ผลของการฉีดฮอร์โมนรีคอมบิแนนท์ โบวายน์ โซมาโตโทรปินและระบบพัดลมพ่นละอองน้ำต่อผลผลิตน้ำนมที่สัมพันธ์กับการทำหน้าที่ของไตในการควบคุมของเหลวในร่างกายโคนมพันธุ์ผสมโฮล์สไตน์

นางสาวดลฤดี บุญสนิท

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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THE EFFECTS OF RECOMBINANT BOVINE SOMATOTROPIN AND MISTY-FAN COOLING SYSTEM ON MILK PRODUCTION RELATING TO RENAL FUNCTION IN REGULATION OF BODY FLUIDS IN CROSSBRED HOLSTEIN CATTLE

Miss Dolrudee Boonsanit

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Thesis Title	THE EFFECTS OF RECOMBINANT BOVINE SOMATOTROPIN AND MISTY-FAN COOLING SYSTEM ON MILK PRODUCTION RELATING TO RENAL FUNCTION IN REGULATION OF BODY FLUIDS IN CROSSBRED HOLSTEIN CATTLE
By	Miss Dolrudee Boonsanit
Field of Study	Animal Physiology
Thesis Advisor	Professor Narongsak Chaiyabutr, Ph.D.
Thesis Co-Advisor	Professor Somchai Chanpongsang, D.V.M.

Accepted by the Faculty of Veterinary Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

M. Techakumphu .... Dean of the Faculty of Veterinary Science
(Professor Mongkol Thechakumphu, Ph.D.)

THESIS COMMITTEE

Kris Angkanaporn .... Chairman
(Associate Professor Kris Angkanaporn, Ph.D.)

Narongsak Chaiyabutr .... Thesis Advisor
(Professor Narongsak Chaiyabutr, Ph.D.)

Somchai Chanpongsang .... Thesis Co-Advisor
(Professor Somchai Chanpongsang, D.V.M.)

Chollada Buranakarl .... Examiner
(Professor Chollada Buranakarl, Ph.D.)

.... External Examiner
(Professor Chanvit Vajarabukka, Ph.D.)

ดลฤดี บุญสนิท : ผลของการฉีดฮอร์โมนรีคอมบิแนนท์ โบวายน์ โซมาโตโทรปินและระบบพัดลมพ่นละอองน้ำต่อผลผลิตน้ำนมที่สัมพันธ์กับการทำหน้าที่ของไตในการควบคุมของเหลวในร่างกายโคนมพันธุ์ผสมโฮล์สไตน์. (THE EFFECTS OF RECOMBINANT BOVINE SOMATOTROPIN AND MISTY-FAN COOLING SYSTEM ON MILK PRODUCTION RELATING TO RENAL FUNCTION IN REGULATION OF BODY FLUIDS IN CROSSBRED HOLSTEIN CATTLE) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: ศ.น.สพ.ดร. ณรงค์ศักดิ์ ชัยบุตร, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ.น.สพ. สมชาย จันทร์ผ่องแสง, 153 หน้า.

โคนมท้องแรก พันธุ์ผสม 87.5% โฮล์สไตน์ จำนวน 20 ตัวถูกใช้เพื่อศึกษาผลของการเสริมฮอร์โมนรีคอมบิแนนท์ โบวายน์ โซมาโตโทรปิน (rbST) และความเย็นโดยพัดลมพ่นละอองน้ำ ต่อผลผลิตน้ำนม และการตอบสนองของฮอร์โมน ที่สัมพันธ์กับการทำหน้าที่ของไตในการควบคุมของเหลวในระยะต่างๆของการให้นม แม่โคในแต่ละการทดลองจะถูกแบ่งออกเป็น 2 กลุ่ม โคในกลุ่มแรกเลี้ยงในโรงเรือนแบบผูกยืนโรงอย่างเดียว (NS) เป็นโคไม่ถูกทำให้เย็น และโคในกลุ่มที่ 2 อยู่ในโรงเรือนที่มีการเสริมระบบพัดลมพ่นละอองน้ำ (MF)เป็นโคถูกทำให้เย็น ในแต่ละระยะของการให้นมโคทั้งสองกลุ่มจะได้รับการฉีด ฮอร์โมน รีคอมบิแนนท์ โบวายน์ โซมาโตโทรปิน ขนาด 500 มก. เข้าใต้ผิวหนัง ทุกๆ 2 สัปดาห์ จำนวน 3 ครั้ง ผลการทดลองพบว่า ค่าเฉลี่ยดัชนีความชื้นในโรงเรือน อยู่ที่ 80 ถึง 84 ทั้งในโรงเรือน NS และ MF แต่อุณหภูมิร่างกายและอัตราการหายใจของ โคถูกทำให้เย็น จะต่ำกว่า โคในกลุ่มไม่ถูกทำให้เย็น ส่วนปริมาณน้ำนม การกินอาหาร และน้ำ ของ โคถูกทำให้เย็น ที่ไม่ได้ฉีด rbST มีแนวโน้มที่จะสูงกว่าโคไม่ถูกทำให้เย็น สำหรับโคที่ได้รับฮอร์โมนรีคอมบิแนนท์ โบวายน์ โซมาโตโทรปิน พบว่าปริมาณน้ำนม อัตราการไหลของเลือดไปยังต่อมน้ำนม น้ำในร่างกาย การกินอาหาร การกินน้ำ อุณหภูมิร่างกาย อัตราการหายใจ จะสูงขึ้นอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับก่อนได้รับ rbST ในแต่ละระยะของการให้นม ในช่วงการเสริม rbST ทั้งใน โคถูกทำให้เย็น และ โคไม่ถูกทำให้เย็น พบการเพิ่มขึ้นของฮอร์โมน อินสุลิน-ไลค์ โกรท์ แฟคเตอร์ I (IGF-I) ควบคู่ไปกับการเพิ่มขึ้นของ ฮอร์โมนอัลโดสเตอโรน แต่ไม่มีผลต่อการเปลี่ยนแปลงอย่างมีนัยสำคัญทางสถิติของ ฮอร์โมนคอร์ติโซล และ วาโสเพรสซิน ในทุกระยะของการให้นม นอกจากนี้ยังพบการลดลงอย่างมีนัยสำคัญทางสถิติของ ฮอร์โมน ไทรอกซีนในระยะแรกของการให้นม การศึกษาการทำหน้าที่ของไตทั้งใน โคถูกทำให้เย็น และ โคไม่ถูกทำให้เย็น ไม่ว่าจะเสริม rbST หรือไม่ พบว่าไม่มีผลต่อการเปลี่ยนแปลงของ renal hemodynamic แต่มีผลต่อการลดลงของอัตราการขับปัสสาวะ อัตราการขับทิ้งของอิเลคทรอไลท์ และ osmolar clearance ทั้งใน โคถูกทำให้เย็น และ โคไม่ถูกทำให้เย็นที่ได้รับ rbST สำหรับผลจากการศึกษา lithium clearance ชี้ให้เห็นว่า การเพิ่มขึ้นของการดูดกลับน้ำและโซเดียมเกิดขึ้นในบริเวณ proximal tubule ของไต จากผลการทดลองสรุปได้ว่า การให้ rbST ในการเพิ่มปริมาณน้ำนมโดยผ่านทางการเพิ่มขึ้นของน้ำในร่างกาย และเป็นผลให้มีการเพิ่มขึ้นของปริมาณเลือดที่ไปสู่ต่อมน้ำนมในการนำส่งสารอาหารในการสังเคราะห์เป็นน้ำนม ผลการกระตุ้นของ rbST ต่อการเพิ่มขึ้นของน้ำในร่างกาย อาจผ่านทางการเพิ่มการดูดกลับของ น้ำและโซเดียมที่บริเวณ proximal tubule โดยอาจเป็นผลมาจากการเพิ่มขึ้นของฮอร์โมนอัลโดสเตอโรนและIGF-I ซึ่งอาจจะกระตุ้นผ่านระบบ เรนิน แองกิโอเทนซิน อัลโดสเตอโรน แต่ไม่เกี่ยวกับ ฮอร์โมนวาโซเพรสซิน.

ภาควิชา สรีรวิทยา ลายมือชื่อนิสิต ดลฤดี บุญสนิท

สาขาวิชา สรีรวิทยาการสัตว์ ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก

ปีการศึกษา 2552 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม สมชาย จันทร์ผ่องแสง

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Dolrudee Boonsanit: THE EFFECTS OF RECOMBINANT BOVINE SOMATOTROPIN AND MISTY-FAN COOLING SYSTEM ON MILK PRODUCTION RELATING TO RENAL FUNCTION IN REGULATION OF BODY FLUIDS IN CROSSBRED HOLSTEIN CATTLE. THESIS ADVISOR: PROF. NARONGSAK CHAIYABUTR, PH.D, THESIS COADVISOR: PROF. SOMCHAI CHANPONGSANG, D.V.M., 153 pp.

Twenty, primiparous, 87.5% crossbred Holstein cows were used to evaluate the effect of supplemental recombinant bovine somatotropin (rbST) and mister-fan cooling on milk production and hormonal responses relating to renal function in regulation of body fluids at early, mid and late lactation. Animals in each experiment were divided into two groups. Cows in the first group were housing in the barn under normal shade (NS) as non-cooled cows and cows in the second group were housing under normal shade plus misty-fan cooling system (MF) as cooled cows. Cows in both groups were received subcutaneous injections of 500 mg of rbST every 2 wks three times in each stage of lactation. The results showed that the averaged temperature humidity index (THI) in both NS and MF barns were from 80 to 84, but the rectal temperature (RT) and respiration rate (RR) of cooled cows were lower than those of non-cooled cows. The milk yield, DMI and water intake of cooled cows without rbST had tendency to higher than non-cooled cows. Cows treated with rbST were significantly higher in milk yield, mammary blood flow (MBF), body fluids, dry matter intake (DMI), water intake, rectal temperature (RT) and respiration rate (RR) than the pretreated period in each stage of lactation . 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 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. The lithium clearance study revealed the increases in water and sodium reabsorption in renal proximal tubule. These data can conclude that rbST supplementation is main action to increase milk production which is mediated primarily through higher body fluid and secondary increased MBF to mammary gland for distribution of nutrients for milk synthesis. 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.

Department : Physiology	Student's Signature Dolrudee Boonsanit
Field of Study : Animal Physiology	Advisor's Signature Narongsak Chaiyabutr
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ABBREVIATIONS

AII	Angiotensin II
AChE	Aldosterone acetylcholinesterase
ADF	Acid detergent fiber
ADH	Antidiuretic hormone
ANP	Atrial natriuretic peptide
AT	Ambient temperature
BW	Body weight
bST	Bovine somatotropin
C_{osm}	Osmolar clearance
C_{H2O}	Free water clearance
C_{Li}	Lithium clearance
CP	Crude protein
DAR_{Na}	Distal absolute reabsorption of sodium
DAR_{H2O}	Distal absolute reabsorption of water
DFR_{Na}	Distal fractional reabsorption of sodium
DFR_{H2O}	Distal fractional reabsorption of water
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
ECF	Extracellular fluid
ERPF	Effective renal plasma flow
ERBF	Effective renal blood flow
FENa, FEK, FECl	Fractional excretion of sodium, potassium and chloride respectively
FF	Fitration fraction
FCM	Fat corrected milk
GHD	Growth hormone deficiency

GFR	Glomerular filtration rate
GH	Growth hormone
GHIH	Growth hormone inhibitory hormone
ICF	Intracellular fluid
IGF-I	Insulin like growth factor-I
M_{Na}, M_K and M_{Cl}	Milk sodium, potassium and chloride
M_{osm}	Milk osmolarity
MPF	Mammary plasma flow
MBF	Mammary blood flow
MF	Misty-fan cooling
MY	Milk yield
NDF	Neutral detergent fiber
NKCC2	Renal Na^+, K^+, $2Cl^-$ cotransporter
NS	Normal shade
PAH	Para-aminohippurate
(PAR_{Na})	Proximal absolute reabsorption of sodium
(PFR_{Na})	Proximal fractional reabsorption of sodium
P_{Na}, P_K, P_{Cl}	Plasma concentration of sodium, potassium and chloride respectively
P_{Li}	The concentration of lithium in plasma
Posm	Plasma osmolality
rbGH	Recombinant bovine growth hormone
rbST	Recombinant bovine somatotropin
RH	Relative humidity
rhGH	Recombinant human growth hormone
rhIGF-I	Recombinant human insuline like growth factor

RPF	Renal plasma flow
rpm	Round per minute
RR	Respiratory rate
RT	Rectal temperature
S/F	Spray and fans
SNF	Solid not fat
ST	Somatotropin
T_3	Triiodothyronine
T_4	Thyroxine
T_{Li}	The concentration of lithium in the proximal tubule fluids
TBW	Total body water
td	Dry bull temperature
TDN	Total nutrient digestibility
THI	Temperature humidity index
TMR	Total mixed ration
TNZ	thermoneutral zone
TS	Total solid
UV	Urine flow rate
$U_{Na}V$, U_KV, $U_{Cl}V$	Urinary excretion of sodium, potassium and chloride respectively
wb	Wet bulb temperature

CHAPTER I

GENERAL INTRODUCTION

Dairy herd in tropical countries are mixed exotic breed and crossbred. Crossbred cattle between *Bos Taurus* and *Bos indicus* have been utilized as an efficient tool for the high milking potential of pure Holstein Friesian (HF) breed with the adaptability of native cattle. However, low milk production and short lactation of both pure Holstein Friesian breed and crossbred dairy cattle are still the main problem in the tropic. The regulations of body fluids are different between different types of crossbred HF cattle. The lower efficiency in water retention mechanism and poor adaptation have been reported in 87.5%HF animals in comparison with those of 50%HF in tropical environment (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 to the decrease in mammary blood flow (MBF) coinciding with the decline in the plasma bovine somatotropin (bST) concentration. These changes would account for the short lactation persistency (Chaiyabutr et al., 2000). Mammary blood flow has been known to be a major determinant for milk production which related to body fluids in distribution of substrates supplying to the mammary gland (Davis and Collier, 1985). The genetic is not the only cause of the problem. Other factors can cause the low milk production such as heat stress condition. Important meteorological factors are environmental temperature, humidity, wind and radiation (Armstrong, 1994). Thailand is a tropical country which is characterized as high ambient temperatures and high humidity which may cause excessive heat load in animals. The effect of excessive heat load leads to an increase in body temperature and impairment of physiological function (Hahn et al., 1999). The physiological responses of cattle to excessive heat load are increment of both respiration rate and sweating rate (Fuquay, 1981; Hahn et al., 1999; West, 2003). Body water is known to play a central role in the mechanism of heat dissipation including the process of lactation. Thus, body water loss during heat dissipation in lactating animals may lead to a decrease in milk yield in cattle. The question then arises as to whether either high environmental temperature or genetic itself influences lactation persistency that causes the decline of milk yields as lactation advance.

Somatotropin is a protein hormone produced in the pituitary gland of all vertebrates. The action of bovine somatotropin (bST) concerns with growth and lactation. Dairy cows treated with exogenous bST are able to increase milk production in both normal thermal zone and hot environment (Staples et al., 1988; Johnson et al., 1991). The effect of bST on milk production is thought to be indirectly mediated via insulin like growth factor-I (IGF-I) (Bauman, 1992). In the previous study (Prosser et al., 1990; 1994) found that Infusion of IGF-I into the pudic artery of lactating goat has been shown to increased milk production on the infused side by directly increasing of mammary blood flow. Moreover, GH administration has been reported to stimulate plasma IGF-I and increase extracellular fluid and plasma volume in humans with severe GH deficiency (Johannsson et al., 2002). Thus, the action of IGF-I may be directly involved increase mammary blood flow or indirectly pass by increasing body fluid. A study in crossbred dairy cows on the mechanism of bST on increasing milk production may exert the galactopoietic action in part through increases in total body water (TBW) and extra cellular water (ECW) in association with an increase in mammary blood flow, in distribution nutrients to the mammary gland for milk synthesis (Maksiri et al., 2005; Chaiyabutr et al., 2007). However, long term exogenous rbST in 87.5% lactating HF animals have been shown to increase in IGF-I, mammary blood flow and milk yield in early lactation (Chaiyabutr rt al., 2004). These data did not support a role of an increase in mammary blood flow by the action of bST on milk production as lactation advanced to late lactation. These changes will be considered to be factors that influence lactation persistency. Animals can't maintain their body fluids which may result in the rapid approach of the end of their normal short lactation. However, No data is available for the relationship among body fluid and mammary blood flow relevant to milk synthesis during administrations of bST in crossbred dairy cattle. Although it has been reported in human and rats that GH can affect on a significant increased fluid retention always associate with changes of the kidney function especially hyperfiltration, antinatriuretic and antidiuretic (Ritz et al., 1991; Hirschberq and Kopple, 1992; Dimke et al., 2007).

The kidney is an important organ in the regulation of body fluids and compositions. GH has shown to affect both renal hemodynamic and renal tubular function. The mechanisms of GH appear to be mediated in part via IGF-I (Moller et al., 2000). The role of GH/IGF-I has been demonstrated to stimulate hyperfiltration such as the increases in glomerular filtration rate (GFR) and renal blood flow (RBF) (Ikkos et al., 1956, Falkheden and Sjogren, 1964; Guler et al., 1989; Jaffa et al., 1994). In many mammals, the mechanism of GH on renal tubular function may exert the direct action on renal tubule or indirect effects pass other mediators. The effects of GH on renal tubular function would stimulate solute reabsorption in various segment of tubule including proximal tubule (Guler et al. 1989; Marsh et al., 2001), distal tubule (Moller et al., 1991; Johannsson et al. 2002), which has shown 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). Furthermore, GH directly affect 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). Therefore, the real mechanism of rbST in relation to renal function changes particularly renal hemodynamic and renal tubular function has not yet been elucidated.

The administration of bST in dairy cows has been shown to exhibit a slightly higher rectal temperature during moderate and high ambient temperature (Sullivan et al., 1992). The greater heat stress is reported in some papers when bST administration improve milk yield which is probably due to an increase in heat production and may increase the severity of responses of cows to heat stress (Cole and Hansen, 1993). Furthermore, there was an evidence of the reduction of plasma concentration of triiodothyronine and cortisol in cows supplemented with bST (Manalu et al., 1988;

Johnson et al., 1991). However, it has been suggested that even though bST increases heat production, it also increases heat dissipation (Johnson et al, 1991; West, 1994; Tarazon-Herrera et al., 1999). These results may be leading to unchanged of metabolic hormone. Previous study founded that the elevation of milk production occurred by the effect of bST under heat condition without changes in plasma levels of triiodothyronine (T_3), thyroxine (T_4) and cortisol (Mohammed and Johnson, 1985; Zoa-Mboe et al., 1989). Thus, the conflicting results of rbST on metabolic hormone are interesting for study.

During lactation, water plays an important role because milk compositions composed of about 87% of water. A loss of water and electrolytes in heat dissipation will lead to decrease in milk yield in hot environment. An environmental modification to reduce ambient temperature is still necessary for improvement of productivity in lactating dairy cows. A number of techniques have been used to alleviate the effect of high ambient temperature on lactating dairy cows, for example, shade, fans, sprinklers, and dietary modifications (Beede and Collier, 1986; Huber et al., 1994; Chan et al., 1997). The evaporative cooled cow had lower rectal temperatures, sweating rate and respiration rates than those of non cooled-cows (Armstrong et al., 1993; Chen et al., 1993). A greater milk yield of cooled cows has been reported when compared with those cows receiving only shade (Chen et al., 1993). The reduction of sweating rate and respiration rates in cooled cows will affect to increase in body water pool and plasma electrolytes concentration, which may attribute to an increase in mammary blood flow (MBF) in distribution of nutrients to the mammary gland for milk synthesis. It is known that the regulation of body water will involve both the concentration of sodium ion in ECF and renal tubular sodium handling. Sodium ion concentration exerts as osmotic skeleton and sodium retention will affect to restore water in ECF. The renal function especially renal tubular sodium handling will be other factors involving changes of body fluids. No data are available for the effect of bST administration on the regulation body fluid and mammary blood flow for an increase in milk yield via the renal function in dairy cattle. The roles of bST on lactating performance in crossbred cattle under appropriate controlled environmental conditions have to be investigated.

Therefore, the present study is performed to investigate the various physiological response and the mechanism in response to supplementation of rest under shade with or without misters and fans in different stage of lactation. The effectiveness of supplemental recombinant bovine somatotropin (rbST) and misters and fans cooling on milk production relative to renal function in regulation of body fluids and mammary blood flow were investigated (Chapter IV). Moreover, the alteration of plasma hormone levels for IGF-I, thyroxin (T_4) and cortisol relating to milk production during rbST administration in different stages of lactation of crossbred 87.5% HF under misty-fan cooling system were carried out (Chapter V). To obtain more evidence of this matter, further examinations were manifested to clarify the site and mechanism of renal tubular reabsorption in response to rbST supplementation with and without mister and fan using measurements of lithium clearance including evaluation of vasopressin and aldosterone (Chapter VI). All experiments (Chapter IV-VI) were discussed in the general discussion (Chapter VII).

CHAPTER II

BACKGROUND INFORMATION

Bovine somatotropin

Bovine somatotropin (bST) is a peptide hormone produced by the pituitary gland located at the base of the animal's brain. The secretion of growth hormone is regulated by two peptides; the growth hormone-releasing factor (GFR) causes release of growth hormone and growth hormone inhibitory hormone (GHIH) or somatostatin inhibits the release of growth hormone. Growth hormone is important for growth, development, and other bodily functions of all animals. The recent technology enables new strains of bacteria of an E. coli K-12 bacterium to produce recombinant bST. Recombinant bovine somatotropin has the identical sequence for 190 of the 191 amino acids found in one of the pituitary forms. The only difference is that the amino acid methionine, which is already found in pituitary bST, has been substituted for alanine at the NH2 end of the chain. Based on extensive analysis, recombinant bST and the pituitary bST forms have essentially the same chemical structure and biological activity. However, Bovine somatotropin differs in structure from human pituitary growth hormone and is biologically inactive in the human being. Feeding bST will not exert its action in cows. The hormone bST is a complex protein that immediately broken down into small, inactive amino acids and peptides and rendered ineffective when it enters a cow's digestive system. Bovine somatotropin is cleared rapidly from the blood stream and is not stored in the body. Clearance of bST occurs by normal body mechanisms and involves breaking the protein down to amino acids. Thus, to give daily injections or use a prolonged-release formulation of bST is one need to achieve a sustained increase in milk yield. Several prolonged-release formulations have been administered by subcutaneous injection at time intervals ranging from 2 to 4 weeks (Bauman, 1992; Muller, 1992).

The mechanism of bovine somatotropin

Somatotropin is a homeorhetic controller that shifts the partitioning of nutrients so that more are used for milk synthesis. Thus, rbST administration will increase milk yields in cows. The biological effects of growth hormone can be broadly classified as either somatogenic or metabolic. Effects of growth hormone (GH) on somatogenic, growth hormone stimulate cell proliferation. These effects are indirect action of somatotropin which mediated via IGF-I. The metabolic effects are direct action of somatotropin in absorbing nutrients. This involves coordinating the metabolism of various body organs and tissues. These orchestrated changes in tissue metabolism involve both direct effects on liver and adipose tissues and indirect effects mediated by IGF-I for the mammary gland. These coordinated tissue responses involve the metabolism of all nutrient; carbohydrates, lipids, proteins, and minerals. In addition, it is thought that blood flow to the cow's mammary gland is increased. The blood carries an increased amount of nutrients available for milk production. More nutrients are extracted from the blood by the mammary gland, which improves efficiency of milk production.

Several studies (Barber et al., 1992; Flint et al., 1992) supported that the role of IGF-I are mediators of GH. Injection of IGF-I into the pudic artery has been shown to increase blood flow and milk production in lactating goats (Prosser et al., 1990; Prosser et al., 1994). An increase in plasma IGF-I level, with a concomitant increase in both mammary blood flow and milk yield in late lactation has been shown after exogenous administration of rbST in 87.5% HF cowls (Tunwattana et al., 2003). However, the galactopoietic effect of GH is not associated with the plasma level of IGF-I as lactation advances in 87.5% HF animals (Chaiyabutr et al., 2000). The plasma level of IGF-I has been shown to remain at the same level as lactation advances, despite declining of circulating GH, mammary blood flow and milk yield (Chaiyabutr et al., 2004).

Effect of bovine somatotropin on milk production

Bovine somatotropin (bST) is an important control in the coordination of nutrients to support higher milk production. Initially, changes in the partitioning and oxidation of key precursors such as glucose, amino acids, and free fatty acids provide additional substrate for milk synthesis. The effect of bST on an increase in milk yield fist well documented. The experiment of Bauman and coworker (1985) studied in high yielding cows, after recombinant bST administration would increase of up to 40% in milk production. Milk yield generally respond positively to bST (average 10 to 20%), although the response varied by study. The pattern of response is one where milk yield gradually increase over the first few days of bST treatment and reaches a maximum during the first week. If treatment is terminated, milk yield gradually returns to pretreatment levels over a similar time period. However, when treatment is continued, the increased milk yield is maintained. The result of bST administration is greater peak milk yield and an increased persistency in yield over the lactation cycle (Etherton and Bauman, 1998). Some experimental studies have been done for the effects of bST in high ambient temperature and RH. The means daily milk yields are not changed by bST. The extended period of high ambient temperature and RH may reduce the response to bST treatment (Mollett et al., 1986).

During bST administration under high ambient temperatures, the optimal bST dose may be lower which may be involved interaction of ambient heat load and increased heat production (Kronfeld, 1989). Milk yield responses are increase of 10-15% (~4-6 kg/day), though even greater increase occur when the management and care of the animals are excellent (Bauman, 1992). Thus, the management and care of the animals are still necessary for improvement of productivity in lactating dairy cows treated with bST. The concentrations of milk composition (lactose, fat, protein, minerals and vitamins) are varying as a result of factors such as genetics, breed, nutritional status, season, diet and stage of lactation. The variability in milk composition response to bST treatment was also observed by Chilliard, (1988). The increase in milk fat observed during bST administration has been reported by Mohammed and Johnson (1985). The increase in milk fat is possibly due to the increased pool of precursors for fat synthesis due to increased feed intake (Chilliard, 1988). However, other investigators reported no change in milk fat percentage even though there was an increase in total fat yield (Elvinger et al., 1988; Staples et al., 1988; Zoa-Mboe et al., 1989). Milk protein did not significantly increase in the study of Johnson et al. (1991).

Effect of bovine somatotropin on dry matter intake

Exogenous growth hormone increases the rates of milk production and provides the necessary nutrients in support of this enhanced rate of milk synthesis within the mammary gland. Administration rbST increases milk yield, feed intake do not increase until several weeks after increases in milk yield. During long-term bST administration, feed intake gradually increases to support higher milk production (Peel and Bauman, 1987). The magnitude of the increase in feed intake is dependent upon the increase in milk production, the degree of body condition change, and the nutrient density of the diets. The nutrient partitioning response to bST treatment that supports increased milk yield particularly concerns the preferential oxidation of fatty acids and the sparing of glucose by peripheral tissues. The increased substrate utilization by the mammary gland may in turn provide a stimulus for increasing feed intake (Bauman and Vernon, 1993).

Effect of bST dry matter intake has been shown to increase in a linear fashion with bST dose (West et al., 1990). The greatest intake occurs in cows administered 20 mg bST and it is 13.5% greater than controls. The percentage increase in fat corrected milk (FCM) yield is greater than that observed for DM intake, leading to an improved apparent efficiency of production. Improved apparent efficiency of production has been noted in other studies (Bauman et al., 1985; Eppard et al., 1985; Elvinger et al., 1988; Soderholm et al., 1988). Long-term experiments improves the efficiency in feed utilization for milk production (kg milk/kg feed) during somatotropin administration which appears to relate to the dilution of the feed costs for maintenance in cows higher milk production.

Effect of bovine somatotropin on mammary blood flow

The mechanism of bST for increase in milk production has not been clearly delineated. The increase in mammary blood flow could make a significant contribution to the availability of milk precursors, thus leading to enhanced milk synthesis (Johnson et al., 1991). Control of mammary blood flow (MBF) may be a way to control nutrient partitioning. Cardiac output has been shown to be 10% higher and MBF increase by 35% concurrent with a 21% increase in milk yield in bST-treated cows (Davis et al., 1988). The study in both cows and goats show an increase in the plasma IGF-I level in response to growth hormone treatment (Davis et al., 1988; Gulay et al., 2004). There are abundant IGF-I receptors in bovine mammary tissue. In contrast, attempts to detect ST receptors in bovine mammary tissue have been unsuccessful (Gertler et al., 1984; Akers et al., 1985; Keys et al., 1988). Close arterial infusion of the mammary gland with bST had no effect on milk yield (McDowell et al., 1987). Several investigations show the effect of rbST on mammary circulation is indirect mediated by IGF-I (Capuco et al., 2001). Other researches have demonstrated that the direct effect of IGF-I on an increase in the mammary blood flow and increase in milk production were noted (Etherton and Bauman, 1998). An elevation of both plasma IGF-I concentration and udder blood flow has also been noted in lactating crossbred cows treated with rbST (Tanwattana et al., 2003). Linzell (1974) reported that administration of rbST significantly increased blood flow though the mammary glands and would be achieved in part local vasodilatation, causing in distribution of milk precursors to the gland. These data confirm that mammary blood is major determining factor for supply of nutrient for milk synthesis and follows the pattern of changes of milk yield. An increase in mammary blood flow was apparent in IGF-I administration which correlated with an increase in milk yield. A role for IGF-I is also consistent with observations that IGF-I dramatically increases blood flow to the mammary gland (Prosser and Davis, 1992; Prosser et al., 1995), and this effect appears to be mediated by local production of nitric oxide (Lacasse et al., 1996; Prosser et al., 1996)

Effect of bovine somatotropin on water metabolism

Total body water is estimated to ranges from 75 to 81% of body weight in dairy cattle (Kadzere et al., 2002). Sources of water consist of drinking, feed, and metabolic (oxidation) water. Losses include water in milk, urine, feces, and various types of evaporation. Milk contains about 87 percent water. Water is the most important nutrient for lactating cows. It has been reported that somatotropin plays an important role in water regulation and probably relating to the galactopoietic effect in part through increases in total body water (TBW) and extracellular water (ECW) in association with an increase in distribution nutrients to the mammary gland for milk synthesis (Maksiri et al., 2005 ; Chaiyabutr et al., 2007). It has been reported that supplemented with bST significantly increased body water of animal due to lower body lipids (Chiliard et al., 1991).

The study in human showed that growth hormone deficiency (GHD) is associated with reduction in total body water (TBW) and extracellular water (ECW). In patients with GHD, four weeks of GH therapy appear to increase body weight, TBW and ECW. The physiological responses on GH in many species (human, rat, dog and sheep) have been similarly such as increase in fluid retention which related to IGF-I (Christ et al., 1997). Both GH and IGF-I may have a direct action on renal tubules sodium handling. GH and IGF-I increased reabsorption of sodium and water in distal tubule (Johansson et al., 2002). It caused increasing water retention, but not all studies. In some study, it has been reported that GH may contribute to fluid retention by activation of the renin-angiotensin system (Ho and Kelly, 1991).

However, the mechanism of GH on elevation of fluid retention in dairy cows was not clearly defined. Thus, growth hormone may be therefore affected on fluid retention and may be related to renal function in dairy cows.

Effect of growth hormone on renal hemodynamic

The effect of GH on renal hemodynamics has been evaluated in both human and animals. GH administration causes increased glomerular filtration rate (GFR) and renal plasma flow (RPF) in human, rat and adult sheep (Christiansen et al., 1981; Marsh et al., 2001). It is generally accepted that the effect of GH is due to an increased IGF-I production. Acute infusion of GH has no effect on GFR in humans (Parving et al., 1978). Rats treated with rGH have shown no change in the estimated GFR (creatinine clearance) as well as no change in systemic IGF-I. The report of Hirschberg et al. (1993)

illustrated of the effect of GH on induced IGF-I and stimulation of GFR. The study in healthy adults received a bolus injection of GH, plasma GH levels peaked after 2 h, and then began to decrease back towards baseline. Plasma IGF-I did not change during the first 5 h after GH-injection on the first day, and neither did GFR. By the second day, plasma IGF-I was elevated twofold, plasma GH was back at baseline, and GFR was significantly increased (Hirschberg et al., 1989). In contrast to GH, acute effects of IGF-I on GFR have been observed in humans (Hirschberg et al., 1993) and rats (Hirschberg et al., 1989; Baumann et al., 1992). Similarly, chronic treatment with IGF-I is associated with increased GFR in humans (Guler et al., 1989; Hirschberg et al., 1993) and rats (Caverzasio et al., 1990; Hirschberg et al., 1991; Hirschberg and Kopple, 1992). Both GH and IGF-I treatments raised GFR by reducing arteriolar and efferent resistance and increasing the glomerular ultrafiltration coefficient (Hirschberg, 1993). These findings have been confirmed by Hirschberg and Kopple (1992) that GFR and renal plasma flow were increased during IGF-I infusion. These changes are associated with decreased afferent and efferent arteriolar resistance and thus filtration fraction does not change. The vasodilator nitric oxide is thought to at least partly mediate these effects (Haylor et al., 1991). In addition, the study of Ritz et al., (1991) in healthy volunteers using extractive and recombinant human (rh) GH would increase effective renal plasma flow (ERPF) and GFR by 10% and 15% respectively. Renal response to GH was delayed and occurred at the same time as an increase in plasma IGF-I levels, whereas infusion of rhIGF-I promptly increased GFR and ERPF, indicating that the haemodynamic response of the kidney to GH would be mediated by IGF-I. After onset of the IGF-I infusion, RPF and GFR began to rise and renal vascular resistance fell within 20 min; renal hemodynamics remained elevated for about 2h after cessation of the IGF-I infusion. This finding supports the hypothesis that IGF-I mediates the effects of GH on renal hemodynamics (Hirshchberg and Kopple, 1989).

Effect of growth hormone on renal tubular function

The action of GH on renal tubule is not fully elucidated. In human, GH receptors are expressed in the proximal and loop of Henle. IGF-I receptors are expressed in proximal, distal, and collecting tubules in the apical and basolateral membrane. Many studies have been performed to examine the effects of GH and IGF-I on tubular functions. Giordano and DeFronzo (1995) found that human receiving IGF-I infusion for 3 h. showed a decrease in the fractional urinary excretion of sodium approximately 50%. IGF-I increased the Na channel activity about 3-fold and increased Na channel conductance through IGF-I receptor activation in distal tubule (Gallego et al., 1995). IGF-I does not activate sodium absorption in cortical collecting ducts (Vehaskari et al., 1989). Thus, IGF-I may mediate the effects of GH to Na reabsorption in distal tubule.

It has been reported that the sodium and water retaining rapidly by the effect of GH took place in the distal tubule and mediated by an interaction with the renin-angiotensin-aldosterone system (Ho and Weissberger, 1990; Moller et al., 1991; Johansson et al., 2002). Several studies (Moller et al., 1999; Johansson et al., 2002) have shown that the primary effect may occur by increase in plasma renin, which in turn will increase serum angiotensin II and thereby increase directly the sodium retaining effect in renal tubules. This finding indicates that the rennin-angiotensin-aldosterone system is responsible for the antinatriuretic effect of GH. In contrast, GH administration in GH-deficient patients and normal subjects for 2 weeks founded that the extracellular volume increased but the plasma volume did not change. Plasma renin, angiotensin II and vasopressin did not change, and aldosterone tended to decrease during GH treatment as compared to placebo (Moller et al., 1995). However, plasma atrial natriuretic peptide decreased significantly with administration of GH. These investigators have suggested that GH-induced fall in plasma atrial natriuretic peptide, which may cause or contribute to the reduced natriuresis resulting in sodium and fluid retention. In addition, the study by Dimke et al., (2006) have shown that acute GH administration reduced urinary excretion of electrolytes (Na^+, K^+, Cl^-) which may be involved indirect activation of renal Na^+, K^+, $2Cl^-$ cotransporter in the medulla thick ascending limbs (mTAL).

Bovine somatotropin during hot weather

Effects of bST injection on milk production in dairy cows in hot condition are still conflicting. Several studies reported that exogenous administration of bST in dairy cows enhanced milk yield during hot climatic conditions (Elvinger et al., 1988; West et al., 1990; Johnson et al., 1991). However, the range of response is wide and may reflect the effects of environment specific to each study. Milk yields were 22.7, 18.1, and 13.6 kg/d decreased yield by 5.4, 3.6, and 2.7 kg/d when THI increased from 72 to 81 (Johnson et al., 1963). Cows at high milk production in heat environment had a greater decline of milk yield response than cows at low milk yield. West and coworkers (1990) reported in rbST treated cows exposed to mean maximum and minimum THI of 85.8 and 71.5 that body temperature was increased by 0.2 and 0.3 °C at a.m. and p.m. milkings, respectively. Cows treated with bST increased milk yield, but the response to bST depended on the pretreatment yield. Cows at low milk yield in pretreatment production had a greater milk yield response to bST than cows at higher milk yield production (both in kilograms of milk and percentage increase). Despite the improved milk yield that occurs in response to bST use in hot environments, potential exists for greater heat stress with the use of bST. It is probably due to an increase metabolic activity and heat production associated with higher milk yield (Igono et al., 1985). During heat stress, administration of bovine somatotropin increased milk yield by 4% and heat production by 10%, although energy intake declined further than with heat alone. Even though bST increases heat production, it also increases heat dissipation (Johnson et al., 1991; West, 1994). The bST-treated cows had higher heat production (19 and 25%) and heat loss (36 and 24%) than controls cows under natural environment and hot conditions, respectively. Increased heat production is the range that could be dissipated by the cows during bST treatment (Manalu et al., 1991). Total evaporative heat loss increase for cows in high temperature versus thermoneutral zone and for cows receiving bST. Cows receiving bST and exposed to heat stress conditions perform normally when the additional heat is dissipated. Rectal temperatures of controls and of cows receiving bST have been shown to diverge as the day progressed, reflecting increased heat load from ambient conditions and the inability of cows to dissipate the greater heat production associated with greater milk yield. Feed intake and milk yield are sharply depressed at high temperature versus low RH under similar high ambient temperatures (Ragsdale et al., 1953). A study conducting during summer has been shown that THI was relatively constant, rectal temperatures of cows administered bST were different from those of controls only on those days when RH was elevated (Sullivan et al., 1992). Thus, high milk yield cows exposure to direct sunlight with administration of bST will have greater heat stress and higher rectal temperatures than the un-shaded cows only (Elvinger et al., 1992). However, milk yield responses to bST administration show tend to increase during summer, demonstrating the ability of cows to maintain milk yield with bST despite high environmental temperature.

The response of cow to heat stress depends on the rate of milk yield, an environment to which the cow is exposed and management strategies. Many technologies and practices that apply to manage the high yielding cow exposed to high ambient temperatures, high RH, or both, should affect to the cow administered bST during exposure to these conditions. Management strategies will maintain sufficient DMI to sustain the potentially increased milk yield because of bST technology (West, 1994)

Physiological response in dairy cows to heat stress

The thermoneutral zone (TNZ) of lactating dairy cows is the most comfortable environment temperature and prefers ambient temperature between 5 and 25 °C. The normal body temperature of a dairy cow is about 38.61 °C. Body temperature is usually maintained by thermoregulatory system within 1 °C under normal ambient conditions that do not expose to heat stress (Bligh, 1973). When temperature is above the upper critical value which is exceeded about 26 °C, the cow begins to show signs of heat stress. Environment factor influences heat stress of lactating dairy cows such as ambient temperature, humidity and radiant energy. In addition, lactating dairy cow is particularly sensitive to heat stress because of metabolic heat load, as a result of milk synthesis, is proportionate to production levels (Beede and Shearer, 1991). Currently, both ambient temperature and humidity are combined to derive the temperature-humidity index (THI). The temperature humidity index (THI) is commonly used for assessing thermal stress. West (1994) has suggested that THI calculated from dry bulb temperature and relative humidity with values of 70 or less are considered comfortable. When a mean daily THI exceeds 72, lactating dairy cows would be in heat stress. THI above 80 has been shown to cause severe heat stress (Wiersma, 1990) with lactating cows being unable to maintain thermoregulatory mechanisms or normal body temperature.

During heat exposure, cow also responds by increasing heat loss mechanisms. As environmental temperature near the body temperature, the effectiveness of for dissipation of body heat via radiation, conduction and convection will be reduced. From these results, a greater evaporation of moisture from the skin surface and a more rapid respiratory rate is necessary to maintain heat balance. The importance of water as a medium for ridding the body temperature of excess heat through sweating and respiration greatly increases as the ambient temperature rise. However, high relative humidity (RH) will reduce the efficiency of evaporative cooling. Thus, high environmental temperature, often coupled with high RH, can overwhelm the cow's cooling capability, and body temperature rises. Body responses of the cow to temperature above the TNZ are varied. These responses include raised respiration rates and rectal temperature (Omar et al., 1996), panting, drooping, reduce heart rates and profuse sweating (Blazquez et al., 1994), decreased feed intake and milk production. Lemerle and Goddard (1986) reported that the respiration rate would begin to increase above a THI value of about 73 and it would probably increase steeply at THI values > 80, although rectal temperature only increased when THI was greater than 80. Maia et al., (2005) reported that lactating cows dissipated heat by evaporation from the skin surface with sweating (85%) and the remainder was due to respiratory system by panting during exposure to high temperature and humidity (Kibler and Brody, 1952; Finch, 1986; Maia et al., 2005).

Physical responses to heat stress in dairy cows appear to be breed-specific (Finch, 1986). The *Bos indicus* and other tropical breeds are less responsive to thermal stress than *Bos taurus* cattle. The differences in response to heat stress between cattle breeds are attributed to varying levels of adaptability to hot environments. Sharma et al. (1983) has shown that within *Bos taurus* dairy cattle breeds, the Jersey is less sensitive to thermal stress than the Holstein-Friesian.

Effect of hot environment on milk production and dry matter intake in dairy cows

High environmental temperatures are costly in terms of reduced milk yield. Environmental conditions such as temperature and humidity are interrelated. The combined effects must be considered on intake and milk yield. THI may describe more precisely the effects of the environment on the cow's ability to dissipate heat. In Europe, milk yield of cow has shown slight decrease when the THI exceed 72 and decrease sharply when THI exceed 76. The effect of hot environments on cow performance is apparently mediated through the body temperature of the cow. The mean daily temperature had the greatest effect on milk yield and rectal temperature when compared with minimum and maximum temperatures (Kabuga and Sarpong, 1991). Each 1 °F increase in body temperature above 101.5 °F resulted in 4 and 3 lb decreases in milk yield and TDN intake, respectively (Johnson et al., 1963). Holter and coworkers (1996) reported that the minimum daily THI was more closely correlated with DMI than maximum THI in Jersey cows. Reductions in DMI commenced when minimum THI exceeded 56 to 57 and continued until THI reached 72. In addition, Maust et al. (1972) reported that DMI and milk yield for cows in mild-lactation were reduced by hot weather condition. It is possible that in early lactation, cows rely on body stores for a portion of the nutrients for production whereas in late lactation, cows consume fewer total nutrients.

Water metabolism of dairy cows during hot weather

In dairy cattle, total body water is estimated to ranges from 75 to 81% of body weight (Kadzere et al., 2002). Sources of water consist of drinking, feed, and metabolic (oxidation) water. Water losses include water in milk, urine, feces, and various types of evaporation. Among environmental factors, temperature and humidity are considered to be important in controlling water intake in lactating dairy cows. Water is the most important nutrient for lactating cows subjected to heat stress. Milk contains about 87 percent water, and water is critical form for dissipation of excess body heat. Water intake is highly correlated with milk yield and DMI (correlation coefficients of 0.94 and 0.96, respectively) (Dado and Allen, 1994).

Heat stress simultaneously influences both energy and water metabolism (Silanikove, 1992). Ruminants exposed to environmental heat appear to increase their total body water content and the volume of extracellular fluid as well as the rate of water turnover (Macfarlane, 1965; Seif et al., 1973). An increases plasma and extracellular fluid volume in proportion to the thermoregulatory requirement of the stressed cows.

Effect of cooling on heat stress, intake and milk yield in dairy cows

Environmental modification may be reducing severe heat stress in dairy cattle such as shade, water spray and fan and evaporative cooling (Armstrong et al., 1994). Shading in a hot, humid climate has been shown to reduce rectal temperature by 2% to 4.1%, respiration rate by 29% to 60%, improved DMI by 6.8% by 23.2%, and improved milk yield by 9.4% to 22.7% compared with un-shaded cows (Schneider et al., 1984; Mallonee et al., 1985; Schneider et al., 1986). The use of evaporative cooling and spray and fans (S/F) can prevent heat stress. The study of Abelardo and coworker (2004) for the effect of two different cooling systems on physiological responses during the summer revealed that the maximum temperature humidity index during the trial was from 73 to 85. Both spray and fans and evaporative cooling system would reduce rectal temperature and respiratory rate and increase in the comfort of cows. The implementation of a cooling program can increase in milk production and improved reproductive performance (Ryan and Boland, 1992; Armstrong, 1994). Similarly, the study of Chen and coworker (1993) reported that cows under shade plus evaporative cooling had a higher milk yield (2.5 kg/day) and DMI, but lower rectal temperature and respiration rate than cows under shade only.

Effect of heat stress on renal function

During high ambient temperatures, potassium ion loss through skin secretions may be substantial because the major inorganic constituents of bovine sweat are K_2CO_3 and $KHCO_3$. Increased sweating with marked increases in K^+ ion loss through skin secretions has been observed during elevated ambient temperature from 35 and 45 °C (Johnson, 1967). Cow exposure to high temperatures show renal compensation with decreasing rate of urine flow and increasing urinary excretion of Na^+ and renal conservation of K^+ (Collier et al., 1982). Reduction in the plasma aldosterone level has been observed during heat stress, which indicates the K status (El-Nouty et al., 1980; Beede et al., 1982). El-Nouty and cowoker (1980) reported that reduction of plasma aldosterone were apparent in Holstein cows subjected to 35 °C of ambient temperature, which was associated with a significant reduction in serum Na level, increased urinary Na^+ excretion, and decreased K^+ in serum and urine. Reduced plasma aldosterone aids in conservation of K at the expense of Na. However, hot environment showed no alteration in glomerular filtration rate (GFR) resulting from autoregulatory mechanism of kidney. In contrast, renal blood flow had a tendency to increase during prolonged heat expose (Alverez and Johnson, 1973).

Renal tubular handling of water and sodium

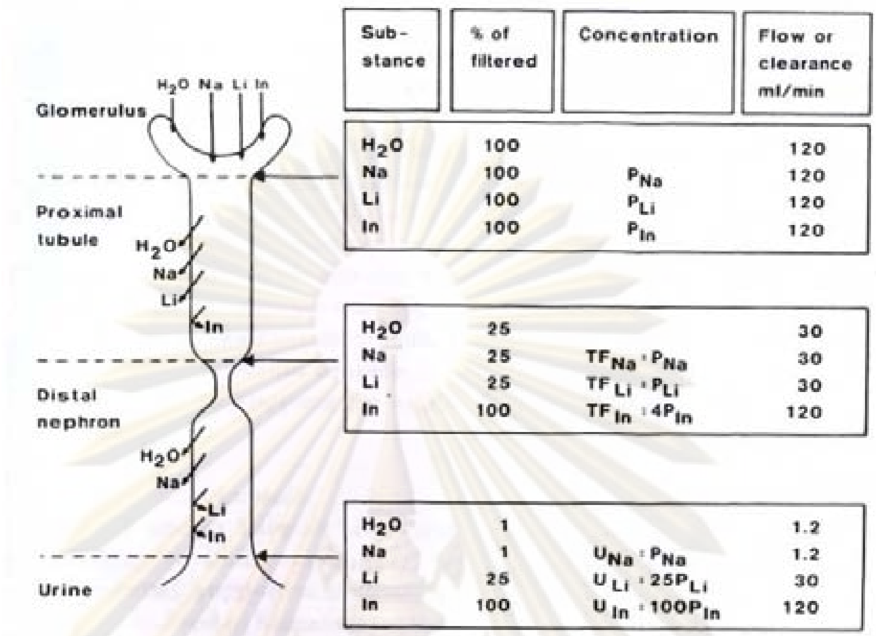
The reabsorption of Na plays a major role to regulate body electrolyte and H_2O homeostasis. Reabsorption of most of the tubular sodium occurs in the proximal tubule. About 65% percent of sodium is reabsorbed in the proximal tubules because of the active transport of sodium ion or Na^+, K^+ ATPase pump in the proximal tubular epithelial cells. When the sodium ion is reabsorbed, the positive charge of the sodium ions causes passive diffusion or co-transport of negative ions such as chloride ions. Then the cumulative reabsorption of ions creates an osmotic pressure difference that move water though the membrane. The epithelial cell is so permeable to water that almost the same identical proportion of water and sodium ions is reabsorbed. For loop of Henle, about 27 percent of sodium is reabsorbed, leaving 8 percent to enter the distal tubules. In segment of the loop is almost impermeable to water. Therefore, the concentration of sodium ions in tubular fluid falls quite low in the ascending limb before it enters the distal tubules. Sodium reabsorption in the distal tubule is controlled mainly by the concentration of aldosterone.

Lithium clearance (C_{Li})

Lithium is a monovalent alkali cation which includes Na^+ and K^+. Li^+ enters into the cells (Radomski et al., 1950; Schou, 1958). The lithium clearance technique is an indirect method for the estimation of delivery of sodium and water from the proximal straight tubules to the loops of Henle. Lithium ion is reabsorbed in the proximal tubules in the same proportion as sodium ion and water. No Li^+ reabsorption or secretion occurs in distal nephron (Thomsen, 1984; 1990). The amount of lithium is delivered from the straight proximal tubules ($V_{prox} \times TF_{Li}$) is equal to the amount is excreted in the urine ($V \times U_{Li}$).

$$C_{Li} = \frac{U_{Li} \times V}{P_{Li}} \quad \text{is equal to} \quad \frac{TF_{Li} \times V}{P_{Li}}$$

Where; TF_{Li} and P_{Li} are the concentration of lithium in the proximal tubule fluids and plasma, respectively. Since $TF_{Li} = P_{Li}$ in the proximal tubules. It can be seen that $C_{Li} = V_{prox} = C_{Na\ prox}$, is presented in figure 2.1. Figure 2.1 shows the percentage of unreabsorption of the substance (Li^+, Na^+, In and H_2O), the concentration of the substance, and its flow or clearance immediately after the filtration, at the end of the proximal tubule and in the urine.



Sub-stance	% of filtered	Concentration	Flow or clearance ml/min
H_2O	100		120
Na	100	P_{Na}	120
Li	100	P_{Li}	120
In	100	P_{In}	120
H_2O	25		30
Na	25	$TF_{Na} = P_{Na}$	30
Li	25	$TF_{Li} = P_{Li}$	30
In	100	$TF_{In} = 4P_{In}$	120
H_2O	1		1.2
Na	1	$U_{Na} = P_{Na}$	1.2
Li	25	$U_{Li} = 25P_{Li}$	30
In	100	$U_{In} = 100P_{In}$	120

Figure 2.1 Tubular reabsorption and rejective of lithium in comparison with that of water, sodium and inulin (Thomsen, 1984).

The fractional delivery of lithium at the end of the proximal tubules is equal to the fractional delivery of sodium and water at proximal tubule. Li^+ is not normally present in body in significant amounts and may be passively transported through Na^+ channel or actively reabsorbed in the same manner as Na^+ transport process (Holstein-Rathlou, 1990). The common technique used to evaluate proximal and distal tubular reabsorption of substance is micropuncture. The study of Hayslett and Kashgarian (1979) demonstrated that Li^+ was reabsorbed in the proximal tubule in the same extent as Na^+ and H_2O by using micropuncture technique, which the $(TE/P)_{Li}$ ratio being 1.0 at the end of proximal convoluted tubules, and no Li^+ reabsorption took place in distal convoluted tubule and collecting ducts. However, the tubular segments between the late proximal convoluted and the early distal tubules are not accessible to micropuncture. The tubular reabsorption in the loop of Henle can not be measured separately. Hayslett and Kashgarian, (1979) suggested that the quantitative reabsorption of lithium between the late proximal convoluted and the early distal tubules accords with the notion that lithium is reabsorbed to the same extent as sodium and water in the straight proximal tubules and that no measureable amount of lithium is reabsorbed in the loop of Henle. The lithium clearance is measured the reabsorption of water and sodium in the proximal tubules and in the distal nephron separately.

Effect of heat stress on hormonal control

Hormones associated with metabolism tend to be reduced in plasma of chronically heat-stressed cows. These include thyroid hormone [thyroxine (T_4) and triiodothyroxine (T_3)] and growth hormone. As a general observation, it is well known that heat acclimation decreases endogenous levels of thyroid hormone (Horowitz, 2001), which is probably an attempt to reduce metabolic heat production in the cow. Growth hormone (bST) may play important roles in acclimation through improved insensible heat loss and regulation of sweat gland function (Manulu et al., 1991). Secretion rate of growth hormone was declined in hot temperature. Reduction in concentration of these hormones may be associated with metabolic adaptation of cattle to chronic thermal stress (Collier et al., 1982). In addition, cortisol response to heat stress challenge is often variable. West et al., (1998) reported that the effects of increasing fiber content of the diet on the response to hot, humid weather reported a linear increase in plasma cortisol.

Effect of growth hormone on plasma aldosterone concentration

Aldosterone is a steroid hormone. It is the major mineralcorticoid and it therefore controls electrolyte levels, blood pressure and hydration. Aldosterone is synthesized from corticosterone by oxidation of the C-18 methyl group to form an aldehyde in the zona glomerulosa of the adrenal cortex. Aldosterone is normally released in response to angiotensin II and potassium. Aldosterone acts on the distal tubules and collecting ducts of the kidney to cause the conservation of sodium, secretion of potassium, increased water retention, and increased blood pressure. The overall effect of aldosterone is to increase reabsorption of ions and water in the kidney. Aldosterone has three main actions:

1. Aldosterone act on the nuclear mineralocorticoid receptors (MR) within the principal cells of the distal tubule and the collecting duct of the kidney nephron, it increases the permeability of the apical (luminal) membrane to potassium and sodium and activates the basolateral Na^+/K^+ pumps, stimulating ATP hydrolysis leading to phosphorylation of the pump and a conformational change in the pump exposes the Na^+ ions to the outside. The phosphorylated form of the pump has a low affinity for Na^+ ions, hence reabsorbing sodium (Na^+) ions and water into the blood, and secreting potassium (K^+) ions into the urine. (Chloride anions are also reabsorbed in conjunction with sodium cations to maintain the system's electrochemical balance.)
2. Aldosterone act on the collecting duct via intercalated cells to secret H^+, regulating plasma bicarbonate (HCO_3^-) levels and its acid/base balance.
3. Aldosterone may stimulate vasopressin (ADH) by posterior pituitary gland in central nervous system which serves to conserve water by direct actions on renal tubular reabsorption.

Control of aldosterone release from the Adrenal Cortex, is involved renin-angiotensin system. Angiotensin II acts synergistically with potassium, and the potassium feedback is virtually in operative when no angiotensin II is present. A small portion of the regulation resulting from angiotensin II must take place indirectly from decreased blood flow through the liver due to constriction of capillaries. When the blood flow decreases, aldosterone was destructed by liver enzymes.

Effect of GH treatment on alteration of aldosterone was also examined in humans and rats (Ho and Weissberger, 1990; Moller etal., 1991; Moller etal., 1997; Hansen et al., 2001). After GH-administration, Plasma aldosterone is either reported to increase or remain unchanged (Venning et al., 1956; Wyse et al., 1993; Hayes et al., 1997; Johannsson et al., 2002). However, GH administration of adrenalectomized rats for three days showed an immediate decrease in sodium excretion, in the same manner as normal rats (Stein et al., 1952). Moreover, within 24h after GH-administration, sodium retention is observed in a bilaterally adrenalectomized patient (Biglieri et al., 1961). It therefore seems unlikely, that either the acute and chronic effects of GH are mediated through aldosterone, although modification of aldosterone levels may be involved in the compensatory changes during long term GH-treatment.

Effect of growth hormone on plasma arginine vasopressin or antidiuretic hormone (ADH)

Vassopressin is a 9 amino acid polypeptide with a disulfide bond. In most mammals, the natural hormone is arginine vasopressin. Vasopressin or antidiuretic hormone (ADH) promotes the renal reabsorption of solute-free water in distal convoluted tubules and collecting duct. ADH increases cyclic adenosine monophosphate (cAMP) at the tubule which increases water permeability at the luminal surface resulting in increased urine osmolality and decreased urine flow. The mechanism by which heat causes a rise in ADH is unclear. Wyndham et al. (1968) suggested that the immediate stimulus to ADH secretion might be the redistribution of blood from the center to the periphery of the animal's body, perhaps by massive dilatation of the skin vessels. The consequent alteration of the tone of the volume-receptors in the heart or main veins might elicit ADH secretion (Johnson et al., 1969). Forsling et al. (1976) found that ADH secretion was suppressed by simultaneous cooling of several parts of the central nervous system containing temperature-sensing neurons. This suggests at least some connection between neurons responsive to temperature and those concerned with releasing ADH from the neurohypophysis. The hyperthermia in bovine is associated with significant increase in ADH with little or no effect on urine output.

It has been reported in healthy adults that the effect of short GH- administration did not change systemic AVP concentrations (Ho and Weissberger, 1990; Moller et al., 1991). Moreover, chronic GH-administration in children with GHD showed no change in urinary cAMP levels (Gertner et al., 1979; Chipman et al., 1980). These results suggest that chronic GH-administration stimulates renal transport mechanisms independent of AVP. The similar result appears to be the case in the acute setting. The study of Biglieri et al., (1961) have shown that GH injection in a patient with diabetes insipidus showed the same acute anti-natriuretic and anti-kaliuretic responses as normal controls. According to the phosphorylation of Ser256 and changes in subcellular distribution of AQP2, this is a hallmark of vasopressin action (Nielsen et al., 1995; Fushimi et al., 1997; Katsura, et al., 1997; Nishimoto et al., 1999; Christensen et al., 2000; Zelenina et al., 2000; Nielsen et al., 2002). The study in rats for 5 h after rGH administration has been shown of unchanged in the relative abundance of Ser256-phosphorylated AQP2 as well as the apical abundance of AQP2 (Dimke et al., 1996). These results suggest that vasopressin is unaltered during acute GH-administration (Dimke et al., 2007).

Effect of growth hormone and hot weather on plasma thyroxine (T_4) concentration

Thyroxine or 3':5' tetraiodothyronine (T_4) is the major hormone secreted by the follicular cells of the thyroid gland. Thyroid secretion is controlled by a thyroid-stimulating hormone (TSH), which is secreted by anterior pituitary hormone. TSH is proteolysis of the thyroglobulin, which causes release of T_3 and T_4 into blood. Thyroxine is formed by the molecular addition of iodine to the amino acid tyrosine. T_4 is transported in blood, with 99.95% of the secreted T_4 being protein bound, principally to thyroxine-binding globulin (TBG), and, to a lesser extent, to transthyretin and serum albumin. T_4 is involved in controlling the rate of metabolic processes in the body and influencing physical development.

In cattle, serum T_3 and T_4 were reduced in cows moved from thermoneutral to heat stress condition (Mohammed and Johnson, 1985). High environmental temperatures reduced thyroid activity, leading to decreased plasma concentrations of T_3 (Magdub et al., 1982). The mechanisms by which hot condition depress thyroid function are unclear because of varied information on $T_4$and T_3 and in blood plasma of cattle under heat stress. There is no evidence of both reduced number of binding sites or decreased excretion of T_4 and T_3 in lactating cattle under heat stress (Vanjonack and Johnson, 1975; Hart et al., 1978, Valtorta, 1979). In contrast with the study of Magdub et al., (1982) founded that the reduction in plasma T_4 and T_3 was positively correlated with excretion of T_4 and T_3 during heat condition. This result suggests that environmental heat reduced synthesis of both hormones. The decreased in T_3 and T_4 plasma hormones concentrations may be associated with metabolic adaptation of cattle to chronic thermal stress. For bST administration, Capuco et al. (1989) demonstrated that exogenous bovine somatotropin (ST) increased the activity of thyroxine-5'- mondeiodinase in mammary tissue of cows. The decreased ST concentration trend observed in TS could control monodeiodhation, leading to elevated T_4 and diminished T_3 concentrations in the short term until T_4 synthesis is reduced. The decreases in plasma concentration of thyroxine observed in bST-treated cows under heat condition (Johnson et al., 1991) are contrary to results of several studies reported that no consistent changes in plasma concentration of thyroxine in response to growth hormone under heat stress (Mohammed and Johnson, 1985; Zoa-Mbae et al., 1989).

Effect of growth hormone on plasma cortisol concentration

Cortisol is a corticosteroid hormone or glucocorticoid produced by the adrenal cortex, that is part of the adrenal gland (in the zona fasciculata and the zona reticularis of the adrenal cortex). It is usually referred to as the "stress hormone" as it is involved in response to stress. Cows exposed to heat stress had increased serum cortisol (Wise et al., 1988).

Effect of rbST on cortisol had reported in many studies. Serum cortisol was not affected by rbST (Gullermo et al., 1990; West et al., 1991), although the responses of heat-stressed cows to bST treatment varied among reports. Johnson (1991) has shown that somatotropin reduced plasma concentrations of cortisol under hot and normal environment. Because increased glucose production may cause a decrease in plasma cortisol, these reduced concentrations of cortisol were probably due to glucose-sparing effect of bST (Vanon, 1988). In addition, IGF-I receptor are expressed in the adrenal gland of humans and bovines. Thus, IGF-I infusion may induce cortisol secretion. Dipaliraha and coworker (2007) have studied the adrenocortical effects of IGF-I in guinea pig, which showed that prolonged IGF-I administration raised the plasma concentration of cortisol, It is possible that IGF-I may act by activating the hypothalamic-pituitaryadrenal axis and the renin-angiotensin system.

The effect of somatotropin on plasma insulin like growth factor I (IGF-I) concentration

Insulin-like growth factor 1 (IGF-1) or calls somatomedin C is polypeptide protein hormone, structurally similar to proinsulin. IGF-1 consists of 70 amino acids in a single chain with three intramolecular disulfide bridges. IGF-1 has a molecular weight of 7649 daltons. IGF-I synthesized in the liver. In human, plasma concentrations of IGF-I are regulated by growth hormone, insulin, age and nutritional state. Growth hormone and insulin are the main regulators of hepatic IGF-I production. In cattle, treatment with bST increases concentrations of IGF-I in plasma (Bilby et al., 2004) and milk (Torkelson et al., 1988; Prosser et al., 1989). Concentrations of IGF-I in plasma begin to increase about 6-12 hr after the initial bST injection and reach maximum concentrations in approximately 48 hr (Cohick et al., 1989). IGF-I has a specific receptor, which is structurally and functionally very similar to the insulin receptor. Specific IGF receptors were presented on many cell types in many tissues. Receptors IGF-I were presented in bovine mammary tissue and increase in number during lactogenesis (Dehoff et al., 1988). Glimm et al. (1988) found that lactating cows treated with exogenous bST increased IGF-I in mammary tissue. Further, IGF-I stimulated DNA synthesis in bovine mammay tissue in vitro (Baumrucker and Stemberger, 1989). Thus, effects of bST at the mammary gland may be mediated by IGF-I (Peel and Bauman, 1987). In the lactating dairy cow, nutritional status plays a key role in the regulation of somatomedins and their binding proteins (Clemmons and Underwood, 1991; McGuire et al., 1992b). Restriction of dry matter intake or undernutrition has no effect on basal blood concentrations of IGF-I, but administration of bST results in a less dramatic increase in circulating IGF-I than when animals have an adequate nutritional status (Breieret et al., 1988; Ronge and Blum, 1989; McGuire et al., 1992a).

CHAPTER III

MATERIALS AND METHODS

Animals preparation

The experiments were conducted in the training farm of Faculty of Veterinary Science, Nakornpathom province. Twenty primiparous, 87.5% lactating crossbred Holstein cattle were used for two experiments of ten animals each. The experiments in Chapter IV and Chapter V were performed in year 2006. During year 2007 to 2008, the experiment was performed in Chapter VI. On each specified experiment, ten lactating crossbred Holstein cattle with 60±1 days postpartum at start of experiment were divided into two groups of five animals each. Animals in the first group were housed under normal shaded barn (NS) as non-cooled cows and animals in the second group were housed under normal shaded barn plus misters and fans (MF) as cooled cows. The open-sided barn with a tiled-roof (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 under normal shade and the second part of barn for cooled cows under shade plus two misters and fans systems. Misters-fans cooling consisted of a 65 cm. diameter blade fan circulating 81 m^3/min of air, with oscillation coverage of 180°. 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 misty-fans for 45 minutes at 15-minute intervals from 06:00 h to 18:00 h. At night, animals were exposed to misters-fans for 15 minutes at 45-minute intervals from 18:00 h to 06:00 h. Ambient temperature and humidity in NS and MF barn 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 using psychometric chart depending on dry and wet bulb thermometer. The temperature humidity index (THI) was calculated according to West (1994), as follow:

THI = td-(0.55-0.55RH) (td-58)

Where; td = dry bulb temperature (°F), and RH = relative humidity.

The experimental studies in both cooled and non-cooled cows were carried out in different stages of lactation (early lactation, mid lactation and late lactation). In each stage of lactation, experiments were divided in pretreatment and treatment periods. Pretreatment in each period was started on each specified day. All injections were administrated at the tail head depression (ischiorectal fossa). 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 of lactation. The experimental studies were performed as follow;

The renal hemodynamics relating to body fluids and mammary blood flow in both cooled and non-cooled cows treated with rbST in different stages of lactation were performed in Chapter IV.

Changes of hormone concentrations relating to milk production in both cooled and non-cooled cows treated with rbST in different stages of lactation were studied in Chapter V.

Alterations of water intake, renal tubular handling of sodium and body fluids of both cooled and non-cooled cows treated with rbST in different stages of lactation were studied in Chapter VI.

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 3.1). Individual feed intake was recorded daily and analyzed for dry matter intake (DMI). Samples of TMR were 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). Feed ingredients and chemical compositions of the TMR diet are shown in Table3.1

Animals were normally milked twice daily at approximately 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 weight.

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.

Table3.1. 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

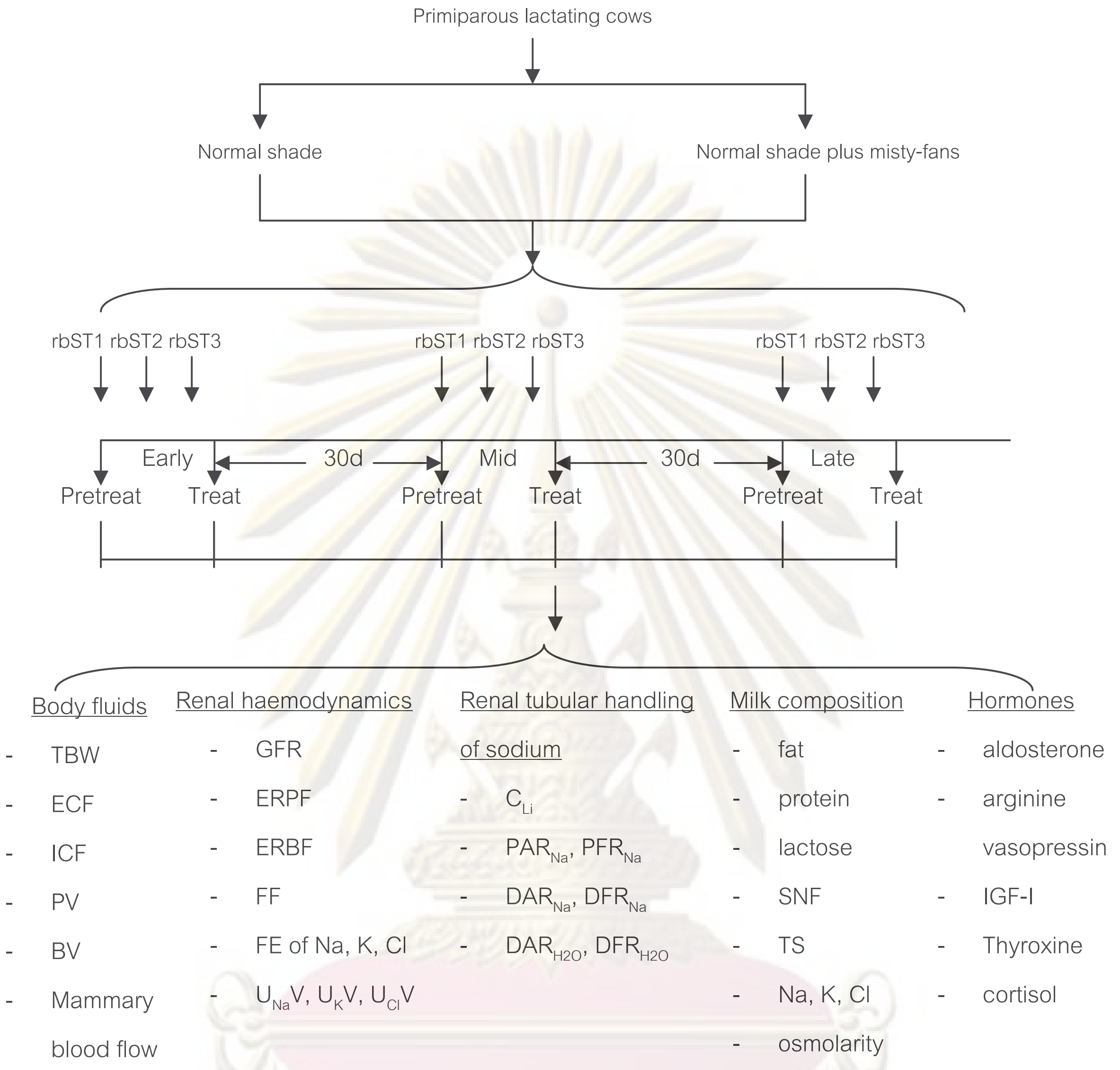


Figure 3.1 Schematic diagrams illustrating the time course of the experiment in each cow supplemented with rbST at different stages of lactation. Pre-treat = timed study for pre-treatment; Treat = timed study for treatment.

Experimental procedures

Determination of mammary blood flow

On the day of both pretreatment and treatment studied mammary blood flow (MBF) in the morning around 9.00-10.00 am.. Cows were inserted with two polymer catheters (i.d. 1.0 mm, o.d. 1.3 mm, L 45 mm; Jelco, Critikon; Johnson & Johnson, UK) into either the left or right milk vein using an intravenous polymer catheter, under local anesthesia. The tip of the catheter was positioned near the sigmoid flexure anterior to the point at which the vein leaves the udder. The other catheter was positioned downstream about 20 cm. Blood flow through half of the udder was determined by measuring of dye T-1824 (Evans blue) dilution by short term continuous infusion and adapted from the method of measuring blood flow in the milk veins of cattle as described previously (Chaiyabutr et al., 1997). The dye was infused by peristaltic pump, at a constant rate of 80 ml/min into milk vein for 10 sec. Before infusion, blood was drawn from downstream in the milk vein as a pre – infusion sample. About 10 sec after starting the infusion, 10 ml of blood was drawn from downstream in the milk vein at a constant rate into heparinized tube. Three consecutive blood samples were taken during dye infusion. The mammary blood flow was therefore calculated by doubling the flow measured in one milk vein. Packed cell volume was measured after centrifugation of the blood in a microcapillary tube. Mammary blood flow was calculated from equation.

$$\text{Mammary plasma flow (MPF; ml/ml)} = \frac{I(C_I-C_V)}{(C_V-C_A)}$$

$$\text{Mammary blood flow (MBF; ml/min)} = \frac{MPF}{1-PCV}$$

Where I = dye infusion rate

CI = concentration of dye infusion

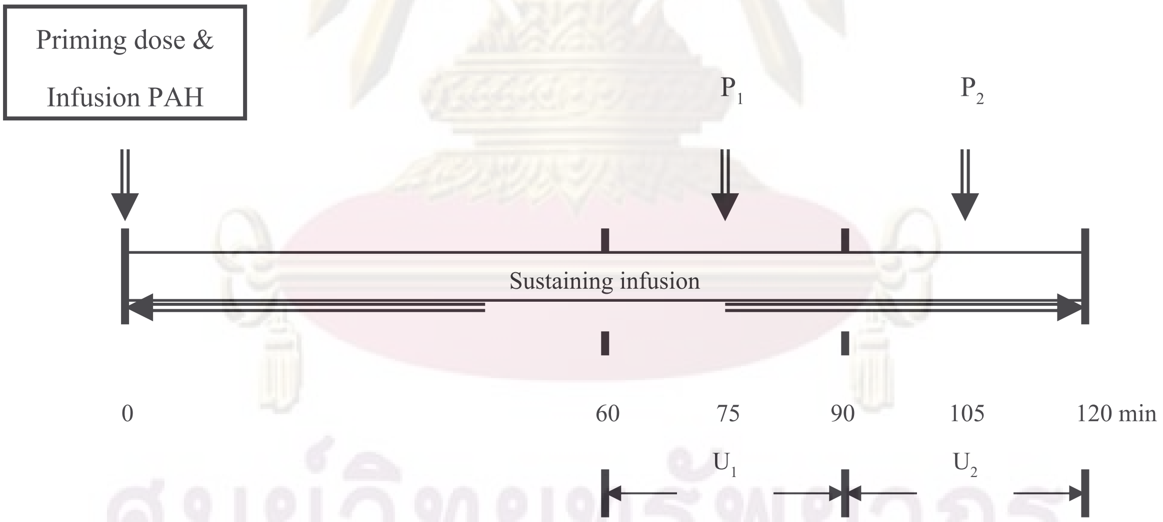
CV = concentration of dye in plasma milk vein

CA = concentration of dye in plasma artery

Determinations of renal function

After the end of study in 2.1, the catheter was inserted into ear vein under local anesthesia with xylocaine for infusion of para-aminohippurate (PAH) solution for renal clearance study. Experiment was started by infusion of priming dose with 20 ml of 2.5% PAH solution via ear vein and follow with infusion of 0.5% PAH in normal saline at the rate of 2 ml/min continuously for 60 minutes to stabilize the plasma PAH concentration. After equilibration period, duplicate of urine collection (U1, U2) along with coccygeal blood sampling at midpoint of urine collection (P1, P2) will be performed. Urine samples were collected 30 minutes via Foley balloon catheter after urine empty bladder. Urine volume was measured after collection. Blood samples were collected for determinations of packed cell volume. Blood was centrifuged and was separated. Plasma and urine samples were kept at -20 °C for analysis of endogenous creatinine, PAH, sodium, potassium, chloride ion and osmolarity. The PAH concentration was determined by the method of Smith (1962). The endogenous creatinine concentration was analyzed by alkaline picrate (Smith, 1956). Sodium and potassium were measured by flame photometer. (Flame photometer 410C, Ciba Corning Inc., USA). Chloride was measured by chlorimeter (Chloride analyzer 925, Ciba Corning Inc., USA). Osmolality was measured by osmometer (Osmometer 3D3, Advance Instrument Inc., USA).

The protocol for renal clearance study was demonstrated as the following;



The renal functions were evaluated by measurements as follow;

- Glomerular filtration rate (GFR)
- Effective renal blood flow (ERBF)
- Effective renal plasma flow (ERPF)
- Filtration fraction (FF)

- Fractional excretion of sodium, potassium and chloride (FE_{Na}, FE_K, FE_{Cl})
- Urinary excretion of sodium, potassium and chloride ($U_{Na}V$, U_KV, $U_{Cl}V$)
- Plasma osmolality (P_{osm})
- Osmolar clearance (C_{osm})
- Free water clearance (C_{H2O})
- Plasma sodium, potassium and chloride (P_{Na}, P_K and P_{Cl})

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

Estimation the rate of water intake values of each animal in each period of experiment was recorded by an average over three days from weighing daily water consumption by water meter.

Animals were measured total body water (TBW), extracellular water (ECW) and plasma volume (PV) in the afternoon (13.00-14.00 h.). 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) via an ear vein catheter was carried out to estimate TBW, ECW, ICF 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 h 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). The preparation for sample counting is achieved by the internal standard technique as described by Vaughan and Boling (1961).

TBW = [standard count (dis/min) × dose (ml)] / [radio activity counts at zero time (dis/min)].

Sodium thiocyanate solution and Evan's blue dye (T-1824) were given for estimate ion of ECW volume and the plasma volume, respectively. Venous blood samples from the jugular vein were taken at 20, 30, 40, 50 and 60 min after dye injection. Blood volume was calculated from the plasma volume and pack cell volume (Chaiyabutr et al., 1980). The measurement method for ECW was modified from the method used by Medway and Kare (1959). Intracellular water (ICW) was calculated by subtracting ECW from TBW.

The parameters were measured as follow;

- Total body water
- Extracellular water
- Intracellular water
- Blood volume
- Plasma volume

Determinations of milk yield, milk compositions, milk electrolytes (Na^+, K^+, Cl^-) concentration and milk osmolality

Milk yield was recorded by milking machine in the morning and afternoon. Milk samples were collected and were kept in 0.1% bronopol (2-Bromo-2-nitropropane-1,3 diol) at 4 °C. Milk samples from morning milking were used to determine milk compositions. Concentrations of milk lactose, milk fat, milk protein, total solid and solid not fat (SNF) were determined using Milkoscan (Milko-Scan 133B, A/S N. Foss Electric, Hillerod, Denmark). The concentrations of electrolytes in milk were estimated for sodium (Na^+) and potassium (K^+) using Flame photometer (410C, Corning, England). The chloride in milk was measured by chlorimeter (Chloride analyzer 925, Ciba Corning Inc., USA). Milk was determined osmotic pressure by osmometer (Osmometer 3D3, Advance Instrument Inc., USA).

The parameters were measured as follow;

- Milk yield
- Milk compositions
    - Lactose
    - Milk fat

- Milk protein
- Total solid (TS) and solid not fat (SNF)
- Milk osmolarity
- Milk sodium, potassium and chloride (M_{Na}, M_K and M_{Cl})

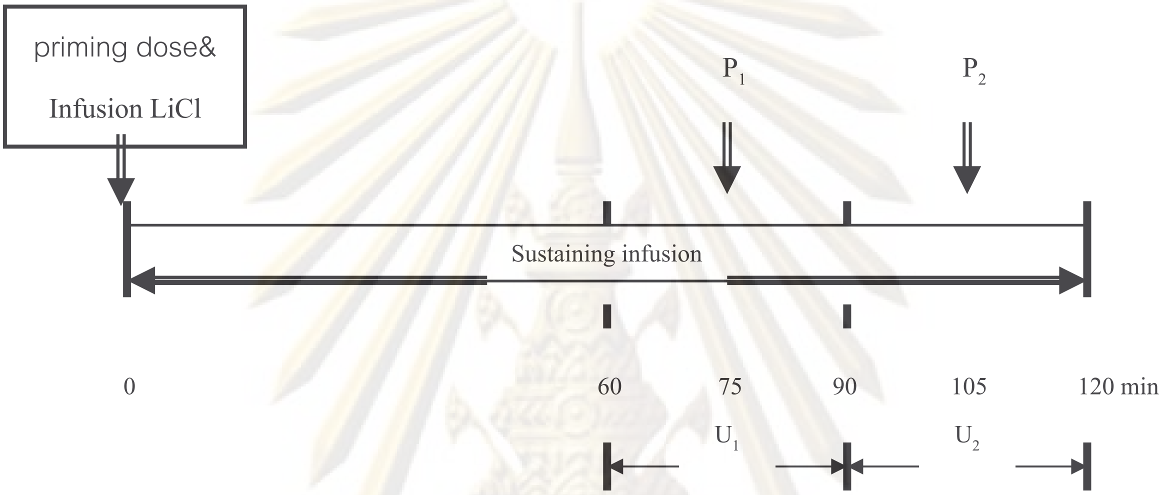
Determinations of renal tubular handling of sodium using lithium clearance (C_{Li})

C_{Li} method was used to determine proximal tubular reabsorption of Na^+ and H_2O. On the basis of the assumption that Li is absorbed solely in the proximal tubules and to the same extent as Na and water, Li clearance (C_{Li}) equals the output of isotonic fluid from the proximal tubule (Thomsen and Leyssac, 1987). C_{Li} was similarly determined of renal clearances.

On the specified day of both pretreatment and treatment period in chapter VI, 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. After inserting catheter into ear vein, Lithium clearance was started by giving of priming dose 20 ml of 5% lithium chloride 20 ml (5.0g/100 ml of normal saline) and 20 ml of 2.5%PAH (2.5g/100ml of normal saline), followed by infusion of 0.5% PAH and 1.25%lithium chloride in normal saline at the rate of 2 ml/min continuously for 60 minutes to stabilize plasma lithium and PAH concentration by a peristaltic pump (Eyela, MP-3, Tokyo, Rikakikai, Japan) throughout the experimental study. During equilibration, 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. After equilibration period, two time of urine collection (U1, U2) along with jugular blood sampling at midpoint of urine collection (P1, P2) was performed. Each urine collection period was 30 minutes. Urine volume was measured for cylinder. Blood samples were collected for determinations of packed cell volume. Blood was centrifuged and was separated.

Plasma and urine were kept at -20 °C for analyses of creatinine, PAH, lithium, sodium, potassium, chloride and osmolarity. The magnitude of proximal Na^+ and H_2O reabsorption were showed by calculation according to Thomsen (1984); Thomsen and Shirley (1990).

The protocol for lithium clearance study was demonstrated as the following;



The renal sodium handing was evaluated by measurements as follow;

- 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})
- Distal fractional reabsorption of water (DFR_{H2O}).

Determination of the plasma aldosterone concentration

The plasma aldosterone was measured by Cayman' s Aldosterone EIA Kit. Cayman' s Aldosterone EIA Kit is a competitive assay which based on the competition between aldosterone and a aldosterone acetylcholinesterase (AChE) conjugate for a limited amount of Aldosterone Monoclonal Antibody. The amount of aldosterone acetylcholinesterase (AChE) conjugate that is able to blind to the aldosterone Monoclonal Antibody was inversely proportion to the concentration of aldosterone. This antibody-aldosterone complex binds to goat polyclonal anti-mouse TgG that has been previously attached to the well. The plate was washed to remove any unbound and then Ellman s Reagent was added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. Aldosterone values were expressed as picrogram per milliliter.

Determination of the plasma vasopressin concentration

Plasma vasopressin was determined by Assay Designs' Vasopressin Enzyme Immunoassay (EIA) kit. EIA kit is a competitive immunoassay for the quantitative determination of vasopressin in plasma. Vasopressin is assayed by a competitive immunoassay. This assay uses a polyclonal antibody to vasopressin to bind, in a competitive manner, in a competitive manner, the vasopressin in sample or standard. Assay procedure, standard diluents were added in the NSB and Bo wells and then Standard and sample were added into the appropriate well. Vasopressin conjugate and Antibody were added in every well. After a simultaneous incubation (4°C) for 24 hours, the plate was washed to remove excess reagent and substrate was added, followed by incubated at 37 °C for 1 hour. After short incubation, Stop solution was added to every well for stop reaction and yellow color generated read on a microplate reader at 405 nm. Vasopressin values were expressed as picrogram per milliliter.

Determination of the plasma IGF-I concentration

Plasma IGF-I was determined by the IMMULITE® analyzer. The IMMULITE® analyzer is automated chemiluminescent immunoassays for the quantitative determination of the plasma IGF-I concentration. The assay utilized a monoclonal murine anti-IGF-I antibody on the solid phase-a polystyrene bead. The bead was coincubated with pretreated sample for 30 minutes and, after removal of unbound material by a centrifugal wash procedure. It was incubated in subsequent 30 minute incubation with alkaline phosphatase-conjugated polyclonal rabbit anti-IGF-I antibody. IGF-I values were expressed as nanogram per milliliter.

Calculation

Calculation for renal hemodynamics and renal function

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) $= (ERPF \times 100)/(100 - Hct)$

Filtration fraction (FF) $= (GFR \times 100)/ERPF$

Osmolar clearance (C_{osm}) $= U_{osm} \times V / P_{osm}$

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

Fractional excretion of electrolyte (e) $= \{(U_e \times V) \times 100\}/(P_e \times GFR)$

Urinary electrolyte excretion (U_xV) $= U_x \times V$

Calculation for C_{Li} method

Lithium clearance (C_{Li}) $= U_{Li} \times V / P_{Li}$

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

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

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

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

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

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

Statistical analysis

Data for milk yield, DMI and water intake in each lactating period were adjusted for covariate effects using mean values at 14 d before the pretreatment study. The stages of lactation (early, mid and late) were separated analysis. 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. Statistic significance was declared at $P<0.05$. Other parameters in each stage of lactation were presented as the mean±SEM. The statistical analyses were performed using the 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 $p<0.05$.

CHAPTER IV

Effects of supplemental recombinant bovine somatotropin (rbST) and misty and fan cooling on renal function in relation to body fluids regulation at 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 (Staples et al., 1988; Johnson et al., 1991). 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 misty and fan 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 (MF). 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 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. 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 were 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).

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.

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.

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 the 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 a 2 h equilibration period of infusion, the experiments were carried out on duplicate urine samples collected over an accurately timed period about 15-20 min. To ensure each collection was accurate, the urine sampling was started after the bladder was voided. Coccygeal blood sampling was performed at the midpoint of urine collection. 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 measure 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 subtraction of the rate of urine flow (V) from osmolar clearance (C_{osm}).

Determinations of water intake, total body water, extracellular fluid, intracellular 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. Total body water (TBW), extracellular fluid (ECF), intracellular fluid (ICF), plasma volume and blood volume were determined in each animal as described in Chapter III.

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).

Determinations of milk yield, milk composition, milk electrolytes (Na^+, K^+, Cl^-) concentration and milk osmolality

Milk yield, milk composition, milk electrolytes (Na^+, K^+, Cl^-) concentration and milk osmolality were determined in all experimental groups as described in chapter III.

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 and temperature humidity index

The mean values of ambient temperature in NS and MF barns are shown in Table 4.1 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 ($p<0.05$), while relative humidity in the MF barn was significantly higher than in the NS barn throughout the periods of study.

Rectal temperature, respiration rate, dietary dry matter intake and water intake

Effects of supplemental rbST and MF cooling on rectal temperature, respiration rate, dietary dry matter intake (DMI) and water intake are shown in Table 4.2. The mean values of both respiratory rate and the rectal temperature of cows under MF were significantly lower than 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 stages of lactation. The DMI of cows housed in the MF barn with or without treatment of rbST was significantly higher than of cows housed in the NS barn in all stages of lactation. Cows treated with rbST under either MF or NS showed significantly increased DMI compared with the pre-treatment period. The highest values of DMI were apparent in cooled cows 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 increased water intake compared with the pre-treatment period. There was no evidence of interaction of cooling system and rbST treatment on water intake.

Milk yield, mammary plasma flow, mammary blood flow and body weight

The alteration of milk yield, mammary plasma flow, mammary blood flow (MBF) and body weight are shown in Table 4.3. 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 of cows treated with rbST was significantly higher in all stages of lactation. Cows treated with rbST under either MF or NS showed significantly increased MBF and MPF compared with the pre-treatment period. There was no evidence of interaction of cooling system and rbST treatment on both MBF and MPF. The body weights of cows housing in MF were significantly higher than those of cows housing in NS. The weight gain of cows treated with rbST in both cooled and non-cooled cows showed significant increases in stepwise in all stages of lactation.

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

Effects of supplemental rbST and MF cooling on total body water (TBW), extracellular fluid (ECF), intracellular fluid (ICF), plasma volume (PV), blood volume (BV) and hematocrit are shown in Table 4.4. 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 MF cooling did not affect the level of TBW. Mean values of ECF, ICF, PV and BV, either as absolute values or as a percentage of body weight, in cows without rbST showed no significant differences between NS and MF barns in all stages of lactation, except that cows in MF barn showed higher absolute PV values during late lactation. The absolute values of ECF, PV and BV markedly increased during rbST treatment in all stages of lactation. The values of ECF, PV and BV as a percentage of body weight of cows with rbST treatment tended to increase, but there were no significant differences as compared with the pre-treatment period. The absolute values of ICF and as a percentage of body weight were significantly increased ($P<0.05$) after rbST treatment in all stages of lactation. Neither rbST treatment nor MF cooling affected the hematocrit values.

Renal hemodynamics of crossbred Holstein cows supplemented with rbST

Effects of supplemental rbST and MF cooling on renal hemodynamics are shown in Table 4.5. No significant changes of GFR, ERPF, ERBF and FF were apparent in cows during rbST treatment or under the application of MF fans cooling in all stages of lactation. The rate of urine flow tended to decrease in cows treated with rbST by an average 14.9% compared with the pre-treatment period under either MF or NS barn conditions.

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

Effects of supplemental rbST and MF cooling on urinary and fractional (FE) excretion of electrolytes, osmolar clearance and free water clearance are shown in Table 4.6. The urinary and fractional excretion of sodium tended to decrease during rbST administration in cows housed in either NS or MF barns. The significant effect of rbST on decreases in both urinary and fractional excretion of sodium was apparent ($p<0.01$) in mid-lactation. Potassium excretion was decreased ($p<0.05$) during rbST treatment in early and mid-lactation, and tended to decrease in cows treated with rbST administration under MF 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 was not statistically different ($p>0.05$) 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 MF throughout 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 4.7. There were no changes in the concentrations of plasma Na^+, K^+, Cl^- and osmolarity in cows treated with rbST or when housed in the MF barn.

Table 4.1 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[1]
		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.

[1] P-values for the effects; MF =Misty-fan cooling effect

Table 4.2 Rectal temperature, respiration rate, dietary dry matter intake (DMI) and water intake in animals treated with rbST under normal shade (NS) and misty-fans cooling (MF) at different stages of lactation.

	Lactating period	Treatments					Effects[1]		
		NS	NS+rbST	MF	MF+rbST	SEM	MF	rbST	MFxrbST
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
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

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 4.3 Milk yield, mammary plasma flow (MPF), mammary blood flow (MBF) and body weight in animals treated with rbST under normal shade (NS) and misty-fans cooling (MF) at different stages of lactation.

	Lactating	Treatments					Effects[1]		
	period	NS	NS+rbST	MF	MF+rbST	SEM	MF	rbST	MFxrbST
Milk yield (kg/d)	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
MPF (ml/min/100kg)	Early	596.6	621.3	556.5	677.1	30.4	0.923	0.044	0.154
	Mid	431.1	533.8	424.5	540.3	47.7	0.999	0.051	0.894
	Late	364.6	542.9	424.8	485.0	30.2	0.984	0.004	0.086
MBF (ml/min/100kg)	Early	802.9	801.4	713.8	871.2	43.8	0.926	0.113	0.108
	Mid	566.2	690.6	548.3	695.6	61.4	0.949	0.058	0.857
	Late	485.4	717.5	554.1	623.1	41.1	0.872	0.006	0.083
Body weight (Kg)	Early	348.8	367.6	387.6	408.2	7.3	0.006	0.027	0.905
	Mid	365.4	367.4	408.2	440.2	6.4	0.002	0.030	0.048
	Late	386.6	376.6	455.2	445.4	2.3	0.004	0.002	0.966

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

	Lactating	Treatments					Effects[1]		
	period	NS	NS+rbST	MF	MF+rbST	SEM	MF	rbST	MFxrbST
TBW	Early	262.7	312.2	280.6	323.9	7.92	0.426	0.001	0.709
(L)	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	Early	74.5	83.5	72.1	76.6	2.81	0.520	0.026	0.800
(L/100kg)	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	Early	97.9	104.3	111.1	123.1	3.79	0.171	0.010	0.086
(L)	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	Early	27.67	27.83	26.02	30.26	0.64	0.852	0.013	0.018
(L/100kg)	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
ICW	Early	156.31	208.48	162.49	186.96	8.85	0.679	0.003	0.156
(L)	Mid	169.68	192.91	146.59	205.79	11.02	0.782	0.004	0.185
	Late	160.11	207.18	150.39	199.97	14.48	0.687	0.010	0.933
ICW	Early	43.91	54.58	43.83	48.94	2.38	0.467	0.011	0.276
(L/100 kg)	Mid	44.05	51.19	37.76	50.23	3.25	0.435	0.017	0.435
	Late	39.84	53.20	34.94	48.14	3.82	0.271	0.008	0.984
PV	Early	18.85	20.88	17.00	19.55	1.11	0.185	0.047	0.627
(L)	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	Early	5.36	5.48	4.44	5.07	0.28	0.211	0.226	0.401
(L/100kg)	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	Early	25.36	26.80	23.20	25.82	1.33	0.437	0.166	0.668
(L)	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	Early	7.18	7.03	6.27	6.82	0.36	0.385	0.594	0.356
(L/100kg)	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 4.5 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 misty-fans cooling (MF) at different stages of lactation.

		Treatments					Effects[1]		
	Lactating period	NS	NS+rbST	MF	MF+rbST	SEM	MF	rbST	MFx rbST
GFR	Early	188.0	192.2	170.8	150.6	12.9	0.130	0.552	0.371
(ml/min/100kg)	Mid	217.6	236.4	193.2	181.6	7.4	0.201	0.638	0.073
	Late	217.6	236.4	193.2	181.6	7.4	0.201	0.638	0.073
ERPF	Early	636.8	568.6	581.8	530.4	34.7	0.423	0.123	0.815
(ml/min/100kg)	Mid	650.0	710.4	632.8	549.8	48.0	0.087	0.820	0.174
	Late	650.0	710.4	672.8	629.8	47.5	0.609	0.859	0.308
ERBF	Early	863.0	730.8	755.8	699.6	47.8	0.420	0.084	0.450
(ml/min/100kg)	Mid	895.0	918.8	814.9	813.1	79.8	0.201	0.894	0.877
	Late	895.0	918.8	814.9	813.1	79.8	0.201	0.894	0.877
FF	Early	30.06	33.73	29.63	25.68	1.08	0.121	0.897	0.008
(%)	Mid	33.45	33.73	29.31	29.82	1.94	0.383	0.845	0.952
	Late	33.45	33.73	29.31	29.82	1.94	0.383	0.845	0.952
Urine flow	Early	4.96	3.59	4.19	3.41	0.71	0.678	0.170	0.691
rate	Mid	4.11	3.80	3.95	3.07	0.57	0.784	0.326	0.634
(ml/min/100kg)	Late	4.63	4.39	2.51	3.62	1.26	0.237	0.739	0.607

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 4.6 Urinary electrolyte excretion, fractional excretion of electrolytes, osmolar clearance and free water clearance in animals treated with rbST under normal shade (NS) and cooling with misty-fans (MF) at different stages of lactation.

	Lactating	Treatments					Effects[1]		
	period	NS	NS+rbST	MF	MF+rbST	SEM	MF	rbST	MFxrbST
Na$^+$ excretion	Early	120.0	72.8	126.1	90.9	16.2	0.776	0.034	0.721
(μmol/min/100kg)	Mid	171.1	141.6	150.4	93.9	11.3	0.431	0.005	0.266
	Late	273.8	136.2	96.2	116.9	53.1	0.160	0.303	0.174
Fractional Na$^+$	Early	0.46	0.26	0.53	0.51	0.09	0.398	0.245	0.360
excretion	Mid	0.55	0.42	0.58	0.38	0.05	0.956	0.007	0.496
(%)	Late	0.83	0.41	0.35	0.50	0.14	0.219	0.397	0.083
K$^+$ excretion	Early	458.4	398.3	465.9	359.2	15.4	0.710	0.001	0.168
(μmol/min/100kg)	Mid	442.5	402.3	504.1	291.4	40.4	0.736	0.014	0.065
	Late	418.6	351.2	341.0	329.0	46.2	0.273	0.415	0.565
Fractional K$^+$	Early	52.36	48.00	61.84	48.75	4.20	0.520	0.071	0.328
excretion	Mid	46.11	36.77	57.67	36.87	4.32	0.552	0.008	0.221
(%)	Late	45.72	34.49	37.63	39.16	5.59	0.720	0.411	0.287
Cl$^-$ excretion	Early	137.8	80.2	91.4	63.4	29.8	0.323	0.189	0.633
(μmol/min/100kg)	Mid	99.6	107.0	154.8	99.1	28.8	0.597	0.427	0.306
	Late	181.2	87.6	95.7	91.5	28.3	0.463	0.123	0.153
Fractional Cl$^-$	Early	0.53	0.44	0.81	0.55	0.13	0.379	0.235	0.544
Excretion	Mid	0.82	0.35	0.47	0.52	0.14	0.696	0.165	0.097
(%)	Late	0.72	0.45	0.55	0.50	0.15	0.755	0.332	0.477
Osmolar	Early	6.69	4.30	4.49	3.66	0.48	0.106	0.010	0.142
clearance	Mid	6.09	5.36	5.63	3.68	0.40	0.163	0.010	0.167
(ml/min/100kg)	Late	6.01	5.12	4.66	4.17	0.40	0.139	0.119	0.627
Free water	Early	-1.74	-0.71	-0.30	-0.25	0.83	0.404	0.536	0.572
clearance	Mid	-1.98	-1.56	-1.68	-0.61	0.84	0.690	0.401	0.705
(ml/min/100kg)	Late	-1.38	-0.73	-2.15	-0.55	1.27	0.836	0.403	0.720

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

	Lactating	Treatments					Effects[1]		
	period	NS	NS+rbST	MF	MF+rbST	SEM	MF	rbST	MFx rbST
Plasma Na$^+$ (mEq/l)	Early	139.4	139.6	138.0	139.2	0.79	0.570	0.400	0.543
	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$^+$ (mEq/l)	Early	4.76	4.56	4.44	4.54	0.09	0.345	0.602	0.142
	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$^-$ (mEq/l)	Early	101.4	100.2	97.6	100.0	1.23	0.380	0.637	0.180
	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 osmolarity (mOsm/kg)	Early	275.0	272.4	275.8	277.0	2.53	0.333	0.789	0.473
	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

In the present study, the temperature-humidity index (THI) derived from ambient temperature and humidity taken in both barns ranged from 79-85 throughout all 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 (Armstrong, 1994), indicating that application of misters and fans was not sufficient to completely eliminate heat stress in cows in the present study. However, THI might not accurately reflect heat stress in MF 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 rectal temperatures and respiratory rates of cooled cows at the period of hottest daily temperatures (1300 to 1400 h) showed a partial alleviation of heat stress from MF cooling, which was confirmed by an increase in milk production compared with non-cooled animals throughout all stages of lactation. However, administration of exogenous bovine somatotropin in crossbred HF animals under high environment temperature could increase milk yield (Chaiyabutr et al., 2007), indicating that thermal stress alone was not an extra-mammary factor causing a reduction in milk production in crossbred HF animals.

An increase in milk yield accompanying elevations of TBW, ECF, blood volume and PV in rbST treated cows with or without MF cooling throughout lactation confirmed the previous reports of Maksiri et al. (2005) and Chaiyabutr et al. (2007) that exogenous rbST in the crossbred cow exerts a galactopoietic action, in part, through increases in body fluids and MBF in distribution of nutrients to the mammary gland for milk synthesis. Similar results for increases in both milk secretion and MBF during administration of exogenous growth hormone were also reported in goats (Hart et al., 1980) and *Bos taurus* cows (Davis et al., 1988). The marked increase in MBF has been shown to be associated 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 is thought to be indirectly mediated via IGF-I (Bauman, 1992), since infusion of IGF-I into the pudic artery of lactating goats has been shown to increase MBF and milk yield on the infused side (Prosser et al., 1990; 1994). However, in the present study, the pattern of progressive decline in milk yield as lactation advanced was apparent even with a higher level of MBF during rbST administration under conditions with or without MF. The decline in milk yield as lactation advances without facilitating MBF during rbST treatment could be attributed to a local change within the mammary gland.

The action of rbST can affect higher blood flow to the mammary gland, but it seems unlikely to affect blood flow to the kidneys, despite a high level of TBW and ECF during rbST administration. With respect to renal hemodynamics, no alterations of glomerular filtration rate (GFR), effective renal plasma flow (ERPF), effective renal blood flow (ERBF) and filtration fraction (FF) were apparent in rbST-treated cows housed in NS or MF barns at all stages of lactation. The different action of rbST on renal blood flow compared to the mammary gland indicates that the kidneys were able to regulate RBF and GFR constantly during experimental periods. It is probable that the kidneys of the ruminant respond differently from the mammary gland to the high level of endogenous IGF-I, secretion of which could be inferred during rbST administration in crossbred cows (Chaiyabutr et al., 2005). The action of IGF-I may appear directly in the blood vessels, but be more pronounced in the mammary gland of ruminants. The present findings seemingly contradict studies of differences among species in the effect of IGF-I on hyperfiltration rate in the kidney. An infusion of IGF-I, or recombinant human IGF-I, has been shown to decrease renal vascular resistance and increased GFR and RBF both in man (Guler et al., 1989; Hirschberg et al., 1993; Jaffa et al., 1994; Giordano and Defronzo, 1995) and rat models (Inishi et al., 1997). The different response in ruminants is an interesting finding that deserves further investigation.

In the present results, the effect of rbST on kidney function would more directly influence the renal tubular part of the nephron rather than change renal hemodynamics. The GFR and filtered load for Na^+, K^+ and Cl^- ions (GFR× plasma ion concentration) of rbST-treated cows remained constant, while the absolute values of urinary excretion of these ions, including FE_{Na}, FE_K and $FE_{Cl,}$ decreased as compared with pre-treatment values under either NS or MF barn conditions. On the other hand, these results obviously indicate an elevation of renal tubular reabsorption of ions in rbST-treated cows. These changes are similar to studies in a rat model by Dimke et al. (2007) which showed that the urinary excretion of sodium, potassium, and chloride ions and urine flow rate decreased in rats treated with growth hormone. An increase in renal tubular reabsorption of sodium ion was also reported in Lewis dwarf rats treated with somatotropin (Wyse et al., 1993). However, the mechanism of action of growth hormone on kidney function is still unsettled. Physiologically, the renal tubular reabsorption of ions is generally known to be under hormonal control. Several studies demonstrated that growth hormone increased sodium and water reabsorption via stimulation of the renin-angiotensin-aldosterone system (Ho and Weissberger, 1990; Cuneo et al., 1991; Herlitz et al., 1994; Moller et al., 1995; 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). The interaction of these hormones on kidney function in ruminants is still speculative.

In the present findings, the increase, and thereby restoration, of body fluids in rbST-treated animals might not be the result of water consumption, although water intakes were higher in rbST-treated cows as compared with non-treated cows. Higher water intake would be attributed to the higher DM intake during the treatment of rbST (MacFarlane et al., 1959). According to the classical view, an increased plasma sodium concentration and plasma osmolality will stimulate vasopressin secretion and thirsty which leads to enlarged plasma volume. However, in the present results, both the plasma sodium concentration and plasma osmolality of cows were maintained constant during rbST supplementation in NS or MF barns. This is likely explained by the fact that sodium ion is the osmotic factor of ECF and water is required in proportion to the amount of body fluids produced. Thus, the secretion of vasopressin acting on water reabsorption from the distal tubules and the collecting ducts of the kidneys might not be expected to occur to save water and thereby increase the ECF in rbST-treated cows. No significant change of the C_{H2O} values was also independent of any direct effect of the rbST on free-water formation. In the present results, the observed decrease in excretion of electrolytes during supplemental rbST would create lower osmotic diuretic effect resulting in the decline in rate of urine flow. These findings would be supported by estimation of C_{osm} which was decreased during supplemental rbST. It is known that the sodium ion is the main cation in the ECF and it plays the dominant role in 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 would increase the number of electrolytes in the body composition. This is a part of the effects of enlarged body fluid volume arising from its colligative properties with exerting osmotic forces for retaining body water. It would be an explanation for an increase in body fluid volume during rbST administration.

In conclusion, cows supplemented with rest and housed under misters and fan cooling could increase milk yield in all stages of lactation. Application of misters and fans alone did not affect kidney function in terms of both renal hemodynamics and electrolyte excretion. An increase in body fluid volume during rest supplementation appears partly due to changes in renal tubular function by stimulating reabsorption of electrolytes without changes in renal hemodynamics. Further studies are required for a better understanding of the mechanisms of exogenous bovine somatotropin on segmental tubular sodium handling in the kidney in relation to aldosterone and vasopressin activation in the regulation of body fluid volume in crossbred dairy cattle.

CHAPTER V

Effects of supplemental recombinant bovine somatotropin (rbST) and misty-fan cooling on milk production relating to changes in plasma hormonal concentrations of thyroxine, cortisol and IGF-I in different stages of lactation of crossbred Holstein cattle

Introduction

Meteorological conditions in the tropical countries like Thailand are generally high ambient temperatures and high relative humidity. Chronic stress of hot environment lowered productive efficiency in dairy cattle both directly and indirectly. The low milk production and short lactating period of exotic breeds and cross-bred animals is still the main problem for dairy farming in the tropics. Dairy cattle adapt to heat stress with variety of hormonal and metabolic response. The responses by which heat stress influences milk production is partly explained by reduced dry matter intake (DMI) together with changes in bodily functions. Nutrients and hormones from blood will be the factors affecting physiological signals received by the mammary gland in sustaining milk synthesis. There are a number of studies for metabolic hormones e.g. thyroxine (T_4), growth hormone and glucocorticoid, which have been shown to decrease in chronically heat stress cattle (Collier et al., 1982b; Magdub et al., 1982).

Somatotropin is one hormone (amongst many) which is known to play a role of the interaction between genetic potential and nutrition and milk production (Gulay & Hatipoglu 2005; Settivari et al. 2007). Previous studies by Chaiyabutr et al., (2000) have been shown that a rapid reduction of milk yield coincided with the decline in the plasma somatotropin (ST) concentration during lactation advanced to mid and late lactation in 87.5% HF animal. These changes would account for the short lactation persistency.

There are a number of studies have shown that the high milk yield cows treated with bST probably lead to increase heat production (West, 1994), which caused reduction in plasma concentration of triiodothyronine and cortisol (Manalu et al., 1988; Johnson et al., 1991). However, there are some conflicting results that increased milk yield by the effect of bST under high temperatures without changes in plasma levels of triiodothyronine (T_3). thyroxine (T_4) and cortisol (Mohammed et al., 1985; Zoa-Mboe et al., 1989). It has been suggested that even though bST increases heat production, it also increases heat dissipation (Johnson et al, 1991; West, 1994; Tarazon-Herrera et al., 1999). An increase in milk production has been reported in cows treated with recombinant bovine somatotropin (rbST) under both normal thermal zone and hot environment (Staples et al., 1988; Johnson et al., 1991). There is a need to study the mechanism of action of rbST on changes in metabolic hormones in crossbred dairy cattle under high temperatures.

There are a number of studies for environmental modifications that can alleviate severe heat stress in dairy cattle, for example, water spray and fans (Armstrong, 1993), evaporative cooling (Armstrong et al., 1985; Armstrong et al., 1988; Ryan et al., 1992).It has been reported that cows under cooling system had higher in milk yield when compared with those of cows under only shade in the tropic (Chen et al., 1993). An environmental modification to improved efficiency of heat dissipation is still necessary for high producing cow especially in rbST treated cows. Changes in endocrine function are also involved of changes in milk yield in hot environment. Correa-Calderon et al., (2004) found that the thyroxine and cortisol concentrations in milk were high by the effect of cooling system. Both thyroxine and cortisol are important hormones which involved thermogenesis and animal's adaptation to a hot environment. The increase in milk production after bST administration would be attributed to an increase in the plasma level of IGF-I (Chaiyabutr et al., 2005). Thus, administration of bST and cooling system in lactating dairy cows may affect to alteration of these hormones which probably involve in milk production. Therefore, the present studies were designed to determine the effect of supplemental rbST and the misty-fan cooling on milk production relating to changes in the plasma levels of IGF-I, thyroxine and cortisol in 87.5 %crossbred Holstein cattle.

Materials and methods

Animal managements

Ten, first lactation, non pregnant, 87.5% lactating Holstein Friesian dairy cattle were selected randomly into two groups of five animals each. All animals were housed in open-sided barn with a tiled-roof during experimental period. 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 other 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). The ambient temperature of NS and MF barns were recorded during the daytime (13.00 hour), using a wet and dry bulb thermometer. The relative humidity of NS and MF barns were calculated by psychrometric chart depending on wet and dry bulb temperature. Temperature humidity index (THI) was calculated from dry bulb temperature and relative humidity according to West (1994) where: THI = td-(0.55-0.55RH) (td-58) with td = dry bulb temperature (°F), and RH = relative humidity.

Rectal temperature and respiration rate of individual animals were determined at the same time as recording ambient temperature around 13.00 hour. Rectal temperature (RT) of each cow was measured by electric thermometer. Respiration rates (RR) of each cow was measured by counting flank movement. Animals were normally milked at around 6.00 am. and 5.00 pm. using a milking machine and milk production were recorded dairy. The present study, the procedures were carried in accordance with the principle and guidelines of the Faculty of Veterinary Science, Chulalongkorn University

Experimental procedures

The experiment in each group was studied during early, mid and late lactation. The pretreatment study was started on each specified days (days 75 post-partum of early lactation, days 135 post-partum of mid lactation, days 195 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. Injections were administrated at the tail head depression (ischiorectal fossa). 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.

On each specified day, before and after rbST injection, blood samples from the jugular vein were taken in the morning (9.00 h). The plasma was separated by centrifugation at 3,000 rpm for 15 min and kept at -70 $^{\circ}$C for measurements of thyroxine (T_4), cortisol and insulin like growth factor I (IGF-I) concentration. Milk samples were collected by milking machine in the morning and were kept in 0.1% bronopol (2-Bromo-2-nitropropane-1,3 diol) at 4 $^{\circ}$C for determinations of lactose, fat and protein, total solid (TS) and solid not fat (SNF) concentrations, sodium, potassium, chloride concentration and osmolarity.

Determinations of the plasma hormone concentrations

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). The concentration of plasma thyroxine was determined by using an electrochemiluminescence immunoassay (ECLIA) (Roche Diagnostics GmbH, USA) by Elecsys and cobas e immunoassay analyzers (Indianapolis, IN, USA). Plasma cortisol concentrations were measured by using a chemiluminescence immunoassay in an immulite analyzer (DPC, Los Angeles, CA)

Determinations of the concentration of milk composition

Milk samples from morning milking were used to determine milk compositions. Concentrations of milk lactose, milk fat, milk protein, total solid (TS) and solid not fat (SNF) were determined using Milkoscan (Milko-Scan 133B, A/S N. Foss Electric, Hillerod, Denmark). Milk Na^+ and K^+ concentrations were determined by flame photometer (Flame photometer 410C, Ciba Corning Inc., USA). Milk Cl^- concentration was determined by Chloridometer (Chloride analyzer 925, Ciba Corning Inc., USA) and milk osmolality was determined by osmometer (Osmometer 3D3, Advance Instrument Inc., USA).

Statistical analysis

The stages of lactation (early, mid and late) were separated analysis. Ambient temperature, relative humidity, temperature humidity and the values of milk yield in lactation curve were presented as the mean±SE. Statistical significant difference between groups was determined by the paired sample t-test. Statistic significance was declared at $P<0.05$. Data for rectal temperature, respiration rate, plasma hormones, milk yield and milk composition in each stage of lactation were presented as the mean±SEM. 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 significant difference was indicated at P-value<0.05.

Results

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

The mean ambient temperature, relative humidity and relative humidity index, respiratory rate and rectal temperature are presented in table 5.1 and table 5.2, respectively. Relative humidity was elevated in MF barn, while ambient temperature and temperature humidity index (THI) were significantly decreased (P<0.05) in MF barn in comparison with NS barn. However, THI in both barns had values ranging 79-85 throughout stages of lactation. The mean values of both respiratory rate and the rectal temperature of cows were significantly higher (P<0.05) after rbST supplementation. In contrary, respiratory rate and the rectal temperature of cows in MF barn were lower than those of cows in NS barn in all stages of lactation.

Plasma insulin like growth factor I, cortisol and thyroxine concentrations.

Effects of supplemental rbST and cooling on alterations of plasma hormones (IGF-I), cortisol and thyroxine are presented in Table 5.3. The mean values of the plasma IGF-I concentration were increased (P<0.05) in both cooled and non-cooled cows during rbST supplementation, while no significant changes by the effects of mist and fan cooling in all stages of lactation. The supplemental rbST and mist and fans cooling did not affect to the mean values of the plasma cortisol concentration . Recombinant bovine somatotropin (rbST) supplementation caused the significant decrease ($P<0.05$) of the plasma thyroxine concentration in both cooled and non-cooled cows in early lactation but not in mid and late lactations. The plasma thyroxine concentration of cooled cows had tendency to be higher than those of non-cooled cows in all stages of lactation.

Milk yield and milk compositions

Effects of supplemental rbST and misty-fan cooling on milk yield and milk composition are shown in Table 5.4. Lactation curve are presented in figure 5.1. The milk yields of cooled cows were slightly higher than those of non-cooled cows. Milk yield showed significantly higher ($P<0.01$) during rbST administration in all stages of lactation. The value of milk yield in early lactation had highest in cows under MF barn and treated with rbST treatment. There were no significant different in the concentration of electrolyte (Na^+, K^+, Cl^-) and milk osmolarity by effects of rbST supplementation and mist and fans cooling system. Milk protein, lactose, TS and SNF concentration in cow treated with rbST and housing either NS or MF barns were not significantly difference in all stage of lactation. Milk fat of cows treated with rbST was significantly increased in early and mid lactation, while it had a tendency to increase in late lactation.

Table 5.1 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.

	Lactating period	Treatments		P-value[1]
		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.

[1] P-values for the effects; MF =Misty-fan cooling effect

Table 5.2 Rectal temperature (RR) and respiration rate (RT) in animals treated with rbST under normal shade (NS) and misty-fans cooling (MF) at different stages of lactation.

	Lactating	Treatments					Effects[1]		
	period	NS	NS+rbST	MF	MF+rbST	SEM	MF	rbST	MFxrbST
Rectal	Early	38.8	39.0	38.0	38.2	0.07	0.001	0.023	0.886
temperature	Mid	39.4	39.9	38.6	38.9	0.13	0.005	0.011	0.245
(°C)	Late	39.1	39.3	38.5	38.8	0.10	0.057	0.023	0.565
Respiration	Early	72.0	78.0	54.0	63.2	2.52	0.001	0.017	0.544
Rate	Mid	70.4	73.4	52.8	55.0	1.22	0.001	0.065	0.751
(breath/min)	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 5.3 The value of plasma insulin like growth factor I (IGF-I), thyroid and cortisol concentrations in animals treated with rbST under normal shade (NS) and misty-fans cooling (MF) at different stages of lactation.

	Lactating	Treatments					Effects[1]		
	period	NS	NS+rbST	MF	MF+rbST	SEM	MF	rbST	MFxrbST
IGF-I	Early	118.2	196	87.4	114.8	18.76	0.301	0.023	0.216
(ng/ml)	Mid	115.6	183.2	112.2	218	32.72	0.644	0.029	0.575
	Late	128.2	350.9	124.1	220.4	41.5	0.221	0.005	0.166
Cortisol	Early	2.15	2.24	1.87	1.74	0.65	0.457	0.972	0.868
(µg%)	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	Early	8.08	7.26	10.64	8.52	0.51	0.518	0.021	0.237
(µg%)	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; MF =Misty-fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

Table 5.4 Milk yield and milk compositions in animals treated with rbST under normal shade (NS) and misty-fans cooling (MF) at different stages of lactation.

	Lactating	Treatments					Effects[1]		
	period	NS	NS+rbST	MF	MF+rbST	SEM	MF	rbST	MFx 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
Milk compositions:									
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
Na+	Early	29.80	31.20	27.20	27.60	1.31	0.250	0.449	0.670
(mEq/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
(mEq/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
(mEq/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

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

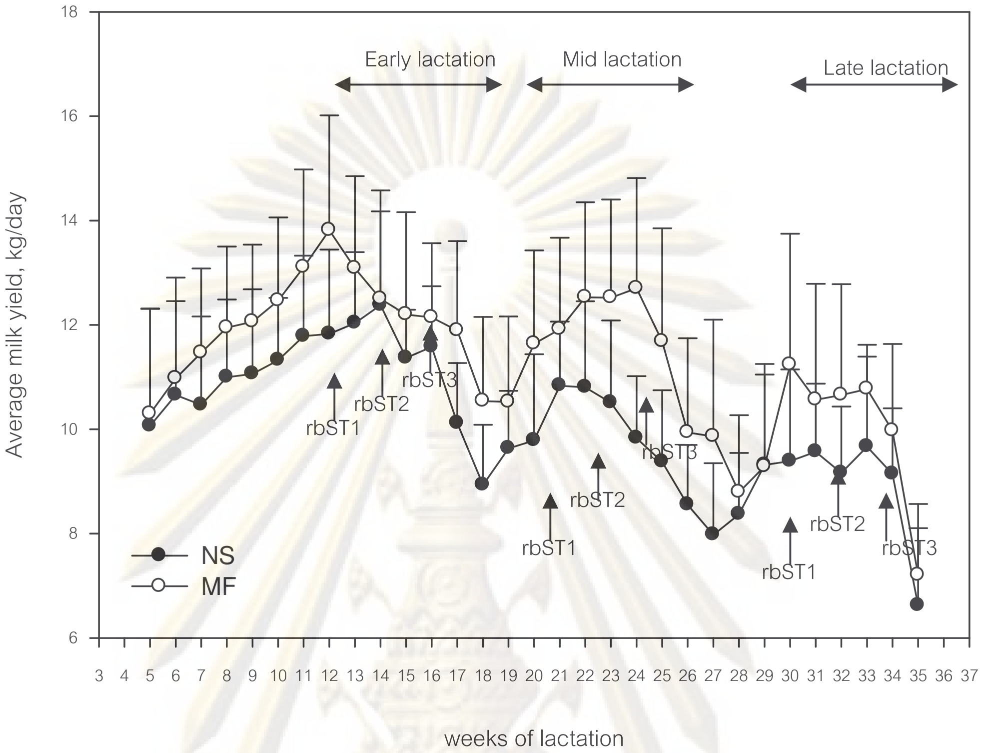


Figure 5.1 Pattern of lactation curve during early, mid and late lactation response to rbST supplementation in crossbred lactating cows housing in normal shade (NS) and shade plus misty-fans cooling system (MF). All values are means±SE, n=5.

Discussion

The present study was performed to investigate whether an increase in milk production during rbST supplementation and application of misters and fans relate to changes in plasma hormone levels of IGF-I, thyroxine (T_4) and cortisol in different stages of lactation. The milk yields were increased throughout lactation by the effect of rbST administration especially in early lactation (fig 5.1). The milk yields of cow treated rbST in early lactation were highest when compared with another period of lactation. The higher of milk production would be a consequence of increase in heat production (Manalu et al., 1988). Thyroid hormones are the hormone associated with that thermogenesis. Thus, increasing of heat production may depress secretion of thyroid hormone which may assist the animal to control body temperature better to maintain homeothermy (Johnson et al., 1991). This result probably accounted for a reduction of plasma Thyroxine (T_4) concentration in early lactation during rbST administration 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 mean fT_4 level, resulting an increased conversion of thyroxine (T_4) to triiodothyronine (T_3) in peripheral tissues (Losa et al ., 2008). The reduction of thyroxine remaining within the normal range does not induce hypothyroidism (Portes et al., 2000) and are not mediated pass 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. The results of this study agree with those reported by Deresz (1987), who found higher plasma thyroxine concentration in cooled cows during hot summer, but differences were not significant. Similarly, Correa-Calderon et al. (2004) observed that milk thyroxine concentration of cooled cows were significantly higher than control cows. In the present results, a lower rectal temperature was observed in cows in the MF barn. These results may be related to higher levels of plasma thyroxine concentration in MF barn consequently the cows increased milk yields. The study of Yousef et al., (1967) in cattle suggested that the response of thyroid to the stimulus of cold may be retarded by vasoconstriction in the thyroid gland. This would prevent the TSH from reaching a concentration sufficient to cause a rapid increase in thyroid secretion rate.

Cortisol is the major adrenal corticoid secreted in cattle which enable animals to tolerate stressful condition (Christison and Johnson, 1972). Some previous studies reported that plasma cortisol levels were reduced in prolong heat exposure in both 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 higher plasma cortisol resulted in a marked elevation in 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 cortisol concentrations did not change by rbST or mist-fans cooling which indicates that cortisol did not play a role in responses to rbSt treatment or mist-fans cooling on increased body fluid in present study. These findings agree with studies reported by Peel et al., (1982, 1983) and West et al., (1991). However, it should be noted by various authors that the levels of adrenocorticortropin (ACTH) increased although the level of cortisol did not differ in rbST-treated cows (Adriaens et al., 1995), high-producing cows (Shayanfar et al., 1990) and 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, bST 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 exogenous 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 rbST on increasing of milk productions requiring IGF-I as a mediator or nutrient partitioning affects. 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 (Clemmonsa 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; Jousan et al., 2007; Settivari et al., 2007).

The effect of misters and fans cooling and decrease in THI in MF barn in the present study can increase comfortable of animals as indicated by the decrease of body temperature and increase in milk yield. Similarity, Correa-Calderon et al., 2004 studied in cows received spray and fans or evaporative cooling system during summer that the decrease in rectal temperature were associated with reduced in cortisol levels, the increased in triiodothyronine (T_3) levels and the increased in milk yields.

In the present study, THI values were averaged 79 to 85 which would be moderate heat stress for lactating cows in normal shade barn and shade plus misters and fans barn. The effect of chronically heat stress cattle had tended to be reduced 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 this 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 100% Holstein lactating cows

Milk protein and lactose content did not alter by the effect of rbST throughout the experiment. These results are in agreement with previous studies (Gallo and Block, 1990; West et al., 1990; Downer et al., 1993), but it is partly in contradiction with the results reported by other authors who demonstrated an increase in milk protein content (Richard et al., 1985; Soderholm et al., 1988). Milk fat was the only parameter in milk compositions which was significantly increased in cows treated rbST in this study. An increase in milk fat content was observed in negative energy cows injected with bST (Eppard et al., 1985), which resulted from the increased yield of milk and the trend for an increased percentage of fat in milk (Bremmer et al., 1997). The increase in milk fat could relate to an increase in the mobilization of long-chain fatty acids from body reserves (McDwell et al., 1987), which in contrast to milk fat of cows in positive energy balance did not influenced by bST administration (Bauman and McCutcheon, 1984). However, many factors involved that affected the quantity and composition of milk produced in cattle including genetics, breed, stage of lactation, age, diet composition, nutritional status, environment, and season. These similar factors also affect the milk compositions of cows treated with bST (Etherton and Bauman, 1998).

In conclusion, an increased of milk yield after rbST treatment did not change milk compositions in cows under either NS or MF barns. The elevation of IGF-I concentration after rbST treatment in the present study confirm an indirect action of rbST on increasing milk production would mediate via IGF-I. The highest of milk yield in cows treated rbST during early lactation may be lead to increase in heat production which may be caused the decrease of plasma thyroxine concentration in cooled and non-cooled cows. However, no alterations of plasma cortisol concentrations were observed in supplemental rbST or mister and fan cooling, indicate that cows did not become stress. This result suggests that 87.5% HF cows can adapt to both hot condition and rbST treatment which supported by lower rectal temperature and respiration rate.

CHAPTER VI

Effects of supplemental recombinant bovine somatotropin and misty-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 (Ritz et al., 1991; Hirschberq and Kopple, 1992; Dimke et al., 2007). The increases in glomerular filtration rate (GFR) and renal blood flow(RBF) have been shown in animals given exogenous growth hormone and insulin-like growth factor 1(IGF-1) (Ikkos et al., 1956, Falkheden and Sjogren, 1964; Jaffa et al., 1994). Previous study in chapter IV has shown that rbST did not affect RBF and GFR. It appeared only decreases in the urinary excretions of sodium, potassium and chloride ions in 87.5% HF under misters and fans in all stages of lactation. 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 75 post-partum of early lactation, day 135 post-partum of mid lactation and day 195 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, intracellular 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. Intracellular fluid (ICF) was calculated by subtracting ECF from TBW. 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.).

Determinations of the concentration of milk composition

Milk samples from morning milking were used to determine milk compositions. Concentrations of milk lactose, milk fat, milk protein, total solid and solid not fat (SNF) were determined using Milkoscan (Milko-Scan 133B, A/S N. Foss Electric, Hillerod, Denmark). Milk Na^+ and K^+ concentrations were determined by flame photometer (Flame photometer 410C, Ciba Corning Inc., USA). Milk Cl^- concentration was determined by Chloridometer (Chloride analyzer 925, Ciba Corning Inc., USA) and milk osmolality was determined by osmometer (Osmometer 3D3, Advance Instrument Inc., USA).

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) $= (ERPF \times 100)/(100\text{-}Hct)$

Filtration fraction (FF) $= (GFR \times 100)/ERPF$

Osmolar clearance (C_{osm}) $= U_{osm} \times V/P_{osm}$

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

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

Urinary electrolyte excretion (U_eV) $= U_e \times V$

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}) $= U_{Li} \times V/P_{Li}$

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

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

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

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

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

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

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 and temperature humidity index

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 6.1. 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.

Dietary dry matter intake, water intake, milk yield, body weight, rectal temperature and respiration rate

Effects of supplemental rbST and misty-fan cooling on dietary dry matter intake (DMI), water intake, milk yield, body weight, rectal temperature and respiration rate are presented in Table 6.2. 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. 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.

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

Effects of supplemental rbST and misty-fan cooling on total body water (TBW), extracellular fluid (ECF), intracellular fluid (ICF), plasma volume (PV), blood volume (BV) and hematocrit are presented in Table 6.3. 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 lactation were increased in cows treated rbST in NS and MF barns but not for mid and late lactation. The absolute values and relative values of ICF showed no significant differences in both cooled and non-cooled cows during rbST supplementation. 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 misty and fan cooling on renal hemodynamics osmolar clearance, plasma osmolarity and free water clearance are presented in Table 6.4. 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 misty and fan cooling on urinary and fractional (FE) electrolytes excretion and plasma electrolytes concentration are presented in Table 6.5. 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 misty-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 Table6.6. 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.6.1. 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.

Milk compositions

Milk compositions in animals treated with rbST under normal shade (NS) and misty-fan cooling (MF) at different stages of lactation are shown in table 6.7. Milk protein, lactose, TS and SNF concentration in cows treated with rbST and housing either NS or MF barns were not significant different in all stage of lactation. Milk fat in rbST-treated cows significantly increased ($P<0.05$) when compared with pretreatment period, while the milk fat concentration of cooled and non-cooled cows without rbST did not difference in all stages of lactation. There were no significant differences in the concentration of milk electrolyte (Na^+, K^+, Cl^-) and milk osmolarity by effects of rbST supplementation and mist and fans cooling system.

Table 6.1 Ambient temperature, relative humidity and relative humidity index in animals treated with rbST under normal shade (NS) and misty-fans cooling (MF) at different stages of lactation.

	Lactating period	Treatments		P-value[1]
		NS	MF	
Ambient temperature (°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
Relative humidity (%)	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
Temperature humidity index (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

Mean±S.E.

[1] P-values for the effects; MF =Misty-fan cooling effect

Table 6.2 Dietary dry matter intake (DMI), water intake, milk yield, body weight, respiration rate and rectal temperature in animals treated with rbST under normal shade (NS) and misty-fans cooling (MF) at different stages of lactation.

	Lactating	Treatments					Effects[1]		
	period	NS	NS+rbST	MF	MF+rbST	SEM	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
Respiration rate (RR)	Early	72.00	77.00	54.00	62.50	2.94	0.001	0.061	0.573
	Mid	69.25	71.75	51.00	54.50	1.19	0.004	0.045	0.689
	Late	69.25	78.25	50.50	56.50	1.49	0.001	0.002	0.352
Rectal temperature (ºC)	Early	38.80	39.03	37.95	38.08	0.08	0.001	0.060	0.533
	Mid	39.50	39.90	38.73	39.03	0.10	0.014	0.012	0.628
	Late	38.90	39.10	38.43	38.80	0.13	0.070	0.063	0.515

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 6.3 Total body water (TBW), extracellular fluid (ECF), intracellular fluid (ICF), Plasma volume (PV), blood volume (BV) and packed cell volume (Hct) in animals treated with rbST under normal shade (NS) and misty-fans 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
ECW	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	102.8	96.40	102.3	112.5	13.58	0.733	0.893	0.563
ECW	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	25.00	23.05	25.59	27.34	3.59	0.680	0.978	0.625
ICW	Early	204.2	226.0	180.6	211.1	11.85	0.389	0.069	0.723
(L)	Mid	187.2	193.7	144.1	171.2	10.61	0.085	0.165	0.371
	Late	183.1	217.2	164.4	176.2	32.55	0.286	0.507	0.744
ICW	Early	52.01	57.37	48.31	54.16	2.91	0.427	0.103	0.937
(L/100kg)	Mid	46.84	48.29	36.47	42.82	2.80	0.098	0.213	0.415
	Late	44.38	51.73	40.25	42.87	7.72	0.323	0.542	0.77
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	0.11	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	6.05	7.33	6.09	7.90	0.25	0.693	0.001	0.343
(L/100kg)	Mid	7.30	7.93	6.57	7.83	0.27	0.564	0.013	0.285
	Late	6.51	7.52	7.01	7.57	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 6.4 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 misty-fans cooling (MF) at different stages of lactation.

	Lactating period	Treatments				SEM	Effects[1]		
		NS	NS+rbST	MF	MF+rbST		MF	rbST	MFxrbST
GFR (ml/min/100kg)	Early	189.1	202.8	202.6	233.0	10.1	0.660	0.072	0.442
	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 (ml/min/100kg)	Early	603.0	610.3	623.0	701.8	25.2	0.671	0.138	0.205
	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 (ml/min/100kg)	Early	846.3	854.3	863.6	976.1	27.0	0.705	0.067	0.101
	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 rate (ml/min/100kg)	Early	6.65	3.98	6.33	3.40	1.05	0.427	0.037	0.909
	Mid	5.66	3.49	3.74	2.56	1.22	0.065	0.221	0.700
	Late	4.57	3.40	3.53	2.17	0.39	0.528	0.017	0.816
Osmolar clearance (ml/min/100kg)	Early	7.54	5.62	7.48	4.74	0.47	0.700	0.003	0.423
	Mid	7.31	4.17	6.02	4.61	0.69	0.732	0.016	0.255
	Late	5.53	4.48	5.57	3.83	0.39	0.779	0.012	0.418
Free water clearance (ml/min/100kg)	Early	-1.05	-1.64	-1.30	-1.00	0.69	0.802	0.504	0.790
	Mid	-1.65	-0.68	-2.28	-2.04	0.87	0.345	0.511	0.687
	Late	-0.97	-1.08	-2.04	-1.66	0.45	0.599	0.778	0.597
Plasma osmolarity (mOsm/kg)	Early	274.8	274.0	272.3	273.5	1.85	0.705	0.897	0.607
	Mid	276.0	270.8	274.5	271.5	1.78	0.938	0.060	0.552
	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 6.5 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 misty-fans cooling (MF) at different stages of lactation.

	Lactating	Treatments					Effects[1]		
	period	NS	NS+rbST	MF	MF+rbST	SEM	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 6.6 Lithium clearance, aldosterone, vasopressin and insulin like growth factor 1 (IGF-1) in cows treated with rbST under normal shade (NS) and shade plus misty-fans cooling (MF) at different stages of lactation.

	Lactating	Treatments					Effects[1]		
	period	NS	NS+rbST	MF	MF+rbST	SEM	MF	rbST	MFxrbST
C_{Li}	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
PAR_{Na}	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
PFR_{Na}	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
DAR_{Na}	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
DFR_{H2O}	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

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 6.7 Milk compositions in animals treated with rbST under normal shade (NS) and misty-fans cooling (MF) at different stages of lactation.

	Lactating	Treatments					Effects[1]		
	period	NS	NS+rbST	MF	MF+rbST	SEM	MF	rbST	MFx rbST
Protein	Early	3.84	4.59	3.39	3.79	0.47	0.120	0.265	0.719
(gm%)	Mid	3.77	3.48	3.59	3.69	0.17	0.972	0.571	0.281
	Late	3.83	3.58	3.57	3.59	0.26	0.648	0.677	0.633
Fat	Early	3.69	4.85	3.74	4.39	0.35	0.701	0.042	0.495
(gm%)	Mid	3.54	4.80	3.61	4.24	0.35	0.522	0.037	0.405
	Late	3.97	4.29	3.32	4.70	0.27	0.862	0.020	0.097
Lastose	Early	4.33	4.13	4.98	4.81	0.33	0.200	0.594	0.974
(gm%)	Mid	4.99	4.68	4.21	5.11	0.36	0.641	0.446	0.148
	Late	5.08	5.02	4.92	4.59	0.21	0.110	0.394	0.537
SNF	Early	8.68	9.53	9.07	8.85	0.19	0.683	0.152	0.034
(gm%)	Mid	9.46	8.86	8.50	9.49	0.38	0.773	0.624	0.113
	Late	9.73	9.39	9.19	8.16	0.45	0.201	0.181	0.475
TS	Early	12.38	13.23	12.53	13.37	0.72	0.881	0.287	0.993
(gm%)	Mid	13.22	13.44	12.11	13.73	0.57	0.590	0.155	0.262
	Late	14.78	14.40	12.73	12.70	0.78	0.389	0.805	0.831
Na+	Early	33.75	42.25	32.25	36.25	5.51	0.597	0.300	0.697
(mEq/l)	Mid	34.00	35.25	33.50	36.00	2.44	0.979	0.471	0.806
	Late	37.50	37.25	43.00	50.75	9.85	0.389	0.717	0.699
K+	Early	38.80	33.20	38.50	37.05	2.44	0.470	0.199	0.428
(mEq/l)	Mid	36.28	38.05	34.95	35.80	2.52	0.398	0.621	0.861
	Late	35.98	37.05	42.65	30.28	4.66	0.991	0.271	0.199
Cl-	Early	32.25	30.75	40.00	36.00	1.80	0.124	0.177	0.513
(mEq/l)	Mid	33.25	32.25	33.00	30.00	3.06	0.773	0.538	0.755
	Late	33.50	34.25	31.25	35.25	4.14	0.936	0.587	0.708
Osmolarity	Early	331.0	294.8	284.5	297.0	22.10	0.422	0.610	0.312
(mOsm/kg)	Mid	299.0	337.3	360.0	311.3	38.40	0.713	0.896	0.301
	Late	291.8	321.3	330.5	288.5	20.26	0.892	0.768	0.128

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

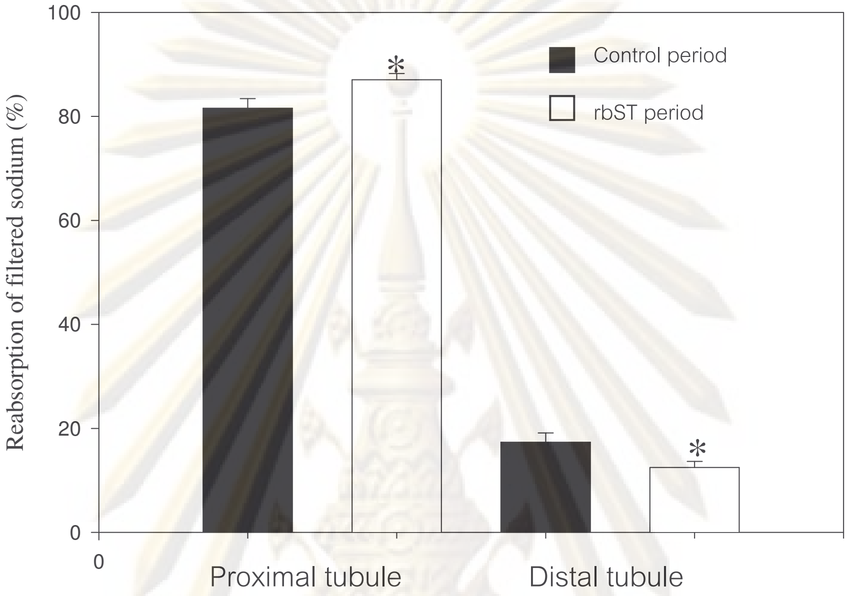


Figure 6.1 Summary of the percent of reabsorption of filtered sodium at the proximal and distal tubules during the control period and the period of supplemental rest in all stages of lactation. The calculation of the percent of reabsorption of filtered sodium at proximal and distal tubule were performed using $(PAR_{Na}*100)/(GFR*P_{Na})$ and $(DAR_{Na}*100)/(GFR*P_{Na})$, respectively. P-values of control period vs. rbST-treated period using paired t-test, * $P < 0.01$ (n=30).

Discussion

It has been found that the application of misty-fans caused a decrease ambient temperature in the barn about 1-2°C throughout the experiment. The present results are similar to chapter IV that the temperature-humidity index (THI) in both barns had values ranging 80-84. 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 misty-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 supplemented with rbST confirmed results in chapter IV.

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 results in chapter IV 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 et al., 1989; Hirschberg and Kopple, 1993). Infusion of IGF-1 has shown to increase GFR and RBF (Guler et al., 1989; Giordano and Defronzo, 1995) 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 results presented in chapter IV. 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 (C_{osm}) 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 (Wyse et al., 1993; Lampit et al., 1998). 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 was 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.

No changes in milk composition including the concentration of protein, lactose, TS and SNF were apparent in both cooled and non-cooled cows treated with rbST, although both rbST supplementation and application of misty-fan cooling increased milk yield. It is similar to most researchers apparently reports the fact that rbST administration does not alter milk composition (Gallo and Block, 1990; West et al., 1990; Downer et al., 1993). However, in present study supported previous experiment that administration of bST showed significantly increased milk fat compared with the pre-treatment period. An increased fat content in milk by rbST supplementation in negative energy balance cows would relate to an increase in the mobilization of long-chain fatty acid from body reserves (McDowell et al., 1987). Moreover, no significant changes in milk electrolytes (Na^+, K^+ and Cl^-) concentrations and milk osmolarity were apparent in both cooled and non-cooled cows supplemental rbST in the present study. This may be indirectly reflected no apparent adverse effects of rbST supplementation under mist-fan cooling.

It is concluded that cows supplemented with rbST under misters and fans 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.

CHAPTER VII

GENERAL DISCUSSION

The studies in this thesis were performed to clarify the mechanisms of actions rbST on regulation of body fluids relating to renal functions and milk production in crossbred Holstein cattle under misty and fans cooling system. The results are presented in Chapter IV-VI.

It is recognized that the comfortable zone of excotic dairy cows is between 5 and 25 °C or THI lower than 72 (McDowell, 1972; Amstrong, 1994) with the maximum productivity (Folk, 1974). Above the threshold level (25 °C, THI 72), dairy cows would increase in respiration rate (RR) and rectal temperature (RT) associated with a decline of milk production (Bitman et al., 1984, Amstrong, 1994). The experimental results in Chapter IV-VI show that ambient temperature and temperature-humidity index (THI) in both NS and MF barns had values ranging 28-34 °C and 80-84, respectively, which these parameters exceeded the threshold of heat stress. It might be considered that animals in both groups exposed to moderate heat stress (Fuquay, 1981). However, the application of misty-fans cooling (MF) in the present study caused a significantly decrease ambient temperature about 1-2°C, decreases in respiration rate and rectal temperature and improved milk production which showed a partial alleviation of heat stress in cooled cows. Therefore, THI value of MF barn might not accurately reflect heat stress, since the misty fan cooling delivers a pressurized spray with considerable air movement above the cow's back, resulting in higher humidity.

The action of rbST on milk production has been elucidated in all experimental studies. The milk yield in crossbred lactating cows housing in both NS and MF barns were significantly increased after rbST supplementation by an average 18.05% when compared with the pretreatment period. These findings agree with several reports for an increase in milk production in rbST-supplemented cows (West, 1994; Etherton and Bauman, 1998; Gulay and Hatipoglu, 2005). The milk yields in all stages of lactation remained greater during rbST supplementation in both cooled and non-cooled cows. However, lactation curves of cooled cows without rbST had tendency to be higher than those of non cooled cows (Figure 7.1).

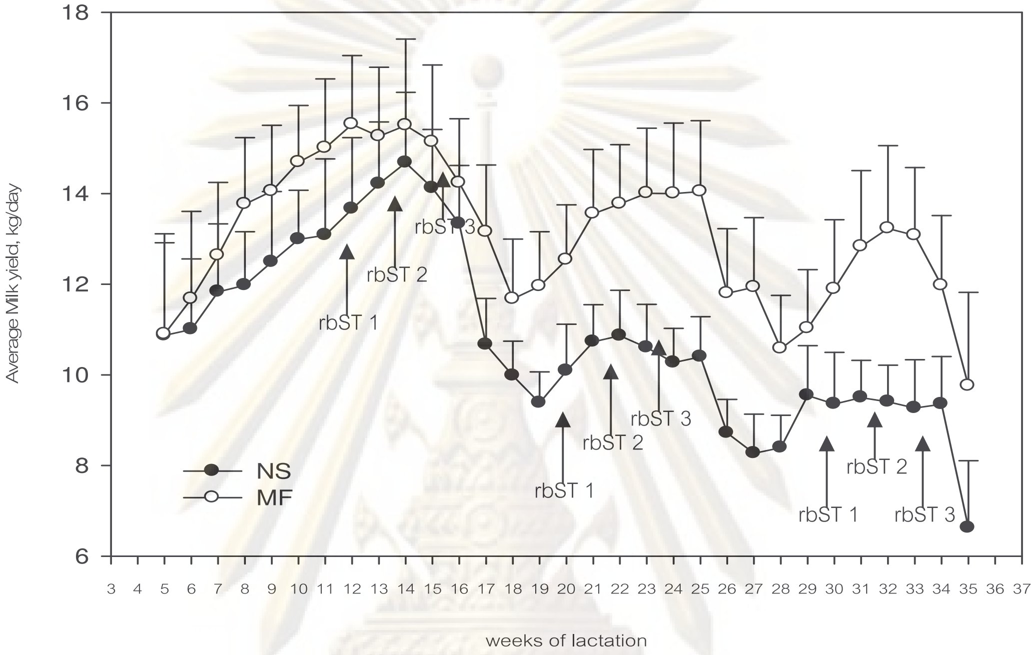


Figure 7.1 Pattern of lactating curve during early, mid and late lactation in response to rbST supplementation in crossbred lactating cows housing in normal shade (NS) and shade plus misty-fans cooling system (MF). All values are means±SE, n=10.

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 non cooled cows. These findings are agreed to the report of 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. The higher of milk production in cows treated with rbST were probably a consequence of increased heat production (Manalu et al., 1988) which confirmed by increasing of level of rectal temperature (RT) in rbST treated cows (Chapter IV-VI). An increase in heat production may depress endocrine functions, e.g. secretion of thyroid hormone which may assist the animal to keep body temperature better to maintain homeothermy (Johnson et al., 1991). The reduction of the plasma thyroxine (T4) concentration was apparent in early lactation by the effect of rbST administration (Chapter V). Even though there were numerically higher rectal temperature and respiration rate in cooled and non-cooled cows treated with rbST, but physiological significance as a sign of additional thermal stress was not evident in rbST-treated cows. Animals still maintained higher feed intake and milk production. Crossbred HF animals treated with bovine somatotropin under high environment temperature could increase milk yield (Chaiyabutr et al., 2007). Moreover, the plasma cortisol did not change in lactating cows treated with rbST either cooled or non-cooled cows (Chapter V). It has been reported that a high plasma cortisol level enables animal to tolerate stressful condition in cattle (Christison and Johnson, 1972). Thus, the present findings indicate that crossbred cattle containing 87.5% Holstein genes using in the present study have a high heat tolerance than exotic *Bos taurus* breeds alone.

Dry matter intake (DMI) is known to be a major source of nutrients for synthesized milk production in dairy cows. An increase in milk production was positively correlated to dry matter intake during rbST supplementation (Murphy, 1992). An increase in dry matter intake will correlate with an increase in water consumption (MacFarlane et al. 1959; Silanikove, 1987; Moallem et al., 2000). Cows with supplemental rbST had the high DMI and water intake associated with greater milk yield (Chapter VI). These findings indicate that the increase in DMI and water intake would distribute the nutrients and water to the mammary gland for sustaining high milk production. The rbST treated cows increased DMI (average 13.39 %) for supported average 18.32% of an increase milk yield from pretreatment period (Chapter VI). It indicates that energy output in milk was greater than energy consumed in food in the rbST-treated cows. The water intake of cows treated with rbST has shown to be higher when compared with the pretreatment period by average 16.71%. This increment coincided with the increases in body fluid compartments, such as TBW, plasma volume and blood volume in both cooled and non-cooled cows treated with rbST in all stages of lactation (Chapter IV and VI). Thus, an increase in water intake would be partly led to a higher water reserve by an influx of water via the GI tract. However, mechanisms for expansion of body fluid by the effect of rbST supplementation would depend on the coordinated action of multiple factors. Thus, the increase in body fluids and thereby restoring body fluids in rbST treated animals might not be the result of water consumption only. In addition, an increase in body weight in cooled and non-cooled cows treated with rbST would be possibly related to the effect of increase in water retention (Tyrrell et al., 1988).

Mammary blood flow is known to be a major determining factor for supply of nutrients for milk synthesis. It has been noted that galactopoietic effect of rbST on mammary gland function is partly through increases in body fluids and mammary blood flow (MBF) in distribution nutrients to the mammary gland for milk synthesis (Maksiri et al., 2005; Chaiyabutr et al., 2007). A significant increase in mammary blood flow was apparent in cooled and non-cooled cows treated with rbST when compared with pretreatment period. This finding is similar to those reports in B. Taurus cows (Davis et al., 1988) and goats (Hart et al. 1980). However, in the present study, the pattern of progressive decline in milk yield level as lactation advances were observed in cooled and non-cooled cows treated with rbST even though a higher level of MBF. The continued decline in the milk yield as lactation advances during rbST treatment without facilitating of MBF would attribute to a local change within the mammary gland. Moreover, an increase in the MBF has been shown to associate with an increase in the level of plasma IGF-I (Chapter V) in cooled and non-cooled cows treated with rbST, which was similar to the result of previous study (Chaiyabutr et al., 2005). Bauman et al., (1999) reported that an increase milk production occurred in IGF-I Infusion into the mammary gland, but not bST. In goat, Infusion of IGF- I into the mammary gland has been shown to increase in MBF and milk yield on the infused side (Prosser et al., 1990; 1994). Thus, these findings indicate that rbST exerts the galactopoietic action indirectly by mediated via IGF-I.

The effects of rbST supplementation and mist-fan cooling system on renal functions are shown in chapter IV and VI. 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 effect on renal hemodynamic (GFR, ERPF, ERBF and FF), despite a higher level of body fluids (Chapter IV and VI) in cooled and non-cooled cow treated with rbST in all stages of lactation. The present findings in Chapter IV and VI 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 VI) 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. An increase in sodium reabsorption via stimulation of the IGF-I, renin-angiotensis-aldosterone system and suppression of atrial natriuretic peptide were noted in GH administration (Cuneo et al., 1991; Wyse et al., 1993;; Herlitz et al., 1994; Moller et al., 1997; Lampit et al.,_1998; Moller et al., 2000). However, the mechanism underlying the effect of these hormones on the kidney function during rbST supplementation is still unsettled. Furthermore, it has been reported that the mechanism of action of growth hormone on sodium and fluid retention might be primarily due to a direct renal tubular but not to be due to enhanced aldosterone secretion (Hoffman et al., 1996). In the molecular study, 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), were not directly mediated by the hormone (Dimke et al., 2007).

In chapter VI, 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). The study in fetal sheep has been shown that 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) reported that. 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. Therefore, an increase in sodium reabsorption in the proximal tubule is most likely explained by the indirect effect of rbST. In contrast to the study in human, a direct action of GH/IGF-I took place mainly in the distal nephron (Johannsson et al. 2002).

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 present study 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 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 free water formula because of no 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.

Milk compositions including protein, lactose, SNF, TS (chapter V and VI) were not different among groups, although both rbST supplementation and application of mist-fan cooling increased milk yield. It is similar to most researchers apparently reports the fact that rbST administration does not alter milk composition (Gallo and Block, 1990; West et al., 1990; Downer et al., 1993). Previous study found that administration of bST in positive energy balance cows did not change milk protein and milk fat (Peel and Bauman, 1987). In contrast, milk fat of cows in present study was increased by supplemented rbST. Previous study founded that negative energy balance cows showed increased milk fat and decreased milk protein during bST injection, which would related to an increase in the mobilization of long-chain fatty acid from body reserves (McDowell et al., 1987). This finding can conclude that using of rbST exerting galactopoietics in the present study was not affected to the composition of milk produced expected increased milk fat may be occur rbST stimulated mobilization of long-chain fatty acid from body reserves. Moreover, the quantity and compositions of milk in cows supplemented with bST would be regulated by many factors including breed, stage of lactation, age, diet composition, nutritional status, environment, and season (Etherton and Bauman, 1998). In the present study, no significant changes in milk electrolytes (Na^+, K^+ and Cl^-) concentrations and milk osmolarity were apparent in both cooled and non-cooled cows supplemental rbST. This may be indirectly reflected no apparent adverse effects of rbST supplementation under mist-fan cooling.

In conclusion, the present study demonstrates that the effects of supplemental rbST and mist-fans cooling could improve milk production which did not change milk composition in all stages of lactation. It indicated that persistency of lactation was also improved by rbST supplementation. The mechanism based on the effects of rbST supplementation and cooling in 87.5% lactating crossbred Holstein cattle are proposed in fig. 7.2. The rbST supplementation is main action to change body function in present study. In the present findings, a role of rbST exerts galactopoietic action is mediated primarily through higher body fluid and secondary increased mammary blood flow to mammary gland for milk synthesis. The stimulatory effects of rbST on expansion of body fluid occurred in 87.5% lactating crossbred Holstein cattle via changes in the renal function. An interesting possibility is that supplementation of rbST stimulated renal tubular function by stimulating solute reabsorption, but not changing renal hemodynamic. The results form lithium clearance study suggest that the increased renal tubular sodium and water reabsorption took place mainly in the proximal tubule during rbST supplementation, possibly as a result of the increase in plasma levels of IGF-I and aldosterone which may involve a stimulation of renin–angiotensin–aldosterone system, but the mechanism does not seem to be mediated via vasopressin hormone. These results may simply reflect one mechanism of rbST on increasing of water retentions. Another possibility is that the increased feed intake and water intake response to rbST supplementation may be caused increase in body fluids which partition the distribution of nutrients to the mammary gland for milk synthesis. Furthermore, application of mist-fan cooling to dairy cows in the present study reliably reduced exposure to conditions of heat stress because of lower RT and RR in cooled cows. The reduction in RT in cooled cows seems to be stimulated thyroxin secretion. The reduction in heat stress in cooled cows was sufficient to significantly increase feed intake and water intake which resulted in a higher in body fluid lead to greater milk production throughout the experimental period compared with non-cooled cows. An increase in milk yield by application of mist-fan cooling is not due to changed renal function. Even though, THI of cooled and non-cooled still higher than upper critical temperature but did not affect on plasma cortsol concentration because effects of rbST supplementation and mist-fan cooling increased water retention, thereby making animals more thermotolerance to heat stress. In addition, the highest of values of milk yield in early lactation may be lead to increased heat production which may be caused depression of synthesis thyroid hormone in present study.

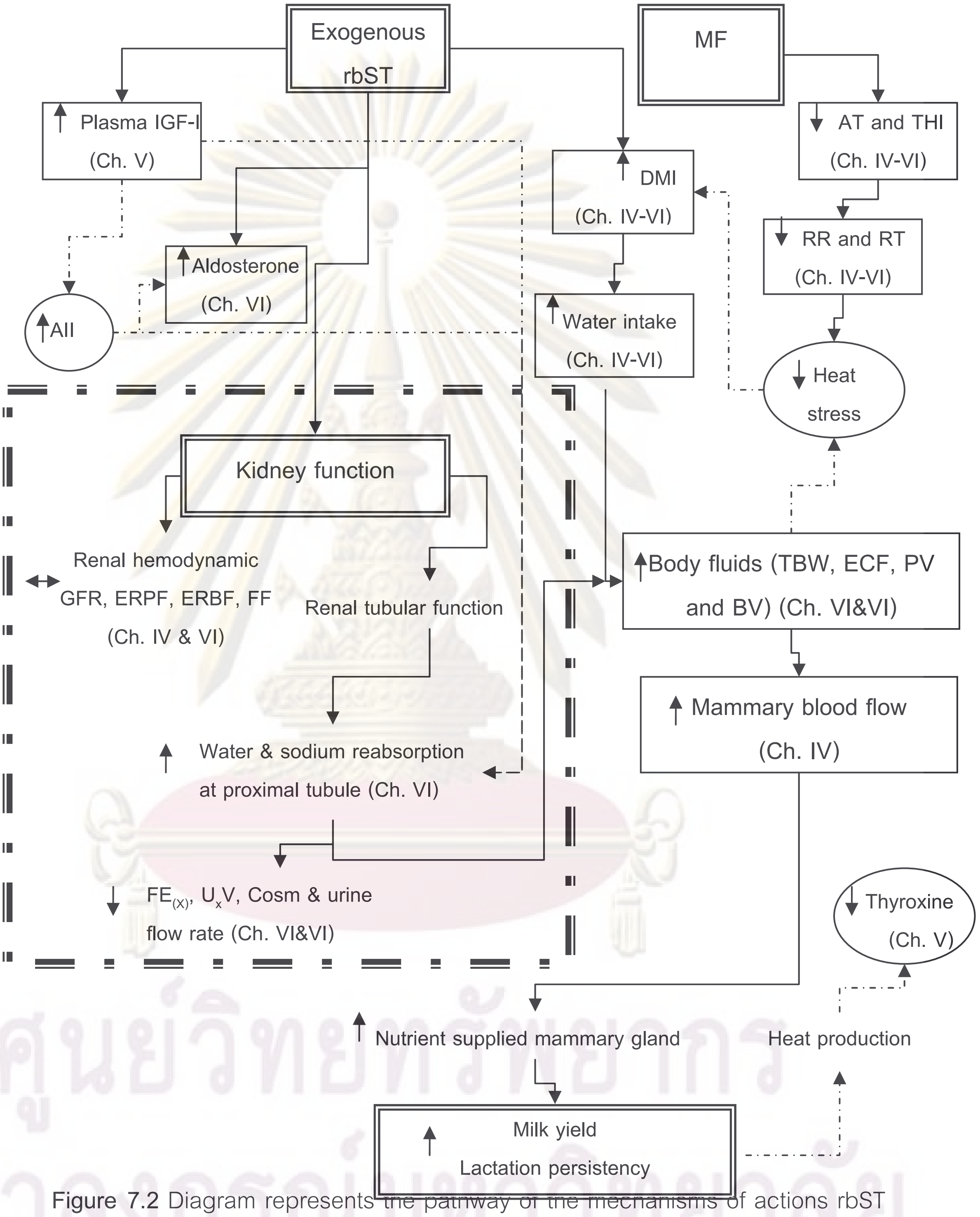


Figure 7.2 Diagram represents the pathway of the mechanisms of actions rbST supplementation and mist-fans cooling system (MF) on regulation of body fluid relating to renal function and milk production in crossbred Holstein cattle, solid arrows represent demonstrated pathways whereas dashed arrows represent speculative pathways.

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APPENDIX

PUBLICATIONS

1. Boonsanit, D., Chanpongsang , S., Chaiyabutr, N., 2010. 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. Asian-Aust. J. Anim. Sci. (in press).
2. Boonsanit, D., Chanpongsang , S., Chaiyabutr, N., 2010. 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. Research in Veterinary Science (submitted).

BIOGRAPHY

Miss Dolrudee Boonsanit was born on October 2, 1978 in Chumporn, Thailand. She graduated Bachelor degree from Faculty of Veterinary Science, Khon Kaen University and received the degree of Doctor of the Veterinary Medicine in 2002. She graduated with a Master of Science in Physiology at the department of Physiology, Faculty of Veterinary Science, Chulalongkron University in 2005.