The Thai Journal of Veterinary Medicine

Volume 40
Issue 2 *June, 2010*

Article 8

6-1-2010

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

Siravit Sitprija

Somchai Chanpongsang

Narongsak Chaiyabutr

Recommended Citation

Sitprija, Siravit; Chanpongsang, Somchai; and Chaiyabutr, Narongsak (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," *The Thai Journal of Veterinary Medicine*: Vol. 40: Iss. 2, Article 8.
Available at: https://digital.car.chula.ac.th/tjvm/vol40/iss2/8

Original Article

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

Siravit Sitprija[1*] Somchai Chanpongsang[2] Narongsak Chaiyabutr[1]

Abstract

Two groups of five crossbred 87.5% Holstein cattle were housed in normal shade only (NS) as non-cooled cows and in shaded with misty-fan cooling (MFC) as cooled cows. The cows were treated with recombinant bovine somatotropin (rbST) in early, mid and late lactation with three consecutive injections of rbST 500 mg of rbST (POSILAC) in every 14 days. During the study, ambient temperature at the hottest period daily (1400 hr) in the MFC barn was significantly lower, while relative humidity was higher than that of the NS barn. The temperature humidity index (THI) in both barns ranged from 80.7-85.5 throughout the periods of study. Cows in the MFC barn showed a lower rectal temperature and respiration rate as compared with cows in the NS barn. Milk yield significantly increased in both cooled and non-cooled cows treated with rbST in each stage of lactation. The high milk yield in both groups of animals declined as lactation advanced to late lactation. Increases in mammary blood flow (MBF) 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 stage of lactation. The mean arterial plasma concentrations for glucose, acetate, β-hydroxybutyrate and triacylglycerol were unchanged but an increase in plasma free fatty acid concentrations in 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 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. These results suggest that the effect of rbST supplementation on milk yield in each stage of lactation of either cooled or non-cooled cows is due to changes in the relative rates of delivery and uptake of nutrients by the mammary gland. The rate of decline in milk yield as lactation advances would be consequences in local changes for biosynthetic capacity within the mammary gland in the utilization of substrates occurring in both cooled and non-cooled cows whether supplemental rbST or not.

Keywords: crossbred Holstein cattle, mammary blood flow, mammary gland, misty fan cooling nutrients, rbST

[1]*Department of Veterinary Physiology,* [2]*Department of Animal Husbandry, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand.*
Corresponding author E-mail: *Siravit Sitprija@yahoo.com*

Thai J. Vet. Med.2010 40(2): 195-206.

บทคัดย่อ

ผลของความเย็นและการเสริมรีคอมบิแนนท์โบวายโซมาโตโทรปิน ต่อปริมาตรของเหลวภายในร่างกาย อัตราการไหลของเลือดสู่ต่อมน้ำนมและการรับสารอาหารโดยต่อมน้ำนม ในระยะต่างๆของการให้นมของโคนมพันธุ์ผสมโฮลสไตน์

ศิรวิทย์ สิตปรีชา[1*] สมชาย จันทร์ผ่องแสง[2] ณรงค์ศักดิ์ ชัยบุตร[1]

โคนมพันธุ์ผสมโฮลสไตน์ 87.5% 2 กลุ่ม กลุ่มละ 5 ตัวเลี้ยงในโรงเรือนปกติ (NS) และโคที่เลี้ยงในที่เย็นในโรงเรือนที่มีพัดลมพ่นละอองน้ำ (MFC) โคทุกตัวจะได้รับการเสริมรีคอมบิแนนท์ โบวายโซมาโตโทรปิน (rbST) ด้วยการฉีด rbST ติดต่อกัน 3 ครั้ง ครั้งละ 500 มิลลิกรัม ห่างกันทุก ๆ 14 วัน ในระยะต้น ระยะกลาง และระยะท้ายของการให้นม ในช่วงบ่ายที่เป็นช่วงที่ร้อนที่สุดอุณหภูมิแวดล้อมในโรงเรือน MFC จะต่ำกว่าโรงเรือนปกติ อย่างมีนัยสำคัญ แต่ความชื้นสัมพัทธ์จะสูงกว่าโรงเรือนปกติ ดัชนีอุณหภูมิความชื้นสัมพัทธ์ (THI) ทั้งสองโรงเรือนอยู่ในช่วง 80.7-85.5 ตลอดระยะการศึกษา โคที่เลี้ยงในโรงเรือนที่มีพัดลมพ่นละอองน้ำจะมีอุณหภูมิวัดที่ทวารหนักและอัตราการหายใจต่ำกว่ากลุ่มโคที่เลี้ยงในโรงเรือนปกติ อัตราการหลั่งน้ำนมจะเพิ่มขึ้นอย่างมีนัยสำคัญในกลุ่มโคที่ฉีด rbSTในทุกระยะของการให้นม อัตราการหลั่งน้ำนมที่สูงในระยะแรกในโคทั้ง 2 กลุ่มจะลดลงเมื่อเข้าสู่ระยะท้ายของการให้นม การเพิ่มอัตราการไหลของเลือดสู่ต่อมน้ำนมร่วมไปกับการเพิ่มขึ้นของปริมาณน้ำในร่างกาย ปริมาณน้ำนอกเซลล์ ปริมาณเลือดและปริมาณพลาสม่าในโคนมทั้ง 2 กลุ่มที่ได้รับ rbST ตลอดระยะการให้นม ความเข้มข้นของกลูโคส อะซีเทต เบต้าไฮดรอกซี่บิวตาเรต และ ไตรกลีเซอไรด์ ในพลาสมาของเลือดแดง ไม่พบการเปลี่ยนแปลง แต่ความเข้มข้นของกรดไขมันอิสระจะเพิ่มขึ้นในโคที่เลี้ยงทั้งโรงเรือนปกติและโรงเรือที่มีความเย็นเมื่อให้ rbST อัตราการใช้กลูโคสและไตรกลีเซอไรด์โดยต่อมน้ำนมจะเพิ่มขึ้นโดยเฉพาะในระยะกลางและระยะท้ายของการให้นม ขณะเดียวกันไม่พบการเปลี่ยนแปลงในความแตกต่างของความเข้มข้นของสารระหว่างเลือดแดงและเลือดดำ (A-V difference) และสัดส่วนการใช้สารโดยต่อมน้ำนมในโคนมทั้งสองกลุ่มที่ให้ rbST ส่วนค่าความแตกต่างความเข้มข้นของสารอาหารระหว่างเลือดแดงและเลือดดำ (A-V difference) และสัดส่วนการใช้และอัตราการใช้ของอะซีเตทและเบต้าไฮดรอกซี่บิวตาเรตไม่พบการเปลี่ยนแปลงในช่วงที่มีการให้ rbST ในโคนมทั้งสองกลุ่ม ผลจากการศึกษาชี้ให้เห็นว่าผลการให้ rbST ต่อการหลั่งน้ำนมในแต่ระยะของการให้นมทั้งในโคที่เลี้ยงในที่ร้อนหรือที่เย็นเป็นผลจากการเปลี่ยนแปลงการนำสารอาหารและอัตราการใช้สารอาหารโดยต่อมน้ำนม อัตราการหลั่งน้ำนมลดลงเมื่อเข้าช่วงระยะท้ายๆของการให้นมเป็นผลต่อเนื่องจากการเปลี่ยนแปลงของความสามารถในการสังเคราะห์น้ำนมของต่อมน้ำนมกับการใช้สารอาหารภายในต่อมน้ำนมที่ลดลงไม่ว่าจะให้ rbST หรือไม่

คำสำคัญ: โคนมพันธุ์ผสมโฮลสไตน์ อัตราการไหลของเลือดสู่ต่อมน้ำนม ต่อมน้ำนม พัดลมพ่นละอองน้ำ สารอาหาร rbST

[1] *ภาควิชาสรีรวิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปทุมวัน กรุงเทพฯ10330*

[2] *ภาควิชาสัตวบาล คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปทุมวัน กรุงเทพฯ10330*

***ผู้รับผิดชอบบทความ E-mail:** Siravit Sitprija@yahoo.com*

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., 2000[a]). 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. 2007[a]). 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 180ºC. 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 min at 15-min intervals from 0600 hr to 1800 hr. At night, animals were exposed to MFC for 15 min at 45-min intervals from 1800 hr to 0600 hr. 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 (°C), db = dry bulb temperature (°C) (McDowell, 1972).

On each specified day of each lactating period, at around 0900 hr, 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°C. Arterial and venous plasma samples were collected and frozen at -20°C 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 1100-1200 hr 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 sec. About 10 sec 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 1300 hr 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 titrated 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 hr subsequent to the injection of titrated water (^3H_2O) for determination of TBW. Total body water (TBW) was determined in each animal by dilution techniques using titrated water as previously described (Chaiyabutr et al., 1997). TBW = (standard count (dis/min) x 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 x 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 Figure 1 and 2. Average values of ambient temperature in the barn during the daytime (1400 hr) 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 hr). 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.

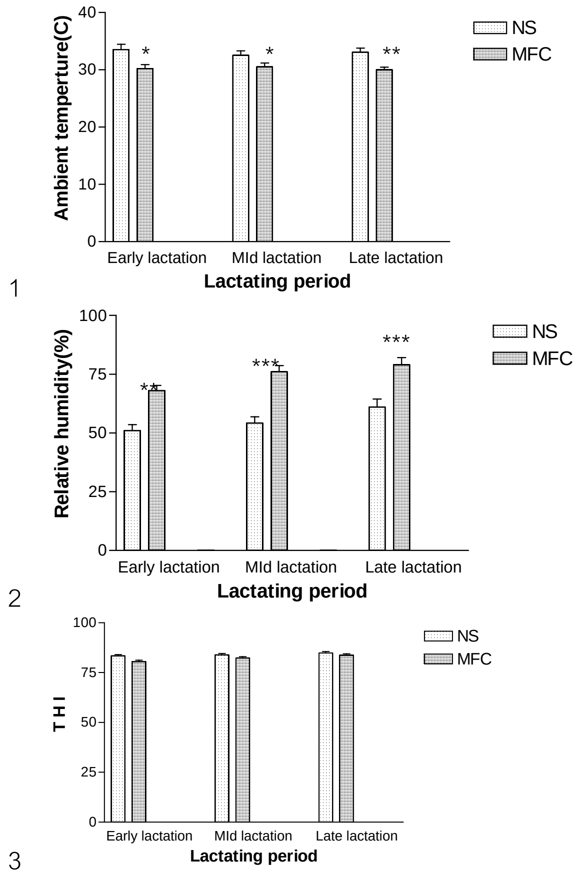

Figure 1. Ambient temperature, relative humidity and temperature humidity index (THI), measuring at 1400 hr in normal shade (NS) barn and NS barn with misters and fans (MFC) at different stages of lactation. (unpaired *t*-test, $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$).

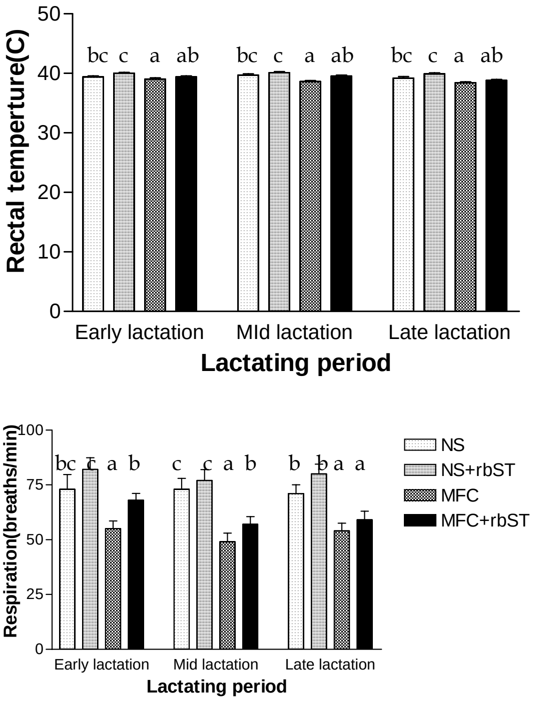

Figure 2. Rectal temperature and respiration rate measurement at 1400 hr in cows treated with rbST housing under NS and MFC barn at each stage of lactation. (Duncan's test; means in each stage of lactation with different superscripts (a, b, c) differ significantly, $p<0.05$).

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.

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

	Stages of lactation	NS		MFC			[1]Effect		
Parameters		Pre	rbST	Pre	rbST	SEM	MFC	rbST	MFC*rbST
TBW (l)	Early	254.4	295.9	277.7	309.2	6.23	0.273	0.001	0.441
	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 (l/100kg)	Early	71.6	78.1	74.8	82.8	2.48	0.369	0.019	0.764
	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 (l)	Early	92.87	106.4	108.1	123.2	3.00	0.029	0.001	0.805
	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 (l/100kg)	Early	26.2	28.1	29.2	33.0	0.87	0.081	0.010	0.319
	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 (l)	Early	18.8	20.6	17.8	19.5	0.95	0.364	0.104	0.937
	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 (l/100kg)	Early	5.3	5.4	4.8	5.2	0.25	0.378	0.274	0.618
	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 (l)	Early	24.3	26.4	23.5	25.0	1.32	0.548	0.205	0.819
	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 (l/100kg)	Early	6.8	6.9	6.4	6.7	0.35	0.532	0.477	0.758
	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 lactation	NS		MFC			[1]Effect		
Parameters		Pre	rbST	Pre	rbST	SEM	MFC	rbST	MFC*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
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
MPF (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
MBF/milk (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	358.8	380.8	360.2	373.8	6.48	0.893	0.025	0.535
	Mid	382.4	383.2	381.8	411.4	4.17	0.586	0.007	0.009
	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, MFCxrbST: interaction effect of MFC and rbST

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.

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 lactation	NS		MFC			[1]Effect		
Parameters		Pre	rbST	Pre	rbST	SEM	MFC	rbST	MFC*rbST
Glucose: Plasma conc (μmol/ml)	Early	3.73	3.51	3.64	3.48	0.10	0.883	0.098	0.763
	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 (μmol/ml)	Early	0.66	0.67	0.76	0.61	0.08	0.858	0.485	0.261
	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 (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
Acetate Plasma conc (μmol/l)	Early	650.47	541.77	437.13	468.93	54.27	0.215	0.499	0.232
	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 (μmol/l)	Early	363.67	305.43	273.07	293.57	48.20	0.661	0.706	0.438
	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 (μmol/min)	Early	1534.4	1358.5	1212.0	1663.5	247.8	0.99	0.593	0.241
	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 (μmol/l)	Early	850	872	652	697	63.1	0.143	0.610	0.860
	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 (μmol/l)	Early	312	266	224	269	49.53	0.268	0.992	0.385
	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 (μmol/min)	Early	1262.4	1083.9	912.9	1253.8	178.6	0.781	0.661	0.184
	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

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.

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 (μmol/l)	Early	156.89	164.27	199.99	287.49	38.02	0.239	0.247	0.323
	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 (μmol/min)	Early	-59.65	-122.58	73.11	462.00	155.5	0.105	0.325	0.184
	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 (μmol/l)	Early	159.25	179.71	195.07	201.93	17.29	0.702	0.452	0.704
	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 (μmol/l)	Early	42.92	32.33	58.39	70.21	6.48	0.209	0.927	0.122
	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 (μmol/min)	Early	167.65	136.88	238.33	388.11	44.04	0.162	0.214	0.075
	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 et al. (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 (Tanwattana et.al., 2003; Chaiyabutr et al., 2005; Maksiri et al., 2005). 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 (Hart et al. 1980; Davis et al. 1988). 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., 2000^{b}), 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.

Acknowledgement

This work was partly supported by The Thailand Research Fund (grant# BRG498004). S. Sitprija is the recipient a grant from Faculty of Science, Mahidol University, Thailand.

References

Armstrong, D.V. 1994. Heat stress interaction with shade and cooling. J. Dairy Sci. 77: 2044-2050.

Bauman, D.E. 1992. Bovine somatotropin: review of an emerging animal technology. J. Dairy Sci. 75: 3432-3451.

Bauman, D. E. and Griinari, J.M. 2003. Nutritional regulation of milk fat synthesis. Ann. Rev. Nutri. 23: 203-227.

Bauman, D.E., Peel, C.J., Steinhour, W.D., Reynolds, P.J., Tyrell, H.F., Brown, A.C.G. and Haaland, G.L. 1988. Effect of bovine somatotropin on metabolism of lactation dairy cows: Influence of rates of irreversible loss and oxidation of glucose and nonesterified fatty acids. Nutr. J. 118: 1031-1040.

Bickerstaffe, R., Annison, E.F. and Linzell, J.L. 1974. The metabolism of glucose, acetate, lipids and amino acids in lactating dairy cows. J. Agri.. Sci. 82: 71-85.

Chaiyabutr, N., Faulkner, A. and Peaker, M. 1980. Effects of starvation on the cardiovascular system, water balance and milk secretion in lactating goats. Res. Vet. Sci. 28: 291-295

Chaiyabutr, N., Komolvanich, S., Sawangkoon, S., Preuksagoon, S. and Chanpongsang, S. 1997. The regulation of body fluids and mammary circulation during late pregnancy and early lactation of crossbred Holstein cattle feeding on different types of roughage. J. Anim. Physiol. Anim. Nutri. 77: 167-179.

Chaiyabutr, N., Preuksagorn, S., Komolvanich, S. and Chanpongsang, S. 2000^a. Comparative study on the regulation of body fluids and mammary circulation at different states of lactation in crossbred Holstein cattle feeding on different types of roughage. J. Anim. Physiol. Anim. Nutr. 83: 74-84.

Chaiyabutr, N., Komolvanich, S., Preuksagorn, S. and Chanpongsang, S. 2000^b. Comparative studies on the utilization of glucose in the mammary gland of crossbred Holstein cattle feeding on different types of roughage during different stages of lactation. Asian-Aust. J. Anim. Sci. 13: 334-347.

Chaiyabutr, N., Thammacharoen, S., Komolvanich, S. and Chanpongsang, S. 2005. Effects of long-term administration of recombinant bovine somatotropin on milk production and insulin like growth factor-I and insulin in crossbred Holstein cows. J. Agri. Sci. (Cambridge) 143: 311-318.

Chaiyabutr, N., Thammacharoen, S., Komolvanich, S. and Chanpongsang, S. 2007. Effects of long-term exogenous bovine somatotropin on water metabolism and milk yield in crossbred Holstein cattle. J. Agri. Sci. (Cambridge) 145: 173-184.

Chaiyabutr, N., Chanpongsang, S. and Suadsong, S. 2008. Effects of evaporative cooling on the regulation of body water and milk production in crossbred Holstein cattle in a tropical environment. Int. J. Biometeorol. 52: 575-585.

Chan, S.C., Huber, J.T., chen, K.H., Simas, J.M. and Wu, Z. 1997. Effects of ruminally inert fat and evaporative cooling on dairy cows in hot environmental temperatures. J. Dairy. Sci. 80: 1172-1178.

Collier, R.J., MCnamara, J.P., Wallace, C.R. and Dehoff, M.H. 1984. A review of endocrine regulation of metabolism during lactation. J. Anim. Sci. 59: 498-510.

Davis, S.R. and Collier, R.J. 1985. Mammary blood flow and regulation of substrate supply for milk synthesis. J. Dairy Sci. 68: 1041-1058.

Davis, S.R., Collier, R.J., McNamara, J.P., Head H.H. and Sussman, W. 1988. Effects of thyroxine and growth hormone treatment of dairy cows on milk yield, cardiac output and mammary blood flow. J. Anim. Sci. 66: 70-79.

Fike, J.H., Staples, C.R., Sollenberger, L.E., Moore, J.E. and Head, H.H. 2002. Southeastern pasture-based dairy systems: housing, posilac, and supplemental silage effects on cow performance. J. Dairy Sci. 85: 866-878.

Forsyth, I. and Hayden, T.J. 1977. Comparative endocrinology of mammary growth and lactation. In: Comparative Aspects of Lactation. M. Peaker (ed.), Symposia of the Zoological Society of London., No 41. London: Academic Press. 135-163.

Forsyth, I.A. 1996. The insulin- like growth factor and epidermal growth factor families in mammary cell growth in ruminants: action and interaction with hormones. J. Dairy Sci. 79: 1085-1096.

Fullerton, F.M., Fleet, I.R., Heap, R.B., Hart, I.C and Mepham, T.B. 1989. Cardiovascular responses and mammary substrate uptake in Jersey cows treated with pituitary-derived growth hormone during late lactation. J. Dairy Res. 56: 27-35.

Fuquay, J.W. 1981. Heat stress as it affects animal production. J. Anim. Sci. 52: 164-174.

Gertler, A., Cohen, N. and Maoz, A. 1983. Human growth hormone but not ovine or bovine growth hormones exhibits a galactopoietic prolactin-like activity in organ culture from bovine lactating mammary gland. Mol. Cell. Endrocrin. 33: 169-182.

Handerson, A.J. and Peaker, M. 1983. Arterial plasma composition during compensatory increase in milk secretion in goat: relation to rate limitation. Q. Jl. Exp. Physiol. 68: 203-208.

Hanwell, A. and Peaker, M. 1977. Physiological effects of lactation on the mother. In: Comparative Aspects of Lactation. M. Peaker (ed.) Symposia of the Zoological Society of London No. 41. London: Academic Press 279-312.

Hart, I.C., Lawrence, S.E. and Mepham, T.B. 1980. Effect of exogenous growth hormone on mammary blood flow and milk yield in lactating goats. J. Physiol. 308: 46-47.

Johnson, H.D., Li, R., Manula, W., Spencer-Johnson, K.J., Becker, B.A., Collier, R.J. and Baile, C.A. 1991. Effects of somatotropin on milk yield and physiological responses during summer farm and hot laboratory conditions. J. Dairy Sci. 74: 1250-1262.

Kirchgessner M, Windisch W, Schwab W, Muller HL. 1991. Energy metabolism of lactating dairy cows treated with prolonged-release bovine somatotropin or energy deficiency. J. Dairy Sci. 74: 35-43.

Leng, R.A. and West, C.E. 1969. Contribution of acetate, butyrate, palmitate, stearate and oleate to ketone body synthesis in sheep. Res. Vet. Sci. 10: 57-63.

Linzell, J.L. and Mepham, T.B. 1974. Effect of intramammary arterial infusion of essential amino acids in the lactating goat. J. Dairy Sci. 41: 101-109.

Madon, R. J., Martin, S., Davies, A., Fawcett, H.A.C., Flint D.J. and Baldwin, S.A. 1990. Identification and characterization of glucose transport proteins in plasma membrane and golgi vesicle-enriched fractions prepared from lactating rat mammary gland. Biochem. J. 272: 99-105.

Maksiri, W., Chanpongsang, S. and Chaiyabutr, N. 2005. Relationship of early lactation and bovine somatotropin on water metabolism and mammary circulation of crossbred Holstein cattle. Asian-Aust. J. Anim. Sci. 18: 1600-1608.

McDowell, R.E. 1972. The animal body in warm environments. In: Improvement of Livestock Production in Warm Climates. W.H. Freeman (ed) San Francisco, CA. 65.

McDowell, G.H., Gooden, J.M., Leenanuruksa, D., Jois, M. and English, A.W. 1987. Effects of exogenous growth hormone on milk production and nutrient uptake by muscle and mammary tissues of dairy cows in mid-lactation. Aust. J. Biol. Sci. 40: 295-306.

Medway, W. and Kare, M.R. 1959. Thiocyanate space in growing domestic fowl. Am. J. Physiol. 196: 873-875.

Mepham, T.B. 1993. The development of ideas on the role of glucose in regulating milk secretion. Aust. J. Agric. Res. 44: 508-522.

Miller, P.S., Reis, B.L., Calvert, C.C., DePeters E.J. and Baldwin, R.L. 1991. Patterns of nutrient uptake by the mammary glands of lactating dairy cows. J. Dairy Sci. 74: 3791-3799.

Moallem, M.U., Dahl, G.E., Duffey, E.K., Capuco, A.V., Wood, D.L., McLeod, K.R., Baldwin, R.L. and Erdman, R.A. 2004. Bovine somatotropin and rumen-undegradable protein effects in prepubertal dairy heifers: Effects on body composition and organ and tissue weights. J. Dairy Sci. 87: 3869-3880.

Murphy, M.R. 1992. Symposium: Nutritional factors affecting anima water and waste quality. J. Dairy Sci. 75: 326-333.

Prosser, C.G. 1988. Mechanism of the decrease in hexose transport by mouse mammary epithelial cells caused by fasting. Biochem. J. 249: 149-154.

Reid, R.L. and Hinks, N.T. 1962. Studies on the carbohydrate metabolism of sheep, XVIII. The metabolism of glucose, free fatty acid, ketones and amino acids in late pregnancy and lactation.

Aust. J. Agri.. Res. 13: 1112-1123.

Shirley, J.E., Emerry, R.S., Convey, E.M and Oxender, W.D. 1973. Enzymic changes in bovine adipose and mammary tissue, serum and mammary tissue hormonal changes with initiation of lactation. J. Dairy Sci. 56: 569-574.

Soderholm, C.G., Otterby, D.E., Linn, J.G., Ehle, F.R., Wheaton, J.E., Hanson, W.P. and Annexstad, R.J. 1988. Effects of recombinant bovine somatotropin on milk production, body composition, and physiological parameters. J. Dairy Sci. 71: 355-359.

Sandles, L.D., Sun, Y.X.D., Cruz, A.G.C., McDowell, G.H. and Gooden. J.M. 1988. Responses of lactating ewes to exogenous growth hormone: short and long-term effects of productivity and tissue utilization of key metabolites. Aust. J. Biol. Sci. 41: 357.

Schultz, L.H. 1974. Ketosis. In: Lactation. vol. II. B.L. Larson and V.R. Smith (eds.). New York and London: Academic Press. 318-354.

Sullivan, J.L., Huber, J.T., Denise, S.K., Hoffman, R.G., Kung, L. Franson, S.E. and Madsen, K.S. 1992. Factors affecting response of cows to biweekly injections of sometribove. J. Dairy Sci. 75: 756-763.

Tarazon, H.M., Hubaer, J.T., Santos, J., Mena, H., Unzo, L. and Nussio, C. 1999. Effect of bovine somatotropin and evaporative cooling plus shade on lactation performance of cows during summer heat stress. J. Dairy Sci. 82: 2352-2357.

Tanwattana, P., Chanpongsang, S. and Chaiyabutr, N. 2003. Effects of exogenous bovine somatotropin on mammary function of late lactating crossbred Holstein Cows. Asian-Aust. J. Anim. Sci. 16: 85-96.

Tyrrell, H.F., Brown, A.C., Renolds, P.J., Haaland, G.L., Bauman, D.E., Peel, C.J. and Steinhour W.D. 1988. Effect of bovine somatotropin on metabolism of lactating dairy cows: energy and nitrogen utilization as determined by respiration calorimetry. J. Nutr. 118: 1024-1030.

Wang, AS., Jan, D.F., Chen, K.J., Yang, D.W. and Fan, Y.K. 2004. Dietary supplementation of increased milk fat percentage without affecting ruminal characteristics in Holstein cows in a warm tropical environment. Asian-Aust. J. Anim. Sci. 17: 213-220.

West, J.W. 1994. Interactions of energy and bovine somatotropin with heat stress. J. Dairy Sci. 77: 2091–2102.

West, C.E., Annison, E.F. and Linzell, J.L. 1967. Plasma free fatty acid uptake and release by the goat mammary gland. Biochem. J. 102: 23P.