ผลของการทำให้เย็นด้วยระบบพัดลมพ่นละอองน้ำต่อการตอบสนองทาง สรีรวิทยาเกี่ยวกับระบบย่อยอาหารของโคพันธุ์ผสมโฮลสไตน์ระยะให้นม ที่ได้รับการฉีดฮอร์โมนโบวาย โซมาโตโทรปิน ในสภาพอากาศเขตร้อน

นาง วิไลพร จันทร์ไชย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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EFFECTS OF MISTY-FAN COOLING SYSTEM ON THE PHYSIOLOGICAL RESPONSES IN RELATION TO DIGESTIVE PROCESS OF LACTATING CROSSBRED HOLSTEIN CATTLE TREATED WITH BOVINE SOMATOTROPIN IN THE TROPICS

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วิไลพร จันทร์ไชย : ผลของการทำให้เย็นด้วยระบบพัดลมพ่นละอองน้ำต่อการตอบสนองทาง สรีรวิทยาเกี่ยวกับระบบย่อยอาหารของโคพันธุ์ผสมโฮลสไตน์ระยะให้นม ที่ได้รับการฉีดฮอร์โมน โบวาย โซมาโตโทรปินในสภาพอากาศเขตร้อน. (EFFECTS OF MISTY-FAN COOLING SYSTEM ON THE PHYSIOLOGICAL RESPONSES IN RELATION TO DIGESTIVE PROCESS OF LACTATING CROSSBRED HOLSTEIN CATTLE TREATED WITH BOVINE SOMATOTROPIN IN THE TROPICS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ศ.น.สพ.ดร. ณรงค์ศักดิ์ ชัยบุตร, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ.น.สพ. สมชาย จันทร์ผ่องแสง, 119 หน้า.

การศึกษาผลของการใช้ ระบบพัดลมพ่นละอองน้ำ ทำความเย็นให้กับแม่โคนมพันธุ์ผสม สายเลือดโฮลสไตน์ 87.5% ที่ได้รับการฉีดฮอร์โมน โบวาย โซมาโตโทรปิน (rbST) การศึกษาใช้แม่โคสาว จำนวน 18 ตัว สำหรับ 4 การทดลอง แม่โคในแต่ละการทดลอง ถูกแบ่งออกเป็น 2 กลุ่ม จำนวนเท่าๆ กัน และจัดให้เลี้ยงในโรงเรือนแบบผูกยืนโรงที่ถูกกั้นแยกออกจากกันเป็นสองด้านด้วยแผ่นโลหะสูงจากพื้นจรดหลังคา กลุ่มแรกเลี้ยงอยู่ในด้านที่ไม่ติดตั้งระบบทำความเย็น (NS) กลุ่มที่สองเลี้ยงอยู่ในด้านที่ติดตั้งระบบทำความเย็นด้วยพัดลมพ่นละอองน้ำ (MF) แม่โคทุกตัวได้รับการฉีดฮอร์โมน rbST ขนาด 500 มก เข้าใต้ผิวหนัง โดยให้ห่างกันทุกๆ 14 วัน ติดต่อกัน3 ครั้งในแต่ละระยะการให้นม แม่โคทั้งสองกลุ่มได้รับการจัดการ การให้อาหาร น้ำ และการจัดการอื่นๆ เหมือนกันตลอดระยะการทดลอง ผลการทดลองพบว่าการใช้ระบบพัดลมพ่นละอองน้ำสามารถลด อุณหภูมิ และดัชนีอุณหภูมิความชื้นภายในโรงเรือน MF ลงได้ มีผลทำให้ อัตราการหายใจและอุณหภูมิร่างกายของแม่โคกลุ่ม MF ต่ำลง นอกจากนี้ยังพบว่า โรงเรือน MF สามารถช่วยลดผลกระทบจากสภาพอากาศร้อนที่มีต่อระบบย่อยอาหารของโค โดยมีส่วนทำให้อัตราการไหลผ่านของอาหารเร็วขึ้น จึงทำให้โคสามารถกินอาหารได้เพิ่มขึ้น การตอบสนองของแม่โคต่อฮอร์โมน rbST และการปรับอุณหภูมิแวดล้อมด้วยระบบ MF ทำให้การกินอาหารเพิ่มขึ้นแล้ว ยังมีผลต่อการเพิ่มผลผลิตจากการหมักอาหารของจุลินทรีย์ในกระเพาะหมัก ได้แก่ กรดไขมันระเหยได้ แอมโมเนียไนโตรเจน รวมถึงการสังเคราะห์จุลินทรีย์โปรตีนเพิ่มขึ้นด้วย

ในการทดลองครั้งนี้ยังพบว่าแม่โคที่ฉีดฮอร์โมน rbST และปรับอุณหภูมิแวดล้อมด้วยระบบ MF มีการกินน้ำเพิ่มขึ้นสัมพันธ์กับการเพิ่มขึ้นของอาหารที่กินได้ ซึ่งเป็นสาเหตุส่วนหนึ่งของการเพิ่มน้ำในทางเดินอาหาร และมีการไหลผ่านของน้ำจากกระเพาะหมักไปยังกระเพาะส่วนอื่นและลำไส้เพิ่มขึ้น ส่วนอิทธิพลของโรงเรือน ระบบ MF พบว่ามีผลต่ออัตราการดูดซึมน้ำผ่านผนังกระเพาะหมักเพิ่มขึ้น จากผลดังกล่าวจึงเป็นสาเหตุสำคัญที่ทำให้มีการเพิ่มขึ้นของน้ำในร่างกาย จากการศึกษาครั้งนี้ยังพบว่ามีการลดลงของระดับฮอร์โมนเลบติน (Leptin) ในพลาสมาพร้อมกับการกินอาหารได้เพิ่มขึ้น ซึ่งพบในโคที่ฉีดฮอร์โมน rbST และปรับอุณหภูมิแวดล้อมด้วยระบบ MF จากผลการศึกษาชี้ให้เห็นว่า rbST มีส่วนเกี่ยวข้องกับการสร้างน้ำนม ผ่านทางการเปลี่ยนแปลงของน้ำในร่างกาย สัมพันธ์กับการควบคุมน้ำในทางเดินอาหาร และการทำงานของกระเพาะหมัก ทำให้มีสารอาหารที่ถูกส่งไปยังต่อมน้ำนมเพิ่มขึ้น และบางส่วนของน้ำในร่างกายยังถูกใช้ไปในกลไกควบคุมความร้อนในร่างกาย การฉีดฮอร์โมน rbST ยังมีผลต่อการควบคุมการกินอาหาร โดยแสดงบทบาทผ่านการขับหลั่งฮอร์โมนเลบตินอีกด้วย

ภาควิชา......สรีรวิทยา ลายมือชื่อนิสิต__________
สาขาวิชา ...สรีรวิทยาการสัตว์ ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก__________
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KEY WORD: MISTY-FAN COOLING / BOVINE SOMATOTROPIN / DIGESTIVE PROCESS / CROSSBRED HOLSTEIN CATTLE / TROPICS

Wilaiporn Chanchai: EFFECTS OF MISTY-FAN COOLING ON THE PHYSIOLOGICAL RESPONSES IN RELATION TO DIGESTIVE PROCESS OF LACTATING CROSSBRED HOLSTEIN CATTLE TREATED WITH BOVINE SOMATOTROPIN IN THE TROPICS. THESIS ADVISOR: PROF. NARONGSAK CHAIYABUTR, Ph.D, THESIS COADVISOR: PROF. SOMCHAI CHANPONGSANG, D.V.M., 119 pp.

The investigations for the effects of misty-fan cooling and supplemental of rbST on physiological responses in relation to digestive process of crossbred 87.5% Holstein gene were performed. Eighteen primiparous cows were used for four experiments. Cows in each experiment were divided into two groups and assigned under the normal shaded barn (NS) as non-cooled cows and shaded barn with misty-fan cooling (MF) as cooled cows. The NS barn was separated from MF barn by longed metal sheet wall from floor to roof. Each cow was injected subcutaneously with 500 mg of rbST in every 14 days for 3 consecutive doses in each stage of lactation. Cows were fed the same total mix ration *ad libitum* and water was freely offered. The experimental results demonstrated that an application of MF cooling could reduce ambient temperature (AT) and temperature humidity index (THI). A low respiratory rate (RR) and rectal temperature (RT) were occurred in cooled cows. The marked effects of MF cooling could reduce the negative effect of high temperatures on digestive function via an increase in the digesta passage rate resulting in an increase in feed intake. An increase in dry matter intake (DMI) in response to both cooling system and rbST supplementation would be partly attributed to an increase in rumen fermentation with increases in VFA, NH_3N and microbial protein. It was also found that an increase in water intake accompanying with an increase in DMI was apparent in rbST-supplemented cows under misty-fan cooling. An increase in gut water and liquid outflow rate from the rumen were apparent in rbST supplemented cows. The effect of MF cooling influenced to an increase in net water transfer through the ruminal wall. The rbST-supplemented cows under MF barn also showed a high level of water absorption through ruminal wall. These changes would be in part accounted for an increase in total body water (TBW). The low level of plasma leptin concentration accompanying with an increase in DMI were observed in rbST-supplemented cows under MF barn. The present results indicate that the rbST exerts its galactopoietic action, in part, through changes in body fluids associated with increased in gut water regulation and rumen function, which would be the consequence in distribution of nutrients to the mammary gland and for thermoregulatory mechanisms. The effect of exogenous rbST on the regulation of feed intake would play a role via a reduction of leptin secretion.

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Academic Year : 2009 Co-advisor's Signature : Somchai Chanpongsang

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ABBREVIATIONS

ADF	Acid detergent fiber
AT	Ambient temperatures
BCS	Body condition score
BHBA	Beta-hydroxybutyric acid
BUN	Blood urea nitrogen
BW	Body weight
C_0	Concentration at zero time
CP	Crude protein
Cr_2O_3	Chromic oxide
D	Dose
db	Dry bulb temperature
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
DNA	Deoxyribonucleic acid
EB	Energy balance
EBW	Empty body water
ECF	Extracellular fluid
F	Flow rate
F_a	Absorption rate
FCM	Fat corrected milk
FFA	Free fatty acid
F_i	Insorption rate
GI	Gastrointestinal
GW	Gut water
HCl	Hydrochloric acid
HF	Holstein Friesian
HTO	Tritiated water
ICF	Intracellular fluid
IGF-I	Insulin like growth factor-I
k	Flow rate of constant
M	Marker
M.W.	Molecular weight
MBF	Mammary blood flow
MF	Misty-fan cooling
MNF	Microbial nitrogen flow
MUN	Milk urea nitrogen
MY	Milk yield
N	Nitrogen
N_a	Net absorption rate
NDF	Neutral detergent fiber
NE	Net energy
NEB	Negative energy balance
NEFA	Non esterify fatty acid
NE*i*	Net energy intake
NE*l*	Net Energy for lactation
NE*m*	Net Energy for maintenance
NH_3N	Ammonium nitrogen

NS	Normal shaded barn
N_t	Nutrients
OM	Organic matter
PEB	Positive energy balance
PEG	Polyethylene glycol
PRL	Prolactin
rbGH	Recombinant bovine growth hormone
rbST	Recombinant bovine somatotropin
RH	Relative humidity
RLD	Rumen liquid dilution
rpm	Round per minute
RR	Respiratory rates
RT	Rectal temperature
$t_{1/2}$	Half time
T_3	Triiodothyronine
T_4	Thyroxine
TBW	Total body water
TCA	Trichloroacetic acid
THI	Temperature humidity index
TMR	Total mixed ration
TSH	Thyroid stimulating hormone
UV	Ultraviolet
V	Volume
V_0	Volume at zero time
VFA	Volatile fatty acid
wb	Wet bulb temperature
WI	Water intake
WTO	Water turnover
X	Allantoin excretion

CHAPTER I

GENERAL INTRODUCTION

In the tropical countries, dairy herds are mixed exotic breeds and/or cross-bred animals. It is known that an efficient tool for blending the adaptability of endogenous tropical cattle with the high milk potential of exotic breeds is crossbreeding between *Bos taurus* and *Bos indicus* (Chaiyabutr et al., 2007). However, the main problem in dairy farming in the tropics of both exotic breed and cross-bred animals is still low level milk production. Chaiyabutr et al. (2000[a]) suggested that the regulation of milk secretion in crossbred cattle have been shown not only to be inherited but also being thought to be among the causes of differences in metabolic parameters. Heat stress is considered as a limiting factor in dairy production in tropics. The combination of high temperature and humidity by the temperature humidity index (THI) is a main factor that can produce heat stress and negative effect on lactation performance and physiological status of dairy cows (Armstrong, 1994; Kadzere et al., 2002). The physiological responses to high environmental temperature in lactating cows have been well known such as decreases in dry matter intake (DMI) and milk production, increases in rectal temperature and respiratory rate causing water loss by respiration including sweating. Furthermore, Chaiyabutr et al. (2000[b]) reported that milk yield of the 87.5% Holstein cattle decreased rapidly as lactation progress coinciding with a reduction in both mammary blood flow and plasma level of bST. It would be accounted for the short lactation persistency. In another report of Chaiyabutr et al. (2007) founded that long-term supplementation of recombinant bovine somatotropin (rbST) in 87.5% crossbred Holsteins could increase milk yield, but the stimulatory effect of bST on milk production was less in late lactation despite a high level of mammary blood flow. Thus, the question arises as to whether either genetic or high environment temperature influences lactation persistency.

Somatotropin (ST) is one of hormones (among many) which is known to play a role of the interaction between genetic potential and nutrition on milk production (Cheli et al. 1988; Gulay and Hatipoglu 2005; Settivari et al. 2007). The bST may exert galactopoietic action in part through increases in total body water (TBW) and extracellular fluid (ECF) in association with an increase in mammary blood flow

(MBF), which contribute partition nutrients to the mammary gland for milk synthesis (Maksiri et al., 2005 ; Chaiyabutr et al., 2007).

An occurrence of greater heat stress has been reported in some studies after bST administration, which is probably due to an increase in metabolic activity associated with higher milk yield (West 1994; Settivari et al. 2007). Cole and Hansen (1993) have concluded that administration of bST can increase the severity of responses of cows to heat stress without changing milk yield. Several studies have proposed that an increase in milk yields of bST-treated cows remained greater than those of control cows even under heat stress conditions (Johnson et al. 1991; Santos et al. 1999; Gulay and Hatipoglu 2005). West (1991) has suggested that administration of bST to high yielding cows in extremely hot-humid environments would restrict feed DMI and increase heat stress. In addition, Nasser et al. (2003) observed that the bST-treated cows had higher energy expenditure, which increased plasma non-esterified fatty acid (NEFA) including rumen ammonium nitrogen and volatile fatty acid. The mechanisms for an alteration of digestive function in crossbred dairy cattle during exposure to high temperatures are not yet clear, although a reduction in dry matter intake (DMI) is partly known as one of mechanism responses to heat stress with a consequence of the reduction of milk yield. There are inconsistent results in regarding to the relationship between high environmental temperatures and digestive function. Some reports showed a reduction in diet digestibility by excess THI which related to a decline in ruminal activity through the depression of rumen cellulolytic activity (Bernabucci et al., 1999). Both slower passage rate and longer mean retention time of digesta have been shown in cow exposure to high ambient temperatures (Christopherson and Kenedy 1983; Silanikove 1992). An increase in diet digestibility has also reported in dairy cows exposure to hot environment (Collier et al., 1982; Mathers et al., 1989).

However, the interaction effects between thermal stress and the role of exogenous rbST on the lactation performance in crossbred lactating cattle are not yet clear. Somatotropin is known to play a role in responsible for galactopoietic and contributing to homeostasis and homeorhesis in ruminants (Bauman and Currie 1980). Administration of bST to lactating cows under hot environment can increase milk yield (West et al., 1991). The rbST-treated cows under hot environment have been shown to increase heat production approximately 25 % over the control cows (West 1994). Johnson et al. (1991) reported that somatotropin increased the efficiency of feed conversion into milk without any significant changes in body weight and body temperature. These results suggest that there is no consistent conclusion in the interaction effects between thermal stress and the role of bST on lactation performance. Little is known about the modification of the effect of somatotropin on body function concerning digestive function in crossbred cattle. In other ruminating animals, an increase in the rate of liquid flow from the rumen during heat exposure has been reported in buffalo and goat (Chaiyabutr et al., 1987; Silanikove and Tadmor, 1989). Although bovine somatotropin is known to influence body nutrient availability and utilization, little is known about the mechanism whereby exogenous bovine somatrotopin exerts it effects on rumen function and milk production in hot conditions. Thus, the initial work of this thesis was concerned with determining the effectiveness of misty-fan cooling and supplemental rbST on rumen function, feed intake and milk production of crossbred Holstein cattle (Chapter IV).

Inconsistent results regarding the relationship between high environmental condition and digestive function have been observed by several studies. Bernabucci et al. (1999) showed a reduction in diet digestibility through a decline in rumen cellulolytic activity when animal exposure to excess THI. Rumination, reticular motility and blood flow to rumen epithelium are depressed during animal exposed to heat stress condition (Attenberry and Johnson, 1968; Hales et al., 1984; Aganga et al., 1990). Additionally, slower passage rate and longer mean retention time of digesta have been shown in cows exposure to high ambient temperature (Christopherson and Kenedy, 1983; Silanikove, 1992). In contrast, other research groups have shown an increase in diet digestibility in dairy cows exposed to hot environment (Collier et al., 1982; Mathers et al., 1989). Shibata and Mukai (1979) have suggested that an increase in diet digestibility would increase total volatile fatty acids leading to increase in heat production. These results would be attributed to increase in body temperature of cows under high temperatures. The studies of digestive function and milk yields of cows during rbST supplementation under high ambient temperature in relation to diet digestibility, digestion kinetics and milk production of crossbred Holstein cattle in the tropics were clarified in Chapter V.

It is known that an increase in water absorption occurs at lower GI-tract for heat dissipation mechanism during heat exposure. In view of the impact of the study of Chaiyabutr et al. (2007) that showed an increase in total body water in rbST-treated crossbred cows. Moreover, the water content of digesta in the rumen and its volume have been shown to increase in heat stress goats which serve as a water reservoir to counterbalance the effect of heat stress (Silanikove, 1992). In other ruminating animal e.g. buffalo, an increase in plasma volume and blood volume during acute and short term heat exposure has been reported (Chaiyabutr et al., 1987). It might be possible that an increase in water absorption occurs at lower gastrointestinal tract would be used for heat dissipation mechanism during heat exposure. An alteration of gut water relates to the regulation of body fluid and mammary blood flow of rbST treated-lactating animal in hot environment has not been clear. The question then arise whether management strategies using rbST supplementation in cows under high temperatures will minimize the effect of heat stress with changes in the flow rate of digesta from the rumen in maintaining sufficient nutrients for increased milk yields. More data are required to study the changes of gut water relating to body fluid and milk production of crossbred Holstein cattle in the tropics (Chapter VI)

A reduction in dry matter intake is partly known as one of mechanism responses to heat stress with a consequence of the reduction of milk yield. It is recognized that the most of nutrients for milk synthesis will be obtained by feed intake. Reduction in dry matter intake (DMI) in cow exposed to hot environment will be a cause of insufficient substrates for milk synthesis. However, there are many factors that regulate feed intake, such as quantity and quality of feed stuff, feeding management, environment, hormone and physiological state of the cows. One of those factors, leptin, peptide hormone synthesis from adipose tissue, plays an important role to regulate feed intake and energy balance (Chillard et al., 2000; Thomas et al., 2001). Therefore, the possible explanation for the decrease in DMI of heat stress animals might be, in part, mediated by increasing plasma leptin concentration. The results of Bernabucci et al. (2006) have suggested that variations of gene expression of leptin and leptin receptors in adipose tissue in lactating dairy cows are directly induced by photoperiod. Accorsi et al. (2005) have reported that the leptin level is influenced by changes in the environmental temperature and/or daylight, as in summer month. However, an evidence for the plasma leptin concentration relation to hot environment in dairy cattle has not yet been clear. An interaction among hormones is usually complex in animals during exposure to high hot-humid environment. The rbST has been known to play a role to increase milk yield which may mediate via either decrease in the plasma leptin concentration resulting in increase in DMI or the reduction of heat stress with low ambient temperature would enhance the response of rbST increasing in DMI. In the Chapter VII, the effect of misty-fan cooling system and supplemental rbST on plasma hormones and metabolite concentrations in relation to feed intake and milk production was elucidated in crossbred Holstein cattle under hot environment.

The overall interaction effects of misty-fan cooling system and rbST supplementation on physiological changes of digestion in relation to milk production in lactating crossbred Holstein cattle are discussed in Chapter VIII. The methodology used in this work is described in Chapter III. One chapter is devoted to the previous work concerned with ruminating animals (ChapterII).

CHAPTER II

LITERATURE REVIEW

Since the main emphasis of the work described in this thesis has been directed towards the dairy cattle. This review is largely concerned with function in ruminating animals, although comparisons with others are made where appropriate.

Effect of hot environments on dairy production

Dairy production in tropical countries, particularly Thailand, where a typical hot-humid environment, has been affected a number of factors, for example, a lower genetic potential for milk production in indigenous cattle, high ambient temperature with high humidity and a low quality of forage. It is recognized that crossbreeding of *Bos taurus* and *Bos indicus* has been chosen as an efficient tool for blending the adaptability of endogenous tropical cattle with the high milk potential of exotic breeds. Most of dairy herd in Thailand is crossbred cow. However, low milk yield is still the main problem. Milk production and reproduction proficiency of lactating dairy cows are greatly diminished when they exposed to hot environment. Therefore, several ways have been used to improve milk production in the tropics including modified feeding program and physical management.

Physiological changes under hot environmental temperature

The thermal environment is a major factor that can negatively affect milk production of dairy cows, especially in animals of high genetic merit. Important meteorological factors affecting milk production are temperature, humidity, wind, radiation, and photoperiod (Armstrong, 1994). Various combinations of temperature and humidity can produce the same temperature humidity index (THI). For example, when low temperature as 24 °C can cause stress to animals if the humidity is high (90%), but high temperature as 38 °C will not cause heat stress to animal if the humidity is low (<10%). Thus, a temperature humidity index (THI) is a single value representing the combined effect of air temperature and humidity for determination the level of thermal stress. Mader et al. (2007) have suggested that a severity of heat stress is quantified by using different THI values (normal <74; alert 74-79; danger 79-

84; emergency>84). The dairy cow subjects to heat stress when heat load is greater than heat dissipation capacity causing a rise in body temperature. Effects of heat stress on physiological response have been reported, for example; increases in respiration rate, water intake and sweating, decreases in dry matter intake, milk production and poor reproductive performance; slow rate of feed passage and decrease in blood flow to internal organs, (Chaiyabutr et al., 2000^b; Kadzere et al., 2002; Abeni et al., 2007).

Effects of hot environment on feed intake and milk production

High humidity can affect the heat loss of dairy cattle under high temperatures and thereby the performance of animal falls markedly in hot, humid summer. Moreover, heat production will influence feed intake level, which in turn affects the production. The sudden responses of cattle to heat stress are apparent with increases in water intake and the breathing rate. A reduction of feed intake causing a reduction of metabolic heat production will associate with digestive and metabolic processes (Abeni et al., 2007). A large body size and high metabolic rate of lactating dairy cows are extremely sensitive to heat stress with a reduction of feed intake leading to reductions in digestive and metabolic processes (Bertoni, 1998). The reduction of metabolic heat production during exposure to high ambient temperatures has been suggested by Johnson et al. (1991) that animal attempts to counteract the greater heat load from a hot environment in controlling body temperature.

Cows exposure to high temperatures for extended periods (at THI above 80 for 5 to 6 wk) have been shown to produce 25 to 35% less milk coinciding with a decrease in feed intake (Huber et al., 1994). In addition, heat stress of lactating cows dramatically reduces roughage intake and rumination. Such decreases in roughage intake have been shown to contribute to decreases in VFA production and an alteration in the ratio of acetate to propionate (Huber et al., 1994). Therefore, a reduction in dry matter intake (DMI) will account for decreases in nutrients available for milk synthesis and a number of lactation parameters are affected (Moody et al., 1971; Bauman and Currie, 1980; Collier et al., 1982; Smith et al., 1993).

Effects of hot environment on digestive function

An alteration of the dynamic characteristics of digestion is recognized as a possible mechanism through which heat stress can affect the nutrition of animals

(Beede and Collier, 1986). Under thermoneutral conditions, reduction of DMI is generally associated with an increase in diet digestibility and a decrease in rumen passage rate (Van Soest, 1994). Conversely, it has been reported that under hot conditions, diet digestibility and rumen passage rate were not affected by a reduction in DMI (Attebery and Johnson, 1968; Warren et al., 1974; Lipke, 1975; Miron and Christopherson, 1992). Increases in diet digestibility in animals exposure to hot environments have been reported in dairy cattle (NRC, 1981). In contrast, several studies have reported that negative or no relationships were apparent between exposure to high ambient temperature and diet digestibility in either dairy cattle (McDowell et al., 1969; NRC, 1981; Mathers et al., 1989) or small ruminants (Silanikove, 1985; Lu, 1989).

Both slower passage rates and longer mean retention times of digesta have been described for dairy cows under hot environments when compared with cows kept under thermal comfort conditions (Miron and Christopherson, 1992; Silanikove, 1992). Previous studies have suggested that the variation in the rate of passage of digesta would be a major cause of change in digestibility by heat-stressed ruminants. However, hot environment can affect digestibility in a time-dependent fashion, suggesting an adaptation of the digestive tract to hot environments (Miron and Christopherson, 1992; Bernabucci et al., 1999). A reduction in feed particle passage rate would cause the feed expose to microbial activity for a longer time, which leads to increase in digestibility. However, several studies have revealed that high temperatures do not depress passage rate or rumen activity via feed intake (Attenbery and Johnson, 1968; Silanikove, 1985; Warren et al., 1974). Microbial efficiency is positively correlated with the rate of passage for organic matter and starch. Rapid passage rate may decrease microbial turnover in the rumen, enhancing microbial efficiency (Oba and Allen, 2000; Oba and Allen, 2003[b]). Although, ruminal fermentation in cattle has the ability to metabolize cellulose materials, it also utilizes non-protein nitrogen and harvest microbial protein as source of amino acids (Hungate, 1966). However, the fermentation also produces ammonia, methane and heat. The heat inclement from ruminal fermentation can be advantageous in cool climates, but it will be another heat load on the animal if the ambient temperature is high. (Russell, 2007)

In addition, the impact of heat stress on performance is in part due to specific behavioral responses not only leading to reduction in dry matter intake but also the physiological responses leading to decreased blood flow to the internal organs, particularly gastrointestinal organ. It will lead to decrease in nutrient uptake as well as an increase in maintenance requirements (Gwatibaya et al., 2007).

Environmental modification to reduce heat stress in dairy cattle

Beede and Collier (1986) have proposed three management strategies to reduce thermal environmental stress, namely: 1) physical modification of the environment, 2) genetic development of heat-tolerant or less heat sensitive breeds, and 3) improved nutritional management schemes. The physical modification of the environment is a common way to selected for alleviated effect of hot environment in dairy cows such as fans, sprinkler and cooling pad system (Beede and Collier, 1986; Huber et al., 1994; Chan et al., 1997).

Heat stress of dairy cattle can be alleviated by artificial cooling methods. The degree of improvement varies with the type of system provided, climate, and production of the cows (Armstrong, 1994). Shade may be cost effective for reducing the effect of heat stress. Cooling methods for cows managed in pasture systems include cooling ponds, fixed or mobile shade structures, and trees. There are the most effective shade producers; they combine protection from the sun with the radiation sink effect created by cooled leaves evaporating moisture (Armstrong, 1994). Fans and sprinklers technologies for heat stress abatement in confined-housing production systems have advanced in the past decade but remain limited for animals on pasture (Fike et al., 2002). Gallardo et al. (2005) reported that RT and RR decreased in response to evaporative cooling with sprinklers and fans before milking. Some types of cooling system can be considered after the more routine practices are taken care of. The most economical cooling method is evaporative cooling using spray jets or mini-sprinklers and fans. In sprinkler and fan cooling, the sprinklers create droplets that wet the cow's hair coat to the skin. Fans are then used to force air over the cow's body causing evaporative cooling to happen on the skin and hair coat. Air movement is needed in fairly humid climates to provide enough drier air above the skin to do a good job of evaporating the water.

Several studies have shown that evaporative cooled cows have lower rectal temperatures and respiration rates than those of non cool cows. A greater DMI for cooled cows have shown to be associated with higher milk production (2.5 kg/d of increase) in comparison with cows receiving only shade (Armstrong et al., 1993;

Chen et al., 1993). Chen et al. (1993) have reported that lower rectal temperature and respiratory rate, but higher DMI and milk yields (2.5 kg/d) for cows receiving shade plus evaporative cooling than for cows under shade only. Fike et al. (2002) reported that housing cows during the day with fans and sprinklers effectively reduced heat stress as indicated by lower body temperatures and respiration rates including increase in 4% FCM (0.9 kg/d) production in response to daytime evaporative cooling.

Hormonal control of mammary function and milk production

The capacity of milk producing cow is determined by a combination of the growth and development of the mammary gland. The changes in mammary cell numbers (mammogenesis) and in milk yield per cell (galactopoiesis) are regulated by both galactopoietic hormones and local mammary factors. Therefore, the maintenance of lactation is at least partly controlled by a group of hormones collectively known as galactopoeitic hormonal complex. The hormonal complex includes prolactin, growth hormone (somatotropin), thyroid hormone, and glucocorticoids.

In most non-ruminants and ruminants, prolactin is one component of a hormonal complex that regulates galactopoeisis. The role of prolactin in cows is ambiguous. Inhibition of prolactin in cows and goats had little effects on milk yield (Koprowski and Tucker, 1973; Peters and Tucker, 1978) when compared with rodents and rabbits (Hennighausen and Robinson, 1997 and 1998). Growth hormone is well known as an essential hormone for maintaining lactation (galactopoeitic) in dairy cattle. Growth hormone coordinates changes in body tissues and physiological processes that support increase in synthesis of milk composition in the mammary gland. Peel et al. (1981) demonstrated that growth hormone plays a key role in regulating nutrient use during lactation. Even at peak milk production, high-yielding cows can be manipulated to partition more nutrients toward milk synthesis. Thus, growth hormone is a homeorhetic control in the coordination of the metabolism of body tissues to support mammary needs.

Glucocorticoids at physiological levels is galactopoeitic, however, at high doses it inhibits lactation. Cortisol is the predominant endogenous glucocorticoid in cattle whose major function at the mammary gland is to cause differentiation of the lobule-alveolar system. This glucocorticoid-induced differentiation is essential to allow prolactin to later induce synthesis of milk proteins (Mills and Topper, 1970). Exogenous thyroxine is well known to be temporarily galactopoietic. The cause of failure to maintain elevated secretion of milk is unknown, although iodine toxicity of the iodinated casein fed has been suggested as a possible cause (Kahl et al., 1991). Injection of thyroid hormone into cow increases milk production for a short period of time (several weeks) but administration of thyroid hormones to animal for more than 7 weeks has been shown no effect on milk yield (Blaxter et al., 1949).

Estrogen is also observed to be involved in initiating lactation in the periparturient period. Estrogen act in at least two ways to initiate lactation: firstly, it cause release of prolactin from the anterior pituitary gland into blood (Nagasawa et al., 1969), which in turn, will initiate lactation; and secondly, estrogen increases the number of prolactin receptors in mammary cells (Sheth et al., 1978), which is another lactogenic event. Estrogen administered in very low doses is galactopoietic. However, higher doses have inhibitory effects (Bruce and Ramirez, 1970). A combination of estrogen and progesterone is more inhibitory effect than given estrogen alone. Progesterone alone has no effect on galactopoeisis because there are no progesterone receptors in the mammary gland during lactation.

Although, control of lactation is clearly regulated by several hormones, however, a local factor such as milk removal is also important. Nursing or milking is also stimulus triggers release of galactopoietic hormones (especially prolactin) which may stimulate the next round of secretory activity. If milk removal is not maintained there is no stimulation for prolactin release.

An overview of somatotropin hormone

Somatotropin is a protein hormone synthesized in and secreted from the anterior pituitary gland. Somatotropin secretion is regulated by two well-characterized hypothalamic peptides that act to stimulate (growth hormone-releasing factor; GRF) or inhibit (somatostatin) release of bovine somatotropin from the pituitary gland (Tuggle and trenkle, 1996). Somatotropin contains 191 amino acids. Bovine somatotropin and porcine somatotropin share a high degree of amino acid sequence similarity 90% (Bauman and Vernon, 1993; Etherton et al., 1993). Somatotropins are fairly species specific, however human and bovine somatotropins are quite different. About 35% of the amino acids in human somatotropin differ from those of bovine somatotropin (Carr and Friesen, 1976; Lesniak et al., 1977; Moore et al., 1985; Fetrow, 1999). Somatotropin orchestrates many diverse physiological processes so that more nutrients can be used for lean tissue accretion (during growth) or milk synthesis (during lactation). Somatotropin is a homeorhetic control that affects numerous target tissues in ways that are highly coordinated to affect marked changes in nutrient partitioning among these tissues. The biological effects of somatotropin can be broadly classified as either somatogenic or metabolic. The somatogenic effects are those in which somatotropin stimulates cell proliferation. These effects are mediated by IGF-I (Rechler and Nissley, 1990). Many of the metabolic effects are a direct action of somatotropin that involved a variety of tissues and the metabolism of all nutrients; carbohydrate, lipid, protein, and minerals. These coordinated changes in tissue metabolism alter nutrient partitioning and thus play a key role in increasing growth performance or milk yield.

Recombinant bovine growth hormone (rbGH) or recombinant bovine somatotropin (rbST) refers to bovine growth hormone that is manufactured in a laboratory using genetic technology. This synthetic hormone is marketed to dairy farmer to increase milk production. To make recombinant bovine somatotropin, the plasmid of a bacterium is cut by enzymes, and then combined with a cow's DNA. It is reintroduced to the bacterium, placed in a fermentation tank and allowed to multiply, then separated and purified before delivery to the farmer (Roush, 1991). This modern technology permitted the development of recombinant bovine somatotrophin (rbST), which provide an unlimited source of ST for research and, potentially, for commercial application. This allows researchers to conduct many studies using rbST. From various researches, it was found that exogenous ST could increase milk production of dairy cows at least 10% to 15% (Bauman, 1992; Santos et al., 1999; Gulay and Hatipglu, 2005). This rbST can be injected into the dairy cow to augment her naturally produced levels of this hormone, enhancing milk production but requiring additional feed and other inputs to achieve increased milk production. However, obtaining a milk yield response to rbST does not require special diets or different feed ingredients. The rbST-treated cows need adequate amounts of balanced diet that contains all nutrients necessary for supporting expected milk production (Buaman, 1992). Furthermore, milk from rbST-treated cows has been approved to safety for human consumption.

Role of somatotropin hormone on milk production

Administration of exogenous ST has been shown to enhance lactational performance in mammals ranging from laboratory animals to humans (Milsom et al., 1992; Bauman and Vernon, 1993; Gunn et al., 1996). In the case of farm animals, a milk yield response has been demonstrated with ST treatment in pigs, sheep, goats, and cows. The majority of research has involved dairy cows, and bST has been approved for commercial use in 25 countries. Milk yield response to bST has been observed for all dairy breeds and in animals of different parity and genetic potential (Burton et al., 1994; NRC, 1994; Hartnell, 1995). In general, response is negligible in early lactation before peak yield, so bST use is over the last 80% of the lactation cycle. Typical milk yield responses are increases of 10–15% (4–6 kg/day), although even greater increases occur when the management and care of the animals are excellent (Chilliard, 1989; Bauman, 1992; NRC, 1994).

Bovine somatotropin increases milk yield with no change in milk composition, unless there is energy deficiency. It provokes high milk yield by increasing overall blood supply to mammary gland, altering glucose metabolism (lactose synthesis in the mammary gland), enhancing lipolysis and increasing lipogenesis in the mammary gland (supply of lipid precursors), and also modifying protein metabolism (supply of amino acids). As a result, milk yield increases and the composition of milk do not change from normal (Davis and Collier, 1985; Cheli et al., 1998; Santos et al., 1999).

The major factor affecting the magnitude of milk response to bST is the quality of management. Milk production responses to bST are not dependent on special diets or unique feed ingredients, but animals must receive adequate amounts of a balanced diet (Etherton and Bauman, 1998; Gulay and Hatipglu, 2005). Substantial milk yield (MY) responses have been observed on diets ranging from pasture to the more typical total mixed ration (TMR) diets. Overall, daily nutrient requirements are increased by an amount equal to the increase in milk. The productive efficiency (milk per unit of feed) is improved because a greater proportion of the nutrient intake is used for milk synthesis (Cooker and Otterby, 1991; Bauman, 1992; Patton and Heald, 1992; NRC, 1994; McGuire and Burman, 1997). Thus, additional feed inputs can be committed entirely to milk production. If the cow is not fed to support additional production, it will not make more milk. The hormone cannot force production, nor does it "burn out" cow (Fetrow, 1999).

In addition, many factors such as cow comfort, hygiene, proper ventilation, heat abatement in hot and humid weather, water, and general health, can contribute to the ability of the cow to respond to the exogenous somatotropin. Most of the management issues are the same as for any effort to improve milk production. Responses are highest on well managed dairies. The most critical need is nutrition level. Cows have to be fed adequate amounts of a well balanced diet in order to respond in production. Proper housing to assure good cow comfort, good ventilation, and adequate hygiene will improve cow health and feed intake. (Fetrow,1999). Furthermore, Chaiyabutr et al. (2007) have shown that the bST exerts galactopoitic action in crossbred Holstein cattle, in part, through increase in total body water, empty body water and extracellular water in association with an increase in mammary blood flow, which partitions the distribution of nutrient to the mammary gland for milk synthesis.

The use of rbST under hot environment

The major challenge for high producing dairy cows in hot climates is to dissipate heat produced by metabolic processes. Metabolic heat production increases as the productive capacity of dairy cows improves. Cows yielding 18.5 and 31.6 kg/d of milk have been shown to produce 27.3 and 48.5% more heat, respectively, than dry cows (Purwanto et al., 1990). The greater heat production would be associated with an approximate 49% increase in milk energy secretion, and increased heat production is balanced with greater heat dissipation. Cows administered bST under hot conditions produce about 25% more heat than cows not receiving bST (Manalu et al., 1991).

Somatotropin injection has been shown to increase both rectal temperatures and respiratory rates (Staples et al., 1988, Zoa-Mboe et al., 1989). However, a study in bST-treated cows under controlled environmental conditions shows no changes in body temperature. Lactating dairy cows treated with bST have shown to increase heat production, whereas cattle in hot environments decrease heat production (Tyrrell et al., 1988). With these facts, Johnson et al. (1991) hypothesized that bST could alleviate the decreased milk production attributed to hot environments; however, the magnitude of responses may be less than under thermoneutral conditions. Furthermore, the increased heat production in bST-treated cows in hot environments may not be great enough to alter the cow's ability to maintain homeothermy, thus, cows injected with bST will continue to have greater and more efficient milk production in a hot environment. The assessment of the interactive effects of bST and a hot environment on lactating performance of dairy cows requires controlled environmental conditions. Any factors that decrease in dry matter intake can reduce the milk response to bST.

During summer in a subtropical environment, slight increases in milk yield and 3.5% FCM production have been observed in bST-treated cows. Such increases are not accompanied by changes in feed intake and milk fat percentage (Staples et al., 1988; Zoa-Mboe et al., 1989). In controlled laboratory conditions, heat-stressed lactating cows injected with purified bST have been shown to increase in milk yield without significant changes in feed intake (Mohammed and Johnson, 1985). Several studies have reported that somatotropin supplementation under heat conditions did not affect plasma concentrations of triiodothyronine (T_3). thyroxine (T_4), cortisol, prolactin (PRL), and insulin, although the responses of heat-stressed cows to bST treatment varied among reports (Chaiyabutr et al., 2000[a]).

Role of leptin in ruminants

Leptin is a protein hormone secreted predominantly by adipocytes. The leptin gene is also expressed in mammary gland, the stomach, and skeletal muscle including placenta and fetal tissues in ruminants (Chilliard et al., 2005) and in other species (Ahima and Flier, 2000). Leptin plays a central role in the regulation of energy homeostasis and food intake, through an action on the hypothalamus and peripheral targets. Results of Bernabucci et al. (2006) have suggested that variations in gene expression of leptin and leptin receptors in adipose tissue of lactating dairy cows are directly induced by photoperiod. A possible prolactin-mediated effect of photoperiod on adipose tissue leptin modulation has been established in lactating dairy cows. Inoue et al. (2005) have suggested that a large number of hormones have been shown to play a role in the regulation of energy homeostasis and body weight in animals. The regulation of dry matter intake in lactating dairy cattle can influence to enhance performance and improve animal health and welfare. Prediction in voluntary dry matter intake is complex and influenced by numerous factors relating to the diet, management, housing, environment and the animal. Leptin inhibits feed intake and down-regulates adipose tissue deposition (Halaas et al., 1995; Morrison et al., 2001) and negatively correlate with the amount of nonesterified fatty acids, which reflects the amount of fat mobilization (Block et al., 2001). Furthermore, there is evidence that leptin positively influences fertility (Liefer et al., 2003).

The satiety effects of leptin have been observed ewes with administration of recombinant human leptin for 3 days. This treatment causes a decrease in voluntary dry matter intake by approximately one third of normal intake. However, these effects are lost when animals are underfed, although leptin is administered (Henry et al., 1999; Morrison et al., 2001). This indicates that another signal blocks the effect of leptin on feed intake when the body is negative energy balance. In addition, Gualillo et al. (1999) and Menendez et al. (2003) have proposed that prolactin and thyroid stimulating hormone (TSH) can stimulate release of leptin from white adipose tissue. It indicates that leptin can be modulated in response to either increases or decreases in ambient temperature, although no definitive research exists to provide evidence for this relationship. In summer months, when both environmental temperatures and daylight are the highest, cows will show high level of plasma leptin but do not show any energy deficit and no change of body weight (Accorsi et al., 2005).

CHAPTER III

MATERIALS AND METHODS

This thesis is based on four experimental studies. The experiments were conducted at Nakornpathom training farm of Faculty of Veterinary Science, Chulalongkorn University in 2006 to 2008. Details of materials and methods in all studies are described in this Chapter.

Animals, housing and managements

Eighteen primiparous, non-pregnant crossbred cattle, containing 87.5 % Holstein (HF) genes, age 36±2 months, average body weight 358±32.5 kg and body condition score (BCS) 2.5±0.5 were used for the experiment. The first-ten cows with averaged 60±1 days in milk (DIM) were used for experimental studies in Chapter IV, V, VI (series I) and VII. The other eight cows were used for studies of water transfer from rumen in mid stage of lactation in Chapter VI (series II). Cows in each experimental study were assigned randomly into two groups, equal number of animals in each group. During the study period, cows in both groups were housed in open-sided with a tiled-roof and tie-stall barn (Fig. 3.1). The barn (16 m long x 7 m wide x 3.5 m high) was separated into two parts by a metal sheet wall (3.5 m high). The first part (8 m long x 7 m wide x 3.5 m high) was arranged for animals in normal shade (NS) and the second part of barn equipped with two misty-fan cooling system (MF) (Masterkool, Thailand) for cooled animal. Each system 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 MF for 45 min at 15-min intervals from 06.00 to 18.00 hour. At night, animals were exposed to MF for 15 min at 45-min intervals from 18.00 to 06.00 hour.

Cows in both groups were fed with the total mixed ration (TMR) which was formulated according to NRC requirements (NRC, 2001) for 10-15kg producing cows. The TMR diet and ingredients are shown in Table 3.1. The TMR was offered twice daily at 110% of *ad libitum* consumption at 06.00 and 17.00 hour throughout the experimental period. Water was given to cows *ad libitum*. Dry matter intake

(DMI) of each cow was measured daily by weighing the TMR offered and subtracting that refused. Cows were normally milked at around 06.00 and 17.00 hour using a milking machine and milk production was recorded daily

Table 3.1 Feed ingredients and chemical compositions of the diet

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

Figure 3.1 The open-side, tie stall barn installed misty-fan cooling system (right) and normal shaded barn (left) used in this study

The ambient temperature at NS and MF barns were recorded using a wet and dry bulb thermometer. The relative humidity at NS and MF barns were read by psychometric chart depending on wet and dry bulb temperature. Ambient temperatures and humidity were measured during the daytime, three days before beginning of the first injection of rbST (pre-treatment period) and three days after the 3rd injection of rbST (treatment period) in each stage of lactation. Average values were considered to be the mean values of all measurements taken throughout periods of study. A temperature-humidity index (THI) was calculated from the average ambient temperature of dry and wet bulb temperatures according to McDowell (1972), as follow:

$$\text{THI} = 0.72\,(\text{wb}+\text{db}) + 40.6$$

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

Body weight (BW) of all animals were recorded by weighing monthly throughout experimental periods. Milk samples were collected from morning milking. The 60 mL of milk sample was preserved with 300 μL bronopol (2-Brom-2-nitro-1,3-propandiol)

(0.02 w/w) and kept at 4°C for determination of milk compositions determination by using Milkoscan (Milko-Scan 133B; A/S N. Foss Electric, Hillerod, Denmark).

Experimental design

An overview of certain parts of the experimental designs in the experimental studies in chapter IV-VII, is presented in Fig. 3.2. The procedures used in the present study were carried out in accordance with the principles and guidelines of the Faculty of Veterinary Science, Chulalongkorn University. These guidelines were formulated to comply with international standards and are in accordance with the principles and guidelines of the National Research Council of Thailand.

Cows using in this experiment either in NS or MF barn were studied in three consecutive stages of lactation: early lactation, 60-90 DIM; mid lactation, 120-150 DIM; and late lactation, 180-210 DIM. (Fig. 3.2). Each stage of lactation, two periods of study were performed for pre-treatment of rbST (Pre-treat) and treatment of rbST (Treat). The study at pre-treatment was conducted at the first day of each stage of lactation. At the end of the pre-treatment, within the same day, the cow was received the first dose injection of 500 mg rbST (POSILAC, Monsanto, USA) at the tail head depression. Subsequently, two additional injections of rbST were given at two-week intervals. Thereafter, within two days after receiving the 3rd rbST injection the treatment study (Treat) was carried out. This schedule within the experimental period was followed for each of the three stages of lactation. During the last 30 days after the end of each stage, no experiments were conducted. This allowed effects of handling, sampling and rbST to be return to normal before start of the next periods. It has been demonstrated in dairy cattle by Kirchgessner et al. (1991) that during the first week after injection of bST, milk yield increased sharply and almost returned to control level within the next 3 weeks. It is known that milk yield of dairy cow gradually decline after peak of lactation. The short time of the experiment in each stage of lactation (for 30 days between pre-treatment and treatment) in the present study might not suspect to influence of milking day on the measurement for milk yield in each stage of lactation.

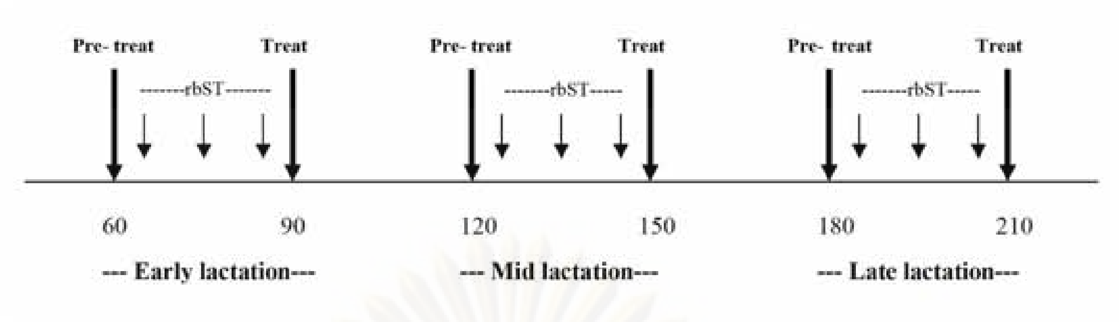

Figure 3.2 Schematic diagrams illustrating the time course of the experiment in each cow supplemented with rbST at different stages of lactation. Pre-treat = timed study for pre-treatment; Treat = timed study for treatment.

Determination of digestibility and passage rate

Chromic oxide (Cr_2O_3) was used as an external indigestible marker to estimate nutrient digestibility and passage rate of digesta in the total gastro-intestinal tract of each cow. Cow was given 10 gram of Cr_2O_3 /day containing in gelatin capsules. Cr_2O_3 was divided to two portions and was given orally to animal twice a day at 06.00 and 18.00 hour for 10 days. In determination of the diet digestibility, fecal grab samples were collected three times a day from day 8 to day 10 after dosing so that nine samples were taken for each cow. Nine fecal samples were pooled and dried for chemical compositions analysis. After the last dose of chromic oxide, individual fecal grab samples were collected at 0, 4, 8, 12, 16, 20, 24, 30, 36, 44, 50, 62, 68, 74, and 96 hours after dosed. The chromic oxide content of the sample was determined by a colorimetric method which modified from Kimura and Miller (1956). The analysis of the sample for chromic oxide content was performed, in brief, as follows; one gram of feces sample was transferred into 100 mL volumetric flask. The 200 uL of 2.5% sodium molybdate and 10 ml of nitric acid were added. The solution was gently boiled on a hot plate for 10-15 min. The flask was removed to cool in room temperature and then added 5 mL of 70% Perchloric acid. The solution was strongly heated again for 10 min or until complete oxidation. The flask was removed to cool for 5 min and further heating for 2-3 min or the solution in the flask change the color to yellow or orange. The flask was allowed to cool and made volume up to 100 mL with distilled water. The solution was allowed to silica settle. The absorbance of clear solution was read at 440 nm. The concentration of chromic oxide content in the sample solution was determined using standard curve preparing from known amount of chromic oxide solution (1-10 mg).

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

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

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

Mean retention time (h) = 1 / k

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

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

Dry matter digestibility (%) = $100 - [\ (M_{\text{in feed}} / M_{\text{in faeces}}) \times 100\ ]$

Nt digestibility (%) = $100 - [(M_{\text{in feed}}/M_{\text{in faeces}}) \times (Nt_{\text{in faeces}}/Nt_{\text{in feed}}) \times 100]$

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

Efficiency of feed utilization = FCM / DMI

Where; FCM = 4 % fat corrected milk (kg/cow/day), DMI = dry matter intake (kg/day)

Determination of rumen fermentation and ruminal microbial production

On specified days, a sample of rumen fluid was collected from each animal using a stomach tube connected to a vacuum pump. Samples were taken 2.5 hour after morning feeding for determination volatile fatty acids. The pH was measured immediately after collection using a pH meter (Orion Model 420A; Orion research Inc. Boston, USA) equipped with a glass electrode (pH-TRIOD^{TM} Orion). The ruminal fluid samples were strained immediately through two layers of cheesecloth and preserved by adding 6 N HCl. The preserved ruminal fluid samples were frozen at -20°C prior to volatile fatty acids (VFA) and ammonium nitrogen (NH_3-N) analyses. Ruminal fluid samples were analyzed for VFA by a gas chromatography and NH_3-N by phenyl-hypochlorite reaction (Weatherburn, 1967). The concentration of acetate, propionate and butyrate are expressed as mmol/l. The acetate:propionate ratio (A:P ratio) was calculated from their concentration.

Milk samples were collected from morning milking and fat-free milk was obtained by centrifugation at 3000 rpm for 15 min at 4 °C. Protein in fat free-milk samples using for the determination of the milk urea concentration were extracted with 10% TCA and the concentration of milk urea were determined by the diacetylmonoxime method (Coulombe and Favreau, 1963). Protein in fat free-milk samples using for determination of the milk allantoin concentrations were extracted with 5% uranyl acetate and the milk allantoin analysis was carried out by a colorimetric method according to Young and Conway (1942).

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

$$MNF = 173+3.35X+ 0.28MY$$

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

Determinations of plasma volume and body fluid

In each animal per measurement, the injection of 20 mL of sodium thiocyanate solution (10 g/100 mL normal saline) and 20 mL of the Evans blue dye (T-1824) (0.5 g/100 mL normal saline) were given via an ear vein catheter to estimate extra cellular fluid (ECF) volume and the plasma volume, respectively. Venous blood samples from the jugular vein were taken at 20, 30, 40 and 50 min after dye injection. Dilution of dye at zero time was determined by using a semi-logarithmic concentration on time extrapolation. Blood volume was calculated from the plasma volume and packed cell volume. The measurement method for ECF was modified from Chaiyabutr et al. (2007). The total body water (TBW) was determined in each animal by tritiated water (^3H_2O, HTO) dilution techniques. A single dose of 3000 μCi/ animal of carrier-free tritiated water were injected intravenously. Blood samples were collected 1, 2, 3, 4, 5, 6, 7, 18, 24, 36 and 48 hours subsequent to the injection. The preparation for sample counting and calculation of total body water (TBW) and empty body water (EBW) was described by Chaiyabutr et al. (2007). Intracellular fluid (ICF) was calculated by subtracting ECF from TBW. Plasma and ruminal osmolality was measured using the freezing point depression method (Advance Osmometer model 3, USA).

Determination of ruminal liquid dilution and gut water kinetics

The ruminal liquid dilution rate and water kinetics measured by using 40 g of polyethylene glycol (PEG; M.W. 4000) diluted in 150 mL of water and 1000 μCi of tritiated water (HTO), were infused into the rumen at 08.00 hour via the rumen catheter. Concurrent blood and ruminal fluid samples were taken directly via each catheter every ten minutes for the first 60 min and then 2, 3, 5, 8, 11, 14, 18 and 24 hours after the infusion. Samples were prepared and transferred into counting tubes and count for an appropriated time. On the ruminal samples, PEG was determined by turbidimetric method according to Hyden protocol (Grimaud and Doreau, 2003) for ruminal fluid measurement. Rumen liquid volume (V) can be calculated from dose of PEG added into the rumen (D) as follow:

$$V = D/C_0.$$

The rate of liquid flow from the rumen (F) can be calculated from rumen liquid volume and the half-life time ($t_{1/2}$) of PEG transferred to the lower gut (T):

$$F = 0.693 \times V / t_{1/2}.$$

The biological half-life time ($t_{1/2}$) of PEG was determined from the slope of the linear regression line obtained from plot on semi-logarithmic paper of the PEG concentration against time.

The transfer of HTO from the rumen to plasma was modified by the method of Holtenius (1989). The HTO activity in plasma and ruminal fluid was measured by liquid scintillation counter (PACKARD A230, Canberra Company). The log concentration was plotted against time. The zero time concentration of HTO (C_0) was estimate by extrapolation to zero time by using linear regression. The volume at zero time (V_0) and equilibrated time at 300 min (V_{300}) were calculated by the concentration of marker (HTO) injected into the rumen divide by concentration of HTO in ruminal fluid at zero time and equilibrated time respectively. The rate constant of HTO (k) was derived from 0.693 divided by the half life time ($t_{1/2}$) of HTO. The absorption rate (F_a), insorption rate (F_i) and net absorption rate(N_a) of water through the rumen were calculated as follows;

$F_a = V \times k$

$F_i = N_a - (V_0 - V_{300}) / 300$

$N_a = F_a \times 300$

Plasma and ruminal osmolality was measured using the freezing point depression method (Advance Osmometer model 3, USA).

Determination of plasma hormones and metabolites

Venous blood samples from the jugular vein will be taken at 11.00-12.00 hours by venipuncture on specified day of each period, the first day before 1st rbST injection and two day after 3rd rbST injection in each lactation period. Blood samples were placed into heparinized test tube and place on ice. Plasma was harvested by centrifugation at 3,000 rpm for 10 min and stored at -20 °C until analysis for hormone and metabolite. Plasma glucose concentrations were measured using enzymatic oxidation in the presence of glucose oxidase (Glucose liquicolor, Wiesbaden, Germany). The plasma concentration of acetate was assayed by the acetic acid UV-method (R-Biopharm, Darmstadt, Germany). Plasma free fatty acid was determined by colorimetric after plasma extraction with chloroform, heptane and methanol and 1(2-Thaiazolyzazo)-2-naphthol solution (Wang et al., 2004). Plasma β-hydroxybutyrate concentrations were assayed using an enzymatic reaction in the presence of β-hydroxybutyrate dehydrogenase (R-Biopharm, Darmstadt, Germany) and plasma triglyceride concentration was determined by enzymatic colorimetric test

(Triglyceride liquicolor, Wiesbaden, Germany). Plasma leptin concentrations were determined using a radioimmunoassay kit specific for multi-species hormone (Linco Research, Inc., USA). The concentration of IGF-1 in plasma was measured by using a chemiluminescence immunoassay in an immulite analyzer (DPC, Los Angeles, CA).

Statistical analysis

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

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

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

A remain physiological parameters and environmental parameters were also analyzed by the similar model, but the covariate effect was excluded. Means values were used to evaluate the effect for all variables. Statistical significance was declared at $P<0.05$ and trends were declared at $0.05 < P \leq 0.10$.

CHAPTER IV

EFFECTS OF MIST-FAN COOLING AND SUPPLEMENTAL RECOMBINANT BOVINE SOMATOTROPIN ON DIET DIGESTIBILITY, DIGESTION KINETICS AND MILK PRODUCTION OF CROSSBRED HOLSTEIN CATTLE IN THE TROPICS

INTRODUCTION

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

Many technologies are required to improve milk production of dairy cattle in the tropics. Environmental modification is the most common approach to increase milk production with alleviation of severe heat stress in dairy cattle, for example, fans and sprinklers (Fike et al., 2002), evaporative cooling systems (Chan et al., 1997). It has been shown that cows cooled with spray and fans had greater milk yields than those cooled with evaporative cooling systems (Armstrong et al., 1994). In addition to environmental modification, other technologies can increase milk production in dairy cattle, for example the application of exogenous bovine somatotropin has been reported to minimize the effects of heat stress and potentially increase milk yields (West, 1994). However, few data are available on the knowledge of the relation between action of exogenous somatotropin and effect of high temperatures on milk production in crossbred Holstein cows in the tropics. Somatotropin is known to play a role in galactopoiesis and contribute to homeostasis and homeorhesis in ruminants (Bauman and Currie, 1980). Administration of bST to lactating cows in a hot environment can increase milk yield (West et al., 1991), but such cows also increase heat production by approximately 25 % over the value in control cows (West, 1994). Johnson et al. (1991) reported that somatotropin increased the efficiency of feed conversion into milk without any significant changes in body weight and temperatures. These results show that there is no consistent conclusion about the interaction effects between thermal stress and bST on lactation performance. Little is known about how somatotropin modifies digestive function in crossbred cattle. In other ruminants, an increase in the rate of liquid flow from the rumen during heat exposure has been reported in buffalo and goat (Chaiyabutr et al., 1987; Silanikove and Tadmor, 1989). It is believed that increased water absorption occurs in the lower GI-tract to increase heat dissipation during heat exposure. In view of an increase in total body water in rbST-treated cows (Chaiyabutr et al., 2007), it is necessary to establish whether rbST supplementation in cows maintained at high temperatures will minimize the effects of heat stress and whether the flow rate of digesta from the rumen will maintain sufficient nutrients to sustain the potentially increased milk yields. It is therefore important to study the digestive function and milk yields during bST supplementation under high ambient temperature in crossbred dairy cattle. Therefore, the objectives of the current study were 1) to evaluate the effect of providing crossbred cattle with housing under shade with or without mister-fans and 2) supplementation of cows with rbST or not during period of lactation (early, mid and late lactation). Measures to evaluate the effectiveness of these treatments were feed intake, digestion kinetic, diet digestibility, efficiency of feed utilization and milk production.

MATERIALS AND METHODS

Ten primiparous, non-pregnant crossbred cattle were used for the experiment. They were assigned randomly into two groups of five animals each. During the study period, cows in both groups were housed in an open-sided barn. The barn was separated into two parts by a metal sheet wall. The first part was arranged for cows in normal shade (NS) and the second part of the barn was equipped with two pedestal mist-fan cooling systems (MF) for cooled cows. Cows in both groups were fed with the total mixed ration (TMR) which was formulated according to NRC requirements (NRC, 2001) for cows producing 10-15kg milk/day. The TMR was offered twice daily *ad libitum* throughout the experimental period. Water was given to cows *ad libitum*. Dry matter intake (DMI) of each cow was measured daily by weighing the TMR offered and subtracting that refused. Cows were normally milked by using a milking machine two times per day and milk production was recorded daily.

The ambient temperature at NS and MF barns were recorded using a wet and dry bulb thermometer. The relative humidity at NS and MF barns were read by psychrometric chart depending on wet and dry bulb temperature. A temperature-humidity index (THI) was calculated from the average ambient temperature of dry and wet bulb temperatures according to McDowell (1972). Rectal temperature (RT) of each cow was measured by electronic thermometer and respiratory rates (RR) was measured by counting flank movements. Body weights (BW) of all animals were recorded by weighing monthly throughout experimental periods. The experiment in each group was divided into 3 periods, namely early- (60-90 DIM), mid- (120-150 DIM), and late lactating periods (180-210 DIM). The details of protocol for rbST administration in each stage of lactation were described in Chapter III.

Chromic oxide (Cr_2O_3) was used as an external marker to estimate nutrient digestibility and passage rate of digesta in the total gastro-intestinal tract of each cow. Cows were given 10g of Cr_2O_3 /day contained in gelatin capsules. Cr_2O_3 was divided into two portions and was given orally to animals twice a day at 06.00 and 18.00 hour for 10 days. Fecal grab samples were collected three times a day from day 8 to day 10 after dosing. Nine fecal samples were pooled and dried for analysis of chemical composition. After the last dose of chromic oxide, individual fecal grab samples were collected at 0, 4, 8, 12, 16, 20, 24, 30, 36, 44, 50, 62, 68, 74, and 96 hour. The samples were dried in a forced oven dryer at 70 °C for 24 hour, followed by grinding. The chromic oxide content of the sample was determined by a colorimetric method modified from Kimura and Miller (1956) as described in Chapter III. Samples of TMR were collected weekly and pooled for determinations of DM, OM, CP, NDF and ADF.

The statistic analyses were performed using General Linear Model procedures of statistical software package SPSS (SPSS for windows, V14.0; SPSS Inc., Chicago, IL, USA). The model used for each analysis was illustrated in Chapter III. Means values were used to evaluate the effect for all variables. Statistical significance was declared at P<0.05.

RESULTS

AT, RH, THI, RR and RT

Data for ambient temperatures (AT), relative humidity (RH), temperature humidity index, rectal temperature and respiratory rate are shown in Table 4.1. Mean values of measurements at experimental site for AT, RH and THI were highly significantly different (P<0.01) between NS and MF barn. The AT and THI in the NS barn were significantly higher than the MF barn but the RH of MF barn was significantly higher than the NS barn (P<0.01) at all stages of lactation. The respiration rate and rectal temperature of cows under mister and fans were lower than non-cooled cows irrespective of rbST supplementation at all stages of lactation. The rbST-supplemented cows were significantly higher in RR and RT than those of cows without rbST under both NS and MF barns throughout the experimental periods.

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

Data of body weight, feed intake and milk yields are shown in Table 4.2. The mean values of BW for cooled and non cooled cows were not significantly different at all stages of lactation. Supplementation of rbST increased BW of cows in MF barn during early and mid lactation but not for late lactation. DMI (kg/d) and DMI (kg/100 kg BW) of cooled cows without rbST supplementation were on average 13% higher than those for non cooled cows at all stages of lactation. The rbST-supplemented cows showed a significant higher DMI than non rbST-supplemented cows kept either in MF or NS barn.

Milk yield of cooled cows were higher than those of non cooled cows on average by 20.5% but these results were not statistically different at any stage of lactation. Milk yield and 40g/kg FCM of rbST-supplemented cows under MF cooling were significantly higher than those of other groups at all stages of lactation. Efficiency of feed utilization for milk synthesis showed no significant differences between cooled and non cooled cows. Supplemented rbST cows significantly increased the efficiency of feed utilization in early and late lactation but not for mid lactation. However, the interaction effect between mist-fan cooling and rbST supplementation was significantly apparent ($P<0.05$) for an increment in efficiency of feed utilization during mid lactation.

Digestion kinetics and digestibility

Mean values of flow rate, fecal output, half time of Cr_2O_3, passage rate and mean retention time of digesta are shown in Table 4.3. The digesta flow rate of cooled cows were not different ($P>0.05$) from non cooled cows at any stage of lactation. Faecal output of cooled cows tended to increase ($P=0.09$) during early lactation and marked increases were apparent in mid and late lactation as compared with non-cooled cows. The digesta flow rate and fecal output of rbST-supplemented cows were not different when compared with those of non rbST-supplemented cows throughout the experimental period. Under mist-fan cooling, the half time of Cr_2O_3 in the whole digestive tract for the cooled cows was lower than for those of non-cooled cows without rbST supplementation ($P<0.05$) during early and mid lactation but not for late lactation. The half time of Cr_2O_3 significantly decreased during rbST supplementation in cows under both NS and MF barns at all stages of lactation. The digesta passage rate constant (the fraction of total digesta moving per unit of time) in cooled cows was significantly higher than in non cooled cows during early and mid lactation. The magnitude of responses to the effects of rbST supplementation from pretreatment values for digesta passage rate constant was larger in animals with rbST than those of animals without rbST. The rapid digesta passage rate constant and short mean retention time of digesta were significantly apparent in rbST-supplemented cows under either NS or MF barns in all stages of lactation.

Digestibility data are shown in Table 4.4. Digestibility of DM, OM, NDF and ADF of cooled cows were no significantly different from those of non-cooled cow, with or without supplementation of rbST in all stages of lactation. CP digestibility during early and late lactation of cooled cows was not significantly different from those of non-cooled cows, except in mid lactation. No effect of rbST-supplementation on CP digestibility at all stages of lactation.

DISCUSSION

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

Thus the used of misters and fans for cooling cows in the present study does partly alleviate the effect of heat stress. Both DMI and milk yield of cooled cows without rbST supplementation were higher than those of non-cooled cows. These results are in agreement with the report of Chen et al. (1993) that an increase in milk production was about 9% with evaporative cooling over shade alone. Several studies have reported that lactating cows exposed to high environmental temperatures either with (Settivari et al., 2007) or without rbST treatment (Bernabucci et al., 1999; Hirayama et al., 2004), showed a reduction in feed intake and milk yields. However, the present results indicate that rbST is effective during hot weather at all stages of lactation, since non-cooled cows with rbST supplementation consumed additional feed DM for the higher milk production compared to cows in normal shade without rbST. These results agree with earlier findings (Santos et al., 1999; Tarazon et al., 1999; Gulay and Hatipoglu, 2005) that the bST-treated cows improved efficiency of utilization of food for milk production. Another reason for enhancement in DMI during rbST supplementation in non-cooled cows may be that cows were able to regulate their body temperatures within normal range. Although rbST increases heat production it also increases heat dissipation (Johnson et al., 1991). Moreover cows supplemented with rbST increase total body water as shown previously (Chaiyabutr et al., 2007). Increased total body water would be useful in slowing down the elevation in body temperature in hot conditions through evaporative cooling during heat dissipation. These changes would partly affect the DMI.

Increased digestibility with reduced gastrointestinal motility and rate of passage of digesta in cattle exposed to hot conditions has often been reported (e.g. Christopherson and Kennedy, 1983; Mathers et al., 1989). In the present study, non-cooled cows without rbST supplementation and exposure to moderate heat stress (THI>80<86) had lower values for both rate of passage of digesta and DMI, but digestibility did not significantly change compared with cooled cows throughout the entire lactation period. Thus the apparent digestibility of DM (including NDF and ADF) for the whole tract did not change as might have been expected as a result of changes in load and rate of passage of digesta.

The significant differences in the passage rate of digesta, but non-significant differences in diet digestibility between non-cooled and cooled cows without rbST, cannot depend upon the different types or nature of feeds offered (Deinum et al., 1968), since crossbred cows in both groups were fed with a similar TMR diet throughout lactation. However, it might be because the cows had been subjected to a prolonged period of exposure to high temperature since there is evidence that the response changes with time. Bernabucci et al. (1999) observed an increase in digestibility coinciding with decrease in passage rate of digesta during short-term of exposure to excess THI, but not in a long term adaptation.

In addition during prolonged heat exposure, depressed rumen cellulolytic activity (Miaron and Christopherson, 1992) and reduction of blood flow to the rumen epithelium (Hales et al. 1984) may occur in non-cooled cows. These factors would tend to reduce total digestibility. The study of Grimaud and Doreau (2003) indicated that a shorter retention time of digesta would reduce the attraction between digesta particles and ruminal microorganisms, resulting in decreased diet digestibility.

Thus the absence of differences in diet digestibility between non-cooled and cooled cows may be due to differences in rumen cellulolytic activity in cows under different environmental conditions. Depressed rumen cellulolytic activity may not occur in cows during exposure to mist-fan cooling, resulting in no change in total diet digestibility. The rate of passage of digesta for cows supplemented with rbST was higher than for cows without rbST supplementation either in NS or MF barns. This suggests that exogenous rbST could overcome some effects of high temperatures.

However, total dry matter digestibility of cows in the present studies was not affected by rbST supplementation, in agreement with other studies in cows using exogenous bST (Peel et al., 1981; Sechen et al., 1989). A faster rate of passage of digesta is usually given as the reason for the decrease in digestibility (Bernabucci et al., 1999). The lack of such an effect in the present study may be partly explained by changes of GI-tract volume and the level of DMI. Increasing the relative mass of many organs and tissues including GI-tract within the animal has been reported during exogenous bovine somatotropin (Moallem et al., 2004). Such an increased capacity of the GI-tract mass may compensate for the effect of the higher DMI level on the higher passage rate of digesta in rbST supplemented cows. The unchanged total digestibility of dry matter perhaps suggests that more complex processes of regulation exist in the digestive system during rbST supplementation.

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

In conclusion, the application of mist-fan cooling to cows slightly increased feed intake and milk yields, whereas rbST supplementation with or without misters and fans showed more marked increases in both feed intake and efficiency of feed utilization. The differences in the magnitude of responses of digestive functions between the effects of mist-fan cooling and rbST supplementation indicate that the main effects of mist-fans cooling reduce a negative effect of hot environment on digestive function via increase in digesta passage rate, resulting in an increase in feed intake. The response of milk production in crossbred cows to rbST supplementation is enhanced when accompanied by the use of mist-fan cooling. The effect of rbST supplementation was not directly on digestive function. It exerted a galactopoietic effect through increased post-absorptive use and partition of nutrients resulting in their increased efficiency of utilization in the mammary gland for milk synthesis.

Table 4.1 The mean values of ambient temperature, relative humidity, temperature humidity index (THI) measured at 13.00 hour under misty-fan cooling (MF) and normal shade (NS) barns and effects of mist-fan cooling (MF) and supplemental rbST on respiratory rate and rectal temperature of crossbred cows

	NS		MF			Effect^{1,2}		
	Pre	rbST	Pre	rbST	SEM	MF	rbST	MF x rbST
Early lactation								
Ambient temperature (°C)	34	35	33	32	0.6	**	ns	ns
Relative humidity (%)	52	53	71	70	3.2	**	ns	ns
THI	83	85	83	83	0.8	*	ns	ns
Respiratory rate (breath/min)	71	80	53	64	0.3	*	*	ns
Rectal temperature (°C)	39.5	39.9	38.9	39.3	0.16	**	*	ns
Mid lactation								
Ambient temperature (°C)	35	35	30	30	0.5	**	ns	ns
Relative humidity (%)	53	50	78	74	3.1	**	ns	ns
THI	85	85	82	81	0.4	*	ns	ns
Respiratory rate (breath/min)	72	76	52	61	2.7	**	*	ns
Rectal temperature (°C)	39.7	40.1	38.6	39.5	0.13	**	**	ns
Late lactation								
Ambient temperature (°C)	31	34	30	29	0.5	**	ns	ns
Relative humidity (%)	60	64	79	78	1.8	**	ns	ns
THI	84	85	81	81	0.6	**	ns	ns
Respiratory rate (breath/min)	69	80	52	57	1.3	**	*	ns
Rectal temperature (°C)	39.1	39.8	38.4	38.9	0.14	*	*	ns

Pre = pre-rbST supplementation, rbST = rbST supplementation

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

[2] Significance; ns = P> 0.05, * = P <0.05 and ** = P < 0.01.

Table 4.2 Effects of misty-fan cooling (MF) and supplemental rbST on body weight, dry matter intake, milk yield and efficiency of feed utilization of crossbred Holstein cows compared with cows in normal shade (NS) barn.

	NS		MF			Effect[1,2]		
	Pre	rbST	Pre	rbST	SEM	MF	rbST	MF x rbST
Early lactation								
Body weight (kg)	373	381	374	386	1.9	ns	*	ns
Dry matter intake (kg/d)	9.1	10.1	10.9	11.9	0.17	ns	*	ns
Dry matter intake (kg/100 kg BW)	2.4	2.6	2.9	3.1	0.05	ns	*	ns
Milk yield (kg/day)	10.9	12.6	13.6	14.4	0.17	ns	*	*
4% FCM (kg/d)	10.3	13.5	14.1	16.2	0.58	ns	*	ns
Efficiency of feed utilization (kg/kg)	1.1	1.3	1.3	1.4	0.04	ns	*	ns
Mid lactation								
Body weight (kg)	397	397	383	408	4.6	ns	*	*
Dry matter intake (kg/d)	8.3	9.2	9.1	9.8	0.16	ns	*	ns
Dry matter intake (kg/100 kg BW)	2.1	2.3	2.4	2.5	0.06	ns	*	ns
Milk yield (kg/day)	10.4	11.3	11.4	12.9	0.33	ns	*	ns
4% FCM (kg/d)	10.9	11.9	10.7	12.8	0.25	ns	*	ns
Efficiency of feed utilization (kg/kg)	1.4	1.3	1.2	1.3	0.03	ns	ns	*
Late lactation								
Body weight (kg)	396	395	426	423	4.4	ns	ns	ns
Dry matter intake (kg/d)	7.6	7.9	8.4	9.3	0.14	ns	**	ns
Dry matter intake (kg/100 kg BW)	1.9	2.0	2.0	2.2	0.03	ns	**	*
Milk yield (kg/day)	8.2	9.2	10.5	12.2	0.44	ns	*	ns
4% FCM (kg/d)	8.4	9.9	10.2	13.4	0.85	ns	*	ns
Efficiency of feed utilization (kg/kg)	1.1	1.3	1.2	1.4	0.06	ns	*	ns

Pre = pre-rbST supplementation, rbST = rbST supplementation

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

[2] Significance; ns = P> 0.05, * = P <0.05 and ** = P < 0.01.

Table 4.3 Effects of misty-fan cooling (MF) and supplemental rbST on digestion kinetics of crossbred Holstein cows compared with cows in normal shade (NS) barn.

	NS		MF			Effect[1,2]		
	Pre	rbST	Pre	rbST	SEM	MF	rbST	MF x rbST
Early lactation								
Digesta flow rate (Kg/day)	17.6	16.6	19.8	19.9	0.60	ns	ns	ns
Fecal output (KgDM/day)	3.4	3.0	3.7	3.4	0.17	P=0.093	ns	ns
Half time of Cr_2O_3 (h)	26	25	20	18	0.7	*	*	ns
Digesta passage rate constant (h^{-1})	0.03	0.03	0.04	0.04	0.001	*	**	ns
Mean retention time (h)	37.2	35.8	29.0	25.6	0.96	*	*	ns
Mid lactation								
Digesta flow rate (Kg/day)	17.0	17.9	19.9	19.0	0.12	ns	ns	ns
Fecal output (KgDM/day)	3.3	3.3	3.8	3.9	0.15	*	ns	ns
Half time of Cr_2O_3 (h)	27	25	21	20	0.4	*	**	ns
Digesta passage rate constant (h^{-1})	0.03	0.03	0.03	0.04	0.001	*	**	ns
Mean retention time (h)	38.8	35.4	30.2	28.2	0.70	*	**	ns
Late lactation								
Digesta flow rate (Kg/day)	19.1	17.3	15.7	15.1	1.07	ns	ns	ns
Fecal output (KgDM/day)	3.5	3.2	4.1	4.1	0.15	**	ns	ns
Half time of Cr_2O_3 (h)	25	23	22	21	0.1	ns	*	ns
Digesta passage rate constant (h^{-1})	0.03	0.03	0.03	0.03	0.001	ns	*	ns
Mean retention time (h)	35.4	33.0	32.4	30.4	0.70	ns	*	ns

Pre = pre-rbST supplementation, rbST = rbST supplementation

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

[2] Significance; ns = P> 0.05, * = P <0.05 and ** = P < 0.01.

Table 4.4 Effects of misty-fan cooling (MF) and supplemental rbST on digestibility in crossbred Holstein cows compared with cows in normal shade (NS) barn.

	NS		MF			Effect[1,2]		
	Pre	rbST	Pre	rbST	SEM	MF	rbST	MF x rbST
Early lactation								
Total DM digestibility (g/kg)	722	715	744	732	27.7	ns	ns	ns
Diet digestibility (g/kgDM):								
OM	723	718	750	743	28.9	ns	ns	ns
CP	779	772	817	799	29.1	ns	ns	ns
NDF	491	465	487	465	56.6	ns	ns	ns
ADF	414	392	387	413	54.5	ns	ns	ns
Mid lactation								
Total DM digestibility (g/kg)	647	676	720	722	27.0	ns	ns	ns
Diet digestibility (g/kgDM):								
OM	659	684	721	725	29.3	ns	ns	ns
CP	715	752	812	817	21.0	**	ns	ns
NDF	350	367	402	439	69.9	ns	ns	ns
ADF	318	284	360	343	49.9	ns	ns	ns
Late lactation								
Total DM digestibility (g/kg)	691	710	718	739	17.0	ns	ns	ns
Diet digestibility (g/kgDM):								
OM	697	717	723	742	17.4	ns	ns	ns
CP	758	772	780	817	12.2	ns	ns	ns
NDF	447	445	442	472	42.6	ns	ns	ns
ADF	283	331	345	377	10.2	ns	ns	ns

Pre = pre-rbST supplementation, rbST = rbST supplementation

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

[2] Significance; ns = P> 0.05, * = P <0.05 and ** = P < 0.01.

CHAPTER V

EFFECTS OF MISTY-FAN COOLING AND SUPPLEMENTAL rbST ON RUMEN FUNCTION AND MILK PRODUCTION OF CROSSBRED HOLSTEIN CATTLE DURING EARLY, MID AND LATE LACTATION IN A TROPICAL ENVIRONMENT

INTRODUCTION

High environmental temperature and humidity are well known to be a factor associated with reduced milk production in tropical environments (Bohmanova et al., 2007). The interaction effects between thermal stress and the role of bST on lactation performance in crossbred lactating cattle is not yet clear. Many studies have proposed that an increase in milk yields of bST-treated cows remained greater than that of the control cow even in heat stress conditions (Johnson et al., 1991, Santos et al., 1999; Gulay and Hatipoglu, 2005). An occurrence of greater heat stress has been reported in some studies when bST was used, which was probably due to an increase in metabolic activity associated with higher milk yield (West, 1994; Settivari et al., 2007). It has been suggested that even though bST-treated cows increase in heat production; it also increases heat dissipation (Johnson et al., 1991; West, 1994). In ruminating animals exposed to high environmental temperatures, feed intake normally decreases and, consequently, production level is reduced. These data are not conclusive, especially for the role of bST on the compensation of nutrients digestibility in hot conditions. Some authors have reported that an increase in milk production of the bST-treated cow under hot environment was due to either increase in DMI (Staples et al., 1988), while in another study showed no alteration in feed intake (Tyrrell et al., 1988). Many studies also reported inconsistent results for the effect of environmental temperature on the rumen function. A number of experiments showed an increase in total volatile fatty acids (VFA) under high environmental temperature (Martz et al., 1971), while another study showed the decrease in VFA concentrations (Olbrich et al., 1972; Gwatibaya et al., 2007). The changes in ruminal VFA during heat exposure occurs not only from a decline in feed intake but also from the direct effect of heat stress (Olbrich et al., 1972). During heat stress, rumen fermentation would be affected by depression of rumination, gut motility and blood flow to rumen epithelium including the decrease in microbial synthesis (Silanikove, 1992).

Many methods have been used to minimize the impact of thermal stress in maintaining sufficient DMI for the potential increase in milk yields by using passive and active environmental modifications, such as shade, fans, fog misters, sprinklers or evaporative cooling (Ryan et al., 1992; Smith et al., 2006; Bohmanova et al., 2007; Chaiyabutr et al., 2008). Although bovine somatotropin has been known to influence body nutrient availability and utilization, little is known about the mechanism whereby exogenous bovine somatotropin exerts it effects on rumen function and milk production in hot conditions. Therefore, the objective of the present study was to evaluate the effect of supplementation of cross-bred cows with rbST under shade with or without misty-fans cooling on rumen function and milk production.

MATERIALS AND METHODS

Ten primiparous, non-pregnant crossbred cattle were used for the experiment. They were assigned randomly into two groups of five animals each. During the study period, cows in both groups were housed in an open-sided barn. The barn was separated into two parts by a metal sheet wall. The first part was arranged for cows in normal shade (NS) and the second part of the barn was equipped with two pedestal mist-fan cooling systems (MF) for cooled cows. Cows in both groups were fed with the total mixed ration (TMR) which was formulated according to NRC requirements (NRC, 2001) for cows producing 10-15kg milk/day. The TMR was offered twice daily *ad libitum* throughout the experimental period. Water was given to cows *ad libitum*. Dry matter intake (DMI) of each cow was measured daily by weighing the TMR offered and subtracting that refused. Cows were normally milked by using a milking machine two times per day and milk production was recorded daily.

The ambient temperature at NS and MF barns were recorded using a wet and dry bulb thermometer. The relative humidity at NS and MF barns were read by psychrometric chart depending on wet and dry bulb temperature. A temperature-humidity index (THI) was calculated from the average ambient temperature of dry and wet bulb temperatures according to McDowell (1972). Rectal temperature (RT) of each cow was measured by electronic thermometer and respiratory rates (RR) was measured by counting flank movements. Body weights (BW) of all animals were recorded by weighing monthly throughout experimental periods. The experiment in each group was divided into 3 periods, namely early- (60-90 DIM), mid- (120-150 DIM), and late lactating periods (180-210 DIM). The details of protocol for rbST administration in each stage of lactation were described in Chapter III.

Rumen fluid samples were collected at 2.5 hours after morning feeding by using a stomach tube connected to a vacuum pump. The pH was measured immediately after collection using a pH meter. The ruminal fluid samples were strained immediately through two layers of cheesecloth and preserved by adding 6 N HCl. The preserved ruminal fluid samples were frozen at -20°C prior to volatile fatty acids (VFA) and NH_3-N analyses. Ruminal fluid samples were analyzed for VFA by a gas chromatography and NH_3-N by phenyl-hypochlorite reaction (Weatherburn, 1967). The concentration of total VFA were determined as mmol/L and the molar percentages of individual VFA (acetate, propionate and butyrate) were calculated and expressed as mol/100 mol of total VFA. The acetate: propionate ratio (A:P ratio) was calculated from their concentrations.

Milk samples were collected and divided into two portions. One portion of milk (60 mL) was preserved with 300 μL of bronopol (2-Brom-2-nitro-1,3-propandiol;0.02w/w) and kept at 4°C for milk compositions determination. Another portion was used for the determination of the milk urea concentration. The milk urea concentrations were determined by the diacetylmonoxime method (Coulombe and Favreau, 1963). The milk allantoin analysis was carried out by a colorimetric method according to Young and Conway (1942). The microbial synthesis of protein was calculated by an indirect method base upon measuring allantoin output in milk and calculating the predicted microbial nitrogen flow (MNF) according to Timmermans et al. ($2000^{a,b}$) as follows:

$$MNF = 173+3.35X+ 0.28MY$$

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

The statistic analyses were performed using General Linear Model procedures of statistical software package SPSS (SPSS for windows, V14.0; SPSS Inc., Chicago, IL, USA). The model used for each analysis was illustrated in Chapter III. Means values were used to evaluate the effect for all variables. Statistical significance was declared at P<0.05.

RESULTS

Chemical compositions of the diet

The feed ingredients and chemical composition of the total mixed ration (TMR) are shown in Table 3.1(Chapter III). The TMR ingredients were balanced to meet requirements for milk yield of lactating cows between 10-15 kg/d. The diet contained 18%CP, 70%TDN, and 2.67 Mcal/kg DM of ME, which were estimated according to the NRC (2001).

Ambient temperature, relative humidity and temperature humidity index (THI)

The mean values of AT, RH and THI measured in NS and MF barn are shown in Table 5.1. In the morning (09.00 hour), AT and THI were not different between NS and MF barn throughout the experimental periods. AT and THI of MF barn in the afternoon (13.00 hour) were significantly lower ($P<0.01$) than those of NS barn in all stages of lactation. RH at MF barn was higher than that of NS barn in both the morning and the afternoon. Animals housing in the same barn both pre-treatment and treatment with rbST were exposed to a similar environmental condition (AT, RH and THI) throughout periods of study.

Body weight, dry matter intake, respiratory rate and rectal temperature

The effects of rbST and misty fans cooling on changes in the mean values of body weight (BW), dry matter intake (DMI), Respiration rate (RR) and rectal temperature (RT) are shown in Table 5.2. The value of BW of cows in early and late stages of lactation was not significantly different between cooled and non-cooled cows either treatment with rbST or not, except for mid-lactation which BW of cows treated with rbST housing in either NS or MF barns was significantly higher than that of the pre-treated period ($P< 0.05$). However, cows in both groups among treatments gained in weight as lactation progress. DMI of cooled cows were significantly higher than those of non cooled cows during early and mid lactation, but no difference was apparent in late lactation. The values of DMI in rbST-treated cows were higher than those of values in the pre treated period in all stages of lactation. Both RR and RT of cooled cows were significantly lower than those of non-cooled cows in all stages of lactation. Both cooled and non-cooled cows showed significant increases in RR and RT after rbST treatment in all stages of lactation. At the pre-treatment period, cows housing in

MF barn showed lower RR and RT than those of cows in NS barn in both the morning and afternoon.

Milk yield, milk compositions and milk urea nitrogen

Milk yield, 4 % fat corrected milk (FCM), milk compositions and milk urea nitrogen (MUN) are shown in Table 5.3. Milk yield and 4% FCM of cooled cows without rbST supplementation were not significantly different in comparison with non-cooled cows without rbST throughout lactation. The responses of rbST supplementation showed significant increase in milk yields of both cooled and non-cooled cows throughout lactation. No significant differences were apparent for the percentages of protein and lactose between cooled and non-cooled cows with or without rbST supplementation. However, the trend for an increase in the percentage of milk fat was apparent in both groups of cows supplemented with rbST, especially the significant effect was occurred in mid lactation. The concentration of MUN of cooled cows was significantly higher than those of non-cooled cows during early lactation. The trend for an increase in the level of the concentration of MUN in cooled cows was also apparent in mid and late lactation. The rbST-treated cows showed significant increases in the concentration of MUN in all stages of lactation. The concentration and excretion of MUN in cooled cows with rbST supplementation were higher than those of other groups.

Ruminal characteristics

The effects of rbST and misty fans cooling on changes in ruminal characteristics are shown in Table 5.4. Ruminal pH of animals kept in both NS and MF barn either without or with rbST supplementation showed no significant differences in all stages of lactation. The cooling effect did not affect to the concentration of NH_3-N in the ruminal fluid of cows without rbST supplementation. An increase in the ruminal NH_3-N concentration was significantly affected by rbST supplementation in either cooled or non-cooled cows throughout stages of lactation. The total VFA concentration of cooled cows without rbST supplementation showed greater than non-cooled cows during early lactation, ($P<0.05$), while it tended to be higher in mid and late lactation than those of non-cooled cows ($P>0.05$). Total VFA concentrations were significantly increased ($P<0.05$) when cows were supplemented with rbST in either cooled or non-cooled cows in all stages of lactation. The molar proportions of individual VFAs were not significantly affected by both cooling cow and supplementation with rbST in all stages of lactation. The concentration of acetate showed the numerically higher in cooled cows than that of non-cooled cows but there were no statistical differences. There were no differences (P>0.05) in the A: P ratio between cooled and non-cooled cows with or without rbST supplementation or not in all stages of lactation.

Allantoin, and microbial nitrogen production

The effects of rbST and MF cooling system on changes in allantoin and microbial nitrogen flow are shown in Table 5.5. The concentration and excretion of allantoin were not significantly different (P>0.05) between cooled and non-cooled cows in all stages of lactation. The rbST-treated cows were significantly greater in concentration and excretion of allantoin than non-rbST treated cows in all stages of lactation. Cooling cows with MF did not show differences in microbial nitrogen flow from the rumen between cooled and non cooled cows in all stages of lactation. Microbial nitrogen flow from the rumen during rbST supplementation showed high values (P<0.01) in both cooled and non-cooled cows throughout the experimental periods.

DISCUSSION

The environmental temperatures measured in NS and MF barn in the present study showed differences in AT and THI, especially in the afternoon throughout the experimental periods. However, MF barn was not sufficient to completely eliminate heat stress in cows, because the range for AT and THI measured at daytime (09.00 to 13.00 hour) under misters and fans throughout the experimental periods remained higher than the threshold level of comfortable zone, 25 °C and 72 for AT and THI respectively (Armstrong, 1994; Kadzere et al., 2002). The THI taken at both barns were ranging 78-86 throughout stages of lactation. Cows in both groups would be subjected to moderate heat stress (Fuquay, 1981). However, THI might not accurately reflect of heat stress in crossbred lactating cows under MF cooling system that deliver a pressurized spray with considerable fan air movement in the barn, resulting in higher humidity but also causing a cooling effect. In addition, cross-bred dairy cattle using in the present experiment containing *Bos indicus* gene, could have a high heat tolerance than exotic *Bos Taurus* cattle (Nakamura et al., 1993; Pereira et al., 2008).

The cooling of cows under MF was significantly lower in both RR and RT in comparison with those of non-cooled cows which was agree to the study of Avendano-Reyes et al. (2006) using water spray and fans in European cows. It indicates a partial alleviation of heat stress by MF system especially in the afternoon. The RR and RT were increased in rbST-treated cows. These results agree with previous reports (Sullivan et al., 1992; Tarazon et al., 1999; Settivari et al., 2007) that observed an increase in RR and RT for cows treated with rbST. However, the RT of animals in all groups in the present study was higher than those reported for normal range (38.3-38.7 °C) in dairy cattle (Abeni et al., 2007). Although rbST-treated cows in both groups were exposed to high temperatures, they also increased DMI and produced more milk yield as compared with the pre-supplemented period. It would suggest that even though rbST increases heat production associated with high milk yield, it also increases heat dissipation (Johnson et al., 1991; West, 1994). However, cows in both groups gained in weight as lactation progress especially a marked change was apparent in mid lactation of rbST-treated cows under MF barn. During mid-lactation, the energy output in milk and for maintenance of crossbred cows was lower than the energy consumed in the food. It is possible that extra energy consumption would be partitioned to body stored as adipose tissue and muscle resulting in gain of weight. This would agree to the report of Sanh et al. (2002) that the levels of feed intake would account for changes in both milk yield and body weight.

Milk yield of the crossbred cows under MF tended to be higher than those of cows under NS barn (P=0.07). An increase in milk production of the cooling crossbred cows in the present experiment is in accordance with the results reported in cooling *Bos taurus* cows (Tarazon et al., 1999). It is possible that the difference of day and night temperature in the present study would affect on milk yield by the effect of heat stress, which animals were exposed to a high THI during hottest of the day. Furthermore, the percentages of milk compositions were not different in cows housing between NS and MF whether supplemented with rbST or not. These findings indicate that an increase in milk production of cross-bred cows would be governed by both MF and rbST supplementation in all stages of lactation. Similar effects were observed in cooled cow with spray and fan system in combination with rbST for an increase in milk yield comparing to non-cooled and non-rbST treated cows (Keister et al., 2002).

In the present study, the values of ruminal pH (pH 6.49 to 6.77) were higher than those of results (pH 5.8 to 6.7) reported by the others (Rabelo et al., 2003), but it was still within an acceptable range to maintain a healthy rumen environment (NRC, 2001). These results are in agreement with Robinson et al. (1991) that reported for unchanged of ruminal pH in cows treated with bovine somatotropin.

In the present results, the higher ammonia production in the rumen during rbST supplementation would not be due to excessive crude protein feeding for microbial degradation, but it would be due to an increase in DMI. These results are in agreement with the study of Robinson et al. (1991) that bST-treated cows had higher ammonium N concentration than the control cows. The cows supplemented with rbST significantly increased total VFA in the rumen fluid as compared with the pre- treated period. An increase in total VFA in the rumen fluid of rbST-treated cows would not be due to the different types of fermentable contents of the diet, since animals were fed with similar TMR throughout periods of study. An increase in voluntary feed intake of cows responding to rbST supplementation would provide substrates availability for ruminal microbial fermentation resulting in an increase in total VFA in all stages of lactation. The total VFA concentration between cooled and non cooled cows without rbST showed no statistical differences. However, total VFA concentration of cooled cows tended to be higher than those of non-cooled cows by approximately 11 to 27 %. It is possible that animals remained exposed to a high THI level in both NS and MF barn that was greater than threshold value (72) for lactating cows. Prolonged exposure of an animal to high environmental temperature resulting in the reduction of VFA production has been noted (Bandaranayaka and Holmes, 1976). In the present study, the molar proportion of individual VFA was not affected by supplementation of rbST to cows under either NS or MF barns throughout lactation. It is possible that no alteration of molar proportion of rumen VFA appeared to be due to animals feeding with similar TMR throughout periods of study, since Sutton et al. (2003) have shown that the marked changes in the molar proportion of rumen VFA will occur with a wide variety of dietary manipulation. However, cooled cows with or without rbST showed higher molar proportion of ruminal acetate than those of non-cooled cows. It is probably that the low ruminal acetate of non-cooled cows may be attributed to the depression of ruminal cellulolytic activity during exposure to high temperatures (Bernabucci et al., 1999). Thus, an increase in total

VFA production in the rumen during rbST supplementation in both groups would not be a function of rbST treatment per se.

In the present study, no measurement of blood urea nitrogen (BUN) was performed. However, the urea concentration of MUN arises primarily from transfer of urea from the blood, since urea in plasma can freely and rapidly diffuse into milk. The close correlations between BUN and MUN have been reported by several investigators in exotic dairy cows (Gustafsson and Palmquist, 1993; Hof et al., 1997; Jonker et al., 2002) including crossbred dairy cows (Chaiyabutr et al., 1999). Thus, milk urea content can reflect the balance of dietary protein and serve as a feed efficiency indicator. MUN in dairy cows can provide information on total nitrogen losses following absorption of ammonia in the rumen (Butler et al., 1996; Hof et al., 1997). The present results show that the marked increases in the MUN concentration coincided with increases in NH_3-N production in the rumen during rbST supplementation in both cooled and non-cooled cows. This could be explained by the fact that excess ruminal NH_3-N would be absorbed and converted to urea in the liver for either recycling to the rumen via saliva and being excreted in milk and urine (Kohn, 2007). The other possible explanations for the higher MUN in rbST-treated cows may be occurred along with increasing milk yield (Carlsson et al., 1995) and arise in feeding especially nitrogen intake during rbST supplementation (Oltner and Wiktosson, 1983; Eicher et al., 1999). An increase in mammary blood flow during rbST supplementation (Chaiyabutr et al., 2000) may increase in mammary uptake of plasma urea which would attribute to an increase in MUN and decrease in plasma urea level as reported by Cheli et al. (1998) in exotic dairy cows. Furthermore, purine derivatives (allantoin, uric acid, xanthine and hypoxanthine) are indicator for microbial protein production in the rumen. In the present study, an increase in both the concentration and excretion of allantoin in milk after rbST supplementation is in agreement with the study of Schager et al. (2003). Therefore, during supplementation of rbST, the cow had greater microbial nitrogen flow from the rumen than pre-supplement period. From these results, it was suggested that an increase in milk allantoin excretion coinciding with an increase in MNF would be used as an index to predict an increase in rumen microbial protein synthesis. Supplementation of rbST could improve microbial protein synthesis in the rumen of crossbred cows during exposure to high environmental temperature.

In summary, the data from this study show that ambient temperature and THI in both NS and MF barn remain higher than threshold for lactating dairy cows. The application of misty-fan cooling to crossbred Holstein cattle was sufficient to alleviated heat stress in cows with a lower RR and RT during the daytime. The supplementation of rbST under the misty-fan cooling in lactating crossbred Holstein cattle increased both DMI and milk production in all stages of lactation. The galactopoietic effect of bovine somatotropin would explain, in part through an increase feed intake as a resulting with greater total VFA, NH_3N and microbial protein synthesis in the rumen thereby making more substrate available to the mammary gland for milk synthesis

Table 5.1 The environmental measurements for housing with misty-fan cooling (MF) and normal shade (NS) only

	time	NS		MF			Effect[1,2]		
		Pre-treat	Treat	Pre-treat	Treat	SEM	MF	rbST	MF x rbST
Early lactation									
Ambient Temperature(°C)	09.00	27.9	27.4	27.2	27.6	0.24	ns	ns	ns
	13.00	33.7	34.9	31.6	31.7	0.57	**	ns	ns
Relative humidity (%)	09.00	78.8	78.4	83.6	86.6	1.58	*	ns	ns
	13.00	52.0	53.2	70.60	70.2	3.16	**	ns	ns
Temperature humidity index	09.00	78.7	78.0	78.2	79.0	0.26	ns	ns	ns
	13.00	83.3	85.0	82.9	82.9	0.77	*	ns	ns
Mid lactation									
Ambient Temperature(°C)	09.00	28.0	28.5	27.6	26.9	0.30	ns	ns	ns
	13.00	35.3	35.0	30.0	29.8	0.48	**	ns	ns
Relative humidity (%)	09.00	78.6	78.8	85.2	83.6	1.26	**	ns	ns
	13.00	52.8	50.4	78.2	74.0	3.09	**	ns	ns
Temperature humidity index	09.00	79.0	79.6	78.9	77.6	0.40	ns	ns	ns
	13.00	85.5	84.8	81.5	80.8	0.39	*	ns	ns
Late lactation									
Ambient Temperature(°C)	09.00	28.5	28.4	27.1	27.3	0.39	**	ns	ns
	13.00	33.8	33.9	29.5	29.3	0.47	**	ns	ns
Relative humidity (%)	09.00	74.6	75.4	82.2	84.0	1.62	**	ns	ns
	13.00	60.0	64.4	78.6	78.4	1.76	**	ns	ns
Temperature humidity index	09.00	79.1	79.0	77.9	78.4	0.41	ns	ns	ns
	13.00	84.5[a]	85.1	80.9	80.7	0.51	**	ns	ns

Pre = pre-rbST supplementation, rbST = rbST supplementation

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

[2] Significance; ns = P> 0.05, * = P <0.05 and ** = P < 0.01.

Table 5.2 Effects of the application of misty-fan cooling (MF) in comparison with normal shade (NS) on body weight (BW), dry matter intake, respiratory rate and rectal temperature of crossbred Holstein cows supplemented with rbST

lactation		NS		MF			Effect[1,2]		
		Pre-treat	Treat	Pre-treat	Treat	SEM	MF	rbST	MF x rbST
Early	BW (kg)	358	380	373	376	7.3	ns	ns	ns
	Dry matter intake (kg/day)	6.1	7.1	7.2	8.5	0.3	*	**	ns
	Respiratory rate (Breath/min)								
	09.00	40	43	36	39	0.6	**	**	ns
	13.00	71	80	53	64	0.3	*	*	ns
	Rectal temperature (°C)								
	09.00	38	38.8	38.0	38.4	0.07	*	**	ns
	13.00	39.5	39.9	38.9	39.3	0.16	**	*	ns
Mid	BW (kg)	383	388	383	408	4.5	ns	*	*
	Dry matter intake (kg/day)	6.2	7.5	8.7	10.0	0.5	*	*	ns
	Respiratory rate (Breath/min)								
	09.00	41	46	36	40	0.7	*	**	ns
	13.00	72	76	52	61	2.7	**	*	ns
	Rectal temperature (°C)								
	09.00	38.5	38.9	38.0	38.3	0.08	*	**	ns
	13.00	39.7	40.1	38.6	39.5	0.13	**	**	ns
Late	BW (kg)	396	395	426	422	4.4	ns	ns	ns
	Dry matter intake (kg/day)	7.6	7.9	8.3	9.3	0.15	ns	**	*
	Respiratory rate (Breath/min)								
	09.00	40	44	37	41	0.1	*	**	ns
	13.00	69	80	52	57	1.3	**	**	ns
	Rectal temperature (°C)								
	09.00	38.5	38.9	37.9	38.3	0.08	**	**	ns
	13.00	39.1	39.8	38.4	38.9	0.14	*	**	ns

Pre = pre-rbST supplementation, rbST = rbST supplementation

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

[2] Significance; ns = P> 0.05, * = P <0.05 and ** = P < 0.01.

Table 5.3 Effects of the application of misty-fan cooling (MF) in comparison with normal shade (NS) on milk yield, milk compositions and milk urea nitrogen (MUN) of crossbred Holstein cows supplemented with rbST

	NS		MF			Effect[1,2]		
	Pre-treat	Treat	Pre-treat	Treat	SEM	MF	rbST	MF x rbST
Early lactation								
Milk yield (kg/day)	10.0	11.8	11.5	13.1	0.28	ns	**	ns
4%FCM(kg/day)	8.6	10.2	10.5	13.5	0.65	ns	**	ns
Fat (%)	3.1	3.3	3.4	4.3	0.33	ns	ns	ns
Protein (%)	3.2	3.6	3.5	3.6	0.13	ns	ns	ns
Lactose (%)	4.6	4.3	4.8	4.8	0.10	ns	ns	ns
MUN concentration (mg/dL)	14.1	16.7	17.0	20.8	0.76	**	**	ns
MUN excretion (g/day)	1.4	1.9	2.0	2.7	0.05	*	**	ns
Mid lactation								
Milk yield (kg/day)	9.4	10.0	11.4	13.1	0.33	ns	**	ns
4%FCM(kg/day)	9.1	10.8	11.1	14.5	0.78	ns	*	ns
Fat (%)	3.8	4.4	4.2	4.9	0.27	ns	*	ns
Protein (%)	3.8	3.8	4.1	4.3	0.15	ns	ns	ns
Lactose (%)	4.3	4.5	4.8	4.8	0.12	ns	ns	ns
MUN concentration (mg/dL)	15.3	17.6	18.2	20.6	0.74	ns	*	ns
MUN excretion (g/day)	1.4	1.7	2.1	2.7	0.13	ns	*	ns
Late lactation								
Milk yield (kg/day)	8.0	9.6	9.7	12.2	0.43	ns	**	ns
4%FCM(kg/day)	8.6	11.7	9.7	13.8	1.34	ns	*	ns
Fat (%)	4.5	5.3	4.2	4.9	0.56	ns	ns	ns
Protein (%)	4.3	4.0	4.3	4.3	0.17	ns	ns	ns
Lactose (%)	4.3	4.0	4.6	4.7	0.11	ns	ns	ns
MUN concentration (mg/dL)	18.9	21.7	21.7	23.5	0.55	ns	**	ns
MUN excretion (g/day)	1.5	2.1	2.2	2.9	0.12	ns	**	ns

Pre = pre-rbST supplementation, rbST = rbST supplementation

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

[2] Significance; ns = P> 0.05, * = P <0.05 and ** = P < 0.01.

Table 5.4 Effcts of the application of misty-fan cooling (MF) in comparison with normal shade (NS) on rumen fermentation characteristics of crossbred Holstein cows supplemented with rbST

	NS		MF			Effect[1,2]		
	Pre-treat	Treat	Pre-treat	Treat	SEM	MF	rbST	MF x rbST
Early lactation								
Rumen fluid pH	6.6	7.0	6.7	6.6	0.13	ns	ns	ns
NH_3-N (mg/dL)	11.4	12.4	12.8	14.2	0.24	ns	**	ns
Total VFA (mmol/L)	78.8	84.5	97.7	104.0	2.12	*	*	ns
Acetate (mol/100 mol)	64.7	64.0	67.3	65.3	1.11	ns	ns	ns
Propionate (mol/100 mol)	22.3	22.7	20.3	21.2	0.76	ns	ns	ns
Butyrate (mol/100 mol)	12.0	12.0	11.3	12.3	0.93	ns	ns	ns
Acetate:Propionate ratio	3.0	2.9	3.4	3.1	0.15	ns	ns	ns
Mid lactation								
Rumen fluid pH	6.7	6.8	6.5	6.5	0.10	ns	ns	ns
NH_3-N (mg/dL)	13.2	14.2	12.0	13.2	0.52	ns	*	ns
Total VFA (mmol/L)	82.0	91.9	97.7	102.5	4.42	ns	*	ns
Acetate (mol/100 mol)	60.0	64.4	67.3	65.9	1.70	ns	ns	ns
Propionate (mol/100 mol)	23.4	22.2	20.8	21.4	1.32	ns	ns	ns
Butyrate (mol/100 mol)	15.0	11.9	10.7	11.7	0.73	ns	ns	*
Acetate:Propionate ratio	2.8	3.2	3.4	3.2	0.03	ns	ns	ns
Late lactation								
Rumen fluid pH	6.7	6.7	6.7	6.6	0.11	ns	ns	ns
NH_3-N (mg/dL)	12.2	13.4	11.2	12.9	0.16	ns	**	ns
Total VFA (mmol/L)	86.6	90.4	96.6	115.4	2.77	ns	**	*
Acetate (mol/100 mol)	64.1	63.7	66.2	66.9	1.46	ns	ns	ns
Propionate (mol/100 mol)	22.4	22.0	20.7	20.7	0.87	ns	ns	ns
Butyrate (mol/100 mol)	12.2	12.9	11.9	11.3	0.73	ns	ns	ns
Acetate:Propionate ratio	2.9	3.0	3.3	3.4	0.22	ns	ns	ns

Pre = pre-rbST supplementation, rbST = rbST supplementation

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

[2] Significance; ns = $P> 0.05$, * = $P <0.05$ and ** = $P < 0.01$.

Table 5.5 Effects of the application of misty-fan cooling (MF) in comparison with normal shade (NS) on concentrations and excretions of allantoin and microbial nitrogen flow of crossbred Holstein cows supplemented with rbST

	NS		MF			Effect[1,2]		
	Pre	rbST	Pre	rbST	SEM	MF	rbST	MF x rbST
Early lacatation								
Milk allantoin concentration (umol/L)	667	716	634	705	10.3	ns	**	ns
Milk allantoin excretion (mmol/day)	6.7	8.5	7.1	9.1	0.33	ns	**	ns
Microbial Nitrogen flow (g/day)	164	178	164	182	3.0	ns	**	ns
Mid lacatation								
Milk allantoin concentration (umol/L)	691	698	724	804	19.3	ns	*	ns
Milk allantoin excretion (mmol/day)	6.7	7.2	8.0	10.2	0.36	ns	**	*
Microbial Nitrogen flow (g/day)	165	169	174	194	3.3	ns	**	*
Mid lacatation								
Milk allantoin concentration (umol/L)	715	783	798	912	14.9	ns	**	ns
Milk allantoin excretion (mmol/day)	5.8	7.6	7.5	10.7	0.38	ns	**	ns
Microbial Nitrogen flow (g/day)	159	175	174	203	3.3	ns	**	ns

Pre = pre-rbST supplementation, rbST = rbST supplementation

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

[2] Significance; ns = P> 0.05, * = P <0.05 and ** = P < 0.01.

CHAPTER VI

CHANGES IN THE RATE OF WATER TRANSFER FROM THE RUMEN DURING rbST SUPPLEMENTATION IN CROSSBRED HOLSTEIN CATTLE UNDER MISTY-FANS COOLING

INTRODUCTION

Generally, the lactating cows consume large amount of water relating to feed intake, energy metabolism and milk yield (Silanikove, 1987). Under extreme condition, water consumed was increased by 78% in sheep (Markwick, 2007). Water is the most important nutrient in minimizing heat stress because it acts is heat sink; therefore, heat is transferred from the cow's body to ingested water (Gwatibaya et al., 2007). Furthermore, Silanikove (1992) suggested that heat stress goats increased in the water content of digesta in the rumen and its volume serve as a water reservoir to counterbalance the effect of heat stress on rumen motility. It is believed that extra water was used for evaporative cooling during heat stress. The rumen may play an important role on body fluid balance, because it contain a large volume of water approximately 10 to 20% of body weight (Cain et al., 2006; Beede, 2005), a part of water might used for distribution of body heat load. During heat stress, lactating cows use body water as a vehicle in distribution of blood to mammary gland as well as for evaporative cooling during body heat dissipation (Collier et al.,1982). In buffalo, an increase in plasma volume and blood volume during acute and short term heat exposure has been reported (Chaiyabutr et al., 1987).

There is still no physiological evidence to the effect of rbST on alteration of body fluids movement at the gut level in lactating cows. In particular, there is no information whether the rumen is responsible for retaining as much water as possible for fluid retention, although rbST supplementation to the lactating cow can increased in total body water, extracellular fluid and blood volume (Maksiri et al., 2005; Chaiyabutr et al., 2007). The present study hypothesized that rbST supplementation would be involved in the regulation of body fluid by accompanying with changes in the rumen function. To explain the mechanism in regulation of body fluids, this study would emphasize exclusively on the rumen events in the crossbred lactating cows during rbST supplementation under hot condition. The objective of the current studies was to evaluate the effect of providing cross-bred cattle housing under shade with or without misters and fans and supplementation of cows with rbST or not during mid stage of lactation on feed and water intake. The effects of these treatments were measured to evaluate milk production in relation to changes of gut water and body water in relating to the movement of water through ruminal wall of crossbred lactating cows.

MATERIALS AND METHODS

Eighteen primiparous cows were used for two series of experiment. First series, ten cows were used for the body fluid study. Second series, eight cows were used for the water movement through ruminal wall. Cows in each series were assigned randomly into two groups of five animals each. Cows in both groups were housed in an open-sided barn. The barn was separated into two parts by a metal sheet wall. The first part was arranged for cows in normal shade (NS) and the second part of the barn was equipped with two pedestal mist-fan cooling systems (MF) for cooled cows. Cows in both groups were fed with the total mixed ration (TMR) which was formulated according to NRC requirements (NRC, 2001) for cows producing 10-15kg milk/day. The TMR was offered twice daily *ad libitum* throughout the experimental period. Water was given to cows *ad libitum*. Dry matter intake (DMI) of each cow was measured daily by weighing the TMR offered and subtracting that refused. Cows were normally milked by using a milking machine two times per day and milk production was recorded daily.

The ambient temperature at NS and MF barns were recorded using a wet and dry bulb thermometer. The relative humidity at NS and MF barns were read by psychrometric chart depending on wet and dry bulb temperature. A temperature-humidity index (THI) was calculated from the average ambient temperature of dry and wet bulb temperatures according to McDowell (1972). Rectal temperature (RT) of each cow was measured by electronic thermometer and respiratory rates (RR) was measured by counting flank movements. Body weights (BW) of all animals were recorded by weighing monthly throughout experimental periods. Cows using in this experiment either in NS or MF barn were studied in mid lactation, 120-150 DIM (Fig. 6.1). Two periods of study were performed for pre-treatment of rbST (Pre-treat) and treatment of rbST (Treat). The details of protocol for rbST administration in this stage of mid-lactaion were described in Chapter III.

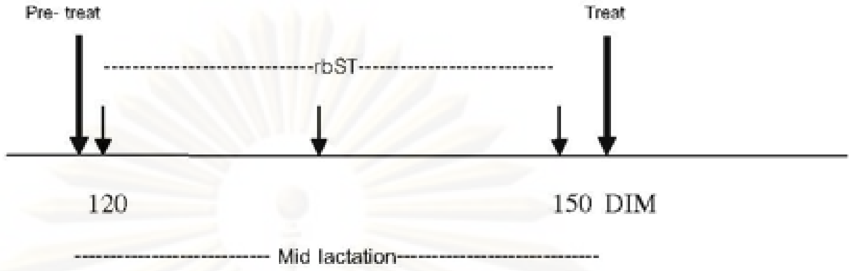

Figure 6.1 Schematic diagrams illustrating the time course of the experiment in each cow supplemented with rbST. Pre-treat = timed study for pre-treatment; Treat = timed study for treatment.

Animal in series I was intravenously injected with solutions containing 20 mL of 10% sodium thiocyanate solution, 20 mL of 5% Evans blue dye (T-1824) and 1 mL of a single dose of 3000 uCi/ animal of carrier-free tritiated water via an ear vein catheter for estimation of extracellular fluid (ECF) volume, the plasma volume and total body water (TBW) respectively. Venous blood samples from the jugular vein were taken at 20, 30, 40 and 50 min after dye injection for ECF and plasma volume determination. Blood samples were subsequently collected at 1, 2, 3, 4, 5, 6, 7, 18, 24, 36 and 48 hour after the injection of tritiated water (^3H_2O) for determination of TBW. Dilution technique of markers at zero time was determined by using a semi-logarithmic concentration on time extrapolation. Blood volume was calculated from the plasma volume and packed cell volume. The method for measurement of ECF was modified as described by Chaiyabutr et al. (2007). The preparation of samples for radioactive counting and calculation of empty body water (EBW), total body water (TBW) and gut water (GT) was described in Chapter III.

Animals in series II were used to determination of ruminal liquid dilution and gut water kinetics. The ruminal liquid dilution rate and water kinetics were measured at 08.00 hour by using 40 g of polyethylene glycol (PEG; M.W. 4000) diluted in 150 mL of water and 900 μCi of tritiated water (HTO), were infused into the rumen via the ruminal catheter(∅ 5 mm). Blood and rumen samples were taken directly via jugular vein catheter and rumen catheter, respectively, in every ten minutes for the first 60 min and then 2, 3, 5, 8, 11, 14, 18 and 24 hour after the infusion. Samples were transferred into counting vials and count for an appropriated time. The concentration of PEG was determined by turbidimetric method according to Hyden protocol (Grimaud and Doreau, 2003) for ruminal fluid measurement. Rumen liquid volume (V) was calculated from the dose of PEG added into the rumen (D) as described in Chapter III.

The statistic analyses were performed using General Linear Model procedure of statistical software package SPSS (SPSS for windows, V14.0; SPSS Inc., Chicago, IL, USA). The model used for each analysis was illustrated in Chapter III. Means values were used to evaluate the effect for all variables. Statistical significance was declared at $P<0.05$.

RESULTS

Environmental parameters, respiration rate and rectal temperature

Environmental parameters, respiration rate (RR) and rectal temperature (RT) are shown in Table 6.1. An ambient temperatures (AT), Relative humidity (RH) and THI measured at 15.00 hour were significant different between NS and MF barn. However, within the same barn, AT were not different between pre-treatment and treatment period. The THI value ranged from 85.06 to 85.51 in NS barn and 80.38 to 80.58 in MF barns. Respiration rate (RR) and Rectal temperature of cooled cow were lower than those of non-cooled cows whether with or without treatment of rbST ($P<0.01$). There were no significant differences in RR and RT between pre-treatment and treatment period.

Body fluids and gut water

Plasma and blood volume, ECF, ICF, TBW, EBW and GW are shown in Table 6.1. Plasma and blood volume were not affected by cooling system ($P>0.05$). Supplementation of rbST to the cows in both NS and MF barn was shown for an increase in plasma and blood volume during the experimental period ($P<0.05$). An ECF and ICF of cows were no significantly different between cooled and non-cooled cows in this experimental period. Treatment of rbST to cows in both NS and MF barn showed no differences of ECF and ICF from pre- treatment period, but there were numerical increases in the level of ECF and ICF after rbST treatment. The cooling system did not affect total body water (TBW) in absolute value or relative value as percentage of body weight. There were significantly different in TBW of cows between pre-treatment and treatment of rbST, but no significant different of TBW in relative values as percentage of body weight. The values of gut water (GW) of all cows in the present study were not affected by both cooling system and supplementation of rbST. It was noted that cooled cows showed significantly higher of GW than non-cooled cows. The high level of gut water was apparent in rbST-supplemented cows.

Body weight, feed intake, water intake and milk yield

Body weight, feed intake, water intake and milk yield are shown in Table 6.2. The cooling system and rbST supplementation were not affected on body weight of cows in this experiment. Cooled cows were greater voluntary feed intake than those of non-cooled cows ($P<0.05$) while DMI of cooled cows tended to be higher than non-cooled cows ($P=0.094$). The supplementation with rbST had effect on inclement of both voluntary feed intake and DMI significantly ($P<0.05$). In the present trial, milk yield of cooled cows were numerically greater than non-cooled cows and they also showed higher milk yield after treated with rbST than that of pre-treated period. Both cooling system and rbST supplementation to cows did not affect on feed efficiency ($P>0.05$). The similar water intakes were apparent in cooled and non-cooled cows, whereas the rbST treated cows had greater water intake than that of pre-treatment period ($P<0.05$).

The liquid dilution rate, ruminal fluid and plasma osmolarity and water movement across ruminal wall

The liquid dilution rate, ruminal fluid and plasma osmolarity and water movement across ruminal wall are shown in Table 6.3. The rumen liquid volume were significant different ($P<0.05$) between cooled and non-cooled cows. Supplementation of rbST did not affect on rumen liquid volume ($P>0.05$). There were no differences in liquid outflow rate from the rumen between cooled and non-cooled cows either with or without rbST treatment, but cooled cows tended to be higher in liquid out flow rate than non-cooled cows ($P=0.094$). Rumen liquid dilution rate as percent per hour and liquid retention time were not significant different among treatment groups ($P>0.05$) in both NS and MF barns. High level of ruminal osmolality was occurred in cooled

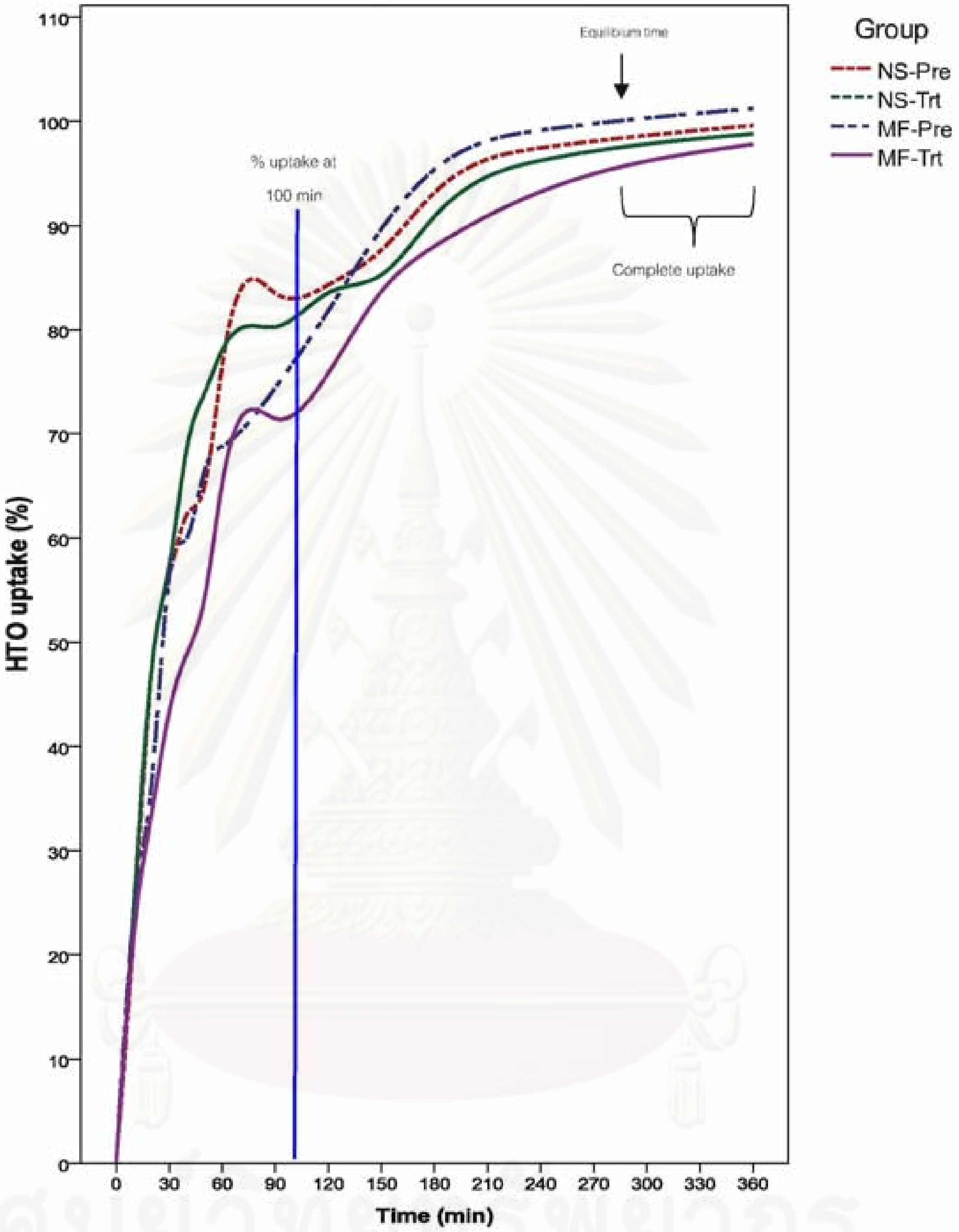

Figure 6.2 The mean values of percentage of uptake of tritiated water (HTO) activity in plasma after intraruminal loading of tritiated water. The studies were: pre-supplemented (Pre) and supplemented of rbST (Trt) under normal shade (NS) and shade plus misty-fans cooling system (MF).

cows, whereas blood osmolality of cooled cows were slightly lower than non-cooled cows (P>0.05). Total water absorption and insorption including net water transfer through the ruminal wall, measured by using HTO technique, were numerically higher for cooled cows than those of non cooled cows.. Supplementation of rbST to cows under both NS and MF barns showed numerically greater of total water absorption, water insorption and net water transfer through the ruminal wall (Table 6.3). These changes were not significantly different among treatment groups.

The tritiated water (HTO) uptake in plasma after intraruminal load is shown in Figure 6.2. The HTO concentration rapidly increased in the first 100 min after loading (about 90% uptake) and the remained markers were completely taken up within 360 min after loading. Similar patterns were shown in both cooled and non-cooled cows without rbST-supplementation. The rbST-supplemented cows housed in MF barn showed a low HTO uptake in comparison to those of cows housed in NS barn. About 70 to 80% of markers were taken up within the first 100 min after loading. During rbSTsupplementation, the rate of HTO transfer from rumen to plasma of non-cooled cows was faster than those of cooled cows. The rbST-supplemented cows housed in NS barn showed the completely HTO uptake within 360 min after loading, while cows housed in MF barn showed about 90% of HTO uptake within 360 min after intraruminal loading.

DISCUSSION

In the present study, An AT and THI value measured in both barns were higher than the comfortable zone for dairy cows (Kadzere et al., 2002). During the experimental period, THI were approximate 85 and 80 in both NS and MF barn respectively. These THI values were in the range of moderate heat stress (Smith et al., 2006). Nevertheless, the utilization of misty-fans system for cooling cows in the present study could alleviate the effect of heat stress which was indicated by lower RR and RT. These results are in agreement with Fike et al. (2002) that cooling cows with fan and sprinkler effectively reduced heat stress as indicated by lower both body temperatures and respiration rates. However, the mean values of RR and RT in the pretreatment period were not statistically significantly different from the period of rbST treatment. It is possible that the high heat gain produced by the effect of rbST-supplementation accompanying with heat dissipation in animals (Johnson et al., 1991).

The plasma volume and blood volume of cooled cows did not differ from non-cooled cows without rbST, while the plasma and blood volume showed significantly higher when cows were supplemented with rbST. The higher water intake in rbST-treated cows might partly account for an increase in plasma volume and blood volume via changes of water tranfer in digestive tract especially in the rbST-treated cows. These results are in agreement with Maksiri et al. (2005) reported that absolute values of plasma volume and blood volume were significantly increased in rbST-treated animals. However, an increase in both plasma volume and blood volume has been shown during acute heat exposure (Chaiyabutr et al., 1987). Cooled cows tended to show numerically greater of TBW, ECF and ICF when compared to non-cooled cows in the present study. However, an increase in the absolute values of TBW was markedly affected during supplemental rbST in both cooled and non cooled cows, but there were no differences in the relative values of TBW as percentage of body weight when compared with pre-treatment period. The high gut water of rbST-treated cows particularly under MF barn would be attributed to the high TBW in the present study. In ruminant, gut water especially in the rumen have 15-20% of the total body water. Louw (1993) has suggested that water can move between body fluid compartments and high value of gut water and high water turnover rate would account for the response of cows in maintaining body temperature during exposure to high temperatures. Thus, high gut water in rbST supplementation in cooled cows accompanying with an increase in TBW would be used for heat dissipation mechanism. These findings support the suggestion that even though bST increases heat production, it also increases heat dissipation (Johnson et al., 1991; West et al., 1991).

The body weights were not significant different between cooled and non-cooled cows with or without rbST supplementation in this study. Therefore, the differences of voluntary feed intake and DMI occurrence in this study would not be the result of the differences of BW. Feed intake and DMI were also markedly increased when cows received rbST. In the present study, cooled cows showed a numerically greater of milk yield than those of non cooled cows. The effect of rbST treatment could increase in milk yield 9.14 and 33.17 % higher than those of pre-treatment period in NS and MF barns, respectively. These findings are in agreement with previous reports (West, 1991; Chen et al., 1993). Cooled cows showed higher feed efficiency than non-cooled cows by average 11.21% in pre-treatment and 32.14 % after rbST treatment. It indicates that misty-fan cooling system showed effectively, in part, to improve milk production, although crossbred lactating cows exposed to high THI in MF barn (THI >72).

Animals exposed to hot environment usually increase water intake (Magdub et al., 1982; Costa et al., 1992; Gwatibaya et al., 2007). In the present study, water intake of the cooled cows tended to be higher than that of non-cooled cows and also increase when cow received rbST. The explanation for high water intake during rbST supplementation under cooling system would be due to the effect of high DMI. The correlation of high water intake with high DMI has been noted (Silanikove, 1987; Moallem et al., 2000). The main component in milk is water about 87 %, which would be another reason for requirement of high water intake for more milk yield in rbST-treated cows under cooling system.

Rumen liquid volume of cooled cows showed significantly greater than non-cooled cows in the present study. An increase in rumen liquid volume also occurred in rbST-supplemented cows under MF barn. These findings suggest that rumen fluid volume about 25% of TBW in cows was kept in the gut for maintenance of body temperature. Since rbST-treated cows under MF barn had heat gain with high level of metabolic rate by both nutrient digestion and milk production. The faster outflow rate of liquid from the rumen to lower gut of rbST-treated cows would account for more water absorption in the lower gut for heat dissipation mechanism. These results are agree with Chaiyabutr et al. (1987) who suggested that an increase in the outflow rate of liquid from the rumen to lower gut occur in heat stress animals for evaporative cooling in heat dissipation mechanism.

In the present study, rumen liquid dilution rate as a percent per hour and retention time were not statistically different among treatment groups. However, cows supplemented with rbST had tendency a high level of rumen liquid dilution rate and short liquid retention time when compared to those of cows without rbST supplementation. The present results may be partly explained by changes of GI-tract volume, since increasing the relative mass of many organs and tissues including GI-tract within the animal have been noted during exogenous bovine somatotropin (Moallem et al., 2004). It would be a possibility that the greater gut volume and/or rumen liquid volume would attribute to the subsequent increase in water intake of rbST-treated cows especially under MF barn. This result is in agreement with Bernabucci et al. (1999) who suggested that high water intake would account for an increase in dilution of rumen contents.

The osmolality of ruminal fluid and plasma were not affected by both cooling system and rbST supplementation in the present study. Animals in both groups were fed with the similar diet which presumably maintained in a similar level of osmolality of ruminal fluid throughout the experimental period. It seems likely that the rate of distribution of water between gut and plasma at the time of sampling would not vary in the present study.

An absorption, insorption and net water transfer through the ruminal wall were numerically greater for cooled cows in the present study. The net water transfer across rumen epithelium increased during rbST administration in both cooled and non-cooled cows. These findings would partly be a cause of an increase in body fluids as blood volume and TBW. The benefit of increasing in body water could be used for milk synthesis and for evaporative cooling mechanism during hot condition. Therefore, the cooled cows with rbST supplementation could produce 48.1% more milk than those of non-cooled cows without rbST supplementation (13.61 vs 9.19 kg/d). Chaiyabutr et al. (2007) have suggested that water loss with an increase in milk yield of the rbST-treated animals would be compensated by a restoration of body water pool (TBW or EBW).

After intra-ruminal loading of HTO, all cows showed rapidly HTO uptake within first 100 min. Murphy (1992) found that water exchange via diffusion was so rapid than the mean residence time of water molecule in the rumen. The HTO uptake into plasma was similar in cooled and non-cooled cows without rbST supplementation, which the completed uptake (100%) was occurred within 360 min after loading except the longer time occurred in rbST-supplemented cows under misty-fans cooling. It is known that the water transports through the ruminal wall is small and generally depend on osmotic pressure gradient between ruminal fluid and plasma (Argyle and Baldwin, 1988). In the present study, cows showed no differences in ruminal fluid and plasma osmolality among treatment groups.

In conclusion, misty-fans cooling system could be alleviated the effect of heat load in rbST-supplemented cows, which lead to enhance DMI, WI and milk production. These experiments demonstrate that the rbST exerts its galactopoietic action, in part, through changes in body fluid associated with regulation in gut water.

Table 6.1 The mean values of ambient environment, respiration rate and rectal temperature measured at 15.00 hour and the mean values of body fluid of crossbred cows with or without rbST treatment during mid lactation under misty-fan cooling (MF) and normal shade (NS) barns.

Parameter	NS		MF			Effect[1,2]		
	Pre	rbST	Pre	rbST	SEM	MF	rbST	MF x rbST
Ambient Temperature (°C)	36	36	29	30	0.8	**	ns	ns
Relative Humidity (%)	52	48	77	74	6.0	**	ns	ns
Temperature Humidity Index	86	85	81	80	0.6	**	ns	ns
Respiration Rate (Breath/min)	69	64	55	54	2.3	**	ns	ns
Rectal temperature (°C)	39.6	39.3	39.0	39.1	0.11	**	ns	ns
Plasma volume (L)	19	20	21	23	1.0	ns	*	ns
Blood volume (L)	25	27	27	31	1.0	ns	*	ns
Extracellular fluid (L)	100	111	116	123	4.5	P=0.081	P=0.073	ns
Intracellular fluid (L)	164	166	150	175	7.6	ns	ns	ns
Total body water (L)	264	277	266	299	6.3	ns	**	ns
Total body water (%BW)	69.2	73.3	69.4	73.8	2.17	ns	ns	ns
Gut water (L)	98	64	70	96	6.6	*	ns	ns

Pre = pre-rbST supplementation, rbST = rbST supplementation

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

[2] Significance; ns = P> 0.05, * = P <0.05 and ** = P < 0.01.

Table 6.2 The mean values of body weight, feed intake, water intake, milk yield and feed efficiency of crossbred cows with or without rbST treatment during mid lactation under misty-fan cooling (MF) and normal shade (NS) barns.

Parameter	NS		MF			Effect[1,2]		
	Pre	rbST	Pre	rbST	SEM	MF	rbST	MF x rbST
Body weight (kg)	402	410	411	416	6.7	ns	ns	ns
Voluntary feed intake (kg/h/d)	21.8	23.0	22.8	30.1	1.67	*	*	ns
Dry matter intake (kg/h/d)	8.5	9.6	8.9	11.8	0.70	ns	*	ns
Dry matter intake (kg/100 kgBW)	2.1	2.3	2.2	2.8	0.20	ns	*	ns
Milk yield (kg/d)	8.5	9.0	10.5	12.4	0.35	ns	**	ns
Feed efficiency (kg milk/ 1 kg feed)	1.1	1.1	1.11	1.2	0.12	ns	ns	ns
Water intake (L/h/d)	71.6	74.5	73.2	80.9	2.08	ns	*	ns

Pre = pre-rbST supplementation, rbST = rbST supplementation

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

[2] Significance; ns = $P > 0.05$, * = $P < 0.05$ and ** = $P < 0.01$.

Table 6.3 Effects of the application of misty-fan cooling (MF) in comparison with normal shade (NS) on liquid dilution rate and water movement across ruminal wall of crossbred Holstein cows treated with rbSTduring mid lactation

Parameter	NS		MFC			Effect[1,2]		
	Pre	rbST	Pre	rbST	SEM	MF	rbST	MF x rbST
Liquid dilution rate								
Rumen liquid volume (L)	62	61	64	75	3.6	*	ns	ns
Liquid outflow rate (L/h)	5.4	5.7	6.0	6.8	0.47	ns	ns	ns
Rumen liquid dilution rate ($\%h^{-1}$)	8.8	9.6	9.3	9.5	0.86	ns	ns	ns
Rumen liquid retention time (h)	12	11	11	11	0.89	ns	ns	ns
Water movement								
Ruminal Osmolality (mOsm/kg)	225	235	249	253	13.5	ns	ns	ns
plasma osmolality (mOsm/kg)	288	282	285	278	3.7	ns	ns	ns
Total water absorption (L/d)	27.08	36.50	36.82	42.48	5.237	ns	ns	ns
Total water insorption (L/d)	0.25	0.29	0.48	0.40	0.106	ns	ns	ns
Net water absorption (L/d)	26.83	36.21	36.34	42.58	5.213	ns	ns	ns

Pre = pre-rbST supplementation, rbST = rbST supplementation

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

[2] Significance; ns = P> 0.05, * = P <0.05 and ** = P < 0.01.

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CHAPTER VII

THE CHANGES OF PLASMA LEPTIN AND METABOLITE CONCENTRATION AND MILK PRODUCTION OF CROSSBRED HOLSTEIN CATTLE SUPPLEMENTAL WITH rbST UNDER MISTY-FAN COOLING SYSTEM

INTRODUCTION

It is recognized that the most of nutrients for milk synthesis will be obtained by feed intake. Reduction in dry matter intake (DMI) in cow exposure to hot environment will be a cause of insufficient substrates for milk synthesis. There are many factors that regulate feed intake, such as quantity and quality of feed stuff, feeding management, hormone and physiological state of cows. Leptin (the adipocyte-derived hormone) is one of factors, which play an important regulator of body mass via its control on feed intake and energy balance (Chillard et al., 2000; Thomas et al., 2001; Inoue et al., 2005). The satiety effects of leptin were also observed in ruminants by administration of recombinant human leptin in ewes for 3 days. This treatment causes a decrease in voluntary dry matter intake to approximately one third of normal intake (Henry et al., 1999). In addition, Morrison et al. (2001) have proposed that leptin represents an important function link between adipose stores and hypothalamic function in ruminants, which regulate multiple physiological processes, including both feed intake and neuroendocrine function. High environmental temperature and/or daylight in the summer month have been shown to stimulate secretion of leptin from adipose tissue (Accorsi et al., 2005). However, few data are available for an influence of rbST supplementation and application of misters and fans on alteration of plasma leptin relation to feed intake in crossbred lactating cows. The objective of the current studies were to 1) to evaluate the effect of providing cross-bred cattle with housing under shade with or without misters and fans and 2) to evaluate the effect of supplementation rbST to cows during three period of lactation (early mid and late lactation). Measures to evaluate the effectiveness of these treatments were alterations of plasma leptin and metabolites in relation to feed intake that supplied nutrients for milk production.

MATERIALS AND METHODS

Ten primiparous, non-pregnant crossbred cattle were used for the experiment. They were assigned randomly into two groups of five animals each. During the study period, cows in both groups were housed in an open-sided barn. The barn was separated into two parts by a metal sheet wall. The first part was arranged for cows in normal shade (NS) and the second part of the barn was equipped with two pedestal mist-fan cooling systems (MF) for cooled cows. Cows in both groups were fed with the total mixed ration (TMR) which was formulated according to NRC requirements (NRC, 2001) for cows producing 10-15kg of milk/day. The TMR was offered twice daily *ad libitum* throughout the experimental period. Water was given to cows *ad libitum*. Dry matter intake (DMI) of each cow was measured daily by weighing the TMR offered and subtracting that refused. Cows were normally milked by using a milking machine two times per day and milk production was recorded daily.

The ambient temperature at NS and MF barns were recorded using a wet and dry bulb thermometer. The relative humidity at NS and MF barns were read by psychrometric chart depending on wet and dry bulb temperature. A temperature-humidity index (THI) was calculated from the average ambient temperature of dry and wet bulb temperatures according to McDowell (1972). Rectal temperature (RT) of each cow was measured by electronic thermometer and respiratory rates (RR) was measured by counting flank movements. Body weights (BW) of all animals were recorded by weighing monthly throughout experimental periods. The experiment in each group was divided into 3 periods, namely early- (60-90 DIM), mid- (120-150 DIM), and late lactating periods (180-210 DIM). In each stage of lactation, the cow received an injection of rbST (POSILAC, 500 mg). The details of protocol for rbST administration in each stage of lactation were described in Chapter III.

The net energy intake and net energy for maintenance and for lactation was calculated from milk compositions and milk yield according to the formula suggested by NRC (2001). The energy balance was calculated from energy intake subtracts by energy for maintenance plus energy output in milk as described in Chapter III.

Venous blood samples from the jugular vein would be taken at 11.00-12.00 hour by venipuncture on specified day of each period, the first day at start experiment and two day after 3rd rbST injection in each period. Blood samples in heparinized test tube were placed on ice. Plasma was harvested by centrifugation at 3,000 rpm for 30 min and stored at -20 °C until analysis for hormone and metabolite. Plasma glucose concentrations were measured using enzymatic oxidation in the presence of glucose oxidase (glucose liquicolor, Wiesbaden, Germany). The plasma concentration of acetate was assayed by the acetic acid UV-method (R-Biopharm, Darmstadt, Germany). Plasma samples were determined for free fatty acid by colorimetry after plasma extraction with chloroform, heptane and methanol and TAN solution (Wang et al., 2004). Plasma β-hydroxybutyrate concentrations were assayed using an enzymatic reaction in the presence of β-hydroxybutyrate dehydrogenase (R-Biopharm, Darmstadt, Germany) and plasma triglyceride concentration was determined by enzymatic colorimetric test (Triglyceride liquicolor, Wiesbaden, Germany). The concentration of IGF-1 in plasma was measured by using a chemiluminescence immunoassay in an immulite analyzer (DPC, Los Angeles, CA). The plasma leptin concentration was determined using a radioimmunoassay kit specific for multi-species hormone (Linco Research, Inc., USA).

The statistic analyses were performed using General Linear Models procedure of statistical software package SPSS (SPSS for windows, V14.0; SPSS Inc., Chicago, IL, USA). The model used for each analysis was illustrated in Chapter III. Means values were used to evaluate the effect for all variables. Statistical significance was declared at $P<0.05$.

RESULTS

AT, RH, THI, RR and RT

An environmental parameters (AT, RH and THI) and physiological parameters (RR and RT) are shown in Table 7.1. Ambient temperature measured in NS barn (range from 33.6 to 34.3 °C) was higher than that of MF barn (range from 29.1 to 30.9°C) throughout experimental periods. Misty-fans cooling caused high relative humidity in the range 73.8-81.2%. The THI were varied in the range 80.2 to 82.9 in MF barn and 82.94 to 85.02 in NS barn, which showed significant differences ($P<0.05$). The AT, RH and THI were not significant different between pre-treatment and treatment period ($P>0.05$) in all stages of lactation. The RR and RT of cows housed in MF barn were significantly lower than those of cows in NS barn. After rbST treatment, the cows showed significant increases in RR and RT when compared with pre-treatment ($P<0.01$) in all stages of lactation.

BW, BCS, DMI, milk yield and feed utilization

The body weight (BW), body condition score (BCS), dry matter intake (DMI), milk yield and feed utilization are shown in Table 7.2. There were no significant different in BW and BCS between cooled and non-cooled cows in all stages of lactation. However, slightly increases in BW and BCS were occurred in rbST-treated cows, especially in mid lactation. DMI of non-cooled cows were lower than those of cooled cows in all stages of lactation. After treatment of rbST, cows showed increase in DMI when compared to pre-treated cows either cooling with misty-fans or not. Milk yield and 4% fat corrected milk (FCM) were numerically greater in cooled cows than those of non-cooled cows but it did not significant difference ($P>0.05$) in all stages of lactation. The rbST-treated cows showed significant increase in milk yield than those of pre-treated cows ($P<0.01$) and also showed a similar pattern in both cooled and non-cooled cows in all stages of lactation. The efficiency of feed utilization was not statistically different by both effects of cooling system and rbST supplementation during early and mid lactation, although it seem to be numerically high in rbST- treated cows.

Energy balance and plasma hormone

Net energy intake (NE*i*), net energy for maintenance (NE*m*), net energy for lactation (NE*l*) and energy balance (EB) including the concentrations of plasma leptin and insulin like growth factor-1 are shown in Table 7.3. The application of misty-fans to cooling cows caused to significantly increase in net energy intake in early and mid lactations. In late lactation, no significant different in NE*i* was occurred in both cooled and non-cooled cows. When cows were supplemented by rbST, the NE*i* were significantly higher than those of pre-treated cows. The energy output as NE*l* were not significantly different between cooled and non-cooled cows ($P>0.05$). However, cows treated with rbST showed significant increase of NE*l* as compared with the pretreated period in all stages of lactation. Furthermore, the energy balance of cows was not significantly different among treatment groups, which relative high positive energy balance in cooled cows.

The plasma leptin concentrations were not significantly different between cooled and non cooled cows without rbST supplementation in early lactation, while the plasma leptin level in cooled cows were significantly low during mid lactation ($P<0.05$) and late lactation ($P<0.01$). The marked reductions of the plasma leptin concentration were apparent when cows received rbST supplementation in all stages of lactation. The plasma insulin like growth factor-1 (IGF-1) concentrations of cows without rbST supplementation were not affected by the effect of misty-fans cooling system. Plasma IGF-1 concentrations of rbST-supplemented cows increased in all stages of lactation.

Plasma metabolites

The plasma concentrations of metabolites for glucose, β-hydroxybutyrate, acetate, triglyceride, free fatty acid and albumin are shown in Table 7.4. Plasma glucose concentrations of cooled cows were numerically higher than those of non-cooled cows but were not statistically different ($P>0.05$). There were no significant differences for the plasma concentrations of acetate, β-hydroxybutyrate, triglyceride and albumin by the effects of cooling and rbST supplementation in all stage of lactation. However, the reduction in the plasma acetate concentration ($P<0.05$) in cooled cows given rbST was apparent in late lactation. The plasma free fatty acid concentrations were numerically increased in rbST-treated cows when compared to pre-treated cows.

DISCUSSION

The application of misty-fans cooling could reduce 3.4-4.5°C of AT and THI which were lower than those of normal shade barn in the present study. Although, relative humidity measured in MF barn were relative high, but it could be partially mitigate heat load, which showing low RR and RT for lactating cows. These findings are in agreement with a number of previous works (Fike et al., 2002; Gallardo et al., 2005). Cows housed in MF barn decreased RR by 12 to15 breaths /min and RT by 0.5°C in comparison with those of cows housed in NS barn. These results might agree with the study of Smith et al. (2006) that using evaporative tunnel cooling can decrease rectal temperatures by 0.6 to 1.0°C and respiration rates by 20 to 30 breaths/min when comparison with traditional cooling strategies. Although, rbST administration is known to increase body heat production, resulting an elevation of RR and RT. The effect of rbST is little for an inclement only 3-5 breaths/min of RR and 0.1-0.4°C of RT from the pre-treated values in both cooled and non-cooled cows. Thus, the supplemental rbST would not be the main cause of heat stress in the present study. These findings might confirm the previous reports by Johnson et al. (1991) and West (1994) who proposed that rbST-treated cow increases heat production, it also increases heat dissipation.

In this study, BW and BCS were not significant different between cooled and non-cooled cows, but the BW of cows in all groups were increased when lactation progress. It would be attributed to the growing effect of primiparous cows used in this experiment. Cooled cows showed greater milk yield as 4% FCM than those of non cooled cows by ranging 12-22% in the pre-treatment and 17-36 % in the treatment period. On the other hand, after treatment of rbST, cows exhibited higher 4% FCM than pre-treatment period by 18-35% of cows housed in NS and 28-42% of cows housed ain MF barn. These results suggest that the supplementation of rbST to lactating cows could improve milk production either cooled cows or not. In addition, application of misters and fans to cows could not only increase in milk production but also enhanced responses to rbST administration with increase in efficiency of feed utilization.

Heat stress animals not only reduce feed intake but also alter endocrine status and increase in maintenance requirement (Collier and Beede, 1985; Collier et al., 2005). In the present study, the plasma leptin concentrations were affected either by cooling system or by rbST treatment. The rbST-treated cows under MF barn showed low level of the plasma leptin concentration accompanying increase in DMI throughout experimental periods. Cows housed in NS barn had an evidence of a high level of the plasma leptin concentration. These results suggest that non-cooled cows exposed to 83-85 THI nearby high heat stress, might increase in the secretion of leptin from adipose tissue, which in agreement with Several studies have proposed that the plasma leptin levels are influenced by environmental temperature and/or daylight, as in summer month (Accorsi et al., 2005; Pollard and Collier, 2004). In general, leptin hormone plays a role not only to regulate feed intake but also regulate energy expenditure in both ruminants and monogastric animals (Chelikani et al., 2003; Inoue et al., 2005). Therefore, an increase in NE*i* was observed in cooled cows given rbST, which would be attributed to an increase in DMI with a low level of the plasma leptin concentration. In the present study, energy consumed by cows among treatment groups were higher than their requirement in the range 6 to 9 and 8 to 13 Mcal/d in cows housed in NS and MF barn, respectively. These results would show a positive energy balance (PEB) in cows. It is known that changes of some blood metabolites, i.e. glucose. FFA and β-hydroxybutyrate (BHBA) could be used as indicators for energy status in lactating cows (Ndlovu, et al., 2007). In the present study, the concentrations of plasma metabolites for glucose, acetate, BHBA and triglyceride of cows showed no statistical differences among treatment groups. However, the low plasma glucose concentrations were apparent in the non-cooled cows as compared with those of cooled cows. This would agree with Roussel et al. (1971) who showed that the decline in plasma glucose level was took place in cattle during hot summer months. In the present study, the plasma FFA concentration was markedly increased after rbST supplementation in cooled cows. An increase in FFA during rbST administration would be the lipolytic activity of rbST per se in adipose tissue (Houseknecht et al., 2000). However, some studies have demonstrated that the decrease in the secretion of leptin was controlled by the concentration of plasma FFA (Chelikani et al., 2003). A negative correlation of leptin with free fatty acid and growth hormone was noted (Accorsi et al., 2005). Even though non-cooled cows showed high level of plasma leptin but there were no differences in plasma glucose and FFA concentrations when compared with cooled cows. The possible reason for this result would be attributed to their positive energy balance in all stages of lactation among treatment groups.

In the present study the plasma IGF-1 level was increased during rbST supplementation in all stages of lactation in both groups. The synthesis and release of IGF-1 is mainly by the liver (Granner, 1996). Mechanisms for regulating the plasma IGF-1level are known to be dependent on the availability in the liver of both bST and some nutritional factors (Clemmons and Underwood, 1991). From the present study, the increase in IGF-1 secretion would appear to be maintained by the availability of exogenous rbST in the liver. Exogenous rbST administration in the present study was sufficient to achieve a satisfactory stimulation of IGF-1 (Bachman et al., 1992). No differences in the nutritional status were apparent among treatment groups. Cows with a lower nutritional state having a lower basal level of IGF-1 (Hodgkinson et al., 1991) or a negative energy balance, have reduced hepatic IGF-1 production (Weller et al., 1994; Ketelsleger et al., 1995), would not be expected to occur in the present study.

The application of misty fans cooling system could reduce the effect of heat stress in lactating crossbred cows and enhance milk production by increasing in feed intake. Cooled cows supplemented with rbST showed greater milk production with weakly sign of heat stress. Therefore, the misty-fans cooling associate with rbST supplementation could enhance milk production by partly an increase in DMI via metabolic signal of the reduction of plasma leptin concentration.

Table 7.1 The respiration rate, rectal temperature of cows and environmental measurements at misty-fan cooling (MF) and normal shade (NS).

Parameter	NS		MF			Effect[1,2]		
	Pre[1]	rbST	Pre	rbST	SEM	MF	rbST	MF x rbST
Early lactation								
Ambient Temperature (°C)	34	34	30	31	0.4	**	ns	ns
Relative Humidity (%)	57	50	76	74	2.0	**	ns	ns
Temperature Humidity Index	85	84	82	83	0.5	**	ns	ns
Respiration Rate (Breath/min)	63	67	48	52	0.7	**	**	ns
Rectal temperature (°C)	39.1	39.2	38.6	39.0	0.07	*	*	ns
Mid lactation								
Ambient Temperature (°C)	34	34	30	30	0.5	**	ns	ns
Relative Humidity (%)	58.	53	81	80	2.5	**	ns	ns
Temperature Humidity Index	84	84	81	82	0.9	**	ns	ns
Respiration Rate (Breath/min)	62	66	47	52	0.3	**	**	ns
Rectal temperature (°C)	39.0	39.4	38.5	38.8	0.07	**	**	ns
Late lactation								
Ambient Temperature (°C)	34	34	30	29	0.3	**	ns	*
Relative Humidity (%)	51	50	77	78	1.1	**	ns	ns
Temperature Humidity Index	83	83	82	80	0.4	**	ns	*
Respiration Rate (Breath/min)	60	64	47	50	0.7	**	**	ns
Rectal temperature (°C)	39.0	39.3	38.5	38.8	0.06	**	**	ns

Pre = pre-rbST supplementation, rbST = rbST supplementation

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

[2] Significance; ns = P> 0.05, * = P <0.05 and ** = P < 0.01.

Table 7.2 Effects of the application of misty-fan cooling (MF) in comparison with normal shade (NS) on body weight, dry matter intake, milk yield and feed efficiency of crossbred Holstein cows treated with rbST

Parameter	NS		MF			Effect^{1,2}		
	Pre^{1}	rbST	Pre	rbST	SEM	MF	rbST	MF x rbST
Early lactation								
Body weight (kg)	358	380	373	376	7.3	ns	ns	ns
Body condition score	2.5	2.6	2.6	2.6	0.06	ns	ns	ns
Dry matter intake (kg/h/d)	6.2	7.1	7.22	8.5	0.31	*	**	ns
Milk yield (kg/d)	10.0	11.8	11.5	13.1	0.28	ns	**	ns
4% fat corrected milk (kg/d)	8.6	10.2	10.5	13.5	0.65	ns	**	ns
Feed efficiency (kg milk/ 1 kg feed)	1.4	1.5	1.5	1.6	0.11	ns	ns	ns
Mid lactation								
Body weight (kg)	383	379	383	408	4.5	ns	*	*
Body condition score	2.6	2.6	2.6	2.7	0.03	ns	*	*
Dry matter intake (kg/h/d)	6.2	7.5	8.7	10.0	0.45	*	*	ns
Milk yield (kg/d)	9.4	10.0	11.4	13.1	0.33	ns	**	ns
4% fat corrected milk (kg/d)	9.1	10.8	11.1	14.7	0.78	ns	*	ns
Feed efficiency (kg milk/ 1 kg feed)	1.5	1.5	1.3	1.5	0.15	ns	ns	ns
Late lactation								
Body weight (kg)	396	395	426	422	4.4	ns	ns	ns
Body condition score	2.6	2.6	2.7	2.7	0.06	ns	ns	ns
Dry matter intake (kg/h/d)	7.6	7.9	8.3	9.3	0.15	ns	**	*
Milk yield (kg/d)	8.0	9.6	9.7	12.2	0.43	ns	**	ns
4% fat corrected milk (kg/d)	8.6	11.7	9.7	13.8	1.34	ns	**	ns
Feed efficiency (kg milk/ 1 kg feed)	1.1	1.5	1.2	1.5	0.13	ns	*	ns

Pre = pre-rbST supplementation, rbST = rbST supplementation

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

[2] Significance; ns = $P > 0.05$, * = $P < 0.05$ and ** = $P < 0.01$.

Table 7.3 Effects of the application of misty-fan cooling (MF) in comparison with normal shade (NS) on blood hormone concentration and energy status of crossbred Holstein cows treated with rbST

Parameter	NS		MF			Effect[1,2]		
	Pre[1]	rbST	Pre	rbST	SEM	MF	rbST	MF x rbST
Early lactation								
Leptin (ng/ml)	2.59	2.38	2.26	2.05	0.077	ns	*	ns
Insulin like growth factor 1 (ng/mL)	118	202	97	185	15.5	ns	**	ns
NE intake (Mcal/day)	16.4	18.8	19.3	22.7	0.83	*	**	ns
NE for Maintenance(Mcal/day)	7.9	8.3	8.2	8.2	0.12	ns	ns	ns
NE for lactation (Mcal/day)	3.2	3.8	4.0	5.5	0.37	*	*	ns
Energy balance (Mcal)	5.3	6.8	7.1	9.0	0.96	ns	ns	ns
Mid lactation								
Leptin	2.53	2.17	2.05	1.88	0.05	*	**	ns
Insulin like growth factor 1(ng/mL)	116	223	142	248	23.6	ns	**	ns
NE intake (Mcal/day)	16.5	20.0	23.3	26.7	1.20	*	*	ns
NE for Maintenance(Mcal/day)	8.3	8.4	8.3	8.7	0.07	ns	ns	*
NE for lactation (Mcal/day)	3.7	4.6	4.5	6.2	0.41	ns	*	ns
Energy balance (Mcal)	4.5	7.2	10.5	11.9	1.35	*	ns	ns
Late lactation								
Leptin	2.48	2.20	2.03	1.84	0.08	**	*	ns
Insulin like growth factor 1(ng/mL)	128	291	124	272	24.4	ns	**	ns
NE intake (Mcal/day)	20.2	21.0	22.1	24.9	0.40	ns	**	*
NE for Maintenance(Mcal/day)	8.5	8.5	9.0	8.9	0.07	ns	ns	ns
NE for lactation (Mcal/day)	3.8	5.2	4.0	5.9	0.38	ns	*	ns
Energy balance (Mcal)	7.9	7.3	9.1	10.0	0.65	ns	ns	ns

Pre = pre-rbST supplementation, rbST = rbST supplementation

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

[2] Significance; ns = P> 0.05, * = P <0.05 and ** = P < 0.01.

Table 7.4 Effects of the application of misty-fan cooling (MF) in comparison with normal shade (NS) on blood metabolite concentration of crossbred Holstein cows treated with rbST

Parameter	NS		MF			Effect[1,2]		
	Pre[1]	rbST	Pre	rbST	SEM	MF	rbST	MF x rbST
Early lactation								
Glucose (mg/dL)	59.0	60.9	68.7	62.2	4.77	ns	ns	ns
Beta-hydroxy butyrate (mg/dL)	11.9	12.1	8.5	9.0	0.89	ns	ns	ns
Acetate (mg/dL)	3.7	3.1	2.4	2.6	0.32	ns	ns	ns
Triglyceride (mg/dL)	11.9	13.4	14.6	15.1	1.29	ns	ns	ns
Free fatty acid (mg/dL)	3.7	3.9	3.2	5.3	0.90	ns	ns	ns
Albumin (g/dL)	4.3	4.2	4.2	4.2	0.06	ns	ns	ns
Mid lactation								
Glucose (mg/dL)	62.3	61.1	63.3	66.1	1.83	ns	ns	ns
Beta-hydroxy butyrate (mg/dL)	10.5	11.4	11.7	9.7	0.74	ns	ns	ns
Acetate (mg/dL)	2.6	2.9	3.6	3.1	0.30	ns	ns	ns
Triglyceride (mg/dL)	15.6	17.2	13.6	15.1	1.79	ns	ns	ns
Free fatty acid (mg/dL)	3.1	4.6	3.3	3.9	0.35	ns	*	ns
Albumin (g/dL)	4.2	4.1	4.1	4.2	0.07	ns	ns	ns
Late lactation								
Glucose (mg/dL)	69.6	61.1	65.9	66.1	3.10	ns	ns	ns
Beta-hydroxy butyrate (mg/dL)	12.3	13.1	9.9	9.8	1.57	ns	ns	ns
Acetate (mg/dL)	4.4	3.9	3.3	2.5	0.46	**	ns	ns
Triglyceride (mg/dL)	14.9	15.7	24.0	18.0	3.61	ns	ns	ns
Free fatty acid (mg/dL)	2.4	3.6	3.0	4.7	0.41	ns	**	ns
Albumin (g/dL)	4.3	4.3	4.2	4.2	0.06	ns	ns	ns

Pre = pre-rbST supplementation, rbST = rbST supplementation

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

[2] Significance; ns = $P > 0.05$, * = $P < 0.05$ and ** = $P < 0.01$.

CHAPTER VIII

GENERAL DISCUSSION

The results of experiments in Chapter IV-VII have shown that an application of misty-fans cooling (MF) to crossbred lactating cows under hot environment could reduce the effect of high heat load in animals and improve milk production. The MF system could significantly reduce ambient temperature (AT) and temperature humidity index (THI) during experimental period. The patterns of changes in AT and THI were similar in all series of experiments (Chapter IV to VII). However, the MF system that delivered mist to the ambient environment would increase in the percentage of relative humidity (RH) as compared with NS barn. Thus, MF system was not sufficient to completely eliminate heat stress in cows during 09.00 to 15.00 hour. The THI measured under the misty fan cooling remained high (THI 77 to 83), whereas the high level of THI in NS barn showed in range 79 to 85. The high level of THI in NS barn would be directly attributed to the high level of AT. The present results indicate that animals housed in both MF and NS barn would subject to moderate heat stress, which the threshold level of comfortable zone has been noted for 25 °C and 72 for AT and THI, respectively (Armstrong, 1994; Kadzere et al., 2002). The effect of misters and fans would be beneficial to alleviate the effect of heat stress in crossbred lactating cows under hot condition, although crossbred dairy cattle using in the present experiment containing *B. indicus* gene, could have a high heat tolerance than exotic *B. Taurus* cattle (Nakamura et al., 1993; Pereira et al., 2008). The low values of both respiration rate (RR) and rectal temperatures (RT) coinciding with high level of DMI and milk yield in cooled cows were apparent. These findings are agreement with earlier reports occurring in animals kept in both warm and tropics countries. Fike et al. (2002) have shown that housing cows during the day with fans and sprinklers effectively reduced heat stress as indicated by lower body temperature and RR. Avendano-Reyes et al. (2006) reported that European cows using water spray and fans were lower RR and RT than non-cooled cows. The other study in cows using evaporative tunnel cooling has also shown to decrease peak daytime RT by 0.6 to 1.0°C and RR by 20-30 breaths/min when compared with traditional cooling strategies (Smith et al., 2006).

In the present study, cows supplemented with rbST would increase in RR and RT. These findings agree with previous reports in *Bos Taurus* cows treated with rbST (Sullivan et al., 1992; Tarazon et al., 1999; Settivari et al., 2007). However, it was noted that RT of cows in the present study (38.5-39.5°C) were mostly higher than the normal range (38.3-38.7 °C) for dairy cattle reported by Abeni et al. (2007). It indicates that rbST could induce heat production in crossbred lactating cows. Although rbST-supplemented cows showed a sign of heat stress with high RR and RT, but they also showed an increase in DMI and milk yield when compared with pre-supplemented period.

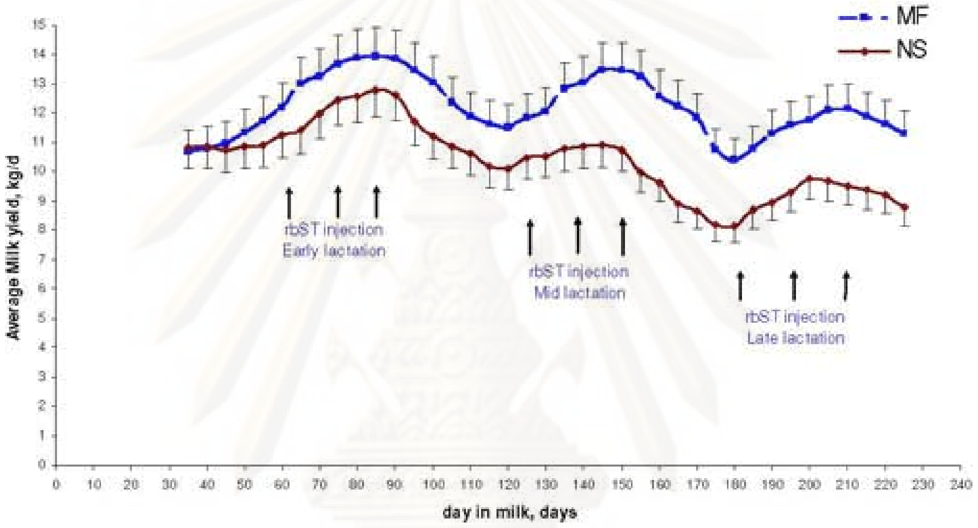

Figure 8.1 Milk yield response to rbST supplementation in crossbred lactating cows housing in normal shade (NS) and shade plus misty-fans cooling system (MF) during early, mid and late lactation

In all experimental studies, the DMI of cooled cows have shown to be higher than those of non-cooled cows by average1.25 kg/d (14.51%) and cooled cows treated with rbST also increased DMI than those of non-cooled cows treated with rbST by average 1.68 kg/d (17.02%). The increases in DMI would make more nutrients available to the mammary gland for increase in milk production by average 1.82 kg/d (16.45%) (the pretreatment between cooled *vs* noncooled cows) and 2.32 kg/d (18.26%) (the rbST treatment between cooled *vs* noncooled cows). In the present study, rbST supplementation could increase milk production by an average 1.23 kg/d (13.30%) in non-cooled cows (pretreatment *vs* treatment) and 1.73 kg/d (16.04%) in cooled cows (pretreatment *vs* treatment). These findings are in agreement with several reports that an increase in milk production is always appeared in rbST-supplemented cows (West, 1994; Etherton and Bauman, 1998; Gulay and Hatipoglu, 2005). A high response of milk yield occurred during rbST supplementation in cooled cows suggesting an efficiency of feed utilization for milk production under MF system. The present findings also demonstrated that the maintenance of lactation persistency in crossbred lactating cows supplemented with rbST (Fig. 8.1) was accompanied by an application of MF system.

The studies of digesta kinetic study in Chapter IV demonstrate that a mean value of DMI from both pretreatment and treatment with rbST in cooled cows was 14.0% higher than those of non-cooled cows. These changes would accompany with the significant increase in the mean value of digesta passage rate (23.1%) of cooled cows as compared with those of non-cooled cows. These results indicate that the faster passage rate of digesta through the digestive tract would partially reduce gut fill which subsequently increased in diet consumption. The mean value of digesta passage rate of crossbred lactating cows housing in both NS and MF barns were increased after rbST supplementation by an average 9.2% when compared with the pretreatment period. The rbST- treated cows under MF barn could increase the digesta passage rate by an average 23.9% when compared with cows under NS barn. It indicates that the effect of MF cooling would superimpose the effect of rbST supplementation on the digesta passage rate.

In general, the faster of digesta passage rate would be a factor causing reduction in diet digestibility (Bernabucci et al., 1999). In the present study, the total DM digestibility of cooled cows was not significantly different from non-cooled cows, although the fast digesta passage rate was occurred in cooled cows and rbST-treated cows. It indicates that the longer retention time of digesta appearing in non-cooled cows might not be sufficient for enhance DM digestibility. The negative effects of higher digesta passage rate on DM digestibility in rbST-treated cows might partly be compensated by an increase in the activity of rumen cellulolytic bacteria. Winsryg et al. (1991) has pointed out that an increase in DMI of rbST-treated cows would subsequently increase in the activity of cellulolytic bacteria.

The effect of misty-fans cooling system on products of volatile fatty acid (VFA) and ammonium nitrogen (NH_3N) in the rumen is reported in Chapter V. The rumen VFA and NH_3N concentrations were not significantly different between cooled and non-cooled cows without rbST. It is probably that both cooled and non-cooled cows were fed in a similar ration of TMR throughout experimental period. In addition, cows under both NS and MF barns were also exposed to moderate heat stress which would affect to rumen fermentation in a similar manner between cooled and non-cooled cows. The study of Bandaranayaka and Holmes (1976) has shown that the reduction of VFA production occurred in animal prolonged exposure to high environmental temperature. The total VFA concentration of cows supplemented with rbST under MF barn was greater than that of rbST-supplemented cows under NS barn by an average 20.8%. An increase in DMI of cows responding to rbST supplementation would provide substrates availability for ruminal microbial fermentation resulting in an increase in total VFA in the present study. However, the concentration of rumen NH_3N was increased during rbST supplementation by an average 9.0% in non-cooled cows and 12.3% in cooled cows. It is in agreement with Robinson et al. (1991) who showed that cows treated with bST had higher rumen NH_3N concentration than the control cows. An increase in the rumen NH_3N production in rbST-treated cows would account for an increase in milk urea nitrogen (MUN). An increase in MUN excretion occurred during rbST administration by average 0.45 g/d in non-cooled cows and 0.70 g/d in cooled cows. It indicates the occurrence of an imbalance of NH_3N and energy for microbial growth in the rumen, since MUN in dairy cows can provide information on total nitrogen losses following absorption of ammonia in the rumen (Butler et al., 1996; Hof et al., 1997). The other possibility for the higher MUN in rbST-treated cows may be occurred along with increasing in mammary blood flow during rbST supplementation (Chaiyabutr et al., 2000[b]); an increase in mammary uptake of plasma urea would attribute to an increase in MUN in the present study.

The concentration and excretion of purine derivatives in urine (allantoin, uric acid, xanthine and hypoxanthine) have been used as an indicator for microbial protein flow from the rumen (Gonda and Lindberg, 1997; Schager et al., 2003). However, Timmermans et al. (2000[a]) suggested that milk allantoin have been a positive relationship with microbial protein flow in dairy cattle. In the present study, the cooling effect of the MF system did not significantly affect to the concentration of allantoin in milk and microbial nitrogen flow (MNF) from the rumen. However, it was found that MNF of cooled cows without rbST supplementation tended to be higher than that of non-cooled cows without rbST supplementation. The marked effect of rbST on MNF was observed in the present study and thereby after rbST supplementation, the MNF increased by an average 11.6 g/d in non-cooled cows and 22.42 g/d in cooled cows. These findings indicate that the effect of MF cooling might partly superimpose in response to rbST in cows. The results in Chapter V can conclude that rbST exerting galactopoietics was not only via an increase in DMI but also via an increase in the rumen fermentation of crossbred lactating cows.

The study for the effect of rbST supplementation and misters and fans on changes in body fluids and water movement through the rumen epithelium are shown in Chapter VI. In general changes in body fluids are usually related to water intake, which is involved by several factors. The increase in water intake is required to maintain water balance and avoid dehydration of animals under heat stress conditions. Several studies have been reported that animal exposed to hot environment would increase in water intake (Magdub et al., 1982; Costa et al., 1992; Gwatibaya et al., 2007). The results in the present study found that water intake in cows housed in MF barn without rbST was higher than those of cows without rbST housed in NS barn by average 1.58 L/cow/d and 6.47 L/cow/d in cow with rbST supplementation. The greater DMI in either cooled cows without rbST (0.4 kg/d) or cooled cows with rbST supplementation (2.1 kg/d) in comparison to non-cooled cows would account for an increase in water intake. These results are in agreement with the studies of Silanikove (1987) and Moallem et al. (2000) that water intake was closely related to DMI. However, it was found that the trend of reduction of gut water (-7.1%) was observed in rbST-treated cows under NS barn while an increase in gut water (36.2%) was observed in rbST-treated cows under MF barn. The differences of these results indicate that more water lost is affected by high temperatures for evaporative heat dissipation process in non-cooled cows under NS barn. An increase in gut water during rbST supplementation may be a larger part accounting for the higher value of total body water in rbST-treated cows. Although, the studies of the liquid outflow rate from the rumen were not statistically different among groups of cows in the present study. However, it was observed that cooled cows showed an increase in liquid outflow rate from the rumen to lower gut by average 0.59 L/h in cows without rbST and 1.1 L/h in cows with rbST supplementation. Nevertheless, the absorption of water through the ruminal wall of rbST-treated cows showed trend to be higher than that of pre-treatment by average 9.42 L/day in non-cooled cows and 5.66 L/day in cooled cows. It indicates that a fast liquid outflow rate by the effect of rbST would partly attribute to reduce the net water absorption through the ruminal wall. These findings in chapter VI would suggest that an increase of TBW in cows with rbST supplementation in the present study could be due to more absorption of water in lower GI tract during fast liquid outflow rate. A small of water transfer from rumen through omasum has been noted (Holtenius, 1989). An increase in milk yield in rbST-treated cows housed in MF barn could be that an increase in the level of total body water, and secondarily the fluids to the mammary gland increases during rbST supplementation.

It is known that feed intake in ruminant is regulated by hormonal level, for example the level of leptin hormone (Accorsi et al., 2005; Bernabucci et al., 2006). An experiment in feed intake regulation by the leptin hormone and changes of blood metabolite are performed in Chapter VII. The findings demonstrate that cows supplementation with rbST showed greater DMI both in non-cooled cows and cooled cows when compared with pre-supplementation period. These changes were accompanied with the reduction of the plasma leptin concentration. These results indicate that the level of leptin hormone influenced in feed intake in ruminants and the level of leptin hormone was also regulated by the effect of exogenous rbST. The high level of leptin inhibiting feed intake and down-regulated adipose tissue deposition has been noted (Halaas et al., 1995; Morrison et al., 2001). There are some reports have shown that the leptin level was also influenced by environmental temperatures and/or daylight, as in summer month (Pollard and Collier, 2004; Accorsi et al., 2005). Cows in the present study exposed to THI over the threshold level of for moderate heat stress in MF barn (THI 81) and high heat stress in NS barn (THI 84.5). Cows housed in NS barn showed high level of the leptin concentration (average 2.39 ng/mL) than those of cows housed in MF barn (2.02ng/mL) (Chapter VII). However, after rbST supplementation the plasma leptin concentration was decreased from pre-supplementation by an average 0.28ng/mL in non-cooled cows and 0.19ng/mL in cooled cows. These results indicate that more reduction of the level of plasma leptin and increase in feed intake took place during rbST supplementation in crossbred lactating cows with application of MF cooling.

There were no statistical changes in plasma metabolite concentrations of cows in this study (Chapter VII). Nevertheless, the tendency for the low level of plasma glucose was observed in non-cooled cows. This result may agree with the observation of Roussel et al. (1971) that the low level of plasma glucose was occurred in cattle during hot summer months. The rbST-treated cows showed an increase in the concentration of plasma free fatty acid (FFA) in cows housed in both NS and MF barns, while no changes in the plasma BHBA concentration were apparent. These results indicate that the dairy cows in the present experiment were in state of energy balance, i.e. the metabolic demands of lactation were met by dietary intake causing in weight gain throughout lactation. An increase in FFA during rbST administration would be the lipolytic activity of rbST per se in adipose tissue (Houseknecht et al., 2000).

In conclusion, the present study provides information regarding the magnitude of responses of digestive functions between the effects of mist-fan cooling and rbST supplementation which are summarized in Fig. 8.2. The mechanisms involved digestive function and milk production in the supplemental rbST under high temperature conditions were elucidated. The main effects of mist-fans cooling could reduce a negative effect of hot environment on changes in digestive function via an increase in digesta passage rate, resulting in an increase in feed intake. However, an increase in rumen fermentation products as VFA and NH_3N would be in part due to an increase in DMI response to both rbST supplementation and MF cooling system. An increase in water intake accompanying with an increase in feed intake was also found in rbST-supplemented cow under-misty fan cooling. It would be suggest that the rbST exerts its galactopoietic action, in part, through changes in gut water regulation associated with changes in body fluid, and secondary the blood flow to the mammary gland for milk synthesis and for to thermoregulatory mechanism. The role of rbST related to water absorption through ruminal wall, making animals more body water retention is particular interest. The present study emphasizes the importance of the changes in water retentions during rbST supplementation under heat stress, thereby making animals more thermotolerance to heat stress. The reduction of plasma leptin concentration was appeared in rbST-treated cows. It would be suggest that exogenous rbST could be in part regulates of feed intake via a reduction of adipose cell (Bauman et al., 1998; Cheli et al., 1998) resulting in a low level of leptin secretion. The present experiment has demonstrated that major changes occur in the digestive process would be the effects of exogenous of rbST accompanying with an application of misty-fan cooling system.

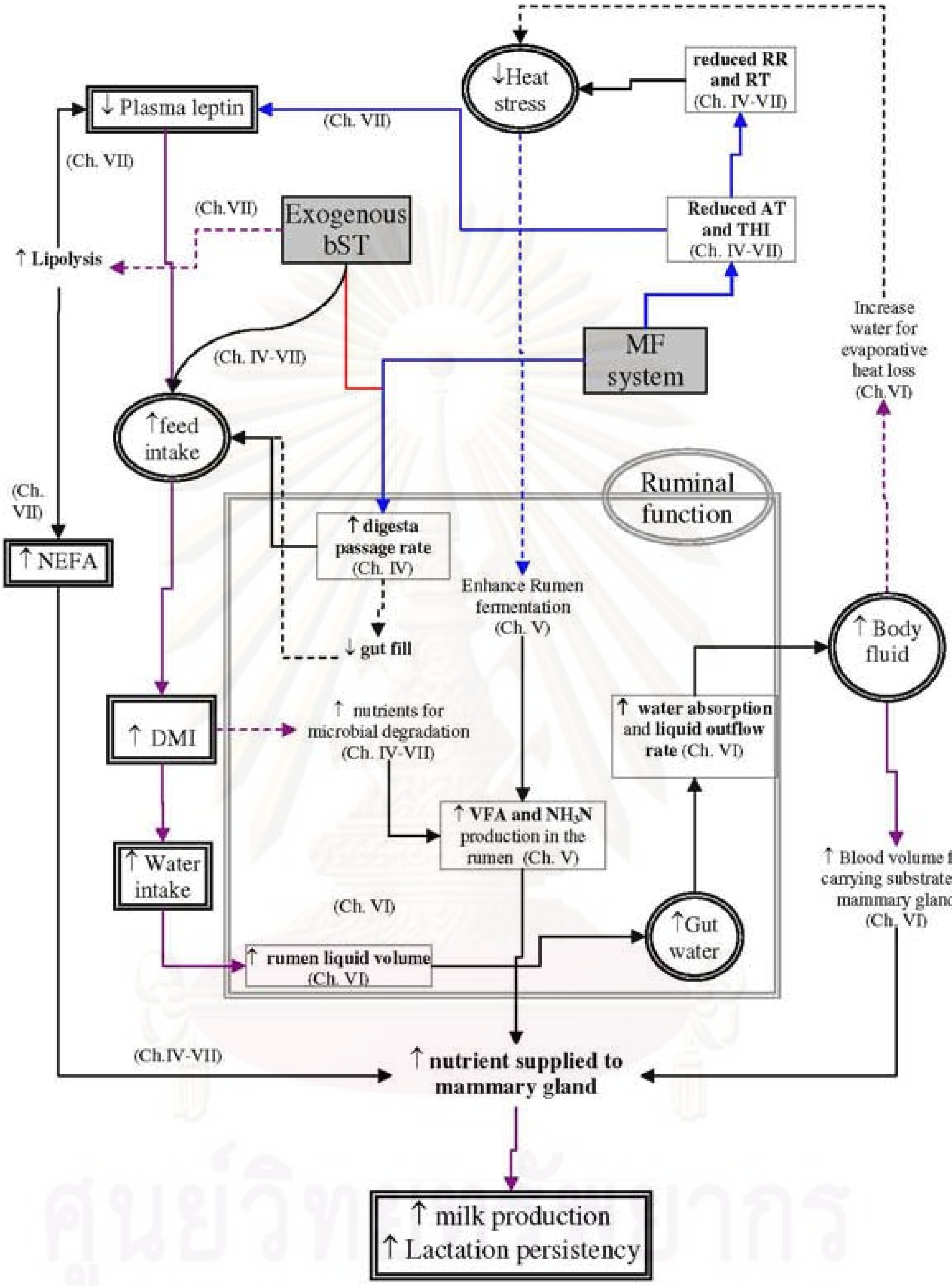

Figure 8.2 Diagram showing the effects of supplemental rbST and misty-fans cooling (MF) on physiological changes in digestive functions. Summary of pathways whereby rbST confers effects, solid arrows represent demonstrated pathways whereas dashed arrows represent speculative pathways.

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APPENDIX

PUBLICATION

1. Chanchai, W., Chanpongsang, S. and Chaiyabutr, N. Effects of misty-fan cooling and supplemental rbST on rumen function and milk production of crossbred Holstein cattle during early, mid and late lactation in a tropical environment. Animal Science Journal, 2009 (in press)

2. Chanchai, W., Chanpongsang, S. and Chaiyabutr, N. Effects of mist-fan cooling and supplemental recombinant bovine somatotropin on diet digestibility, digestion kinetics and milk production of crossbred Holstein cattle in the tropics. Journal of Agricultural Science (Cambridge), 2009 (in press)

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