate the effect of providing cross-bred cattle with housing under shade with or without misters and fans and 2) supplementation of cows with bST or not during three period of lactation (early, mid and late lactation). Measures to evaluate the effectiveness of these treatments were rumen function. milk production and composition.

MATERIALS AND METHODS

Animals, housing and managements

Ten primiparous, non pregnant crossbred cattle, containing 87.5 % Holstein (HF) genes, age 36±2 month, average body weight 358±32.5 kg and BCS 2.5± 0.5 were used for the experiment. The animals with averaged 60±1 days in milk (DIM) at start of trial were assigned randomly into two groups of five animals each. During the study period, animal in both groups were housed in open-sided with a tiled-roof and tie-stall barn. The barn (16 m long x 7 m wide x 3.5 m high) was separated into two parts by a metal sheet wall (3.5 m high). The first part (8 m long x 7 m wide x 3.5 m high) was arranged for animals in normal shade (NS) and the second part of barn equipped with two misters and fans system (MF) for cooled animal. Each system consisted of a 65 cm. diameter blade fan circulating 81 m^3/min of air, with oscillation coverage of 180º. The amount of water discharged from 4 mister spray heads(mounted relative to the fan) was 7.5 L/h and size of mist droplet 0.01 mm. Animals were exposed to MF for 45 minutes at 15-minute intervals from 06:00 h to 18:00 h. At night, animals were exposed to MF for 15 minutes at 45-minute intervals from 18:00 h to 06:00 h. The experiment was performed during two consecutive summer beginning March and continuing to November of the next year. Climatic condition during each time of the year trypically range from slightly cool only in December to very hot and humid April, May, June and July

The total mixed ration (TMR) was fed twice daily for *ad libitum* consumption. The TMR was prepared fresh each day using ingredients in Table 1. Animals were milked twice daily (06.00 and 17.00 hr) by machine and milk weights recorded for each milking. Animals were given water *ad libitum*. Dry matter intake (DMI) of each animal measured daily by weighing the TMR fed and subtracting that refused. Amount fed daily was divided into the two equal portions and one portion fed at 06.00 and 17.00 hr. Samples of both feeds were collected and kept at -20 C for dry matter (DM) determination and chemical analysis. Samples of TMR were analyzed for DM, crude protein and ash using procedures described by AOAC (1990). ADF and NDF were analyzed according to Van Soest and Robertson (1991).

The ambient temperature was recorded using a wet and dry bulb thermometer(Mercury) at NS and MF barns. The relative humidity at NS and MF were read by psychrometric chart depending on wet and dry bulb temperature. Ambient temperatures and humidity were measured during the daytime (09:00, 13:00 and 17:00

h) on two days before beginning of the first injection of rbST and two days after the 3rd injection of rbST in each stage of lactation. Average values were considered to be the mean of all measurements taken throughout periods of study. A temperature-humidity index (THI) was calculated from the average ambient temperature of dry and wet bulb temperatures according to McDowell (1972), as follow:

THI = 0.72 (wb+db) + 40.6

Where ; wb = wet bulb temperature and db = dry bulb temperature expressed in °C

On each specify day during measurements of ambient temperature around 09.00, 13.00 and 17.00 h, each cow was measured rectal temperature by electronic thermometer, respiratory rates was measured by counting flank movements. Body weight (BW) of all animals were recorded by weighing monthly throughout the experimental periods.

Experimental design

The experiment was divided into three periods during the lactation: 1) early,60-90; 2) mid, 120-150; and late lactation, 180-210 DIM. Each of these 3 periods there was a pretreatment period that was 30 d long; pre-treatment began on day one of the period. On the day pre-treatment ended the cow in the two treatment group received their first injection of rbST (POSILAC, 500 mg). Two additional injections of rbST were given at two-week intervals. Within two days after receiving the 3rd injection the treatment measurements were initiated. This schedule within the period was followed for each of the three periods. During the last 30 days of each of the three periods no measures were made or rbST supplemented. This allowed effects of handling, sampling and rbST to be return to normal before start of the next period.

Sample collection and chemical analysis

On specified days, a sample of rumen fluid was collected from each animal using a stomach tube connected to a vacuum pump. Samples were taken 2.5 hr after morning feeding for determination of volatile fatty acids. The pH was measured immediately after collection using a pH meter (Orion Model 420A Orion research Inc. Boston, USA) equipped with a glass electrode (pH-TRIOD™ Orion). The ruminal fluid samples were strained immediately through two layers of cheesecloth and preserved by adding 6 N

HCl. The preserved rumen fluid samples were frozen at -20°C prior to volatile fatty acids (VFA) and NH_3-N analyses. Rumen fluid samples were analyzed for VFA by a gas chromatography and NH_3-N by phenyl-hypochlorite reaction (Weatherburn 1967). The concentration of acetate, propionate and butyrate are expressed as mmol/l. The acetate:propionate ratio (A:P ratio) was calculated from their concentration.

Milk samples were collected from morning milking and devided into two portions. The 60 ml of milk sample was preserved with 300 μL bronopol(2-Brom-2-nitro-1,3-propandiol) (0.02 w/w) was kept at 4°C until analysis for milk compositions by infrared method using Milkoscan (Milko-Scan 133B, A/S N. Foss Electric, Hillerod, Denmark). Another portion for fat-free milk was obtained by centrifugation at 3000 rpm for 15 min at 4°C. Protein in fat free-milk samples using for the determination of the milk urea concentration were removed with 10% TCA and the concentration in milk urea was determined by the diacetylmonoxime method (Coulombe and Favreau 1963). Protein in fat free-milk samples using for determination of the milk allantoin and uric acid concentrations were removed with 5% uranyl acetate and the milk allantoin analysis was carried out by a colorimetric method according to Young and Conway (1942). The uric acid concentration in milk was determined by enzymatic assay (Wolfschoon-Pombo and Klostermeyer 1981)

Calculations

The microbial synthesis of protein was calculated by an indirect method base upon measuring allantoin output in milk and calculating the predicted microbial nitrogen flow (MNF) according to Timmermans et al. (2000) as follows:

$$MNF = 119+11.6X– 3.3MY$$

Where; X = allantoin excretion in milk (mmol/day), MY = milk yield (kg/day)

The net energy intake and net energy for maintenance and for lactation was calculated from milk compositions and milk yield according to the formula suggested by NRC (2001). Then, energy balance was calculated from energy intake subtract by energy for maintenance and energy for lactation as follow:

NE*i* (Mcal/day), = feed energy × DMI

NE*m* (Mcal/day) = $0.08 \times BW^{0.75}$

NE_l (Mcal/kg) = [(0.0929x%Fat) +(0.0547x% Protein)+(0.039x%Lactose)] x MY

Energy balance (Mcal) = NE*i* – (NE*m* + NE*l*)

Where; NE*i* = net energy intake, NE*m* = net energy for maintenance, NE*l* = net energy for lactation, DMI = dry matter intake (kgDM/day), $BW^{0.75}$ = metabolic body weight (kg)

Statistical analysis

Data for BW, milk yield, DMI and NE*i*, in each lactating period were adjusted for covariate effects using mean value of 14 d before starting of the experimental period.The NH_3-N, ruminal pH, total VFA and specific VFA were adjusted for covariate effects using DMI. The stages of lactation (early, mid and late) were separated analysis. The statistic analyses were performed using General Linear Models procedure of statistical software package SPSS (SPSS for windows, V14.0; SPSS Inc., Chicago, IL, USA). The model used for each analysis was:

$$Y_{ijk} = \mu + A_l + H_i + A(H)_{il} + B_j + (HB)_{ij} + A(HB)_{ijl} + Cov_k + e_{ijkl}$$

Where Y_{ijk} = observation, μ = overall mean, A_l= Animal effect H_i= house effect as main plot (*i* = NS, MF), $A(H)_{il}$ = main plot error (animal *l* in house *i*), B_j = treatment effect (rbST) as a split plot (*j* = with and without rbST supplementation), $(HB)_{ij}$ = interaction effect between treatment and house , $A(HB)_{ijl}$ = split plot error (animal *l* in house *i* and treatment *j*), Cov_k = covariate effect and e_{ijk} = residual error.

Other data were also analyzed by the similar model, but the covariate effect was excluded. Mean value were used to evaluate the effect for all variables. Statistical significance was declared at $P<0.05$ and trends were declared at $0.05 < P \leq 0.10$.

RESULTS

Chemical compositions of the diet

The feed ingredients and chemical compositions of ingredients are in Table 1. The TMR ingredients were balance to meet requirements for milk yield of lactating cows range 10-15 kg/d. The diet had 18%CP, 70%TDN, and 2.67 Mcal/kg DM of ME were estimated according to the NRC (2001).

Ambient temperature, relative humidity, temperature humidity index (THI) respiratory rate and rectal temperature

Mean values of measurements at experimental site during periods of studies for daily temperatures, humidity and THI are in Table 2 and the rectal temperature and respiratory rate are in Table 3. Average values of ambient temperature (AT) at 09.00 h during early and mid lactation were not significant different (P>0.05). During late lactation, the AT of MF barn had significantly lower than NS barn (P<0.01) at 09.00 h. Relative humidity(RH) at 09.00 h were significantly different between NS and MF barn in all stags of lactation. Although RH of MF barn was significantly higher than that of NS barn, but the THI at 09.00 h in all stags of lactation were not different between NS and MF barn. The AT, RH and THI at 13.00 and 17.00 h in all stages of lactation were significantly different between NS and MF barn. During experimental studies, the environmental condition of both NS and MF barn had no effect during supplementation with or without rbST in all stags of lactation. The RR and RT at 09.00, 13.00 and 17.00 h of the cows in NS barn were significantly higher than those of cows in MF barn in all stage of lactation. Throughout the experimental period, the rbST-supplemented cows had greater RR and RT than those of non rbST-supplemented cows in all measured time.

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Dry matter intake, energy intake, energy balance and body weight

The effect of misters and fans and rbST supplementation on changes in BW, DMI, NE*i*, NE*m*, NE*l* and EB are in Table 4. Body weights and NE*m* increased as lactation advanced in both cooled and non-cooled cows. These changes showed no significant differences between cooled and non-cooled cows throughout lactation. Changes in BW and NE*m* in mid lactation were greater for animals treated with rbST ($P<0.05$) including interaction effects between cooling and rbST ($P<0.05$). DMI and NE intake were significantly positive affected in animals acclimated to misters and fans in early and mid

lactation (*P<0.05*) but no effects were apparent in late lactation. During rbST supplementation, a significant increase in NE*i* was related to an increase in DMI throughout lactation. The interaction effect of cooling and rbST on DMI and NE*i* was apparent in late lactation. During mid lactation, dry matter intake per 100 kg BW of cooled cows were significantly higher than those of non-cooled cows (*P<0.05*), but not for early and late lactation. Dry matter intake per 100 kg BW of both cooled and non-cooled cows were affected by supplementation of rbST during mid and late lactation. The net energy was used for lactation (NE*l*) in cooled cows tended to be higher than those of non-cooled cows in all stages of lactation and the significant effect was apparent in early lactation (*P<0.05*). The effects of rbST supplementation on increasing NE*l* occurred in both cooled and non cooled cows which coincided with increases in milk yields in all stages of lactation. The cooled cows in the present study had higher the energy balance (EB) than non-cooled cows in mid lactation.

Milk yield and milk compositions

Supplementation of rbST increased milk yield of cows in both groups (*P<0.01*) in all stages of lactation (Table 5). Milk yields of cooled cows without rbST supplementation were higher by 18.81% (+1.72 kg/d) than those of non-cooled cows throughout lactation. The responses of rbST supplementation for milk yields throughout lactation were higher on average 29.41% and 15.53% of cooled and non-cooled cows respectively. Cooling with misters and fans did not affect the concentrations of milk fat and protein throughout the experimental periods. Increases in milk fat percentage were apparent in both cooled and non-cooled cows supplemented with rbST in all stages of lactation and it was markedly apparent in mid lactation (*P<0.05*). Milk composition yields were significantly increased by the effect of rbST supplementation in all stages of lactation. In the present study, 4%FCM was increased in rbST-supplemented cows throughout lactation. An increase in the milk lactose yield did not coincide with the concentration of milk lactose.

Ruminal characteristics

The effects of rbST and misters and fans on changes in ruminal characteristics are shown in Table 6. The mean values of ruminal pH of animals kept in both NS and MF barn either without or with rbST supplementation showed no significant differences in all stages of lactation. However, the values of ruminal pH in the present study were 6.49 to 6.77. The ruminal NH_3-N concentrations were not significantly different between cooled

and non-cooled cows in all stages of lactation. An increase in the ruminal NH_3-N concentration was significantly affected by rbST supplementation in either cooled or non-cooled cows throughout stages of lactation. The concentrations of total ruminal VFA of cooled cows tended to be higher (18.01%) than those of non-cooled cows in all stages of lactation when rbST were not administered. The cooling effect on total VFA and the concentration of acetate were significantly apparent in early lactation but not for mid and late lactation. Cows supplemented with rbST under MF showed interaction effect on increases in total VFA in late lactation. Either cooled or non-cooled cows supplemented with rbST, marked increases in the total ruminal VFA level were apparent as compared with the pre-treated periods in all stages of lactation. These changes coincided with significant increases in the concentration of ruminal propionate. The acetate to propionate ratio was maintained in similar values among stages of lactation. The cooled cows without rbST supplementation in different stages of lactation had no effects on the concentrations of ruminal butyrate and valerate as compared with non-cooled cows. The effect of rbST supplementation significantly increased *<0.05*) in the concentration of butyrate in late lactation and the concentration of valerate in early lactation.

Milk urea, allantoin, uric acid, and microbial nitrogen

The effects of rbST and misters and fans on changes in milk urea, allantoin, uric acid, and microbial nitrogen flow are shown in Table 7. Supplementation of rbST caused significant excretion of milk urea nitrogen (MUN) during the three stages of lactation evaluated but cooling only affected MUN excretion during early lactation (*P<0.01*). The concentrations and excretions of allantoin in milk were increased after rbST supplementation in all stages of lactation. The concentration and excretion of uric acid in milk were not affected by either cooling or rbST supplementation. The rbST supplemented-cows had higher in MNF by average 7.16% in non-cooled cows and 13.11% in cooled cows in all stages of lactation.

Table 1. Feed ingredients and chemical compositions of the Total Mixed Ration (TMR) fed to cross-bred cattle during lactation.

Ingredients	Kg
Pine apple waste	50
Soybean meal	23
Rice bran	3.0
Cotton seed	20
Lime stone	1.4
Di-calcium phosphate	1.4
Sodium bicarbonate	0.3
Potassium chloride	0.1
Mineral and vitamin premix	0.8
Total	100
Chemical composition	
Dry matter (%)	39.1
Organic matter (% DM)	92.7
Crude protein (% DM)	18.0
Acid detergent fiber (% DM)	20.1
Neutral detergent fiber (% DM)	33.9
Total digestible nutrients (% DM)	70.0
Metabolizable energy (Mcal/kg DM)	2.7

Table 2. The environmental measurements of misters and fans (MF) in comparison with normal shade (NS)

Parameter	time	NS		MF			Effect[2,3]		
		Pre[1]	rbST[1]	Pre	rbST	SEM	MF	rbST	MF x rbST
Early lactation									
Ambient Temperature(°C)	09.00	27.90	27.40	27.20	27.60	0.242	0.521	0.842	0.100
	13.00	33.70	34.90	31.60	31.70	0.570	0.000	0.287	0.363
	17.00	34.30	34.10	30.30	30.90	0.426	0.000	0.651	0.375
Relative humidity	09.00	78.80	78.40	83.60	86.60	1.578	0.040	0.434	0.313
	13.00	52.00	53.20	70.60	70.20	3.162	0.002	0.902	0.807
	17.00	56.60	49.60	75.80	73.80	2.037	0.000	0.058	0.255
Temperature humidity index	09.00	78.69	77.97	78.18	78.98	0.263	0.591	0.895	0.021
	13.00	53.30	85.02	82.86	82.93	0.773	0.016	0.254	0.291
	17.00	85.02	83.58	81.78	82.89	0.533	0.002	0.405	0.106
Mid lactation									
Ambient Temperature(°C)	09.00	28.00	28.50	27.60	26.90	0.302	0.090	0.749	0.082
	13.00	35.30	35.00	30.00	29.80	0.476	0.002	0.613	0.919
	17.00	33.70	34.30	29.50	30.00	0.486	0.000	0.334	0.842
Relative humidity	09.00	78.60	78.80	85.20	83.60	1.256	0.009	0.595	0.497
	13.00	52.80	50.40	78.20	74.00	3.089	0.000	0.318	0.779
	17.00	58.00	52.80	81.00	80.20	2.502	0.000	0.250	0.426
Temperature humidity index	09.00	78.98	79.55	78.90	77.75	0.402	0.216	0.495	0.064
	13.00	85.46	84.81	81.50	80.78	0.388	0.019	0.116	0.928
	17.00	84.30	84.16	81.35	81.86	0.877	0.002	0.842	0.721
Late lactation									
Ambient Temperature(°C)	09.00	28.50	28.40	27.10	27.30	0.387	0.007	0.900	0.709
	13.00	33.80	33.90	29.50	29.30	0.468	0.000	0.917	0.757
	17.00	33.60	34.20	30.40	29.10	0.345	0.000	0.340	0.025
Relative humidity	09.00	74.60	75.40	82.20	84.00	1.619	0.010	0.445	0.765
	13.00	60.00	64.40	78.60	78.40	1.761	0.000	0.267	0.228
	17.00	50.60	49.80	76.60	77.80	1.116	0.000	0.862	0.396
Temperature humidity index	09.00	79.05	78.98	77.90	78.40	0.406	0.145	0.610	0.499
	13.00	84.45	85.10	80.85	80.70	0.506	0.000	0.632	0.456
	17.00	82.94	83.58	81.86	80.20	0.373	0.007	0.214	0.015

[1] Pre = pre-treatment of rbST, rbST = treatment of rbST

[2] Effect ; MF =Misters and fans effect, rbST = rbST effect , MF x rbST = interaction effect of MF and bST

[3] Significant ; *ns = P> 0.05 , * = P <0.05 and ** = P < 0.01*

Table 3. Effects of the application of misters and fans (MF) in comparison with normal shade (NS) on respiratory rate and rectal temperature of crossbred Holstein cows supplemented with rbST

Parameter	time	NS		MF			Effect[2,3]		
		Pre[1]	rbST[1]	Pre	rbST	SEM	MF	rbST	MF x rbST
Early lactation									
Respiration rate	09.00	40.0	42.8	35.6	39.20	0.548	0.010	0.000	0.486
	13.00	70.8	80.2	53.2	64.40	0.267	0.016	0.014	0.790
	17.00	63.2	67.2	48.00	51.60	0.735	0.000	0.001	0.792
Rectal temperature	09.00	38.44	38.84	38.04	38.44	0.065	0.021	0.000	1.000
	13.00	39.48	39.98	38.90	39.32	0.161	0.008	0.021	0.810
	17.00	39.06	39.20	38.64	38.96	0.074	0.052	0.015	0.260
Mid lactation									
Respiration rate	09.00	41.20	45.80	36.40	40.40	0.663	0.022	0.000	0.633
	13.00	71.60	76.40	51.60	60.50	2.687	0.000	0.031	0.437
	17.00	62.00	65.60	47.20	51.60	0.283	0.001	0.000	0.195
Rectal temperature	09.00	38.46	38.96	38.00	38.30	0.082	0.024	0.001	0.258
	13.00	39.72	40.08	38.60	39.46	0.130	0.002	0.002	0.090
	17.00	39.04	39.36	38.46	38.80	0.067	0.002	0.001	0.885
Late lactation									
Respiration rate	09.00	40.4	44.	36.8	41.20	0.131	0.046	0.008	0.733
	13.00	69.2	79.6	51.80	57.00	1.275	0.006	0.000	0.076
	17.00	59.6	63.6	47.20	50.40	0.678	0.000	0.001	0.572
Rectal temperature	09.00	38.54	38.86	37.92	38.32	0.080	0.001	0.002	0.629
	13.00	39.10	39.80	38.42	38.90	0.138	0.013	0.003	0.449
	17.00	39.00	39.28	38.52	38.78	0.059	0.003	0.002	0.870

[1] Pre = pre-treatment of rbST, rbST = treatment of rbST

[2] Effect ; MF =Misters and fans effect, rbST = rbST effect , MF x rbST = interaction effect of MF and bST

[3] Significant ; *ns = P> 0.05 , * = P <0.05 and ** = P < 0.01*

Table 4. Effects of the application of misters and fans (MF) in comparison with normal shade (NS) on body weight, dry matter intake, energy intake and energy balance of crossbred Holstein cows supplemented with rbST

Parameter	Lactation period	NS		MF		SEM	Effect[2,3]		
		Pre[1]	rbST[1]	Pre	rbST		MF	rbST	MF x rbST
BW (kg)	Early	357.8	380.3	373.2	375.6	7.33	ns	ns	ns
	Mid	382.8	387.8	383.4	407.8	4.45	ns	*	*
	Late	396.0	395.4	425.8	421.8	4.43	ns	ns	ns
Dry matter intake (kg/day)	Early	6.14	7.05	7.22	8.49	0.31	*	**	ns
	Mid	6.18	7.49	8.72	10.00	0.45	*	*	ns
	Late	7.57	7.88	8.26	9.32	0.15	NS	**	*
Dry matter intake (%BW)	Early	1.73	1.85	1.94	2.28	0.111	ns	ns	ns
	Mid	1.61	1.97	2.28	2.50	0.130	*	*	ns
	Late	1.92	2.01	1.97	2.23	0.040	ns	**	ns
NE intake (Mcal/day)	Early	16.40	18.81	19.27	22.66	0.83	*	**	ns
	Mid	16.50	20.00	23.28	26.71	1.20	*	*	ns
	Late	20.22	21.04	22.05	24.89	0.40	ns	**	*
NE for Maintenance (Mcal/day)	Early	6.57	6.89	6.79	6.82	0.10	ns	ns	ns
	Mid	6.92	6.87	6.93	7.25	0.06	ns	*	*
	Late	7.10	7.09	7.49	7.44	0.05	ns	ns	ns
NE for lactation (Mcal/day)	Early	3.22	3.76	4.02	5.52	0.37	*	*	ns
	Mid	3.65	4.57	4.46	6.16	0.41	ns	*	ns
	Late	3.76	5.23	4.01	5.92	0.38	ns	*	ns
Energy balance (Mcal)	Early	6.61	8.16	8.46	10.32	0.95	ns	ns	ns
	Mid	5.92	8.57	11.89	13.30	1.35	*	ns	ns
	Late	9.35	8.71	10.55	11.53	0.60	ns	ns	ns

[1] Pre = pre-treatment of rbST, rbST = treatment of rbST

[2] Effect ; MF =Misters and fans effect, rbST = rbST effect , MF x rbST = interaction effect of MF and bST

[3] Significant ; *ns = P> 0.05 , * = P <0.05 and ** = P < 0.01*

Table 5 Effects of the application of misters and fans (MF) in comparison with normal shade (NS) on milk yields and milk composition of crossbred Holstein cows supplemented with rbST

Parameters	Lactation period	NS		MF			Effect[2,3]		
		Pre[1]	rbST[1]	Pre	rbST	SEM	MF	rbST	MF x rbST
Milk yield (kg/day)	Early	9.99	11.82	11.51	13.05	0.28	ns	**	ns
	Mid	9.42	10.03	11.37	13.05	0.33	ns	**	ns
	Late	8.02	9.63	9.69	12.17	0.43	ns	**	ns
Fat yield(g/day)	Early	308.04	356.22	392.22	522.26	40.17	*	*	ns
	Mid	352.38	450.14	435.30	616.90	44.22	ns	*	ns
	Late	361.76	522.30	386.66	592.18	79.18	ns	*	ns
Protein yield(g/day)	Early	351.04	416.80	399.44	496.08	14.47	ns	**	ns
	Mid	344.24	381.32	460.62	549.12	17.81	ns	**	ns
	Late	322.82	383.84	406.66	523.20	30.98	ns	*	ns
Lactose yield (g /day)	Early	461.56	520.26	555.40	626.54	15.66	ns	**	ns
	Mid	402.44	445.46	542.58	634.26	20.89	ns	*	ns
	Late	384.36	397.82	445.04	570.44	23.99	ns	**	ns
4%FCM(kg/day)	Early	8.62	10.21	10.49	13.51	0.65	ns	**	ns
	Mid	9.05	10.76	11.08	14.47	0.78	ns	*	ns
	Late	8.63	11.67	9.67	13.75	1.34	ns	*	ns
Milk composition:									
Fat (g%)	Early	3.12	3.26	3.37	4.26	0.33	ns	ns	ns
	Mid	3.84	4.44	4.16	4.93	0.27	ns	*	ns
	Late	4.52	5.30	4.16	4.86	0.56	ns	ns	ns
Protein (g%)	Early	3.17	3.61	3.48	3.63	0.13	ns	ns	ns
	Mid	3.79	3.84	4.09	4.26	0.15	ns	ns	ns
	Late	4.25	4.02	4.30	4.32	0.17	ns	ns	ns
Lactose (g%)	Early	4.62	4.34	4.82	4.79	0.10	ns	ns	ns
	Mid	4.27	4.47	4.79	4.84	0.12	ns	ns	ns
	Late	4.29	4.01	4.58	4.68	0.11	ns	ns	ns

[1] Pre = pre-treatment of rbST, rbST = treatment of rbST

[2] Effect ; MF =Misters and fans effect, rbST = rbST effect , MF x rbST = interaction effect of MF and bST

[3] Significant ; *ns = P> 0.05 , * = P <0.05 and ** = P < 0.01*

Table 6 Effects of the application of misters and fans (MF) in comparison with normal shade (NS) on rumen fermentation characteristics of crossbred Holstein cows supplemented with rbST

Parameter	Lactation period	NS		MF		SEM	Effect[2,3]		
		Pre[1]	rbST[1]	Pre	rbST		MF	rbST	MF x rbST
Rumen fluid pH	Early	6.63	6.96	6.65	6.57	0.13	ns	ns	ns
	Mid	6.70	6.77	6.48	6.49	0.10	ns	ns	ns
	Late	6.67	6.68	6.74	6.60	0.11	ns	ns	ns
NH_3-N (mg/dl)	Early	11.35	12.42	12.80	14.24	0.24	ns	**	ns
	Mid	13.15	14.18	11.94	13.22	0.52	ns	*	ns
	Late	12.17	13.35	11.23	12.90	0.16	ns	**	ns
Total VFA (mmol/l)	Early	78.80	84.54	97.74	104.02	2.12	*	*	ns
	Mid	81.98	91.90	97.66	102.50	4.42	ns	*	ns
	Late	86.62	90.36	96.56	115.40	2.77	ns	**	*
Acetate (mmol/l)	Early	51.34	54.30	52.82	65.78	1.48	*	ns	ns
	Mid	50.62	60.44	65.80	67.46	4.38	ns	ns	ns
	Late	56.04	57.96	64.10	77.58	3.01	ns	*	ns
Propionate (mmol/l)	Early	17.32	18.96	19.82	22.02	0.65	ns	*	ns
	Mid	18.34	19.54	20.26	22.08	0.69	ns	*	ns
	Late	18.98	19.52	19.86	23.60	0.83	ns	*	ns
Butyrate (mmol/l)	Early	9.38	10.26	11.12	12.86	0.94	ns	ns	ns
	Mid	11.70	10.60	10.42	11.92	0.06	ns	ns	ns
	Late	10.48	11.68	11.50	12.94	0.54	ns	*	ns
Valerate (mmol/l)	Early	0.78	1.06	1.04	1.28	0.12	ns	*	ns
	Mid	1.34	1.32	1.18	1.06	0.16	ns	ns	ns
	Late	1.12	1.16	1.08	1.28	0.15	ns	ns	ns
Acetate:Propionate ratio	Early	3.00	2.92	3.36	3.08	0.15	ns	ns	ns
	Mid	2.76	3.20	3.44	3.18	0.03	ns	ns	ns
	Late	2.94	3.00	3.28	3.44	0.22	ns	ns	ns

[1] Pre = pre-treatment of rbST, rbST = treatment of rbST

[2] Effect ; MF =Misters and fans effect, rbST = rbST effect , MF x rbST = interaction effect of MF and bST

[3] Significant ; *ns = P> 0.05 , * = P <0.05 and ** = P < 0.01*

Table 7 Effects of the application of misters and fans (MF) in comparison with normal shade (NS) on urea and purine derivatives concentrations and excretions in milk of crossbred Holstein cows supplemented with rbST

Parameter	Lactation period	NS		MF		SEM	Effect[2,3]		
		Pre[1]	rbST[1]	Pre	rbST		MF	rbST	MF x rbST
MUN concentration (mg/dl)	Early	14.05	16.69	17.00	20.84	0.76	**	**	ns
	Mid	15.31	17.59	18.17	20.62	0.74	ns	*	ns
	Late	18.93	21.73	21.66	23.51	0.55	ns	**	ns
MUN excretion (g/day)	Early	1.41	1.94	1.97	2.67	0.05	*	**	ns
	Mid	1.44	1.70	2.10	2.73	0.13	ns	*	ns
	Late	1.53	2.10	2.16	2.93	0.12	ns	**	ns
Milk allantoin cocentration (mmol/l)	Early	0.67	0.72	0.63	0.71	0.01	ns	**	ns
	Mid	0.69	0.70	0.72	0.80	0.02	ns	*	ns
	Late	0.72	0.78	0.80	0.91	0.01	ns	**	ns
Milk allantoin excretion (mmol/day)	Early	6.69	8.49	7.14	9.14	0.33	ns	**	ns
	Mid	6.66	7.19	7.98	10.21	0.36	ns	**	*
	Late	5.76	7.59	7.48	10.67	0.38	ns	**	ns
Milk uric acid concentration (mmol/l)	Early	0.23	0.24	0.20	0.20	0.02	ns	ns	ns
	Mid	0.20	0.21	0.22	0.20	0.01	ns	ns	ns
	Late	0.27	0.26	0.19	0.21	0.02	*	ns	ns
Milk uric acid excretion (mmol/day)	Early	2.30	2.73	2.25	2.67	0.18	ns	*	ns
	Mid	1.91	2.03	2.44	2.67	0.13	ns	ns	ns
	Late	2.10	2.59	1.80	2.49	0.25	ns	*	ns
Microbial Nitrogen synthesis (g/day)	Early	163.64	178.42	163.86	181.88	3.01	ns	**	ns
	Mid	165.21	169.25	174.02	194.34	3.27	ns	**	*
	Late	159.36	175.30	173.74	202.67	3.26	ns	**	ns

[1] Pre = pre-treatment of rbST, rbST = treatment of rbST

[2] Effect ; MF =Misters and fans effect, rbST = rbST effect , MF x rbST = interaction effect of MF and bST

[3] Significant ; *ns = P> 0.05 , * = P <0.05 and ** = P < 0.01*

DISCUSSION

In the present study, ambient temperatures and THI at 09.00h of all stages of lactation were not significantly different between NS and MF barns, while relative humidity(RH) of MF barn was significantly higher in comparison with NS barn. These results suggest that a change in THI for the potential environmental heat stress would not be dependent on a high relative humidity (Bohmanova et al. 2007). However, MF barn was not sufficient to completely eliminate heat stress in cows, because the range for THI measured at daytime (09.00 to 17.00 h) under misters and fans throughout the experimental periods remained high (77.7 to 82.9), exceeding the threshold level of a THI of 72 according to Abeni et al. 2007; McDowell 1972. Cross-bred dairy cattle using in the present experiment containing *Bos indicus* gene, could have a high heat tolerance than exotic *Bos Taurus* cattle (Nakamura et al. 1993; Pereira et al. 2008). Therefore, THI might not accurately reflect heat stress of cross-bred lactating cows under misters and fans that deliver a pressurized spray with considerable fan air movement in the barn, resulting in higher humidity but also causing a cooling effect.

The cooling of cows under MF was significantly lower in both rectal temperatures and respiratory rates in comparison with those of non-cooled animals. It indicates a partial alleviation of heat stress by MF system, which was sufficient to reduce effective temperature in the crossbred lactating cows during hottest of the day. These results are in agreement with those reported by Avendano-Reyes et al. (2006) using water spray and fans in European cooled cows, which had lower rectal temperatures and respiratory rates than non-cooled cows. However, the measurement of the rectal temperature at daytime of both non-cooled cows and cooled cows in this experiment were still higher than those reported for normal range (38.3-38.7 °C) in dairy cattle (Abeni et al. 2007). An increase in both RR and RT of cross-bred cows in the present study was affected by rbST supplementation in either cooled or non-cooled cows in all stages of lactation. These results would support the study of Sullivan et al. (1992) that rbST-treated cows exhibited slightly higher RT during hot condition, whereas the experiment by Tarazon et al. (1999) has shown that under thermoneutral condition, the RR and RT were not affected by bST supplementation. In the present results, increases in both RR and RT of rbST-supplemented cows would remained expose to heat stress. However, rbST-supplemented cows could produce more milk than non-supplemented rbST cows. It would suggest that eventhough rbST increases heat production associated with high milk yield, it also increases heat dissipation (Johnson et al. 1991; West 1994).

Animals in both cooled and non-cooled cows gained weight throughout the experiment. These changes were correlated to positive energy balance in all stages of lactation (5.9 to 13.3 Mcal/day). These results differed from the study of Tarazon et al. (1999) who observed that weight gain occurred only in cooling of cows. It is probable that the response to cooling and rbST supplementation of cross-bred Holstein cattle using in the present study, might not only increase in milk production but also increased efficiency of food conversion to meat. Animals were fed to allow an adequate replacement of body reserve during study. Milk yield in the first lactation of cross-bred animals in the present study were not as great as that of multiparous cows (Sullivan et al. 1992). Although no differences of energy balance of cows between supplementation with or without rbST were apparent in this experiment, but the rbST supplemented cows trended to have higher levels of energy balance than non supplemented cows(P=0.10). These results are in agreement with the observations recorded by Oldenbroek et al. (1989) in European cattle that the rbST-treated cows had higher net energy intake and energy output in milk than those of control cows. It is possible that cows using in this study at the start of experiment (60 days postpartum) would enter positive energy balance between 6 to 8 weeks after postpartum (Moallem et al 2000).

Although, milk yield showed no statistical differences in cows housing between NS and MF, but milk yield of the cooled cows trended to be higher than those of non-cooled cows. (P=0.07). However, the percentage of increase in milk production responses of the cooling cross-bred cows in the present experiment is in accordance with the results reported with the study in *Bos taurus* cows (Tarazon et al. 1999). It is possible that the difference of day and night temperature in the present study would affect the effects of heat stress on milk yield, which animals were exposed to a high THI during hottest of the day. Both cooled and non cooled cows responses to rbST supplementation by increases in DMI and NEi, consequently increase in milk yield. Furthermore, the percentages of milk compositions were not different in cows housing between NS and MF whether supplemented with rbST or not. The present results indicate that an increase in milk production of cross-bred cows would be governed by both MF and rbST supplementation in all stages of lactation. These results are in accordance with the results reported in cooled cow with spray and fan system in combination with rbST increased milk yield comparing to non-cooled and non-rbST treated cows(Keister et al. 2002). An increase in milk yields of cooled cows in response to rbST supplementation during late lactation (+25.59%) would be attributed to an increase in mammary uptake of

the substrate coinciding with an increase in mammary blood flow during rbST treatment (Tanwattana et al. 2003). A numbers of studies have been reported milk production increased from 14-30% in response to rbST administration alone (Gibson et al. 1992). In the present study, it was noted that an increase in the milk lactose yields did not coincide with the concentration of milk lactose. It is possible that lactose is the osmotic factor of milk synthesis and is required in proportion to the amount of milk produced (Linzell and Peaker 1971). The DMI in both cooled and non-cooled animals was significantly increased during rbST supplementation, while it did not affect on ruminal pH in all stages of lactation. However, the values of ruminal pH in the present study (pH 6.49 to 6.77) were higher than those results (pH 5.8 to 6.7) reported by the others (Rabelo et al. 2003), but it was still within an acceptable range to maintain a healthy rumen environment (NRC, 2001). These results agree with Robinson et al. (1991) which reported that the ruminal pH had no affected by bST treatment.

In the present results, the higher ammonia production in the rumen during rbST supplementation would not be due to excessive feeding a higher level of crude protein for microbial degradation but it would be due to an increase in DMI. These results agree to the study of Robinson et al. (1991) who showed that cows treated with bST had higher ammonium N concentration than the control cows. In the present study, an increase in total VFA in the rumen fluid of rbST treated cows would not be due to the different types of fermentable contents of the diet, since all animals were fed with similar TMR throughout the experimental period. The magnitude of these changes would be due to an increase in the concentrations of ruminal propionate in rbST-supplemented cows accompanying with the effect of cooling in all stages of lactation. Prolonged exposure of an animal to high envoronmental temperature resulting in the reduction of VFA production has been noted (Bandaranayaka and Holmes 1976). Cooled and non cooled cows showed no differences in total VFA concentration in this study. It is possible that animals remained exposed to a high THI level in both NS and MF barn which was greater than threshold value (72) for lactating cows. These differences in total VFA levels would be attributed, in part, by an increase in DMI of rbST-treated cows. According to a marked increase in the concentration of ruminal propionate, the conversion of propionate to glucose in the liver for the precursor of lactose synthesis to increase in milk yields would be further investigated in cross-bred dairy cattle. The concentration of acetate were significantly apparent in early lactation but not for mid and late lactation between noncooled and cooled cows but the acetate to propionate ratio

were maintained in similar values among stages of lactation, suggesting that rbST supplementation have a positive effect on both roughage and concentrate digestion. Thus, an increase in total VFA production in the rumen during rbST supplementation in both groups would be a function of rbST treatment per se and independently in excessive food intake. There are evidences that the highly positive correlations between the plasma urea and milk urea concentration of dairy cows have been noted (Gustafsson and Palmquist, 1993). Milk urea content reflects total nitrogen losses in dairy cows especially the nitrogen surplus in the rumen (Jonker et al 2002). In this study, the marked increase in the MUN concentration coincided with increases in NH_3-N production in the rumen by supplementation of rbST in both cooled and non-cooled cows. This could be explained by the fact that excess ruminal NH_3-N is absorbed and converted to urea in the liver for either recycling to the rumen through saliva or an excretion in milk and urine. It is probable that an increase in mammary uptake of plasma urea coinciding with an increase in mammary blood flow during rbST supplementation (Chaiyabutr et al. 2000) would attribute to an increase in MUN. In contrary to the present findings, Cheli et al. (1998) found the decrease in the plasma urea level in exotic dairy cows during rbST supplementation. Furthermore, purine derivatives (allantoin, uric acid, xanthine and hypoxanthine) are indicator for microbial protein production in the rumen. In the present study, an increase in both the concentration and excretion of allantoin in milk after rbST supplementation is in agreement with the study of Schager et al. (2003). Therefore, during supplementation of rbST, the cow had greater microbial nitrogen flow from the rumen than pre-supplement period. From these results, it was suggested that an increase in milk allantoin excretion coinciding with an increase in MNF would be used as an index to predict an increase in rumen microbial protein synthesis. Supplementation of rbST could improve microbial protein synthesis in the rumen of crossbred cows during exposure to high environmental temperature.

Conclusions

Data from this study show that the application of misters and fans to crossbred Holstein cattle was sufficient to alleviated heat stress, the cooled cows showed a lower RR and RT during the daytime. The ambient temperature and THI in both NS and MF barn remained high than threshold for lactating dairy cows. The supplementation of rbST under the misters and fans in lactating crossbred Holstein cattle increased both DMI and milk production in all stages of lactation. The fermentation for total VFA and NH_3N production in the rumen were greater in rbST-supplemented cows whether under

misty fan cooling or not. The rbST-supplemented cows also increased microbial protein nitrogen flow from the rumen. These results suggest that a part of the response in milk production to rbST in crossbred Holstein cows whether under misters and fans or not, is at changes in ruminal fermentation in supplying of nutrients as precursors for milk synthesis. Further study should be carried out to investigate the passage rate of digesta from the rumen of crossbred Holstein cattle during rbST supplementation under misters and fans.

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CHAPTER XI(Series 11.2)

Effects of mist-fan cooling and supplemental recombinant bovine somatotropin on diet digestibility, digestion kinetics and milk production of crossbred Holstein cattle in the tropics

INTRODUCTION

The low milk production of both exotic and crossbred cattle is still the main problem in dairy farming in the tropics. The combination of high temperature and humidity (as indicated by the temperature humidity index (THI)) is a main factor that can produce heat stress and have a negative effect on lactation performance and physiological status of dairy cows (Armstrong 1994; Kadzere *et al*. 2002). The mechanisms for an alteration of digestive function in crossbred dairy cattle during exposure to high temperatures are not yet clear, although a reduction in dry matter intake (DMI) is known as one of the responses to heat stress leading to,reduction of milk yield. However, there are inconsistent results as regards the relationship between high environmental temperature and digestive function. Some reports showed a reduction in diet digestibility by excess THI which related to a decline in ruminal activity through the depression of rumen cellulolytic activity (Bernabucci *et al.* 1999). Slower passage rate and longer mean retention time of digesta have been shown in response to high ambient temperature (Christopherson and Kenedy 1983; Silanikove 1992). Other results have shown an increase in diet digestibility in dairy cows exposed to hot environment (Collier *et al.* 1982; Mathers *et al.*, 1989). The increase in diet digestibility would result in increase in total volatile fatty acids produced at high temperatures which might lead to increased heat production and result in increased body temperature (Shibata and Mukai 1979).

Many technologies are required to improve milk production of dairy cattle in the tropics. Environmental modification is the most common approach to increase milk production with alleviation of severe heat stress in dairy cattle, for example, fans and sprinklers (Fike *et al.* 2002), evaporative cooling systems (Chan *et al.* 1997). It has been shown that cows cooled with spray and fans had greater milk yields than those cooled with

evaporative cooling systems (Armstrong *et al.* 1994). In addition to environmental modification, other technologies can increase milk production in dairy cattle, for example the application of exogenous bovine somatotropin has been reported to minimize the effects of heat stress and potentially increase milk yields (West 1994). However, few data are available on the knowledge of the relation between action of exogenous somatotropin and effect of high temperatures on milk production in crossbred Holstein cows in the tropics. Somatotropin is known to play a role in galactopoiesis and contribute to homeostasis and homeorhesis in ruminants **(**Bauman and Currie 1980). Administration of bST to lactating cows in a hot environment can increase milk yield (West *et al.* 1991), but such cows also increase heat production by approximately 25 % over the value in control cows (West 1994). Johnson *et al.* (1991) reported that somatotropin increased the efficiency of feed conversion into milk without any significant changes in body weight and temperatures. These results show that there is no consistent conclusion about the interaction effects between thermal stress and bST on lactation performance. Little is known about how somatotropin modifies digestive function in crossbred cattle. In other ruminants, an increase in the rate of liquid flow from the rumen during heat exposure has been reported in buffalo and goat (Chaiyabutr *et al.* 1987; Silanikove and Tadmor 1989). It is believed that increased water absorption occurs in the lower GI-tract to increase heat dissipation during heat exposure. In view of an increase in total body water in rbST-treated cows (Chaiyabutr *et al.* 2007), it is necessary to establish whether rbST supplementation in cows maintained at high temperatures will minimize the effects of heat stress and whether the flow rate of digesta from the rumen will maintain sufficient nutrients to sustain the potentially increased milk yields. It is therefore important to study the digestive function and milk yields during bST supplementation under high ambient temperature in crossbred dairy cattle. Therefore, the objectives of the current study were 1) to evaluate the effect of providing crossbred cattle with housing under shade with or without mister-fans and 2) supplementation of cows with rbST or not during period of lactation (early, mid and late lactation). Measures to evaluate the effectiveness of these treatments were feed intake, digestion kinetic, diet digestibility, efficiency of feed utilization and milk production.

MATERIALS AND METHODS

Animals, housing and managements

Ten primiparous, non-pregnant crossbred cattle, containing 0.875 Holstein (HF) genes, age 36±2 months, average body weight 358±32.5 kg and body condition score (BCS) 2.5±0.50 were used for the experiment. Cows on average had been 60±1 days in milk (DIM) at start of the trial. They were assigned randomly into two groups of five animals each. During the study period, cows in both groups were housed in an open-sided barn with a tiled-roof and tie-stall. The barn (16m long x 7m wide x 3.5m high) was separated into two parts by a metal sheet wall (3.5m high) to ensure no drift of water from the mist treatment could reach to the other half of the barn. The first part was arranged for cows in normal shade (NS) and the second part of the barn was equipped with two pedestal mist-fan cooling systems (MF) which were placed 6 feet from the ground for cooled cows. Each system consisted of a 65 cm. diameter blade fan circulating 81 m^3/min of air, with oscillation coverage of 180º. The amount of water discharged from 4 mister spray heads (mounted relative to the fan) was 7.5 L/h and size of mist droplet 0.01 mm. Animals were exposed to MF for 45 minutes at 15-minute intervals from 0600 to 1800 h. At night, animals were exposed to MF for 15 minutes at 45-minute intervals from 1800 to 0600 h.

Cows in both groups were fed with the total mixed ration (TMR) which was formulated according to NRC requirements (NRC, 2001) for cows producing 10-15 kg milk/day. The TMR diet and ingredients are shown in Table 1. The TMR was offered twice daily at 1.1 of *ad libitum* consumption at 0600 and 1700 h throughout the experimental period. Water was given to cows *ad libitum*. Dry matter intake (DMI) of each cow was measured daily by weighing the TMR offered and subtracting that refused. Cows were normally milked at around 0600 h and 1700 h using a milking machine and milk production was recorded daily

The ambient temperature at NS and MF barns were recorded using a wet and dry bulb thermometer. The relative humidity at NS and MF barns were read by psychrometric chart depending on wet and dry bulb temperature. Ambient temperatures and humidity were measured during the daytime (1300 h), three days before beginning of the first injection of rbST (pre-supplemented period) and three days after the 3rd injection of

rbST(supplemented period) in each stage of lactation. Average values were considered to be the mean values of all measurements taken throughout periods of study.

On each specified day during measurements of ambient temperature around 1300 h, rectal temperature (RT) of each cow was measured by electronic thermometer and respiratory rates (RR) was measured by counting flank movements. Body weights (BW) of all animals were recorded by weighing monthly throughout experimental periods. The procedures used in the present study were carried in accordance with the principles and guidelines of the Faculty of Veterinary Science, Chulalongkorn University. These guidelines were formulated to comply with international standards and are in accordance with the principles and guidelines of the National Research Council of Thailand.

Experimental design

The experiment in each group was divided into 3 periods, namely early- (60-90 DIM), mid- (120-150 DIM), and late lactating periods (180-210 DIM). The pretreatment study was conducted on the starting day of each period. At the end of the pretreatment, within the same day, the cow was injected with the first subcutaneous injection of 500 mg of recombinant bovine somatotropin (rbST) (POSILAC, Monsanto, USA). Subsequently, the animal was injected with two consecutive doses injections of rbST every 2 weeks. Thereafter, within 3 days after the third injection, the effect of rbST supplementation was conducted. During the last 30 days of each period, no experiments were performed. This allowed effects of handling, sampling and rbST to be return to normal before start of the next periods. It has been demonstrated in dairy cattle by Kirchgessner *et al*. (1991) that during the first week after injection of bST, milk yield increased sharply and almost returned to control level within the next 3 weeks.

Sample collection and chemical analysis

Chromic oxide (Cr_2O_3) was used as an external marker to estimate nutrient digestibility and passage rate of digesta in the total gastro-intestinal tract of each cow. Cows were given 10 g of Cr_2O_3 /day contained in gelatin capsules. Cr_2O_3 was divided into two portions and was given orally to animals twice a day at 06.00 and 18.00 h. for 10 days. In determination of the diet digestibility, fecal grab samples were collected three times a day

from day 8 to day 10 after dosing so that nine samples were taken for each cow. Nine faecal samples were pooled and dried for analysis of chemical composition. After the last dose of chromic oxide, individual fecal grab samples were collected at 0, 4, 8, 12, 16, 20, 24, 30, 36, 44, 50, 62, 68, 74, and 96 h. The samples were dried in a forced oven dryer at 70 °C for 24 h, followed by grinding. The chromic oxide content of the sample was determined by a colorimetric method according to Kimura and Miller (1956) using a nitric-perchloric acid oxidation with molybdate catalyst. The concentrations of Cr_2O_3 in the faeces were used for estimation of the passage rate of digesta.

Samples of TMR were collected weekly and pooled for determinations of DM, OM, CP, NDF and ADF. Feed and feces were dried at 55°C in a forced-air oven for 72 h and were analyzed for DM concentration. All dried samples were ground with a Wiley mill (1 mm screen). All feed and feces samples were dried at 105°C for 8 h in a forced-air oven before determination of nutrients (OM, CP, NDF and ADF). The ash concentration of samples was determined by heating at 550°C for 5 h in a muffle furnace. Concentrations of NDF and ADF were determined according to Van Soest *et al.* (1991). Crude protein was determined using the Kjeldahl procedure (AOAC 1990).

Calculation

The semi-logarithm of Cr_2O_3 concentrations in faeces were plotted against time of sampling taken over the period of 4 days after the last dose of Cr_2O_3 administration. The descending portion of the curve was used for regression analysis and the regression line represents the passage rate and half time of Cr_2O_3. The digesta passage rate constant (h^{-1}), mean retention time (h), faecal output (kgDM/d), digesta flow rate (kg/d), concentration of dry matter(DM) and digestibility, and efficiency of feed utilization for milk production were calculated as follows:

Digesta passage rate constant; $k\ (h^{-1}) = 0.693/\ t_{1/2}$

Mean retention time (h) = 1 / k

Faecal output (kgDM/d) = M_{ingested} (g/d) / $M_{\text{conc in faeces}}$ (g/kgDM)

Digesta flow rate (kg/d) = M_{dosed} (kg/d) / $M_{conc.\ in\ digesta}$ (kg/kg of digesta)

Dry matter digestibility (coefficient) = $1 - [(M_{in\ feed} / M_{in\ faeces})]$

N digestibility (coefficient) = $1 - [(M_{in\ feed}/M_{in\ faeces}) \times (N_{in\ faeces}/N_{in\ feed})]$

Where; M = Marker, N= Nutrients, $M_{conc.}$ = marker concentration, $t_{1/2}$ = half time of Cr_2O_3 in the whole digestive tract.

Efficiency of feed utilization = FCM / DMI

Where; FCM = fat corrected milk at 40g/kg (kg/cow/day), DMI = dry matter intake (kg/day)

A temperature-humidity index (THI) was calculated from the average ambient temperature of dry and wet bulb temperatures according to McDowell (1972), as follow:

THI = 0.72 (wb+db) + 40.6

Where; wb = wet bulb temperature and db = dry bulb temperature expressed in °C

Statistical analysis

Data for BW, DMI and milk yield in each lactating period were adjusted for covariate effects using the mean value in the 14 d before onset of the experimental period (46-59 DIM). The statistic analyses were performed using General Linear Model procedures of statistical software package SPSS (SPSS for windows, V14.0; SPSS Inc., Chicago, IL, USA). The model used for each analysis was:

$$Y_{ijk} = \mu + A_l + H_i + A(H)_{il} + B_j + (HB)_{ij} + A(HB)_{ijl} + Cov_k + e_{ijkl}$$

Where Y_{ijk} = observation, μ = overall mean, A_l= Animal effect H_i= house effect as main plot (i = NS, MF), $A(H)_{il}$ = main plot error (animal l in house i), B_j = treatment effect (rbST) as a split plot (j = with and without rbST supplementation), $(HB)_{ij}$ = interaction

effect between treatment and house , $A(HB)_{ijl}$ = split plot error (animal l in house i and treatment j), Cov_k = covariate effect and e_{ijk} = residual error.

The environmental and physiological parameters, digesta passage rate and all nutrients digestibility were also analyzed by a similar model, but the covariate effect was not included. Means values were used to evaluate the effect for all variables. Statistical significance was assessed as $P<0.05$ and trends were declared at $0.05 < P \leq 0.10$. Duncan's new multiple range tests were used to detect the statistical significance different among treatment groups.

RESULTS

AT, RH, THI, RR and RT

Data for ambient temperatures (AT), relative humidity (RH), temperature humidity index, rectal temperature and respiratory rate are shown in Table 2. Mean values of measurements at experimental site for AT, RH and THI were highly significantly different ($P<0.01$) between NS and MF barn. The AT and THI in the NS barn were significantly higher than the MF barn but the RH of MF barn was significantly higher than the NS barn ($P<0.01$) at all stages of lactation. The respiration rate and rectal temperature of cows under mister and fans were lower than non-cooled cows irrespective of rbST supplementation at all stages of lactation. The rbST-supplemented cows were significantly higher in RR and RT than those of cows without rbST under both NS and MF barns throughout the experimental periods.

Body weight, dry matter intake, milk yield and efficiency of feed utilization

Data of body weight, feed intake and milk yields are shown in Table 3. The mean values of BW for cooled and non cooled cows were not significantly different at all stages of lactation. Supplementation of rbST increased BW of cows in MF barn during early and mid lactation but not for late lactation. DMI (kg/d) and DMI (kg/100 kg BW) of cooled cows without rbST supplementation were on average 13% higher than those for non cooled cows at all stages of lactation. The rbST-supplemented cows showed a significant higher DMI than non rbST-supplemented cows kept either in MF or NS barn.

Milk yield of cooled cows were higher than those of non cooled cows on average by 20.5% but these results were not statistically different at any stage of lactation. Milk yield

and 40g/kg FCM of rbST-supplemented cows under MF cooling were significantly higher than those of other groups at all stages of lactation. Efficiency of feed utilization for milk synthesis showed no significant differences between cooled and non cooled cows. Supplemented rbST cows significantly increased the efficiency of feed utilization in early and late lactation but not for mid lactation. However, the interaction effect between mist-fan cooling and rbST supplementation was significantly apparent ($P<0.05$) for an increment in efficiency of feed utilization during mid lactation.

Digestion kinetics and digestibility

Mean values of flow rate, fecal output, half time of Cr_2O_3, passage rate and mean retention time of digesta are shown in Table 4. The digesta flow rate of cooled cows were not different ($P>0.05$) from non cooled cows at any stage of lactation. Faecal output of cooled cows tended to increase ($P=0.09$) during early lactation and marked increases were apparent in mid and late lactation as compared with non-cooled cows. The digesta flow rate and fecal output of rbST-supplemented cows were not different when compared with those of non rbST-supplemented cows throughout the experimental period. Under mist-fan cooling, the half time of Cr_2O_3 in the whole digestive tract for the cooled cows was lower than for those of non-cooled cows without rbST supplementation ($P<0.05$) during early and mid lactation but not for late lactation. The half time of Cr_2O_3 significantly decreased during rbST supplementation in cows under both NS and MF barns at all stages of lactation. The digesta passage rate constant (the fraction of total digesta moving per unit of time) in cooled cows was significantly higher than in non cooled cows during early and mid lactation. The magnitude of responses to the effects of rbST supplementation from pretreatment values for digesta passage rate constant was larger in animals with rbST than those of animals without rbST. The rapid digesta passage rate constant and short mean retention time of digesta were significantly apparent in rbST-supplemented cows under either NS or MF barns in all stages of lactation.

Digestibility data are shown in Table 5. Digestibility of DM, OM, NDF and ADF of cooled cows were no significantly different from those of non-cooled cow, with or without supplementation of rbST in all stages of lactation. CP digestibility during early and late lactation of cooled cows was not significantly different from those of non-cooled cows,

except in mid lactation. No effect of rbST-supplementation on CP digestibility at all stages of lactation.

Table 1 Feed ingredients and chemical compositions of the diet

Ingredients	g/kg [a]
Pineapple waste	500
Soybean meal	230
Rice bran	30
Cotton seed	200
Limestone	14
Di-calcium phosphate	14
Sodium bicarbonate	3
Potassium chloride	1
Mineral and vitamin premix	8

Chemical composition	
Dry matter (g/kg)	391
Ash (g/kgDM)	129
Organic matter (g/kgDM)	872
Crude protein (g/kgDM)	19.17
Acid detergent fibre g/kgDM)	195
Neutral detergent fibre (g/kg DM)	328
Total digestible nutrients (g/kgDM)	700
Metabolizable energy(MJ/kg DM)	11.20

[a] as fed basis

Table 2 The mean values of ambient temperature, relative humidity, temperature humidity index (THI) measured at 13.00 h under mist-fan cooling (MF) and normal shade (NS) barns and effects of mist-fan cooling (MF) and supplemental rbST on respiratory rate and rectal temperature of crossbred cows

	NS		MF			Effect[1]		
	Pre	rbST	Pre	rbST	SEM	MF	rbST	MF x rbST
Early lactation								
Ambient temperature (°C)	34 [b]	35 [b]	33 [a]	32 [a]	0.6	P<0.01	ns	ns
Relative humidity (%)	52 [a]	53 [a]	71 [b]	70 [b]	3.2	P<0.01	ns	ns
THI	83 [ab]	85 [b]	83 [a]	83 [a]	0.8	P<0.05	ns	ns
Respiratory rate (breath/min)	71 [bc]	80 [c]	53 [a]	64 [ab]	0.3	P<0.05	P<0.05	ns
Rectal temperature (°C)	39 [bc]	40 [c]	39 [a]	39 [ab]	0.2	P<0.01	P<0.05	ns
Mid lactation								
Ambient temperature (°C)	35 [b]	35 [b]	30 [a]	30 [a]	0.5	P<0.01	ns	ns
Relative humidity (%)	53 [a]	50 [a]	78 [b]	74 [b]	3.1	P<0.01	ns	ns
THI	85 [b]	85 [b]	82 [a]	81 [a]	0.4	P<0.05	ns	ns
Respiratory rate (breath/min)	72 [c]	76 [c]	52 [a]	61 [b]	2.7	P<0.01	P<0.05	ns
Rectal temperature (°C)	40 [bc]	401 [c]	39 [a]	39 [b]	0.1	P<0.01	P<0.01	ns
Late lactation								
Ambient temperature (°C)	31 [b]	34 [b]	30 [a]	29 [a]	0.5	P<0.01	ns	ns
Relative humidity (%)	60 [a]	64 [a]	79 [b]	78 [b]	1.8	P<0.01	ns	ns
THI	84 [b]	85 [b]	81 [a]	81 [a]	0.6	P<0.01	ns	ns
Respiratory rate (breath/min)	69 [b]	80 [b]	52 [a]	57 [a]	1.3	P<0.01	P<0.01	ns
Rectal temperature (°C)	39 [bc]	40 [c]	38 [a]	39 [ab]	0.1	P<0.05	P<0.01	ns

Pre = pre-rbST supplementation, rbST = rbST supplementation

[1] Effect; MF =Mist-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

The statistical significance of main and interaction effects are shown as P-value, ns = not significance (P> 0.05)

[a,b,c] Means within the same row with different superscripts differ significantly (P<0.05)

Table 3 Effects of mist-fan cooling (MF) and supplemental rbST on body weight, dry matter intake, milk yield and efficiency of feed utilization of crossbred Holstein cows

	NS		MF			Effect[1]		
	Pre	rbST	Pre	rbST	SEM	MF	rbST	MF x rbST
Early lactation								
Body weight (kg)	373	381	374	386	1.9	ns	P<0.05	ns
Dry matter intake (kg/d)	9.1	10.1	10.9	11.9	0.17	ns	P<0.05	ns
Dry matter intake (kg/100 kg BW)	2.4	2.6	2.9	3.1	0.05	ns	P<0.05	ns
Milk yield (kg/day)	10.9	12.6	13.6	14.4	0.17	ns	P<0.05	*
4% FCM (kg/d	10.3[a]	13.5[ab]	14.1 [ab]	16.2 [b]	0.58	ns	P<0.05	ns
Efficiency of feed utilization (kg/kg)	1.12	1.32	1.32	1.39	0.036	ns	P<0.05	ns
Mid lactation								
Body weight (kg)	397	397	383	408	4.6	ns	P<0.05	P<0.05
Dry matter intake (kg/d)	8.3	9.2	9.1	9.8	0.16	ns	P<0.05	ns
Dry matter intake (kg/100 kg BW)	2.1	2.3	2.4	2.5	0.06	ns	P<0.05	ns
Milk yield (kg/day)	10.4	11.3	11.4	12.9	0.33	ns	P<0.05	ns
4% FCM (kg/d	10.9	11.9	10.7	12.8	0.25	ns	P<0.05	ns
Efficiency of feed utilization (kg/kg)	1.35	1.32	1.18	1.31	0.030	ns	ns	P<0.05
Late lactation								
Body weight (kg)	396	395	426	423	4.4	ns	ns	ns
Dry matter intake (kg/d)	7.6	7.9	8.4	9.3	0.14	ns	P<0.01	ns
Dry matter intake (kg/100 kg BW)	1.9	2.0	2.0	2.2	0.03	ns	P<0.01	P<0.05
Milk yield (kg/day)	8.2	9.2	10.5	12.2	0.44	ns	P<0.05	ns
4% FCM (kg/d	8.4[a]	9.9[ab]	10.2 [ab]	13.4 [b]	0.85	ns	P<0.05	ns
Efficiency of feed utilization (kg/kg)	1.11	1.26	1.22	1.41	0.058	ns	P<0.05	ns

Pre = pre-rbST supplementation, rbST = rbST supplementation

[1] Effect; MF =Mist-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

The statistical significance of main and interaction effects are shown as P-value, ns = not significance (P> 0.05)

[a,b,c] Means within the same row with different superscripts differ significantly (P<0.05)

Table 4. Effects of mist-fan cooling (MF) and supplemental rbST on digestion kinetics of crossbred Holstein cows

	NS		MF			Effect[1]		
	Pre	rbST	Pre	rbST	SEM	MF	rbST	MF x rbST
Early lactation								
Digesta flow rate (Kg/day)	17.6	16.6	19.8	19.9	0.60	ns	ns	ns
Fecal output (KgDM/day)	3.4	3.0	3.7	3.4	0.17	P=0.093	ns	ns
Half time of Cr_2O_3 (h)	25.8^{c}	24.8^{b}	20.0^{ab}	17.8^{a}	0.71	P<0.05	P<0.05	ns
Digesta passage rate constant (h^{-1})	0.028	0.029	0.036	0.040	0.0008	P<0.05	P<0.01	ns
Mean retention time (h)	37.2	35.8	29.0	25.6	0.96	P<0.05	P<0.05	ns
Mid lactation								
Digesta flow rate (Kg/day)	17.0	17.9	19.9	19.0	0.12	ns	ns	ns
Fecal output (KgDM/day)	3.3	3.3	3.8	3.9	0.15	P<0.05	ns	ns
Half time of Cr_2O_3 (h)	27.0^{c}	24.6^{b}	20.8^{ab}	19.6^{a}	0.42	P<0.05	P<0.01	ns
Digesta passage rate constant (h^{-1})	0.026	0.029	0.034	0.036	0.0007	P<0.05	P<0.01	ns
Mean retention time (h)	38.8	35.4	30.2	28.2	0.70	P<0.05	P<0.01	ns
Late lactation								
Digesta flow rate (Kg/day)	19.1	17.3	15.7	15.1	1.07	ns	ns	ns
Fecal output (KgDM/day)	3.5	3.2	4.1	4.1	0.15	P<0.01	ns	ns
Half time of Cr_2O_3 (h)	24.6^{c}	23.0^{b}	22.4^{b}	21.0^{a}	0.05	ns	P<0.05	ns
Digesta passage rate constant (h^{-1})	0.029	0.031	0.033	0.034	0.0007	ns	P<0.05	ns
Mean retention time (h)	35.4	33.0	32.4	30.4	0.70	ns	P<0.05	ns

Pre = pre-rbST supplementation, rbST = rbST supplementation

[1] Effect; MF =Mist-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

The statistical significance of main and interaction effects are shown as P-value, ns = not significance (P> 0.05)

[a,b,c] Means within the same row with different superscripts differ significantly (P<0.05)

Table 5 Effects of mist-fan cooling (MF) and supplemental rbST on digestibility coefficient in crossbred Holstein cows

	NS		MF			Effect[1]		
	Pre	rbST	Pre	rbST	SEM	MF	rbST	MF x rbST
Early lactation								
Total DM digestibility	0.722	0.715	0.744	0.732	0.027	ns	ns	ns
Diet digestibility								
OM	0.723	0.718	0.750	0.743	0.029	ns	ns	ns
CP	0.779	0.772	0.817	0.799	0.029	ns	ns	ns
NDF	0.491	0.465	0.487	0.465	0.057	ns	ns	ns
ADF	0.414	0.392	0.387	0.413	0.055	ns	ns	ns
Mid lactation								
Total DM digestibility	0.647	0.676	0.720	0.722	0.027	ns	ns	ns
Diet digestibility								
OM	0.659	0.684	0.721	0.725	0.029	ns	ns	ns
CP	0.715	0.752	0.812	0.817	0.021	P<0.01	ns	ns
NDF	0.350	0.367	0.402	0.439	0.070	ns	ns	ns
ADF	0.318	0.284	0.360	0.343	0.050	ns	ns	ns
Late lactation								
Total DM digestibility	0.691	0.710	0.718	0.739	0.017	ns	ns	ns
Diet digestibility								
OM	0.697	0.717	0.723	0.742	0.017	ns	ns	ns
CP	0.758	0.772	0.780	0.817	0.012	ns	ns	ns
NDF	0.447	0.445	0.442	0.472	0.043	ns	ns	ns
ADF	0.283	0.331	0.345	0.377	0.010	ns	ns	ns

Pre = pre-rbST supplementation, rbST = rbST supplementation

[1] Effect; MF =Mist-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

The statistical significance of main and interaction effects are shown as P-value, ns = not significance (P> 0.05)

DISCUSSION

In the present study, THI (based upon ambient temperature and humidity) were always higher than 72 in the barn for animals of both groups, this value is considered the upper critical THI for lactating dairy cows (Kadzere *et al.* 2002; Smith *et al.* 2006). Animals were therefore always subjected to moderate heat stress throughout experimental periods (i.e. THI = 80.7 to 85.5). Thus the effect of misters and fans for cooling animals in the present study was not sufficient to completely eliminate heat stress. The values of THI might not accurately reflect heat stress when using a mister and fan system for evaporative cooling that result in higher humidity but also causes cooling. Although the mist-fan cooling was not sufficient to adequately reduce THI in the barn, there is a beneficial effect as indicated by a lower RR and RT and also higher milk yield throughout lactation. These results are consistent with the study of Fike *et al.* (2002) that housing cows during the day with fans and sprinklers effectively reduced heat stress as indicated by lower body temperature and respiration rate. In the present study, an increase in milk yield of rbST-supplemented cows was accompanied with an increase in both RT and RR in comparison with cows without rbST supplementation in both NS and MF barns throughout the experimental periods. It indicates that cows increase heat production during rbST supplementation. This observation may support the report of West *et al.* (1991) and West (1994) that rbST-treated cows in a hot environment increased heat production in either high milk producing cows or low milk producing cows.

The used of misters and fans for cooling cows in the present study does partly alleviate the effect of heat stress. Both DMI and milk yield of cooled cows without rbST supplementation were higher than those of non-cooled cows. These results are in agreement with the report of Chen *et al.* (1993) that an increase in milk production was about 9% with evaporative cooling over shade alone. Several studies have reported that lactating cows exposed to high environmental temperatures either with (Settivari *et al.* 2007) or without rbST treatment (Bernabucci *et al.* 1999; Hirayama *et al.*, 2004), showed a reduction in feed intake and milk yields. However, the present results indicate that rbST is effective during hot weather at all stages of lactation, since non-cooled cows with rbST supplementation consumed additional feed DM for the higher milk production compared to cows in normal shade without rbST. These results agree with earlier findings (Santos *et al.* 1999; Tarazon *et al.* 1999; Gulay and Hatipoglu 2005) that the bST-treated cows

improved efficiency of utilization of food for milk production. Another reason for enhancement in DMI during rbST supplementation in non-cooled cows may be that cows were able to regulate their body temperatures within normal range. Although rbST increases heat production it also increases heat dissipation (Johnson *et al.* 1991). Moreover cows supplemented with rbST increase total body water as shown previously (Chaiyabutr *et al.* 2007). Increased total body water would be useful in slowing down the elevation in body temperature in hot conditions through evaporative cooling during heat dissipation. These changes would partly affect the DMI.

An increase in diet digestibility concomitant with the reduction of both gastrointestinal motility and digesta passage rate in cattle exposed to hot condition have often been reported in the literature (Christopherson and Kennedy 1983; Mathers *et al.* 1989). In the present study, non-cooled cows without rbST supplementation and exposure to moderate heat stress (THI>80<86) showed low values of both digesta passage rate and DMI level, while diets digestibility did not significantly change in comparison with cooled cows over the entire lactation period. Whole-tract apparent digestibility of DM including NDF and ADF did not follow the trends expected based upon digesta passage rate and digesta loads. The lack of heat stress effect on increase in digestibility is probably due to time-dependent fashion of heat exposure, which cows have been subjected to the prolonged period of exposure to high temperatures. These results are consistent with findings of Bernabucci et al. (1999), which observed an increase in digestibility coinciding with decrease in passage rate of digesta of animal during the short term of exposure to exceed THI, but not in long term adaptation. During prolonged heat exposure, depressed rumen cellulolytic activity (Miaron and Christopherson 1992) including the reduction of blood flow to the rumen epithelium (Hales et al. 1984) may occur in non-cooled cows, which these factors could not contribute to greater total digestibility. On the contrary, cooled cows alone showed a shorter retention time of digesta without alteration in diets digestibility. The study of Grimaud and Doreau (2003) indicated that a shorter retention time of digesta would reduce the attraction between digesta particles and ruminal microorganisms, resulting in decreased the diet digestibility. However, in the present study, the significant differences in the passage rate of digesta, but non-significant differences in diet digestibility between non-cooled and cooled cows without rbST, would not depend upon the different types or nature of feeds offered (Deinum *et al.* 1968), since

crossbred cows in both groups were fed with a similar TMR diet throughout lactation. The findings for non-significant differences in diet digestibility between non-cooled and cooled cows probably are mediated through differences in rumen cellulolytic activity in cows under different environmental conditions. The less effect on depressed rumen cellulolytic activity may appear in cows during exposure to mist-fan cooling and, hence, would not decrease the total diets digestibility. These findings suggest that an adaptive response in different manners occurred to compensate for normal digestive tract function in crossbred cows either exposure to high temperatures or exposure to mist-fan cooling.

The rate of passage of digesta for cows supplemented with rbST was higher than for cows without rbST supplementation either in NS or MF barn. This suggests that exogenous rbST could overcome the some effects of high temperatures. However, total dry matter digestibility of cows in our studies was not affected by rbST supplementation, which was in agreement to other studies in cows using exogenous bST (Peel *et al.* 1981; Sechen *et al.* 1989). A faster rate of passage of digesta is usually given as the reason for the decrease in digestibility (Bernabucci *et al.* 1999). The inconsistency of these findings in the present study may be partly explained by changes of GI-tract volume and the level of DMI. Increasing relative mass of many organs and tissue including GI-tract within animal has been reported during exogenous bovine somatotropin (Moallem *et al.* 2004). Such an increased capacity of the GI-tract mass may partly compensate for the effect of the higher DMI level on the higher passage rate of digesta in rbST supplemented cows. Higher digesta passage rates at the higher DMI levels would be the result of larger amounts of digesta leaving the reticulorumen during rbST supplementation. A change in the process of the DM digestibility would depend on not only the digesta passage rate but also on both microbial activity and time of contact between microorganisms and particles (Michalet-Doreau, and Doreau 2001). Because of the shorter mean retention time of digesta, microbial digestion in the reticulorumen would be reduced, whereas an increase in cellulolytic bacteria in the rumen occurred during bST supplementation (Winsryg *et al.* 1991); this might be partly compensated by a shift of higher microbial activity in the upper half of the GI tract to the hindgut. Thus, the adaptive of fermentation pattern would account for non-significant differences in total digestibility between cows with and without rbST supplementation. However, unchanged total digestibility of dry matter gives evidence that more complex processs of regulation exist in digestive system during rbST

supplementation. In the present study, rbST-supplemented cows showed high value efficiency of feed utilization. It has been reported by Bauman (1992) that an increase in efficiency of feed utilization for milk production in rbST-supplemented cows is associated with an increase the partition of nutrient to mammary gland. From the present results, it would indicate that the higher milk production from the effect of rbST on efficiency of feed utilization would depend on both an increase in partition of nutrients and post-absorptive use of nutrients for milk synthesis (Sechen et al. 1989).

It is clear that the application of mist-fan cooling to cows slightly increased feed intake and milk yields, whereas the effects of rbST supplementation under either with or without misters and fans showed more marked increases in both feed intake and efficiency of feed utilization. The differences in the magnitude of responses of digestive functions between the effects of mist-fan cooling and rbST supplementation indicate that the main effects of mist-fans cooling could reduce a negative effect of hot environment on digestive function via increase in digesta passage rate, resulting increase in feed intake. The response of milk production in crossbred cows to rbST supplementation would be enhanced accompanying with the use of misty-fan cooling. The effect of rbST supplementation did not directly act on digestive function, it would exert galactopoietic effect through partly increased in both post-absorptive use and partition of nutrients for increased efficiency of feed utilization in providing nutrients to the mammary gland for milk synthesis.

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CHAPTER XII

GENERAL DISCUSSION

The results of the present experiments have shown that an application of misty-fans cooling (MFC) to crossbred lactating cows under high environmental temperatures could reduce the effect of high heat load in animals and improve milk production. The MFC system could significantly reduce ambient temperature (AT) and temperature humidity index (THI) during experimental period. The patterns of changes in AT and THI were similar in all series of experiments (Chapter III to XI). However, the MFC system that delivered mist to the ambient environment would increase in the percentage of relative humidity (RH) as compared with NS barn. The THI measured in barn under the misty fan cooling were higher than that of the level of THI in NS barn. The present results indicate that animals housed in both MF and NS barn would subject to moderate heat stress, which the THI level was higher than the threshold level of 72 (Armstrong, 1994). The effect of misters and fans would be beneficial to alleviate the effect of heat stress in crossbred lactating cows under hot condition, although crossbred dairy cattle using in the present experiment containing *B. indicus* gene, could have a high heat tolerance than exotic *B. Taurus* cattle (Nakamura et al., 1993; Pereira et al., 2008). The low values of both respiration rate (RR) and rectal temperatures (RT) coinciding with high level of DMI and milk yield in cooled cows were apparent. However, cows supplemented with rbST would increase in RR and RT. These findings agree with previous reports in *Bos Taurus* cows treated with rbST (Sullivan et al., 1992; Tarazon et al., 1999; Settivari et al., 2007). Although rbST-supplemented cows showed high RR and RT, but they also showed an increase in DMI and milk yield when compared with pre-supplemented period (Fig.1).

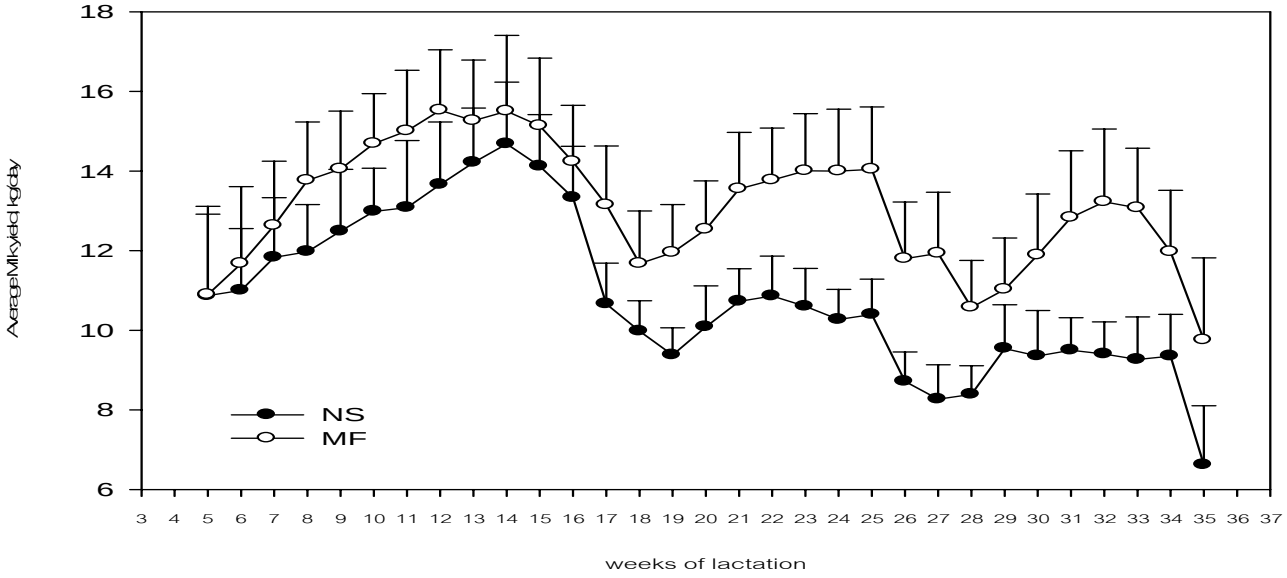


Figure 1. Milk yield response to rbST supplementation in crossbred lactating cows housing in normal shade (NS) and shade plus misty-fans cooling system (MFC) during early, mid and late lactation

A high response of milk yield occurred during rbST supplementation in cooled cows in comparison to non-cooled cows suggesting an efficiency of feed utilization for milk production under MFC system (Fig. 8.1). In the present study, an increase in milk yield in response to rbST was returned to the control level within 30 days after rbST administration in both cooled and noncooled cows. These findings are agreed to the report of Kirchgessner et al., (1991) that during the first week after injection of bovine somatotropin, milk yield increased sharply and almost returned to the control level within the next 3 weeks.

The results in Chapter IV showed the marked increases in blood flow to the mammary gland coincided with an increase in milk yield during rbST supplementation in both cooled and non-cooled cows. These results agree to previous studies by Chaiyabutr and co-worker (2007a) that long-term administrations of rbST showed a marked increase in mammary blood flow throughout lactation. Factors that might affect to increase MBF during supplemental rbST could include an increasing relative mass of many organs and tissue including mammary tissue (Moallem *et al.* 2004) and an

increase in cardiac output (Soderholm et al., 1988) in bST treated cows. However, an increase in MBF does not involve the direct action of somatotropin on the mammary gland (Collier et al., 1984). It would accompany with an increase in circulating levels of IGF-I, which has been shown in either short-term or long-term rbST administration in different stages of lactation in crossbred HF animals (Chaiyabutr et al., 2005; Maksiri et al., 2005;Tanwattana et.al., 2003) including in goat (Davis et al. 1988; Hart et al. 1980). It indicates that rbST plays a role for an increase in MBF requiring IGF-I as a mediator (Forsyth, 1996). However, the lack of effect of higher plasma IGF-I levels on persistency of lactation in rbST treated animals was also noted. (Chaiyabutr et al., 2005).

The supplementation of rbST markedly increased both the absolute values of PV BV, ECF, TOH and EBW in both cooled and non-cooled cows. The water turnover rate was slightly higher coinciding with higher milk yield in both groups of cows supplemental rbST throughout experiment. These findings would relate to the fact that lactation requires more water and more loss of water due to secretion in milk, which is generally known to be about 87% and would account for these phenomena. An increase in ECF leads to an increase in MBF as secondary responses, thereby the increase in MBF drives nutrients supply per se to the mammary gland and increase in milk production in rbST treated cows. The expansion of body fluid in rbST-treated animals 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. However, during lactation advanced to late lactation in both cooled and non-cooled cows, the decline in milk yields were still apparent, although MBF, ECF TOH, were still in high levels during supplemental rbST. These results indicate that an increase in milk yield of dairy crossbred cattle in response to rbST administration will not be sustained for long and is influenced by the stage of lactation. These data suggest that changes in milk production during the progress of lactation in rbST treated animals might not be controlled systematically but also locally within the mammary gland (Chaiyabutr et al., 2005).

It is known that a reduction in dry matter intake (DMI) is partly known as one of mechanism responses to heat stress with a consequence of the reduction of milk yield. The changes in digestive function durings exposure to high environmental temperatures are shown to be inconsistent results. Some reports showed a reduction in diet

digestibility by excess THI relating to a decline in ruminal activity through the depression of rumen cellulolytic activity (Bernabucci et al., 1999). Both slower passage rate and longer mean retention time of digesta have been shown in cows exposure to high ambient temperatures (Christopherson and Kenedy 1983; Silanikove 1992). An increase in diet digestibility has also reported in dairy cows exposure to hot environment (Collier et al., 1982; Mathers et al., 1989). During administration of bST to high yielding cows in extremely hot-humid environments would restrict feed DMI and increase heat stress(West 1991). In addition, Nasser et al. (2003) observed that the bST-treated cows had higher energy expenditure with an increase in plasma non-esterified fatty acid (NEFA) including rumen ammonium nitrogen and volatile fatty acid. However, The interaction effects between thermal stress and the role of exogenous rbST on the lactation performance in crossbred lactating cattle are not yet clear. The present studies of diet digestibility and digestion kinetics in Chapter XI (Series11.1) demonstrate that a mean value of DMI from both pretreatment and treatment with rbST in cooled cows was higher (14.0%) than those of non-cooled cows. These changes would accompany with the significant increase in the mean value of digesta passage rate (23.1%) of cooled cows as compared with those of non-cooled cows. These results indicate that the faster passage rate of digesta through the digestive tract would partially reduce gut fill which subsequently increased in diet consumption. The mean value of digesta passage rate of crossbred lactating cows housing in both NS and MF barns were increased after rbST supplementation when compared with the pretreatment period. The rbST-treated cows under MF barn could increase the digesta passage rate (23.9%) when compared with cows under NS barn. It indicates that the effect of MFC cooling would superimpose the effect of rbST supplementation on the digesta passage rate. The main effects of mist-fans cooling could reduce a negative effect of hot environment on changes in digestive function via an increase in digesta passage rate, resulting in an increase in feed intake. An increase in rumen fermentation products as VFA and NH_3N (Chapter XI, Series11.2) would be in part due to an increase in DMI response to both rbST supplementation and MF cooling system. An increase in water intake accompanying with an increase in feed intake was also found in rbST-supplemented cow under-misty fan cooling. It would be suggest that the rbST exerts its galactopoietic action, in part, through changes in gut water regulation associated with changes in body fluid, and secondary the blood flow to the mammary gland for milk synthesis and for thermoregulatory mechanism. The present study emphasizes the importance of the

changes in water retentions during rbST supplementation under heat stress, thereby making animals more thermotolerance to heat stress. The major changes occur in the digestive process would be the effects of exogenous of rbST accompanying with an application of misty-fan cooling system.

High temperature has been known to influence milk yield, plasma metabolites and endocrine status in maintenance requirement (Collier et al., 2005). Changes of some blood metabolites, i.e. glucose, acetate, FFA and β-hydroxybutyrate could be used as indicator for energy status in lactating cows (Ndlovu, et al., 2007). The results in Chapter IV show that plasma metabolites for glucose, β-hydroxybutyrate, acetate, and triglyceride of cows showed no statistical differences in both cooled and non-cooled cows with or without supplemental rbST. The plasma FFA concentration was markedly increased after rbST supplementation in both cooled and non-cooled cows. An increase in FFA during rbST administration would be the lipolytic activity of rbST per se in adipose tissue (Houseknecht et al., 2000). In the present study the plasma IGF-1 level was increased during rbST supplementation in each stage of lactation in both groups. The synthesis and release of IGF-1 is mainly by the liver (Granner, 1996). Exogenous of rbST in the present study was sufficient to achieve a satisfactory stimulation of IGF-1 (Bachman et al. 1992). In the present study, an increase in plasma IGF-1 level in cows supplemental rbST was not affected by a lower nutritional state (Hodgkinson, Bass & Gluckman, 1991) or a negative energy balance (Weller et al. 1994; Ketelsleger et al. 1995), since no differences in the nutritional status among treatments. During early and mid lactation, plasma insulin levels had tendency to increase during rbST supplementation in both cooled and non-cooled cows. This suggests that the availability of both somatotropin and insulin to the liver would attribute to increase in IGF-I secretion (Luo & Murphy, 1991). The relationship between somatotropin and insulin was not apparent for rbST treated cows in late lactation. However, maintaining the plasma concentration of glucose with high concentrations of insulin during rbST supplementation in both cooled and non-cooled cows may indicate the decrease in the responsiveness of peripheral tissues to high concentrations of insulin. This would spare glucose for insulin insensitive tissues, particularly the mammary gland. The higher of milk production would be a consequence of increase in heat production (Manalu et al., 1988). Thyroid hormones are known to be a calorigenic hormone. Increasing of heat production during supplementation of rbST may depress secretion of thyroid hormone

which may assist the animal to regulate body temperature in maintaining homeothermy (Johnson et al., 1991). This result would account for a reduction of the plasma thyroxine (T_4) concentration in early lactation during rbST supplementation in the present study. It has been reported in patients with GH deficiency that rhGH therapy appears to increase serum T_3 level and decrease in mean of free T_4 level, resulting an increased conversion of thyroxine (T_4) to triiodothyronine (T_3) in peripheral tissues (Losa et al., 2008). However, the effect of rbST on the T_4 levels is varied. During hot and humid weather, the levels of triiodothyronine and thyroxine were not affected by bST administration in Holstein and Jersey cows (West et al., 1991). Johnson et al. (1991) founded in cows treated with rbST that plasma triiodothyronine were significantly decreased while it had no effect on the plasma T_4 level. Cortisol is the major adrenal corticoid secreted in cattle which enable animals to tolerate stressful condition (Christison and Johnson, 1972). Plasma cortisol levels have been shown to reduce in prolonged heat exposure (Ingraham et al.,1979) including during rbST supplementation in both thermoneutral and hot condition (Johnson et al., 1991), and to increase in response to exposure to cool environment (Guerrini and Bertchinger, 1982). The present results showed no changes in plasma cortisol concentrations in both cooled or non-cooled cows with or without rbST. These findings are in agreement with studies reported by Peel et al., (1982, 1983) and West et al. (1991). The endogenous bST levels are naturally higher in early lactation and in high producing cows (Hart et al., 1980; Bonzek et al., 1988; Chaiyabutr et al., 1997). Therefore, rbST may influence in reduced adrenal ACTH responsiveness. It is suggested that this phenomenon is part of the coordinated metabolic adaptations required to support the increases in milk production (Adriaens et al., 1995).

The results in Chapter VIII and Chapter X showed no physiological responsiveness for blood constituents in both cooled and non-cooled cows supplemental rbST. In the present study, selected biochemical, haematological and oxidative stress parameters remained within the published values and did not differ statistically between cooled and non-cooled cows before and after the supplementation of rbST. The results of the present study suggest that rbST is efficacious in increasing milk yield without adverse effects on crossbred cattle. However, among biochemical parameters, the marked decrease in BUN of either cooled or non-cooled cows supplemented with rbST were apparent (Chapter X). The reduction of the plasma urea concentration might not reflect the dry matter crude protein intake which showed higher in cows supplemental rbST. The decrease in the

plasma urea concentration during supplementation of rbST may be due to the effect of the hormone bST via N- actyl glutamate, on carbamyl phophate synthetase, a key enzyme in the urea cycle (Oddy and Linsay,1986). The study in Chapter VIII for the oxidative stress markers in plasma for TBARS, a breakdown product of lipid peroxidation (Nielsen *et al*. 1997) and SH residue, an important marker in reducing equivalent in body fluid were not different between cooled and non-cooled cows before and after the supplementation of rbST. These two oxidative stress markers showed different physiological responsiveness for the concentration of ascorbic acid in the plasma. These results suggest that the oxidation of the SH residue and breakdown product of lipid peroxidation by oxidative stress did not utilize the same mechanism as that of ascorbic acid oxidation. Ascorbic acid in the plasma seems to be sensitive to high environmental temperature stress.

The kidneys are known to be an important organ in regulating both volume and composition of body fluids in maintaining the plasma osmolality through the regulation of water excretion (Lunn and McGuirk, 1990). The regulation of fluid volume and body composition depends on the coordinated action of multiple mechanisms of various hormones in regulating water intake and excretion. The effects of rbST supplementation and mist-fan cooling system on renal functions are shown in Chapter IX (Series 9.1 and 9.2). The renal functions including renal hemodynamic and renal tubular function were not significantly different between cooled and non-cooled cows without rbST. The action of rbST had not significant effects on renal hemodynamic (GFR, ERPF, ERBF and FF), despite a higher level of body fluids (Chapter IV) in cooled and non-cooled cow treated with rbST in each stage of lactation. The present findings in Chapter IX Series 9.1 and 9.2 indicate that rbST supplementation in either cooled or non-cooled cows, causes changes both extrarenal and intra-renal factors mainly in the renal tubular function. It has been reported that several hormones play a role in the increase reabsorption of water and electrolytes in renal tubule, which may lead to body fluids expansion such as GH, IGF-I, rennin angiotensin aldosterone system (RAAS) and cortisol. In the present study, the remained constant of the plasma cortisol concentrations during supplemental rbST in both cooled and non-cooled cows (Chapter V) might not expect to involve body volume expansion, since the high plasma cortisol has been reported to exert water and sodium-retaining activity which was associated with a significant decrease in urinary sodium excretion (Fan et al., 1975; Guerrini and Bertchinger, 1982). However, plasma IGF-I and aldosterone levels (Chapter IX ; Series

9.2) were markedly increased during rbST supplementation. These findings indicate that the changes in reabsorption of water and electrolytes during rbST administration lead to body fluids expansion may mediate via the action of IGF-I or aldosterone, the similar results have been reported in both human and experimental rat. Renal tubular handling of sodium was studied by using the lithium clearance (C_{Li}) technique. It has established that tubular reabsorption of Li is proportion to the reabsorption of Na^+ and H_2O at proximal tubule (Thomsen et al., 1984; 1990). The present results showed increases in the proximal absolute values (PAR_{Na}) and proximal fractional reabsorption of sodium ion (PFR_{Na}). This finding indicated that increased renal tubular sodium reabsorption in response to rbST seems to occur in the proximal tubule. An increase in sodium reabsorption in proximal tubule is likely to involve a direct action of IGF-1 (Guler et al. 1989). IGF-I enhance proximal tubular reabsorption of sodium and stimulated the renin-angiotensin (RAS) system (Marsh et al., 2001). Moreover, growth hormone treatment also activates an increase in the plasma aldosterone concentration coinciding with increases in IGF-I and renin-angiotensis-aldosterone system (Hanukoglu et al., 2001). Thus, it is possible that an increase in sodium reabsorption in proximal tubule induced by rbST supplementation should be the result from the stimulation of IGF-I and AII. It is known that the proximal tubule is a site of action of angiotensin II (AII) and IGF-I on sodium ion reabsorption (Harris and Navar, 1985; Guler et al. 1989). In present study, the mechanisms of increase Na reabsorption in proximal tubule may be a stimulation of angiotensin II (AII) because higher of plasma aldosterone levels by rbST supplementation would be attributable to the action of the renin-angiotensin system (RAS). The primary effect may be increased plasma rennin activity, which in turn will increase angiotensin II.

It has been noted that the increase in both distal absolute (DAR_{Na}) and fractional reabsorption of sodium (DFR_{Na}), were observed in human treated with GH (Hansen et al., 2001). It is interesting that these findings were not similar to the present studies in crossbred cows which DAR_{Na} had tendency to decrease by rbST supplementation. It is possible that an increase in sodium reabsorption in proximal tubule during rbST supplementation may be lead to decreased proximal tubular fluid output, resulting in the decrease in distal absolute reabsorption. However, the distal fractional reabsportion were slightly increased in cooled and non-cooled cows after rbST supplementation. This finding may be due to a reduction in the delivery of sodium via the tubular fluid to macula densa cells may stimulate rennin release which will stimulate angiotensin and

aldosterone production (Guyton, 1991). Thus, an increase in plasma aldosterone in the present study (Chapter IX ; Series 9.2) seems to be a cause of the increase in distal fractional reabsorption of sodium (DFR_{Na}) in rbST-treated cows. In addition, there were no significant changes in DAR_{H2O}, and DFR_{H2O} of cows which coincided with any significant changes in the plasma vasopressin concentration and free water clearance (C_{H2O}) in cooled and non-cooled cows treated with rbST in all stages of lactation. It indicates that the mechanism on water reabsorption in this study might not occur in distal tubule and collecting duct and independent on stimulation of vasopressin. In addition, an increase in the renal tubular reabsorption of electrolytes (Na^+, K^+, Cl^-) responses to rbST supplementation were accompanied by increased water intake, resulted in increased the number of electrolytes and water in body fluids. However, plasma electrolytes concentration and plasma osmolarity did not differ during rbST supplementation, which would reflect a part of the effects of enlarged body fluid volume arising from its colligative properties with exerting osmotic forces for retaining body water. These results suggest that the effect of rbST supplementation to cows housed either in NS or MF barns on body fluid volume expansion is attributable to changes in the rate of electrolyte excretion by the kidney. The increased availability of renal tubular reabsorption of sodium, potassium and chloride ions during rbST treatment was a major factor in retaining body water through its colligative properties in exerting formation of an osmotic force mechanism.

The remainder of the discussion is concerned with changes in intra- mammary factors (Chapter V, VI, and VII). The study of intra-mammary function for the effects of cooling and recombinant bovine somatotropin (rbST) administration on the utilization of glucose in the mammary gland in different stages of lactation in crossbred Holstein cattle is mentioned (Chapter V, Series 5.1). It is known that milk production requires glucose for synthesis of lactose which is essential for milk secretion and glucose moiety of lactose arises directly from plasma glucose (Ebner & Schanbacher 1974). An increase in milk yield without an alteration of the plasma glucose concentration during supplemental rbST in both cooled and non-cooled cows indicates that this requires a substantial increase in supply of glucose to the mammary gland. An increase in mammary blood flow is a factor for glucose uptake by the mammary gland (Linzell 1973), which the rate of mammary plasma flow of cows supplemental rbST significantly increased as compared with the pretreatment period. However, in the

present study, an increase in mammary plasma flow during rbST supplementation in each stage of lactation would not be a major determinant in the mediation of nutrient delivery and uptake by the mammary glands for increase in milk production throughout lactation. Local changes for biosynthetic capacity within the mammary gland would be a factor in identification of the utilization of substrates in the rate of decline in milk yield with advancing lactation (Chaiyabutr *et al*. 2005).

Effects of supplemental rbST and cooling on glucose kinetics and plasma glucose concentrations in both cooled and non-cooled cows maintained over a wide range at different stages of lactation. It indicates that steady state conditions between the rate of gluconeogenesis and the rate of utilization of glucose existed in the body pool of glucose in both groups (Chapter V, Series 5.1). Cows using in the present study were primiparous with low yielding cows. These may reflect the differences of utilization for lactose synthesis between high yielding and low yielding cows.

The reversible turnover rate of [3-^{3}H]glucose (the total glucose entry rate) and the irreversible turnover rate of [U-^{14}C] glucose (the utilization rate of glucose) of cooled cows without rbST were slightly higher than those of non-cooled cows in all stages of lactation. It is probably that the turnover rate of glucose correlated positively with a higher milk yield in cool cows. However, administration of rbST showed non-significant changes in both glucose entry and utilization rates in comparison with those of pretreatment periods in both cooled and non-cooled cows throughout lactation. During studies, both cooled and non-cooled cows with or without supplemental rbST were fed TMR diet to satisfy requirements for metabolizable energy and the body weights increased stepwise throughout lactating periods. It is possible that both cooled and non-cooled cows were in positive energy balance, since irreversible losses of glucose has been shown to increase in cows with negative energy balance (McDowell et al.,1987). The reversible turnover rate of [3-^{3}H]glucose represents the total glucose turnover rate as the ^{3}H is not recycled from products of partial glucose degradation (Katz *et al*.1965). Thus, recycling of glucose-C was estimated by simultaneous injection of [3-^{3}H]glucose and [U-^{14}C]glucose as in the present studies in cooled and non-cooled cows, which showed no differences between the pretreatment and rbST treated period in all stages of lactation. These findings suggest that a constant level of tricarbon units originally derived from glucose being again reincorporated into glucose, which was not affected by either cooling or the supplemental rbST.

The utilization of glucose across the mammary gland during supplemental rbST in both cooled and non-cooled cows at different stages of lactation are complex regulatory mechanisms. It would depend both on the partitioning of blood flow between extra-mammary tissues and local regulation. The results for the mammary uptake of plasma glucose in both groups were not based on changes in A-V concentration differences and extraction ratio of glucose (Chapter V, Series 5.2). An increase in the rate of blood flow to the mammary gland during supplemental rbST in both cooled and non-cooled cows, would be a major determinant of the rate of glucose uptake by the mammary gland. In all stages of lactation, the net mammary glucose uptake increased during supplemental rbST as compared with the pretreatment period in both groups. Glucose extracted by the mammary gland has several possible metabolic fates in mammary epithelial cells that may occur at another level than transmembrane transport (Xiao and Cant, 2003). The glucose uptake by the mammary gland during supplemental rbST and cooling was rate limiting for the transport of glucose to the mammary cell. The high blood flow to the mammary gland during supplemental rbST would decrease the transit time of glucose, thereby reduction for prolonging the contact time between glucose in blood and glucose transporter in mammary epithelial cell (Chaiyabutr *et al*. 2007b). It is possible that an increase in a number of specific glucose transporters at the mammary cell membrane was related with an increase in body protein synthesis during rbST administration, which might proportion to an increase in MBF (Prosser, 1988; Madon et al., 1990). Therefore, the limited transport of glucose into mammary cell would not apparent by these means.

The major of energy source of normal fed ruminants are the volatile fatty acids in the form of acetate and β-hydroxybutyrate. In the present study, mammary arteriovenous concentration differences, mammary extraction and mammary uptake of acetate and β-hydroxybutyrate were not affected during rbST supplementation in different stages of lactation in both cooled and non-cooled cows (Chapter V, Series 5.2). Acetate is known to involve in mammary gland metabolism in either de novo synthesis of short and medium-chain milk fatty acids or generation of ATP and NADPH. It is known that the circulating β-hydroxybutyrate arise mainly from rumen butyrate in the fed animal (Leng and West, 1969), and the principal effect of bST has been shown to increase oxidation of free fatty acids during negative energy balance in high yield lactating cows. An increase in the concentration of plasma β-hydroxybutyrate would be

consistent with an increase in oxidation of free fatty acids (Bauman et al., 1988) e.g. during hepatic ketogenesis due to greater mobilization of fat reserves in starved animals (Schultz, 1974), which were not apparent during rbST-supplementation in both cooled and non-cooled cows

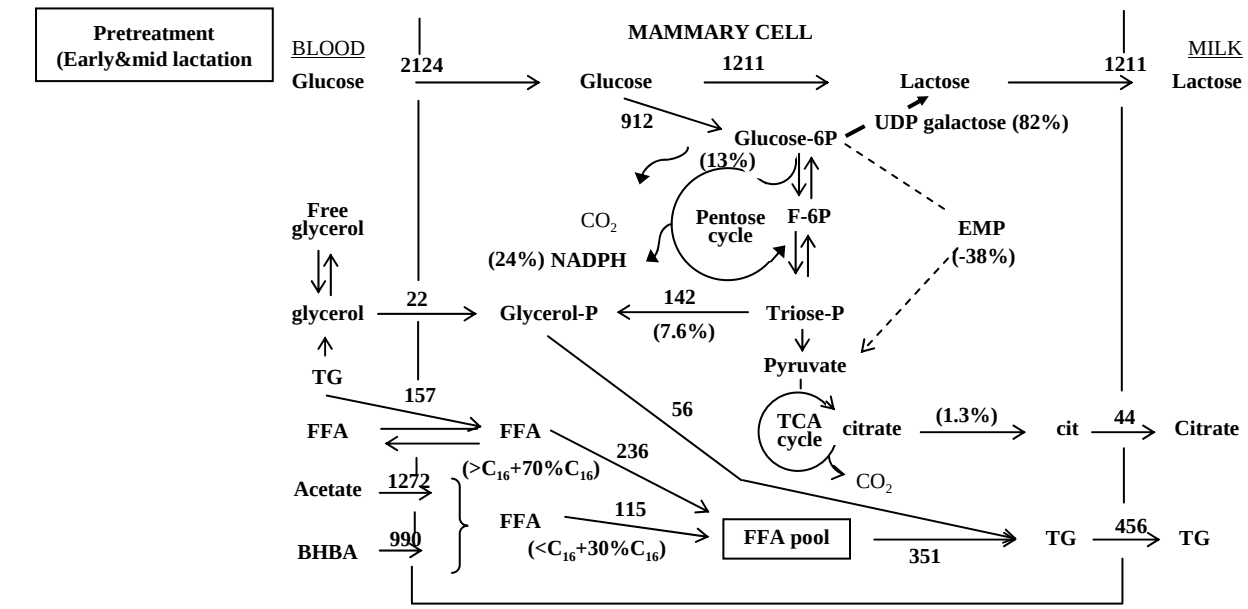
During rbST supplementation the mean values for the arterial plasma concentration of free fatty acids but not for triacylglycerol increased which was more sensitive to alteration than other blood substrates. This phenomenon has been proposed as an indication of under-nutrition (Reid and Hinks, 1962). However, cows in both cooled and non-cooled cows gained weight throughout the experimental periods. A marked increase in milk yield with rbST supplementation without loss of body weight, especially during early lactation, may be due to the fact that cows were offered TMR diet to allow an adequate replacement of body reserves during lactations. Milk yield in the first lactating crossbred cows 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 gain of cows during their first lactation. During early lactation, the metabolic demands of lactation during supplemental rbST in both cooled and non-cooled cows were met by dietary intake, thus no appearance causing mobilization of body tissues as indicated by no alteration of the levels of plasma triglyceride. The marked increases in the plasma concentrations of FFA were apparent in cows supplemental rbST in both cooled and non-cooled cows especially in mid and late stages of lactation are reported in Chapter V (Series 5.2). Thus, the lipolytic activity would be a function of rbST treatment per se in stead of the associated changes in energy balance. The measurement of A-V 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. The high uptake of triacylglycerol by the mammary gland especially significant increase in the late lactation in cows supplemental rbST, which is agree with the results reported by Miller et al. (1991). It is possible that the negative mammary uptakes of free fatty acids may reflect hydrolysis of triacylglycerol, 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 releasing of FFA would be as a result of enzymatic activity of lipoprotein lipase in the mammary tissue which has been reported to be higher in the mammary tissue relative to other tissue (Shirley et al., 1973; Bauman and Griinari, 2003). Local changes for biosynthetic capacity within the mammary gland

would be a factor in identification of the utilization of substrates in the rate of decline in milk yield with advancing lactation.

In the present study, the balance data for the utilization of both short chain and long chain fatty acids were performed by calculating their likely contribution to milk free fatty acids knowing its composition and substracting these values from the measured uptake of the substrates in different stages of lactation of both cooled and non-cooled cows supplemental rbST (Figure 2 and 3). Acetate and β-hydroxybutyrate were grouped together because it is known that they both contribute to the synthesis of milk fatty acids up to and including C_{16} (Annison et al., 1968). It is clear that uptake of milk fat precursors confirm the theory that acetate and β- hydroxybutyrate are major precursor of milk fatty acids. The present studies confirmed the previoius reported that there was a negligible oxidation of free fatty acid by the mammary gland in normal fed cows (Chaiyabutr et al 2007b).

Since glucose cannot synthesize by the bovine mammary gland, which lacking glucose-6-phosphatase (Scott et al., 1976). Glucose plays a crucial role in their metabolism and lactose synthesis, which is formed in Golgi vesicles from a combination of glucose either directly or after phosphorylation to glucose 6-phosphate and conversion to UDP-galactose (Ebner and Schanbacher, 1974). The calculated amount of metabolism of glucose 6-phosphate to the galactose moiety of lactose during supplemental rbST in both cooled and non-cooled cows in different stages of lactation would be sufficient to account for the cytosolic lactose synthesis.The utilization of glucose carbon incorporation to lactose in the udder increased in both early and mid lactation but not for late lactation during supplemental rbST in both cooled and non-cooled cows. The decrease in the metabolism of glucose 6-phosphate to the galactose moiety of lactose as lactation advanced to late lactation in both cooled and non-cooled cows would affect to the lactose synthesis and milk production in both groups (Chapter V Series 5.1) (Figure 3). A low enzymatic activity for lactose synthesis might be expected to appear as lactation advances. According to Davis and Bauman (1974), 50 to 60% of the glucose in the glucose-6-phosphate pool is converted into galactose. Major part of the galactose has been shown to derive from mammary extracted glucose, as well as from glycerol and other metabolic pathways. However, glucose is not the sole carbon source for lactose synthesis but remains the main one. An increase in the glucose concentration in milk representing an increase in glucose concentration in the mammary

Figure 2.The metabolic pathway involved in the metabolism of the precursor of milk in both early and mid-lactation of both cooled and non-cooled cows with and without supplementation of rbST (The value shown are in micromole/min.)



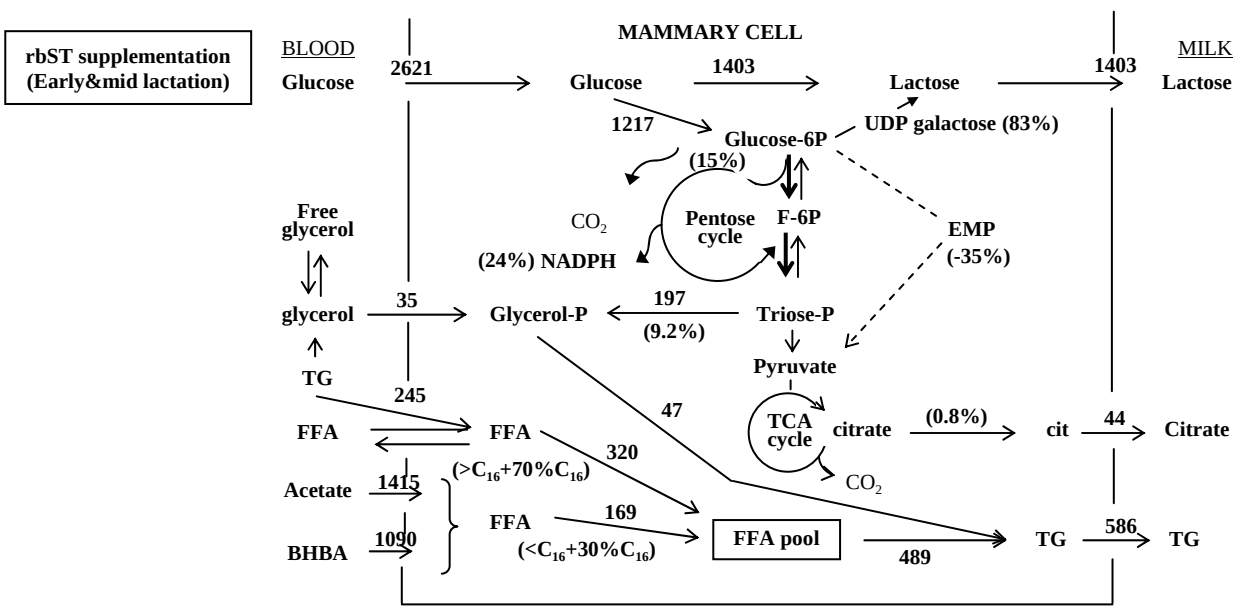
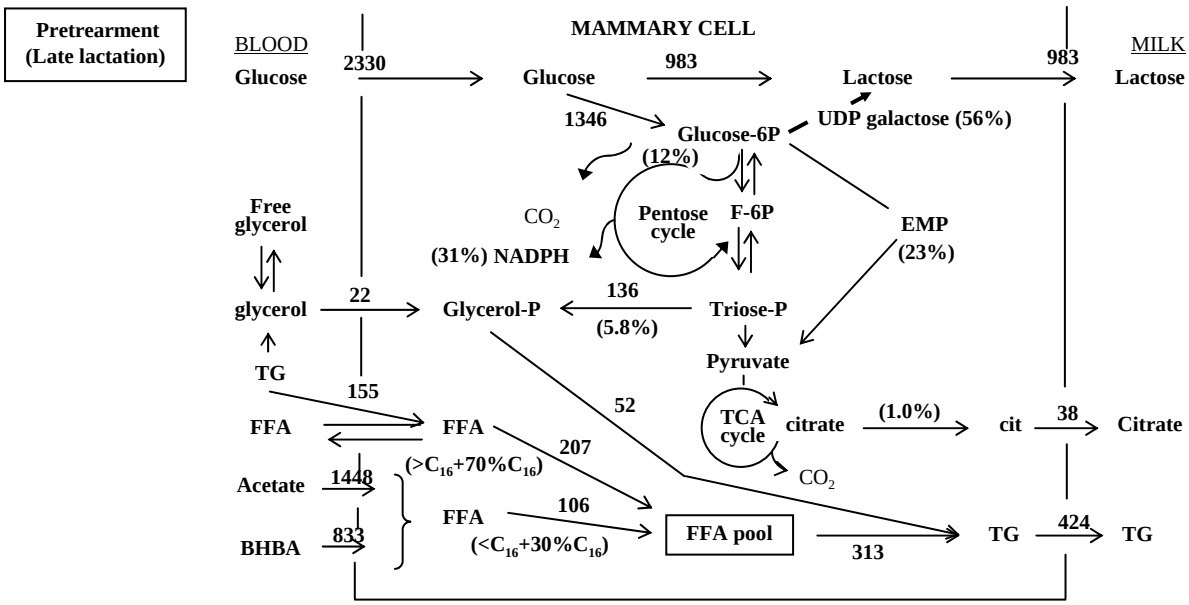
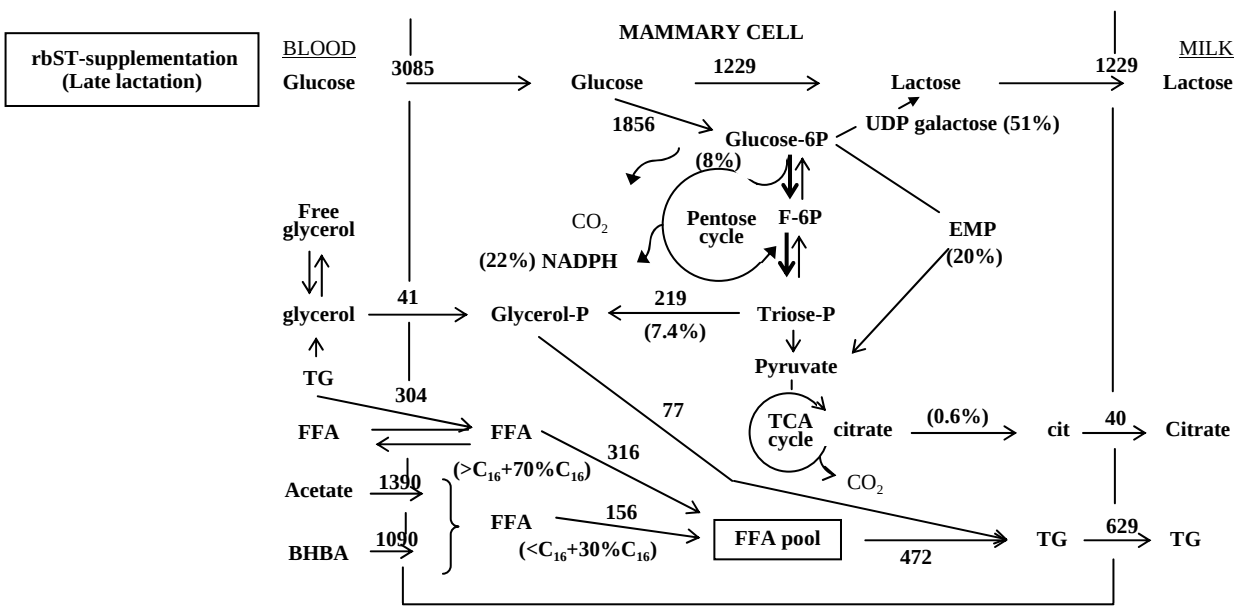


Figure 3. The metabolic pathway involved in the metabolism of the precursor of milk in both early and mid-lactation of both cooled and non-cooled cows with and without supplementation of rbST (The value shown are in micromole/min.)





epithelial cell during prolonged treatment of rbST has been noted (Chaiyabutr et al., 2008a).

It is known that 80–85% of lactose carbon atoms arise from glucose (Faulkner and Peaker, 1987; Bickerstaffe et al., 1974). The quantitative utilization of the glucose taken up by the mammary gland is used directly in the synthesis of lactose, while the remaining of extracted glucose can participate in the supply of ATP (Embden-Meyerhof pathway and the tricarboxylic acid cycle), other portions would be metabolized via the pentose phosphate pathway and secrete in milk glucose. Both cooled and non-cooled cows supplemental rbST had tendency to increase in the milk glucose concentration in early and mid-lactation (Chapter VI). The present studies have shown that glucose 6-phosphate metabolized via the pentose phosphate pathway by average 10-17% throughout lactation in both cooled and non-cooled cows without rbST, while it increased in early and mid-lactation but it decreased in the late lactation after supplemental rbST. These results also agree with prolonged treatment of rbST in crossbred HF cows showing that percentage values of glucose 6-phosphate metabolized via the pentose phosphate pathway were variable in different stages of lactation (Chaiyabutr et al., 2008b). However, the net proportion of the metabolism of glucose 6-phosphate via the pentose cycle pathway was increased during supplemental rbST at early stage of lactation of cooled and non-cooled cows. High metabolism of glucose 6-phosphate in early lactation of rbST treated cows 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 supplementation.

Absolute amount of glucose carbon incorporation to milk lactose was significantly higher during supplemental rbST in early and mid lactation in both cooled and non-cooled cows. As lactation advances of both cooled and non-cooled cows without rbST, high values of both the proportion and absolute amount of glucose carbon incorporation to milk citrate and milk triacylglycerol were apparent, which would be attributed to an increase in proportion of glucose 6-phosphate metabolized via the Embden-Meyerhof pathway and was oxidized in the tricarboxylic acid cycle. During supplemental rbST in each stage of lactation, both the proportion and absolute amount of glucose carbon incorporation to milk triacylglycerol were increased, while amount of glucose carbon incorporation to milk citrate were decreased. These changes can be interpreted in terms of metabolic shifts that are occurring within the mammary epithelial cell, and one might speculate that such changes reflect the high flux of the utilization of glucose carbon by

the mammary epithelial cell through the rate of lactose synthesis and milk production during supplemental rbST. In addition to the use of glucose carbon for milk fat synthesis, the hydrogen from glucose has been shown to be incorporated into milk fat in early and mid lactation in both cooled and non-cooled cows supplemental rbST (Chapter V, Series 5.1), although studies *in vitro* have shown that fatty acid synthesis could occur from the utilization of acetate in the perfused goat udder (Hardwick et al., 1963). It has been known that milk fat is synthesized from fatty acids of both blood lipids and from de novo synthesis within the mammary epithelial cells. However, an increase in milk fat after rbST supplementation was associated with the increased yield of long-chain fatty acids characteristic of plasma free fatty acids. Significant increases in plasma free fatty acids in rbST-treated cows have been published elsewhere (Chaiyabutr et al., 2007b). Thus, the lipolytic activity would be a function of bST treatment per se in stead of the associated changes in energy balance.

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Xiao C, Cant J.P. (2003). Glucose transporter in bovine mammary epithelial cells is an asymmetric carrier that exhibits cooperativity and trans-stimulation**.** Am J Physiol Cell Physiol, 285: C1226-C1234·

Output (publications)

1.Wilaiporn CHANCHAI, Somchai CHANPONGSANG and **Narongsak CHAIYABUTR.** (2010). Effects of misty-fan cooling and supplemental rbST on rumen function and milk production of crossbred Holstein cattle during early, mid and late lactation in a tropical environment. Animal Science Journal 81:230-239 (Impact factor 0.713)

2. W.CHANCHAI, S. CHANPONGSANG AND **N. CHAIYABUTR**. (2010). Effects of cooling and supplemental recombinant bovine somatotropin on diet digestibility, digestion kinetics and milk production of cross-bred Holstein cattle in the tropics. Journal of Agricultural Science (Cambridge) 148, 233–242. (Impact factor 1.471)

3. D. Boonsanit, S. Chanpongsang and **N. Chaiyabutr.** (2010). Effects of Supplemental Recombinant Bovine Somatotropin (rbST) and Cooling with Misters and Fans on Renal Function in Relation to Regulation of Body Fluids in Different Stages of Lactation in Crossbred Holstein Cattle. Asian-Aust. J. Anim. Sci. 23;355-365**.** (Impact factor 0.857)

4. Siravit Sitprija, Somchai Chanpongsang, **Narongsak Chaiyabutr**. (2010). Effects of Cooling and Recombinant Bovine Somatotropin Supplementation on Body Fluids, Mammary Blood Flow, and Nutrients Uptake by the Mammary Gland in Different Stages of Lactation of Crossbred Holstein Cattle. Thai J.Vet. Med.40(2):9-14. (ISI and Scopus)

5. Siravit Sitprija , Somchai Chanpongsang and **Narongsak Chaiyabutr**. (2010). The effects of supplemental bovine somatotropin and cooling on milk production relating to body glucose metabolism and the utilization of glucose by the mammary gland in crossbred Holstein cattle. Acta Veterinaria Scandinavica (Sumitted) (Impact factor 0.9)

6. D. Boonsanit , S. Chanpongsang and **N. Chaiyabutr**. (2010). Effects of recombinant bovine somatotropin and misters and fans cooling on renal tubular handling of sodium in different stages of lactation in crossbred Holstein cattle. Research in Veterinary Science. (Sumitted) (Impact factor 1.384)

The following paper topics will be prepared for submission for publication in International Journal.

7. Effects of shaded cooled animals and recombinant bovine somatotropin (rbST**)** administration on water metabolism and mammary circulation in different stages of lactation in crossbred Holstein cattle. (Journal of Agricultural Science) (Cambridge)

8. Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on the plasma level of insulin like growth factor-1, insulin and plasma metabolites in different stages of lactation in crossbred Holstein cattle. **(**Animal Science Journal).

9. Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on cellular metabolites in milk secretion at different stages of lactation in crossbred Holstein cattle. **(**Animal Science Journal).

10. Effects of shaded cooled animals and recombinant bovine somatotropin (rbST) administration on plasminogen and plasmin system in the mammary gland in different stages of lactation in crossbred Holstein cattle**. (**Animal Science Journal).

11. Indicator of oxidative status in plasma of shaded cooled animals with recombinant bovine somatotropin (rbST) administration in different stages of lactation in crossbred Holstein cattle**. (**Animal Science Journal).

12. Effects of exogenous bovine somatotropin and cooling on hematological and biochemical parameters in different stages of lactation of crossbred Holstein cattle in the tropic (The Journal of Veterinary Medical Science).