ผลของรีคอมบิแนนท์ โบวายน์ โซมาโตโทรปิน และระบบพัดลมพ่นละอองน้ำ ต่อผลผลิตน้ำนมที่สัมพันธ์กับเมแทบอลิซึมของกลูโคสในร่างกายและต่อมน้ำนมของโคนมพันธุ์ผสม โฮล์สไตน์

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THE EFFECTS OF RECOMBINANT BOVINE SOMATOTROPIN AND MISTY-FAN COOLING SYSTEM ON MILK PRODUCTION RELATING TO BODY AND MAMMARY GLAND GLUCOSE METABOLISM IN CROSSBRED HOLSTEIN CATTLE.

Mr. Siravit Sitprija

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Thesis Title	THE EFFECTS OF RECOMBINANT BOVINE SOMATOTROPIN AND MISTY-FAN COOLING SYSTEM ON MILK PRODUCTION RELATING TO BODY AND MAMMARY GLAND GLUCOSE METABOLISM IN CROSSBRED HOLSTEIN CATTLE
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ศิรวิทย์ สิตปรีชา : ผลของรีคอมบิแนนท์ โบวายน์ โซมาโตโทรปิน และระบบพัดลมพ่นละอองน้ำต่อผลผลิตน้ำนมที่สัมพันธ์กับเมแทบอลิซึมของกลูโคสในร่างกายและต่อมน้ำนมของโคนมพันธุ์ผสมโฮล์สไตน์ (THE EFFECTS OF RECOMBINANT BOVINE SOMATOTROPIN AND MISTY-FAN COOLING SYSTEM ON MILK PRODUCTION RELATING TO BODY AND MAMMARY GLAND GLUCOSE METABOLISM IN CROSSBRED HOLSTEIN CATTLE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ศ.นสพ.ดร. ฉรงค์ศักดิ์ ชัยบุตร, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ.นสพ. สมชาย จันทร์ผ่องแสง, 125 หน้า.

โคนมพันธุ์ผสมโฮลสไตน์ 87.5% 2 กลุ่ม กลุ่มละ 5 ตัวเลี้ยงในโรงเรือนปกติ (NS) และโคที่เลี้ยงในที่เย็นในโรงเรือนที่มีพัดลมพ่นละอองน้ำ(MF)โคทุกตัวจะได้รับการเสริมรีคอมบิแนนท์ โบวายโซมาโตโทรปิน (rbST)ด้วยการฉีด rbST ติดต่อกัน 3 ครั้ง ครั้งละ500 มิลลิกรัมห่างกันทุก ๆ 14 วัน ในระยะต้น ระยะกลาง และระยะท้ายของการให้นม ในช่วงบ่ายที่เป็นช่วงที่ร้อนที่สุดอุณหภูมิแวดล้อมในโรงเรือน MF จะต่ำกว่าโรงเรือนปกติ อย่างมีนัยสำคัญ แต่ความชื้นสัมพัทธ์จะสูงกว่าโรงเรือนปกติ ดัชนีอุณหภูมิความชื้นสัมพัทธ์ (THI) ทั้งสองโรงเรือนอยู่ในช่วง 77.8-85.5 ตลอดระยะการศึกษา โคที่เลี้ยงในโรงเรือนที่มีพัดลมพ่นละอองน้ำจะมีอุณหภูมิวัดที่ทวารหนักและอัตราการหายใจต่ำกว่ากลุ่มโคที่เลี้ยงในโรงเรือนปกติ อัตราการหลั่งน้ำนมจะเพิ่มขึ้นอย่างมีนัยสำคัญในกลุ่มโคที่ฉีด rbSTในทุกระยะของการให้นม พบการเพิ่มขึ้นของอัตราการไหลของเลือดสู่ต่อมน้ำนม และระดับของพลาสม่า IGF-I ร่วมไปกับการเพิ่มขึ้นของปริมาณน้ำในร่างกาย ปริมาณน้ำนอกเซลล์ ปริมาณเลือดและปริมาณพลาสม่าในโคนมทั้ง 2 กลุ่มที่ได้รับ rbST ตลอดระยะการให้นม ไม่พบการเปลี่ยนแปลงความเข้มข้นของกลูโคส อะซีเตท เบต้าไฮดรอกซีบิวทีเรต และ ไตรกลีเซอไรด์ ในพลาสม่าของเลือดแดง แต่ความเข้มข้นของกรดไขมันอิสระจะเพิ่มขึ้นในโคที่เลี้ยงทั้งโรงเรือนปกติและโรงเรือนที่มีความเย็นเมื่อให้ rbST อัตราการใช้กลูโคสและไตรกลีเซอไรด์โดยต่อมน้ำนมจะเพิ่มขึ้นโดยเฉพาะในระยะกลางและระยะท้ายของการให้นม ไม่พบการเปลี่ยนแปลงของอัตราการหมุนเวียนกลูโคสในโคนมทั้งสองกลุ่มที่ได้รับ rbST และไม่ได้รับ rbST อัตราการใช้กลูโคสโดยต่อมน้ำนมถูกนำไปใช้ในวิถีของการสังเคราะห์แลคโตส และในวิถีเพนโตสกับการเพิ่ม NADPH เพื่อการสังเคราะห์กรดไขมันในโคนมทั้งสองกลุ่มที่ให้ rbST อัตราการใช้คาร์บอนอะตอมกลูโคสเพิ่มขึ้นในน้ำนมแลคโตสและไขมันนมในโคนมทั้งสองกลุ่มที่ได้รับ rbST ในระยะต้นและระยะกลางของการให้นมแต่ไม่พบการเพิ่มซิเตรทในน้ำนม การลดอัตราการหลั่งน้ำนมเมื่อเข้าช่วงท้ายๆของการให้นมที่พบในโคทั้งสองกลุ่มเมื่อไม่ได้รับ rbST การเปลี่ยนแปลงเฉพาะที่สำหรับความสามารถในการสังเคราะห์ในต่อมน้ำนมอาจเป็นปัจจัยที่กำหนดอัตราการใช้สารตั้งต้นในขณะที่มีการลดลงของอัตราการหลั่งน้ำนมตามระยะเวลาของการให้น้ำนมในโคนมทั้งสองกลุ่ม อัตราส่วนของกลูโคสจะถูกเมแทบอไลซ์เข้าสู่การสังเคราะห์แลคโตสลดลง แต่จะถูกเมแทบอไลซ์เข้าสู่วิถีเอมบ์เดน – เมเยอร์ฮอฟ และวัฏจักรกรดไตรคาร์บอกซิลิคเพิ่มมากขึ้นตามระยะเวลาของการให้น้ำนมในโคนมทั้งสองกลุ่มไม่ว่าจะได้รับ rbST หรือไม่

ภาควิชา สรีรวิทยา ลายมือชื่อนิสิต .....

สาขาวิชา สรีรวิทยาการสัตว์ ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ปีการศึกษา 2552 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

4875955631: MAJOR ANIMAL PHISIOLOGY
KEYWORD: MISTY-FAN COOLING / BOVINE SOMATOTROPIN / GLUCOSE METABOLISM / CROSSBRED HOLSTEIN CATTLE / MILK PRODUCTION

SIRAVIT SITPRIJA: THE EFFECTS OF RECOMBINANT BOVINE SOMATOTROPIN AND MISTY-FAN COOLING SYSTEM ON MILK PRODUCTION RELATING TO BODY AND MAMMARY GLAND GLUCOSE METABOLISM IN CROSSBRED HOLSTEIN CATTLE. THESIS ADVISOR: PROF. NARONGSAK CHAIYABUTR, D.V.M., Ph.D. THESIS CO-ADVISOR: PROF. SOMCHAI CHANPONGSANG, M.S., 125 pp.

Two groups of five crossbred 87.5% Holstein cattle each, were housed in normal shade only (NS) as non-cooled cows and in shaded with misty-fan cooling (MFC) as cooled cows. The cows were supplemented with recombinant bovine somatotropin (rbST) in early, mid and late lactation with three consecutive injections of rbST 500 mg of rbST (POSILAC) in every 14 days. During the study, ambient temperature at the hottest period daily (1400h) in the MFC barn was significantly lower, while relative humidity was higher than that of the NS barn. The temperature humidity index (THI) in both barns ranged from 77.8-85.5 throughout the periods of study. Cows under the MFC barn showed a lower rectal temperature and respiration rate as compared with cows in the NS barn. Milk yield significantly increased in cows treated with rbST in each stage of lactation. Increases in mammary blood flow and plasma level of IGF-I accompanied with increases in total body water (TBW), extracellular fluid (ECF), blood volume (BV) and plasma volume (PV) in both cooled and non-cooled cows receiving rbST in each stages of lactation. The mean arterial plasma concentrations for glucose, acetate, β-hydroxybutyrate and triacylglycerol were unchanged, while the mean arterial plasma concentrations of free fatty acid increased in both cooled and non-cooled cows supplemental rbST. The net mammary glucose and triacylglycerol uptakes of cows in both groups markedly increased in mid and late stages of lactation. Glucose turnover rates were not significant different between cooled and non-cooled cows whether supplemental rbST or not. The glucose taken up by the mammary gland of both non-cooled and cooled cows increased flux through the lactose synthesis and the pentose cycle pathway with significant increases in NADPH formation for fatty acid synthesis during rbST supplementation. The utilization of glucose carbon incorporation into milk appeared to increase in milk lactose and milk triacylglycerol of both cooled and non-cooled cows supplemental rbST during early and mid lactation but not for milk citrate as lactation advances. Milk yield of both cooled and non-cooled cows without rbST decreased as lactation advanced to late lactation. Local changes for biosynthetic capacity within the mammary gland would be a factor in identification of the utilization of substrates in the rate of decline in milk yield with advancing lactation in both cooled and non-cooled cows. The proportion of glucose would be metabolized less for lactose synthesis, but metabolized more via the Embden-Meyerhof pathway and the tricarboxylic acid cycle as lactation advances whether supplemental rbST or not.

Department: Veterinary Physiology Student's Signature ... Siravit Sitprija
Field of Study: Animal Physiology Advisor's Signature ... Narongsak Chaiyabutr
Academic Year: 2009 Co-advisor's Signature... Somchai Chanpongsang

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LIST OF ABBREVIATIONS

AT	Ambient temperature
ATP	Adenosine triphosphate
BHBA	Beta-hydroxybutyric acid
bST	Bovine somatotropin
BV	Blood volume
BW	Body weight
db	Dry bulb temperature
DM	Dry matter
DMI	Dry matter intake
ECF	Extracellular fluid
FCM	Fat corrected milk
FFA	Free fatty acid
GH	Growth hormone
HF	Holstein Friesian
IGFBP	Insulin like growth factor binding protein
IGF-I	Insulin like growth factor-I
MBF	Mammary blood flow
MF	Misty-fan cooling
MPF	Mammary plasma flow
MY	Milk yield
NADPH	Nicotinamide adenine dinucleotide phosphate

NS	Normal sheded barn
NSS	Normal saline solution
PCV	Packed cell volume
PV	Plasma volume
rbST	Recombinant bovine somatotropin
RH	Relative humidity
rpm	Round per minute
RR	Respiratory rates
RT	Rectal temperature
SEM	Standard error of the mean
TBW	Total body water
TDN	Total digestible nutrient
THI	Temperature humidity index
TMR	Total mixed ration
UDP	Uridine diphosphate

CHAPTER I

GENERAL INTRODUCTION

In the tropics, crossbreeding of indigenous and exotic cattle for tropical use has been exploited as an efficient tool for blending the adaptability of native cattle with the high milking potential of exotic breeds resulting in increased milk production (Chaiyabutr et al., 2007[a]). However, the low milk production of both exotic and crossbred cattle is still the major problem in dairy farm in tropical countries. The mechanisms that limit the rate of milk yield and shorter lactation persistency are unknown. Many factors affect milk production in dairy cattle in tropical areas including high environmental temperature and humidity (Kadzere et al., 2002; Mader et al.,2007), lower genetic potential for milk production in indigenous cattle and inadequate supply of food during the dry and hot summer season (Chaiyabutr et al., 2000[a]).

It is known that lactating dairy cows metabolize large amounts of water and are affected rapidly by water deprivation. An increase in water intake during lactation closely match to increase in water secreted in milk (Woodford et al., 1984), which milk composition has about 87% of water (Murphy, 1992). An alteration in bodily function during lactation is apparent; for example, blood volume (Chaiyabutr et al., 1997) and cardiac output (Hanwell and Peaker, 1977) are increased. These changes may effectively alter body fluid and thus circulatory distribution including the blood supply to the mammary gland. The lactating mammary gland receives signals from the rest of body in form of nutrient and hormones from blood. Mammary blood flow is thus a major parameter controlling milk production in a way to carry milk precursors to the mammary gland at the process of milk synthesis. A decrease in blood flow to the mammary gland with a short persistent milk yield during the transition period from early to mid lactation has been noted in the 87.5% HF animal (Chaiyabutr et al., 2000[d]). The control mechanism for mammary blood flow in different stages of lactation in crossbred dairy cattle has not been fully elucidated, although mammary blood flow has been known to be a major determinant for the rate of substrate supply for milk synthesis (Davis and Collier,

1985). Different between animals partitioning abilities are known to be inherited and are thought to be under endocrine control with a homeorrhetic principle in bovine lactation. Bovine growth hormone or somatotropin (bST) is a homeorrhetic hormone connected with growth and lactation, which is known to play a role between genetic potential and nutrition on milk production (Gulay and Hatipoglu 2005; Settivari et al., 2007). *Bos Taurus* animals normally have higher plasma bovine somatotropin during lactation. The importance of bST for maintaining milk output in ruminant is well established (Bauman, 1992). It has been reported that the concentration of plasma bovine somatotropin of 87.5% crossbred Holstein cattle decreased rapidly as lactation progressed to mid and late lactation which coincided with the decrease in mammary blood flow. These decreases could contribute to a reduction in milk yield (Chaiyabutr et al., 2000[a]). Many studies have demonstrated the efficacy of bST for improvement in milk yield (Breier et al., 1991; Burton et al., 1994). Long term exogenous recombinant bovine somatotropin (rbST) in 87.5% crossbred Holstein cattle increased in milk yield which accompanied with an increase in the rate of mammary blood flow, but the stimulant effect for milk yield was less in late lactation despite a high level of mammary blood flow (Chaiyabutr et al., 2007[a]). It is not known which factors are the cause and which factors are the effects for such a reduction and whether a high level of bST increases the metabolic rate (Tyrrell et al., 1988); as such an effect would make thermoregulation in a tropical environment more difficult as lactation advances. These changes were not apparent in crossbred dairy cattle containing 50% Holstein genes (Chaiyabutr et al., 2000[d]). Chaiyabutr and co-worker (2005) reported that long-term administrations of recombinant bovine somatotropin (rbST) showed a marked increase in mammary blood flow throughout lactation, but a short persistency of lactation in rbST treated animals was still similar to the control animals receiving placebo. 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 nutrients partitioning to the mammary gland for milk synthesis (Maksiri et al., 2005; Chaiyabutr et al., 2007[a]). The lack of effect of higher plasma IGF-I levels on persistency of lactation in rbST treated

animals was also noted (Chaiyabutr et al., 2005). During rbST administration in crossbred HF animals, no negative energy balance have been apparent during high peak yield in the early lactation, which differ from those of high yielding cows in temperate countries. Changes in milk production during the progress of lactation in rbST treated animals might not be controlled systematically but also locally within the mammary gland (Chaiyabutr et al., 2005). Therefore, the interaction of genetics and environment in the determination for response of lactating dairy cows to bST administration is not yet clear.

High temperature is another factor which limits milk production of dairy cows. Cows exposure to high temperature will develop heat stress. Heat stress is a multifaceted adaptive response that occurs when an animal's capacity for heat dissipation is exceeded by the heat load. The adaptive responses have important physiological consequences in terms of greatly increased body temperatures and impaired physiological functions including mammary function (Her et al., 1988; McGuire et al., 1989; Lough et al., 1990).

Milk secretion is a continuous process and requires a continuous supply of substrates. It has long been well recognized that the stress of high temperatures lowered productive efficiency in dairy cattle both directly and indirectly. Many studies have been done in attempting to improve dairy productivity by management strategies. Management can minimize the adverse effects of heat stress in cattle. Environmental management is one of these strategies, which can diminish severe heat stress in cattle, for example water spray with fans or evaporative cooling system. Selecting the types of temperature controlling systems and selecting the type of crossbred cattle which are suitable for tropical countries are still further investigated. However, there is less information concerning the profitability of efficient utilization of environmental modification for dairy production in crossbred cattle, although the performance of crossbred animal has been found to differ from pure breeds both in body composition and water turnover rate (Macfarlane and Howard, 1970). Water turnover values in ruminants have been shown to be related to the food and water intake and metabolism of animal (Murphy, 1992)

including exposure to high ambient temperature (Chaiyabutr et al., 1987). Thus, the initial work of this thesis was concerned with determining the effectiveness of misty-fan cooling and supplemental rbST on body fluids, mammary blood flow, milk production and nutrients uptake by the mammary gland in different stages of lactation of crossbred Holstein cattle (Chapter IV).

An increase in production, especially to milk production, necessitates a substantial increase in the glucose requirement of the animal. Glucose is an important intermediary substrate of metabolism in general and is particularly important for lactation. Glucose is utilized by the mammary gland for the biosynthesis of lactose, triacylglycerol and citrate. This has been studies in lactating ruminant in vivo and in the isolated perfuse udder. The role of glucose in regulating milk secretion has been formulated in the theory that lactose secretion can draw water osmotically from the inside of mammary cell to milk (Linzell and Peaker, 1971). This is believed to be a mechanism for increasing milk yield by which bulk water movement occurs into milk. Metabolism of glucose in mammary gland is also important in providing the reducing equivalents required for the de novo synthesis of fatty acids.

The supply of glucose is a principle determinant of the milk yield response to growth hormone. It has been reported that the whole body utilization of glucose is increased and whole body oxidation of glucose is decreased during bovine somatotropin treatment (Bauman et al., 1988). The glucose utilization for biosynthetic pathways in mammary gland of 50% HF animals was maintained in a similar pattern throughout the previous of lactation, while a short persistency of lactation in 87.5% HF animals has been shown to related to a decrease in a lactose biosynthetic pathways (Chaiyabutr et al., 2000[b]). Glucose metabolism in the udder has been reported to be metabolized less for lactose synthesis and the pentose phosphate pathway but metabolized more via the glycolysis pathway as lactation advances during rbST administration in 87.5% HF cows (Chaiyabutr et al., 2000[c]). In crossbred cattle, mechanisms of milk secretion are known to be inherited and are thought to be among the causes of differences in metabolic

parameters. No data are available concerning intra-mammary factors for the utilization of glucose and glucose metabolism in the udders of crossbred Holstein dairy cattle keeping under high environmental temperature. Short persistency of lactation is occurred in 87.5% HF cows, whether by the effect of high ambient temperature or by the less stimulant effect of bovine somatotropin or combination of both of these factors during lactation advances. The effect of rbST administration on an increase in total body water of crossbred Holstein cattle has been noted (Chaiyabutr et al., 2007[a]), which may involve in heat dissipation mechanism. A greater water reserve would not only provide a greater reservoir of soluble metabolites for biosynthesis for milk, but it is useful in slowing down the elevation in body temperature during heat stress. The studies of body glucose metabolism and the utilization of glucose by the mammary gland and milk yields of cows during rbST supplementation under high ambient temperature of crossbred Holstein cattle in the tropics were clarified in Chapter V.

Changes in endocrine function are known to cause of decline in milk yield in a hot environment. Both reduction of environmental temperature and bST administration may have beneficial effects on milk production. However, it does not appear to be the factors responsible for the increase in milk yield as direct effects on the metabolism in the mammary gland have been reported (Chaiyabutr et al., 2008[b]). It is known that in some tissue, the effects of somatrotopin are mediated through the action of IGF-1. Concentrations of IGF-1 increase in responsible to bovine somatrotopin treatment in cows and goats (Prosser et al., 1991; Maksiri et al., 2005, Chaiyabutr et al., 2005). These results have suggested that the effects of bovine somatrotopin on mammary gland are mediated via IGF-I. No report has shown the combined effects of reduction of environmental temperature and bST administration on milk secretion in responsible for the short persistency of milk yield as lactation advance in crossbred Holstein cattle. If changes in the utilization of glucose within the mammary gland are important in the mechanism of action of bovine somatotropin in stimulating milk secretion and if locally action of IGF-I is relevant to this process, the alteration of plasma hormone levels of insulin like growth factor-I including insulin and plasma metabolites would be carried out

by the effect of recombinant bovine somatotropin (rbST) administration in different stages of lactation of crossbred 87.5% HF under misty-fan cooling system (Chapter VI). The results of Chapter IV-VI involving extra-mammary factors and intra-mammary factors relating to body glucose metabolism and utilization of glucose by the mammary gland during bST administration in 87.5% HF animals under misty-fan cooling system will be discussed in the general discussion (Chapter VII). The methodology used in this thesis 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.

The effect of hot environment on milk production

Lactating dairy cows exposed to high ambient temperature, often coupled with high relative humidity (RH) or radiant energy (direct sunlight) usually respond with reduced milk yield. In Israel, the rectal temperature of cows increased during the day as ambient temperature increased to its maximum; milk yield declined with heat stress, but the effect was alleviated when cows were cooled (Her et al., 1988). Early Missouri work (Johnson et al., 1963) showed that cows consumed less feed as ambient temperature and combined ambient temperature and RH were increased, and that cows exposed to high ambient temperatures and low RH had greater intake than cows exposed to similar ambient temperatures but with high RH. In addition, they reported that milk yield and total digestible nutrient (TDN) intake declined as rectal temperature increased (Johnson et al., 1963). Performance of cows is diminished by hot weather, and effects appear to be mediated through elevated body temperature.

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 produced 27.3 and 48.5% more heat, respectively, than dry cows (Purwanto et al., 1990). Cows in hot climates generally produce additional heat relative to cool climates because of the greater physical activity (such as panting) necessary to enhance cooling in hot conditions (Robinson et al., 1986). Cows administered bST under hot conditions has been shown to produce about 25% more heat than cows not receiving bST. The greater heat

production would be associated with an approximate 49% increase in milk energy secretion, and increased heat production would be balanced with greater heat dissipation (Johnson et al., 1991). It is apparent that continued improvements in genetics and management techniques will continue to improve feed intake and milk yield for the dairy cow, contributing to greater metabolic heat production (Manalu et al., 1991).

Environmental temperature, radiant energy, relative humidity, and wind speed contribute to the degree of heat stress or cooling that occurs for the cow. Temperature-humidity index (THI) incorporates the effects of both ambient temperature and RH in an index (NOAA, 1976), which used for determination the level of heat stress. Mader et al. (2007) have proposed that a level of heat stress is quantified by different THI value (normal < 74; alert 74-79; danger 79-84; emergency > 84). Classical work at Missouri (Kibler and Brody, 1953) demonstrated that as ambient temperature increased in the presence of low or high RH, cooling mechanisms shifted from non-evaporative processes (convective, conductive, and radiation) to evaporative (sweating and panting). Combined effects of environmental stressors may be more critical to cow comfort and performance than single measures such as ambient temperature. Holter et al. (1996) reported that minimum THI was more closely correlated with dry matter intake (DMI) than maximum THI, and DMI depressions commenced when minimum THI was in the range of 56 to 59 and maximum THI was in the range of 71 to 73. Multiparous cows have been shown to be more drastically affected than primiparous cows (Holter et al., 1997). Linvill and Pardue, (1992) demonstrated that the correlation coefficient for a stepwise regression analysis in summation of THI values above 74 for the preceding 4 d relating to milk yield was 0.42 in South Carolina. An understanding of the interaction of various environmental factors with lactational performance is necessary so that management techniques and cooling practices can be developed to meet the needs of the high producing cow subject to the effects of hot and humid conditions.

Physiological effects of heat stress on milk production

Numerous physiologic changes occur in the digestive system, acid-base chemistry, and blood hormones during hot weather; some in response to reduce nutrient intake, but many changes occur as a result of strain in the cow. Neurons that are temperature sensitive are located throughout the animal's body and send information to the hypothalamus, which invokes numerous physiological, anatomical or behavioral changes in the attempt to maintain heat balance (Curtis, 1983). During heat stress cows exhibit reduced dry matter intake, decreased milk production, reproduction performance and activity, seek shade and wind, increase respiratory rate, increase peripheral blood flow, water intake and sweating. These responses have a deleterious effect on both production and physiologic status of the cow (Chaiyabutr et al., 2000[d]; Kadzere et al., 2002; Abeni et al., 2007).

Cows that were fed ad libitum in a thermal comfort environment, fed ad libitum in a thermal stress environment, or fed a restricted intake in a thermal comfort environment had similar milk yields for both restricted intake and thermal stress treatments, and mammary blood flow tended to be lower compared with ad libitum fed cows in thermal comfort, suggesting blood flow was responsive to level of DMI (Lough et al., 1990). For cows exposed to similar treatments as those of Lough et al. (1990), portal plasma flow was reduced about 14% for cows in thermal comfort with restricted intake or in thermal stress when compared with thermal comfort, ad libitum fed cows (McGuire et al., 1989). Many studies concluded that a portion of the negative effects of heat stress on milk production could be explained by decreased nutrient intake and decreased nutrient uptake by the portal drained viscera of the cow. Blood flow shifted to peripheral tissues for cooling purposes may alter nutrient metabolism and contribute to lower milk yield during hot weather.

Physical modification of the environment for reduce the effect of heat stress

Although shade reduces heat accumulation from solar radiation there is no effect on air temperature or relative humidity and additional cooling is necessary for lactating dairy cows in a hot, humid climate. Beede and Collier (1986) have proposed that physical modification of the environment, genetic development and improved nutritional management are three management strategies to reduced thermal stress of the environment. The physical modification of the environment is a simple management strategies for minimized the adverse effects of heat stress in dairy cows such as fan, cooling pad system and sprinkler or mister fan cooling system (Strickland et al., 1988; Huber et al., 1994; Chan et al., 1997; Lin et al., 1998). A number of cooling options exist for lactating dairy cows based on combinations of the principles of convection, conduction, radiation, and evaporation. Air movement (fans), wetting the cow, evaporation to cool the air, and shade to minimize transfer of solar radiation are used to enhance heat dissipation. Any cooling system that is to be effective must take into consideration the intense solar radiation, high ambient temperature, and the typically high daytime relative humidity, which increases to almost saturation at night (Armstrong, 1994).

Sprinkler and fan cooling systems generate a large volume of waste water which must be processed. The cooling system used by Strickland et al. (1988) used 454.2 L/cow per d, which totaled 54,504 L/cow for a 120 d cooling season. However when differing rates of water application for cooling were compared, a system using 313.4 L/h (215.9 L/cow per d) cooled cows as well as a system delivering 704.1 L/h (Means et al., 1992). Large droplets from a low-pressure sprinkler system that completely wet the cow by soaking through the hair coat to the skin were more effective than a misting system (Armstrong, 1994). A combination of misters and fans was as effective as sprinklers and fans, which intake and milk yield were similar for the misted cows (Lin et al., 1998). The fan/sprinkler system used about 10 fold more water than the fan/mist system. Thus attention to water delivery rate through nozzle size or the use of fans and misters has

proven effective in cooling cows while using substantially less water than systems evaluated in earlier research.

Evaporative cooling systems use high pressure, fine mist and large volumes of air to evaporate moisture and cool the air surrounding the cow. Because of the evaporation there is little wastewater to process in this type of cooling system, which is beneficial when developing a water budget for the dairy farm. Evaporative cooling systems improve the environment for lactating dairy cows in arid climates, and the reduced air temperature results from the removal of heat energy required to evaporate water (Takamitsu et al., 1987; Ryan et al., 1992). Evaporative cooling can be accomplished by passing air over a water surface, passing air through a wetted pad, or by atomizing or misting water into the air stream. There are questions regarding the effectiveness of evaporative systems in climates with high relative humidity. Evaporative cooling pads was an effective reduction in air temperature of the barn but milk yield was not altered although rectal temperature and respiratory rate were reduced (Taylor et al., 1986). Evaporative cooled cows have lower RT and RR than those of the non cooled cows (Armstrong et al., 1993). The increased of DMI for evaporative cooled cows have shown to be associated with higher milk yield (2.5 kg/d increase) in comparison with shaded cows (Chen et al., 1993). Evaporative cooling with sprinklers and fans effectively reduced heat stress as indicated by lower RT and RR (Gallardo et al., 2005) including increase in 4% FCM (0.9 kg/d) production (Fike et al., 2002).

Somatotropin hormone

Somatotropin is a protein hormone synthesized by the anterior pituitary gland. Secretion from the pituitary gland is regulated by two peptides: growth hormone-releasing factor, which stimulates release, and somatostatin, which inhibits release (Tuggle and Trenkle, 1996). The amino acid sequence for somatotropin is known for many species (Wallis, 1975). Bovine ST produced by the pituitary can have either a 191 or 190 amino acid sequence with either a leucine or valine at position 127 (Wood et al.,

1989; numbering based on the 191 amino acid variant). These represent the four major variants of bST that are produced naturally. Differences in the cleavage of the signal peptide cause the N-terminus to be an alanine (191 amino acid sequence) or a phenylalanine (190 amino acid sequence). Variation between valine or leucine at position 127 is due to differences in gene alleles, and the frequency of these alleles varies for the major dairy breeds (Lucy et al., 1991). Recombinantly derived forms of bST that have been used experimentally can differ slightly from the bST produced by the pituitary gland. Depending on the manufacturing process, from 0 to 8 extra amino acids are attached to the N-terminus of the bST molecule (Juskevish and Guyer, 1990). However, when the same purification techniques are used, recombinantly derived and pituitary-derived bST have similar potencies in various biological test systems (Langley et al., 1987; Wood et al., 1989).

Effect of bovine somatotropin on milk production

Bovine somatotropin (bST) is known as a homeorrhetic hormone connected with both growth and lactation. Milk-yield responses to bST have been reported in all dairy breeds. Milk yield gradually increases over the first few days of bST treatment and reaches a maximum during the first week. If treatment is terminated, milk yield gradually returns to pretreatment levels over a similar time period. However, when treatment is continued, the increased milk yield is maintained. Thus, bST results in a greater peak milk yield and an increased persistency in yield over the lactation cycle (Johnsson and Hart, 1986; Peel and Bauman, 1987). Milk-yield increases after bST treatment are observed in cows of all parities, but the magnitude of the increase in milk yield varies according to stage of lactation (Peel and Bauman, 1987; McBride et al., 1988; Chilliard, 1989). In general, response has been small or negligible when bST is administered in early lactation prior to peak yield. Although a number of reviews have been published on the relationship between the plasma bST concentration and milk yield in both normal and hot environments (West et al., 1991). There are few studies on the mechanisms acting within the body of crossbred cattle concerning the role of bST on water metabolism, in

relation to persistent lactation. Long-term treatment with rbST at different stages lactation shows the effect of rbST on exerting its galactopoietic action through expansion of body fluids in association with an increase in mammary blood flow for milk production (Chaiyabutr et al., 2007[a]). It has been demonstrated that receptors for bST are not apparent on secretory epithelial cells of mammary tissue (Akers, 1985). The mechanism of action of bST on milk production is still a controversial area. The effects of bST on milk production are thought to be indirectly mediated via nutrient partitioning effects or via insulin like growth factor-I (IGF-I) (Bauman, 1992). Some studies support this role. Infusion of IGF-I into the pudic artery of lactating goats has been shown to increase blood flow and milk production on the infused side (Prosser et al., 1990; Prosser et al., 1994), whereas infusion of bST into the mammary artery of sheep did not increase milk yield (Peel and Bauman, 1987). Several other reports, refuting the role of IGF-I as mediators of bST action, have been published (Barber et al., 1992; Flint et al., 1992;Plaut et al., 1993). It has been reported that bST can stimulate milk production under circumstances in which IGF-I does not (Prosser and Davis, 1992). Chaiyabutr et al. (2000[b]) reported that the galactopoietic effect of bST is not associated with the plasma level of IGF-I as lactation advances in 87.5% HF animals. The plasma level of IGF-I has been shown to remain at the same level as lactation advances, despite declining circulating bST, mammary blood flow and milk yield (Chaiyabutr et al. 2004). These data did not support a role for IGF-I in mediating the action of bST on milk production. However, an increase in plasma IGF-I level, with a concomitant increase in both mammary blood flow and milk yield in late lactation, was seen after exogenous administration of rbST in 87.5% HF animals (Tunwattana et al., 2003). Despite a number of studies looking at these differences, there have been few observations about the mechanism of short persistency of lactation in 87.5% HF dairy cattle. Changes in milk production during the progress of lactation in longed-term rbST treated animal have been shown not to be controlled systematically but also locally within the mammary gland. The lack of effect of higher plasma IGF-I levels on persistency of lactation in rbST

treated animals, may be due to changes in the pattern of IGF-I binding proteins and paracrine production inhibiting IGF-I action. (Chaiyabutr et al., 2005).

Effect of bovine somatotropin on glucose metabolism

Glucose is utilized by the mammary gland for the biosynthesis of lactose, triacylglycerol and citrate. This has been studied in lactating ruminants in vivo and in the isolated perfused udder. The role of glucose in regulating milk secretion has been formulated in the theory that lactose secretion can draw water osmotically from the inside of the mammary cells to milk (Linzell and Peaker, 1971). This is believed to be a mechanism for increasing milk yield by which bulk water movement occurs into milk. Metabolism of glucose in mammary glands is also important in providing the reducing equivalents required for the de novo synthesis of fatty acids.

The supply of glucose is a principal determinant of milk yield, since glucose requirement is used for lactose production. The administration of rbST elicited a marked increase in the milk production of crossbred dairy cattle. Elevated responses did not maintain for the duration of the treatment period in rbST treated animals (Bauman, 1992), and that it is influenced by the stage of lactation (Phipps et al., 1991). The low potential for extended persistency of lactation in rbST treated animals appears similar to that which occurs in higher yielding cows (Chase, 1993). However, it has been reported that the whole lactational response to somatotropin might be reduced if treatmet begins very early in lactation (Bauman and Vernon, 1993; Burton et al., 1994). A marked increase in milk yield without an alteration in lactose content during early lactation in rbST treated animals indicates that this requires a substantial increase in supply of glucose to the mammary gland (Bauman and McCutcheon, 1986). Glucose is essential for milk secretion and glucose moiety of lactose arises directly from plasma glucose (Ebner and Schanbacher, 1974). The milk secretion of animals was not dependent on the blood glucose level, since the plasma glucose concentration remained constant over a wide range at different stage of lactation. The marked increase in the mammary blood flow of rbST treated animals (Chaiyabutr et al., 2005) which support a previous conclusion from

a study in cows or goats by Linzell (1973) that glucose uptake is determined mainly by mammary blood flow. An increase in milk yield during bST administration is thought to be determined primarily by lactose secretion (Linzell and Peaker , 1971). Lactose is synthesized in the mammary secretory cell from glucose derived from the blood. The concentration of milk glucose significantly increased which coincided with an increase in milk yield during rbST administration in both early and mid-lactation. This would reflect to the intracellular glucose concentration (Kuhn and White, 1975; Faulkner et al., 1981), since glucose freely permeates across Golgi vesicles and apical membranes of the mammary secretory cells (Faulkner and Peaker, 1987). Mammary cell cannot synthesize free glucose because they lack glucose-6-phosphatase activity (Threadgold and Kuhn, 1979). It is likely that the high concentrations of milk glucose in rbST-treated animals are related to a high rate of glucose uptake by the mammary gland (Chaiyabutr et al., 2008[b]), consistent with the higher mammary blood flow to the mammary gland during rbST administration (Chaiyabutr et al., 2007[b]).

CHAPTER III

MATERIALS AND METHODS

Animals, housing and managements

The experiments were conducted on crossbred cattle containing 87.5% gene of Holstein Friesian. Ten, pregnant heifer crossbred 87.5% Holstein cattle, were selected for the experiment. Animals were randomly divided into two groups of five animals each. During the experimental period, cows in both groups were housed in open-sided with a tiled-roof and tie-stall barn. The barn(16 m long x 7 m wide x 3.5 high) was devided into two parts by metal sheet devision (3.5 m high). Animals in the control group were housed in the first part (8 m long x 7 m wide x 3.5 m high) of normal shade (NS) in individual stall, while animals in the experimental group were housed in shade with using misty-fan cooling system (MF) (Masterkool, Thailand) to reduce the environmental temperature. The MF barn had two sets of misters and fans cooling system, which each system consisted of a 26 inch diameter blade fan circulating 7,200 ft^3/min of air, with oscillation coverage of 180°. The amount of water discharged from 4 spray heads was 7.5 L/h and side of mist droplet 0.01 mm. Animal were exposed to MF for 45 minutes at 15-minute intervals from 0600h to 1800h. At night, animals were exposed to MF for 15 minutes at 45-minute intervals from 1800h to 0600h.

Animals in each group were fed with the total mixed ration (TMR) which was formulated according to NRC requirement (NRC, 2001) for 10-15 kg milk producing cows. The TMR died and ingredients are shown in Table 3.1. Each day, the diet was given at 110% of *ad libitum* consumption at about 0600h and 1700h throughout the experimental period. Water was available at all times. Dry matter intake (DMI) of each cows was measured daily by weighing the TMR offered and subtracting that refused. All animals were normally milked at about 0600 and 1700h using a milking machine and milk production was recorded daily. Milk sample were collected from morning milking.

The 60 mL. of milk sample was preserved with 300 µL. bronopol (2-Brom-2-nitro-1,3-propandiol)(0.02w/w) and kept at 4ºC for determination of milk compositions. Rectal temperature and respiration rate of individual animals were determined at the same time as recording ambient temperature and humidity. The body weight (BW) of animals were recorded by weighing monthly throughout the experiment.

Table 3.1. Compositions of the total mixed ration (TMR) diet.

Ingredients	Kg (as fed basis)
Pine apple waste	50.0
Soybean meal	23.0
Cotton seed meal	20.0
Rice bran	3.0
Lime stone	1.4
Di-calcium phosphate	1.4
Sodium bicarbonate	0.3
Potassium chloride	0.1
Mineral and vitamin premix	0.8
Total	100.0
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/kgDM)	2.7

Ambient temperature of NS and MF barns were recorded using a wet and dry bulb thermometer. The relative humidity of NS and MF barns were read by psychometric chart depending on wet and dry bulb temperature. Ambient temperature and humidity were measured weekly throughout the experiment. Average values were considered to be the mean values of all measurements taken throughout the study period. The temperature humidity index (THI) was calculated from the average dry bulb temperature and relative humidity according to West (1994), as follow:

$$THI = db - (0.55-0.55RH) (db -58)$$

Where : db = dry bulb temperature (°F), and RH = relative humidity.

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

Experimental procedures

The experiments were carried out throughout lactating period in each group. The experiment in each group was divided into 3 stages, namely early- (Days 65-95 postpartum), mid- (Days 125-155 postpartum), and late lactating stages (Days 185-215 postpartum). The pretreatment study was conducted on the starting day of each lactating stage. At the end of the pretreatment, within the same day, the animal was injected with the first dose subcutaneous injection of 500 mg of recombinant bovine somatotropin (rbST) (POSILAC, Monsanto, USA). Subsequently, the animal was injected with two consecutive doses injections of rbST every 2 weeks. Thereafter, within 2 days after the third injection, the treatment study was conducted. The pretreatment, 3 doses of injections, and the treatment periods were performed during the first 30 days and the same procedures were followed for each stage (Figure 3.1). During the last 30 days of

each lactating stage, no experiments were conducted in order to allow the milk yield from the effect of rbST treatment to return to the control level (Kirchgessner et al., 1991).

On each specified day of study, measurements of mammary blood flow, glucose metabolism and the utilization of glucose by the mammary gland were carried out. At around 10.00 h. both ear vein and milk vein were catheterized with the non-radiopaque intravenous catheter, gauge 18G (Surflo, Terumo Europe N.V., Belgium) under local anesthesia for infusion of solution. An arterial blood sample was collected from the coccygeal artery by venipuncture with a # 21 needle into heparinized tube. Blood samples from arterial and mammary venous blood in heparinized tube were kept in crushed ice and then centrifuge at 3000 rpm for 30 min at 4 ºC. Plasma samples were collected and frozen at -40°C in aliquots until time of assays for measurements the concentration of metabolites.

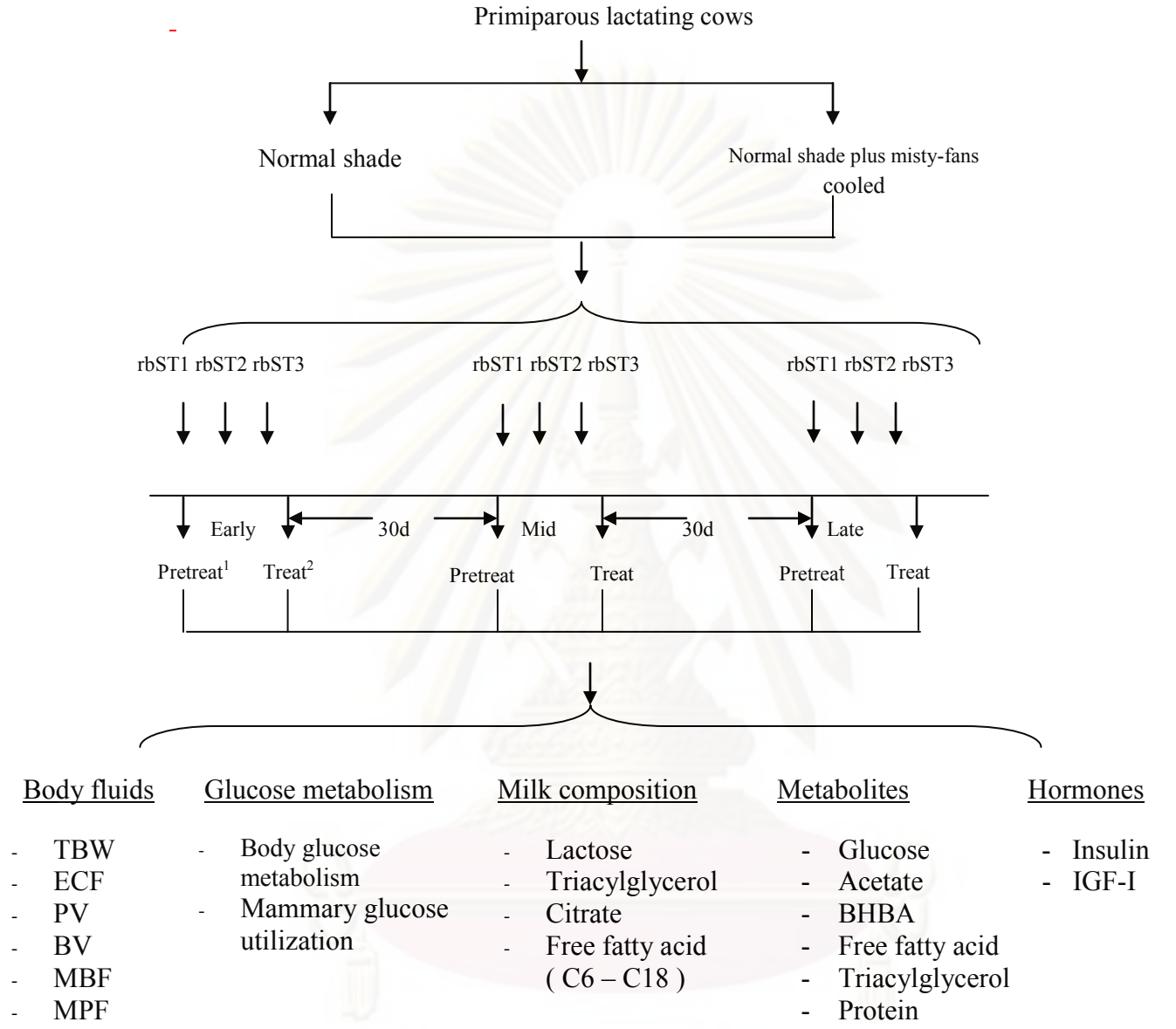


Figure 3.1. Schematic diagrams illustrating the time course of the experiment in each cow supplemented with rbST at different stage of lactation.

Pre-treat[1] = Timed study for pre-treatment ; Treat[2] = Timed study for treatment.

Body fluid measurements

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

Glucose turnover measurements

The study on glucose kinetic and efficiency of glucose utilization by the mammary gland during pretreatment and treatment periods with rbST was performed at different stages of lactation: early, mid and late lactation by using both [U-^{14}C]-glucose and [3-^{3}H]-glucose infusion in animals, of both groups of animals. Glucose kinetic studies of each animal in each lactating stage were carried out as described previously by Chaiyabutr et al. (1998). Briefly, at about 10.00h of the specified day, a priming dose of radioactive glucose in 20 ml of sterile NSS containing 30 μCi [3-^{3}H]-glucose and 15 μCi[U-^{14}C]-glucose was administered intravenously via the ear vein catheter and followed by a continuous infusion of 1 ml/min of NSS (0.9%) containing 0.7 μCi/ml of [U-^{14}C]-glucose and 1.5 μCi/ml of [3-^{3}H]-glucose for 3 h. (Peristatic pump; EYLA Model 3). During the last 1 hour (1200-1300h) of continuous infusion three sets of blood

samples were collected at 20 min intervals. A venous blood sample was collected from the milk vein via a catheter while an arterial blood sample was collected from the coccygeal vessel by venipuncture with a # 21 needle. Blood samples in heparinized tubes were kept in crushed ice for chemical studies. Milk secretion was recorded for the last 1 hour of continuous infusion. Milk samples were used for measurement of radioactive glucose incorporation into other milk components. Milk yield was recorded by weight.

Mammary blood flow measurement

The measurement of the mammary blood flow through half of the udder was performed at around 1100-1200h on the specified day. The duplicated measurements were done by measuring the dilution of dye T-1824 (Evans blue) by a short term continuous infusion as described by Chaiyabutr et al. (1997). In brief, a dye (T-1824) was dissolved in sterile normal saline and diluted to a concentration of 100 mg/L. The solution was infused by a peristaltic pump (Gilson Medical Electronics) at a constant rate of 100 ml/min into the milk vein for 1 min. which could produce adequate mixing of dye with blood. Before infusion, blood was drawn from downstream in the milk vein as a pre-infusion sample. About 10 second after starting the infusion, 10 ml of blood was drawn from downstream in the milk vein at a constant rate into a heparinized tubes. Two consecutive plasma samples were taken during each dye infusion at about 5 min interval. Blood flow of half of the udder was calculated from plasma samples and the value of packed cell volume using the equation derived by Thompson and Thomsom (1977). Quarter milking showed that the yields of the two halves of the udder were similar. Udder blood flow was therefore calculated by doubling the flow measured in one milk vein (Bickerstaffe et al., 1974). Packed cell volume was measured after centrifugation of the blood in a microcapillary tube.

Chemical methods

Radiochemicals for [U-^{14}C]-glucose and [3-^{3}H]-glucose were obtained from the Radiochemical Center, Amersham Bucks, UK. The specific activity of labeled plasma glucose was determined by the method described by Chaiyabutr and Buranakarl (1989).

The plasma glucose concentration was measured using enzymatic oxidation in the presence of glucose oxidase (Human GmBH, Germany). Plasma free fatty acid were determined by colorimetry after plasma extraction with chloroform, heptane and methanol and TAN solution (Wang et al., 2004). Plasma triacylglycerol concentration was determined by enzymatic colorimetric test (Triglyceride liquicolor, Wiesbaden, Germany).

The concentration of milk lactose was determined by spectrophotometry (Teles et al., 1978). Lactose radioactivity was determined after isolation by the hydrolysis method (Wood et al., 1965).

Milk fatty acids was extracted from 1 ml of an aliquot thawed milk in 2 ml of Dole's solution (Dole, 1956), (*iso*-propanol 40: *n*-heptane 10: 1N H_2SO_4 1, v/v) shaking in water bath for 30 min. After 1 ml hexane and 1ml H_2O was added to the vial and shaking, the upper layer containing fatty acids was transferred into two vials for radioactivity assay and determination of milk fatty acids concentration. Milk extraction solution in counting vial with a scintillation cocktail was measured radioactivity of ^{14}C and ^{3}H-fat by liquid scintillation counter (Liquid scintillation analyzer, Tricarb 2300 TR, Packard instrument Co.Inc.USA.). Other portion of milk extraction was used to determine milk fatty acids concentration by colorimetry according to Wang et al. (2004) using chloroform, heptane and methanol and TAN solution. Milk fatty profiles was determined by gas chromatography (GC-2010 Gas Chromatograph, Shimazu) after extraction by chloroform and methanol (Christopherson and Glass, 1969) in comparison with the appropriate internal standard of pentadecanoic acid (C15:0).

The concentration of milk citrate was determined by spectophotometry from tricarboxylic acid filtrate (White and Davies, 1963). Citrate radioactivity was determined after isolation by anion exchange chromatography (Hardwick et al., 1963).

Determinations of plasma hormone concentration

Plasma samples were collected from coccygeal vessel for determinations of hormonal concentrations of IGF-I and insulin. The plasma IGF-I concentration was determined by Chemiluminescence immunoassay using an IMMULITE® Analyzer (IMMULITE IGF-1, Diagnostic Products Corporation, Los Angeles, CA). The plasma insulin concentration was quantified for bovine insulin using ELISA technique (Mercodia Bovine Insulin, Mercodia AB, Sylveninsgatan 8, Uppsala, Sweden).

Calculations

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

$$T = I/G_A$$

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

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

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

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

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

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

where T_3 = reversible turnover of glucose calculated from 3-^{3}H glucose (μmol/min) and

P_{AG} = arterial plasma glucose concentration (μmol/mL).

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

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

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

Milk components output (MO), expressed as μmol/min, was calculated from the equation:

$$MO = Ms \times Cc/1000$$

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

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

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

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

G_A = specific activity of ^{14}C- or ^3H-glucose in arterial plasma at equilibrium (μCi/μmol) and t = time of infusion (min).

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

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

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

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

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

$$G_6^P = U_G - L$$

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

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

$$B = L$$

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

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

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

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

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

$^{14}CO_2$ released from [1-^{14}C]glucose via the pentose phosphate pathway (Katz *et al*., 1974). Metabolism of glucose via PC was therefore calculated from the equation:

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

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

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

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

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

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

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

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

$^{3}H/^{14}C$ lactose = $^{3}H/^{14}C$ in milk lactose relative to $^{3}H/^{14}C$ ratio of 1 in the infusion, $^{3}H/^{14}C$ galactose = 2($^{3}H/^{14}C$ lactose) - ($^{3}H/^{14}C$ glucose),

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

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

Statistical analysis

Data for BW, milk yield, and DMI in each lactating period were adjusted for covariate effects using mean value of 14 d before start of experimental period. The statistic analyses were performed using General Linear Model procedure of statistical

software package SPSS (SPSS for windows, V14.0; SPSS Inc., Chicago, IL, USA). The model used for each analysis was:

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

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

Other data were also analyzed by the similar model, but the covariate effect was excluded. Mean 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 recombinant bovine somatotropin (rbST) administration on body fluid, mammary blood flow, and nutrients uptake by the mammary gland in different stages of lactation of crossbred Holstein cattle under misty-fan cooling (MF) system.

Introduction

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

has not been fully elucidated, although mammary blood flow has been known to be a major determinant for the rate of substrate supply for milk synthesis (Davis and Collier, 1985).

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

Materials and Methods

Ten pregnant, crossbred 87.5% Holstein heifer were selected for the experiment. Cows were randomly divided into 2 groups as control (n=5) and experimental groups (n=5). Animals in the control group were housed in the normal shade barn (NS), while cows in the experimental group were housed in normal shade plus misty-fan cooling system (MF). Cows in both groups were housed in tie stall barns and offered a TMR twice a day in equal portion, around 06.00 and 17.00 throughout the experiment. Each day, the diets were given when cows were milked. Water is available at all time. All animals were weighed monthly throughout the experiment.

Each cow in the control group was performed by the pretreatment study without rbST (NS), and the treatment study with rbST (NS + rbST). In the experimental group, cows in shade plus MF without rbST injection (MF) and treatment with rbST injection (MF + rbST). The pretreatment periods of both groups were performed on days 60, 120, and 180 of early, mid, and late lactation, respectively. The detail for rbST administration procedures in each stage of lactation were described in Chapter III. Rectal temperature and respiration rate of individual cow were determined at the same time as recording ambient temperature. Ambient temperature and humidity were measured weekly throughout the experiment. The temperature humidity index (THI) was calculated according to West (1994). On each specified day of each lactating period, at around 9.00 h, milk vein and ear vein were catheterized with non-radiopaque intravenous catheters, gauge 18G (Surflo, Terumo Europe N.V., Belgium) under local anesthesia for measurements of mammary blood flow through half of the udder and body fluids, respectively as described in Chapter III. Blood samples were collected from the coccygeal artery and milk vein by venipuncture with a # 21 needle into heparinized tubes. Blood samples were kept in crushed ice and then centrifuge at 3000 rpm for 30 min at 4 ºC. Arterial and venous plasma samples were collected and frozen at -20 ºC in aliquots until time of assays for measurements the level of metabolites.

Both coccygeal arterial (A) and milk vein plasma (V) samples were determined for the plasma glucose concentration which was measured by using enzymatic oxidation in the presence of glucose oxidase (Human GmBH, Germany). The plasma concentration of acetate was assayed by the acetic acid UV-method (R-Biopharm, Darmstadt, Germany). Plasma β-hydroxybutyrate concentrations was assayed by using an enzymatic reaction in the presence of β-hydroxybutyrate dehydrogenase (R-Biopharm, Darmstadt, Germany). Plasma free fatty acids were determined by colorimetry after plasma extraction with chloroform, heptane and methanol and TAN solution (Wang et al.,2004). Plasma triacylglycerol concentration was determined by enzymatic colorimetric test (Triglyceride liquicolor, Wiesbaden, Germany). Mammary uptake of metabolites and extraction of metabolites by the mammary gland were calculated as follows; Mammary uptake = mammary plasma flow×arteriovenous differences (A-V); Mammary extraction = (A-V)/A.

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

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

Mean values of measurements at experimental site during periods of studies for daily temperatures, humidities, THI, the rectal temperature and respiratory rate are shown in Table 4.1. Average values of ambient temperature in the barn during the daytime (1400 hours) at NS were significantly higher than that of MF. The relative humidity in MF was significantly higher than that of NS barn. THI values at the MF barn were significantly lower in comparison with NS barn. Cows in both groups exposed to high THI values (80.7 to 85.5) in both barns. Rectal temperature of cooled and non-cooled cows were

significant different whether rbST injection or not. The cooled cows showed lower rectal temperature than non-cooled cows during afternoon (1400 hours). There were significant increases in rectal temperature and respiration rate by the effect of supplemental rbST during the daytime. The cooled cow showed significantly lower respiratory rate than those of non-cooled cows throughout experimental periods.

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

The supplementation of rbST markedly increased both the absolute values and relative values as percentage of body weight of TBW, ECF, PV and BV in each stage of lactation (Table 4.2). The cooling system did not affect TBW, ECF, PV and BV in absolute value or relative value as percentage of body weight. The packed cell volume was not influenced by the supplementation of rbST in both cooled and non-cooled cows.

Change in milk yield, mammary blood flow and body weight

The milk yield, rate of mammary blood flow, plasma flow and body weight in cooled and non-cooled cows are shown in Table 4.3. It is obvious that both cooled and non-cooled cows supplemental rbST increased milk yield, which was significantly higher than that of the pretreatment period, but it decreased as lactation advances. It is obvious that both cooled and non-cooled cows supplemental rbST increased mammary plasma flow and mammary blood flow, which were significantly higher than those of the pretreatment periods. The ratio of mammary blood flow to the rate of milk yield was not affected by the supplementation of rbST in both groups. The body weights of both cooled and non-cooled cows were increased stepwise as lactation advances whether supplemental rbST or not.

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

The mean arterial plasma concentration for glucose were largely unchanged throughout periods of study in both cooled and non-cooled cows whether supplemental

rbST or not (Table 4.4). There were no significant changes in A-V concentration differences and mammary extractions for glucose across the mammary gland throughout the stage of lactation. During rbST supplementation mammary glucose uptake increased in each stage of lactation in both cooled and non-cooled cows. There were significant increases of mammary glucose uptake in rbST treated animals in mid and late lactation. The arterial plasma acetate concentration were unchanged throughout experimental periods in both groups of animals whether supplemental rbST or not. There were no significant changes in A-V concentration differences and mammary extraction for acetate across the mammary gland throughout the stage of lactation. During rbST supplementation, mammary acetate uptake was unaltered as compared with the pretreatment in each stage of lactation in both cooled and non-cooled cows. The means arterial plasma concentration for β–hydroxybutyrate were unchanged between cooled and non-cooled cows whether supplemental rbST or not in each stage of lactation. The A-V differences, mammary extraction and the mammary uptake for β–hydroxybutyrate were not influenced by the supplementation of rbST in both cooled and non-cooled cows.

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

The arterial plasma fatty acid concentrations were increased significantly during mid and late lactation of cows supplementation with rbST in cooled and non-cooled cows (Table 4.5). There were no significant changes in A-V concentration differences, mammary extraction and the mammary uptake of fatty acid across the mammary gland in early and mid lactation, but there were significantly higher in late lactation after rbST supplementation. The mean arterial plasma concentration, A-V differences and mammary extraction for triacylglycerol showed no significant differences between cooled and non-cooled cows whether supplemental rbST or not in each stage of lactation. The mammary uptake of triacylglycerol in cows supplemental rbST had tendency to increase, but a significant increased were apparent in late lactation in both cooled and non-cooled cows.

Table 4.1 Ambient temperature, relative humidity, temperature humidity index, rectal temperature and respiratory rate measurement at 1400h in animals supplementation with rbST housing under normal shade (NS) and misty-fan cooling (MF) at different stages of lactation

Parameters	Stages of Lactation	NS		MF		SEM	[1]Effect		
		Pre	rbST	Pre	rbST		MF	rbST	MF*rbST
Ambient Temperature (°C)	Early	33.9	35.1	31.6	31.8	0.74	*0.001*	0.386	0.473
	Mid	35.3	35.0	30.0	29.8	0.48	*0.002*	0.613	0.919
	Late	33.5	34.1	29.9	29.1	0.35	*0.001*	0.865	0.098
Relative Humidity (%)	Early	49.5	52.8	66.0	68.8	3.28	*0.001*	0.396	0.942
	Mid	52.8	50.4	78.2	74.0	3.10	*0.001*	0.318	0.779
	Late	59.0	63.5	78.5	79.8	2.07	*0.001*	0.214	0.462
Temperature Humidity Index (THI)	Early	83.2	85.2	82.4	82.8	0.89	*0.003*	0.205	0.396
	Mid	85.5	84.8	81.5	80.8	0.39	*0.019*	0.116	0.928
	Late	83.9	85.2	81.4	80.7	0.29	*0.004*	0.316	*0.071*
Rectal Temperature (°C)	Early	39.4	40.0	39.0	39.4	0.21	*0.037*	*0.061*	0.817
	Mid	39.7	40.1	38.6	39.5	0.13	*0.002*	*0.002*	0.090
	Late	39.2	39.9	38.4	38.8	0.16	*0.015*	*0.016*	0.309
Respiration Rate (breath/min)	Early	73.0	82.3	55.5	68.0	4.13	*0.023*	*0.039*	0.708
	Mid	73.6	77.2	49.0	57.6	1.96	*0.001*	*0.018*	0.294
	Late	71.5	80.0	54.3	59.3	1.09	*0.019*	*0.001*	0.159

SEM = Standard error of the mean.

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

Table 4.2 Total body water (TBW), extracellular fluid (ECF), plasma volume (PV), blood volume (BV) and packed cell volume (PCV) in animals supplementation with rbST housing under normal shade (NS) and misty-fan cooling (MF) at different stages of lactation.

Parameters	Stages of lactation	NS		MF			[1]Effect		
		Pre	rbST	Pre	rbST	SEM	MF	rbST	MF*rbST
TBW	Early	254.4	295.9	277.7	309.2	6.23	0.273	*0.001*	0.441
(L)	Mid	262.0	303.1	272.0	326.9	6.97	0.336	*0.001*	0.352
	Late	269.1	320.4	286.9	327.4	10.65	0.467	*0.003*	0.624
TBW	Early	71.6	78.1	74.8	82.8	2.48	0.369	*0.019*	0.764
(L/100kg)	Mid	68.6	79.4	71.2	79.8	2.19	0.624	*0.002*	0.657
	Late	67.3	81.7	67.8	78.4	2.74	0.641	*0.002*	0.501
ECF	Early	92.9	106.4	108.1	123.2	3.00	*0.029*	*0.001*	0.805
(L)	Mid	103.1	114.5	119.1	126.6	4.52	0.050	0.068	0.668
	Late	102.3	112.7	118.6	131.9	3.58	*0.034*	*0.011*	0.693
ECF	Early	26.2	28.1	29.2	33.0	0.87	0.081	*0.010*	0.319
(L/100kg)	Mid	27.0	30.1	31.3	30.9	1.13	0.132	0.252	0.160
	Late	25.8	28.7	28.2	31.5	0.85	0.208	*0.006*	0.842
PV	Early	18.8	20.6	17.8	19.5	0.95	0.364	0.104	0.937
(L)	Mid	18.6	20.1	21.3	24.0	0.79	*0.017*	*0.028*	0.430
	Late	19.8	21.7	23.3	26.0	0.96	*0.007*	*0.042*	0.671
PV	Early	5.3	5.4	4.8	5.2	0.25	0.378	0.274	0.618
(L/100kg)	Mid	4.9	5.2	5.6	5.9	0.23	*0.037*	0.154	0.992
	Late	5.0	5.5	5.5	6.2	0.23	0.152	*0.022*	0.637
BV	Early	24.3	26.4	23.5	25.0	1.32	0.548	0.205	0.819
(L)	Mid	24.2	26.1	27.5	30.7	1.00	*0.016*	*0.034*	0.541
	Late	25.9	28.4	30.0	34.0	1.20	*0.006*	*0.027*	0.535
BV	Early	6.8	6.9	6.4	6.7	0.35	0.532	0.477	0.758
(L/100kg)	Mid	6.4	6.8	7.2	7.6	0.30	*0.039*	0.192	0.860
	Late	6.5	7.2	7.1	8.2	0.27	0.092	*0.012*	0.506
PCV	Early	22.3	21.8	24.1	22.3	0.52	0.423	0.057	0.261
(%)	Mid	23.0	23.1	22.8	21.9	0.56	0.290	0.506	0.407
	Late	23.8	23.6	22.5	23.7	0.60	0.768	0.438	0.300

SEM = Standard error of the mean.

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

Table 4.3 Milk yield, mammary blood flow (MBF), and body weight in animals supplementation with rbST housing under normal shade (NS) and misty-fan cooling (MF) at different stages of lactation.

Parameters	Stages of lactation	NS		MF			[1]Effect		
		Pre	rbST	Pre	rbST	SEM	MF	rbST	MF*rbST
Milk yield (kg/day)	Early	13.39	15.43	14.82	15.84	0.31	0.684	*0.001*	0.140
	Mid	11.13	13.10	13.79	15.73	0.54	0.269	*0.003*	0.549
	Late	10.31	11.77	11.29	15.00	0.61	0.372	*0.003*	0.101
MBF (ml /min)	Early	4969	5222	5242	6555	265	0.524	*0.018*	0.081
	Mid	4142	5053	4133	5434	388	0.821	*0.021*	0.629
	Late	3751	5096	4435	4968	249	0.735	*0.005*	0.141
MPF (ml/min)	Early	3748	4030	3923	5024	186	0.561	*0.006*	0.060
	Mid	3139	3871	3164	4141	303	0.822	*0.023*	0.696
	Late	2817	3843	3389	3792	185	0.676	*0.005*	0.131
MBF/milk yield (L/kg)	Early	535.0	491.6	554.5	583.0	19.29	0.685	0.701	0.100
	Mid	615.8	612.7	473.9	534.9	49.30	0.568	0.573	0.534
	Late	561.9	672.2	589.7	597.5	63.51	0.888	0.391	0.430
Body weight (kg)	Early	358.8	380.8	360.2	373.8	6.48	0.893	*0.025*	0.535
	Mid	382.4	383.2	381.8	411.4	4.17	0.586	*0.007*	*0.009*
	Late	398.2	393.0	425.0	423.0	4.89	0.268	0.483	0.752

SEM = Standard error of the mean.

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

Table 4.4 The arterial plasma concentrations, arteriovenous differences and mammary uptake for glucose, acetate and β-hydroxybutyrate in animals supplementation with rbST housing under normal shade (NS) and misty-fan cooling (MF) at different stages of lactation.

Parameters	Stages of lactation	NS		MF		SEM	[1]Effect		
		Pre	rbST	Pre	rbST		MF	rbST	MF*rbST
Glucose:									
Plasma conc.	Early	3.73	3.51	3.64	3.48	0.10	0.883	0.098	0.763
(µmol/ml)	Mid	3.55	3.40	3.52	3.67	0.10	0.719	0.992	0.159
	Late	3.49	3.52	3.82	3.77	0.09	0.286	0.918	0.646
A-V difference:	Early	0.66	0.67	0.76	0.61	0.08	0.858	0.485	0.261
(µmol/ml)	Mid	0.62	0.58	0.74	0.72	0.07	0.480	0.810	0.605
	Late	0.78	0.86	0.81	0.80	0.08	0.650	0.552	0.352
Extraction (%):	Early	16.7	18.6	19.3	16.9	1.58	0.816	0.984	0.164
	Mid	17.1	16.7	19.6	18.8	1.57	0.461	0.696	0.398
	Late	22.2	24.4	21.5	21.5	1.62	0.901	0.530	0.373
Udder uptake:	Early	2299	2651	2438	2653	212	0.766	0.168	0.632
(µmol/min)	Mid	1879	2437	1881	2745	355	0.624	*0.042*	0.982
	Late	2183	3235	2475	2936	253	0.530	0.051	0.203
Acetate : Plasma conc.	Early	650.47	541.77	437.13	468.93	54.27	0.215	0.499	0.232
(µmol /L)	Mid	462.43	514.47	648.60	540.13	60.66	0.287	0.654	0.222
	Late	668.57	602.77	555.00	439.43	71.09	0.181	0.238	0.735
A-V difference:	Early	363.67	305.43	273.07	293.57	48.20	0.661	0.706	0.438
(µmol /L)	Mid	248.23	316.73	450.87	343.80	64.94	0.239	0.774	0.213
	Late	424.93	408.27	409.10	249.53	52.43	0.384	0.131	0.210
Extraction (%):	Early	52.87	48.88	62.68	57.05	4.99	0.548	0.364	0.874
	Mid	51.38	54.22	68.05	60.89	5.87	0.281	0.722	0.419
	Late	58.35	64.92	66.77	63.26	5.1	0.763	0.772	0.352
Udder uptake:	Early	1534.4	1358.5	1212.0	1663.5	247.8	0.990	0.593	0.241
(µmol /min)	Mid	831.2	1165.5	1512.5	1473.0	260.0	0.234	0.586	0.493
	Late	1377.1	1612.0	1519.3	1169.3	231.3	0.925	0.304	0.076
β-OH-butyrate: Plasma conc.	Early	850	872	652	697	63.1	0.143	0.610	0.860
(µmol/L)	Mid	752	814	883	742	53.3	0.831	0.480	0.093
	Late	874	932	750	744	112.4	0.332	0.823	0.783
A-V difference :	Early	312	266	224	269	49.53	0.268	0.992	0.385
(µmol/L)	Mid	240	252	333	282	26.67	0.366	0.486	0.271
	Late	302	270	220	326	70.52	0.859	0.614	0.356
Extraction (%):	Early	36.99	29.39	34.78	40.36	5.35	0.277	0.855	0.253
	Mid	30.61	29.77	39.48	37.4	4.11	0.105	0.733	0.885
	Late	34.72	26.62	28.51	43.13	5.72	0.346	0.585	0.082
Udder uptake:	Early	1262.4	1083.9	912.9	1253.8	178.6	0.781	0.661	0.184
(µmol /min)	Mid	726.6	882.8	1059.8	1140.1	129.0	0.157	0.386	0.776
	Late	881.5	1043.0	784.3	1137.8	255.8	0.997	0.344	0.717

SEM = Standard error of the mean

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

Table 4.5 The arterial plasma concentrations, arteriovenous differences (A-V), extraction and mammary uptake for free fatty acid and triacylglycerol in animals supplementation with rbST housing under normal shade (NS) and misty-fan cooling (MF) at different stages of lactation.

Parameters	Stages of lactation	NS		MF		SEM	[1]Effect		
		Pre	rbST	Pre	rbST		MF	rbST	MF*rbST
Free fatty acids :									
Plasma conc. (µmol/L)	Early	156.89	164.27	199.99	287.49	38.02	0.239	0.247	0.323
	Mid	133.24	196.31	188.53	204.45	14.82	0.576	*0.029*	0.150
	Late	102.52	153.59	178.58	262.77	17.14	0.147	*0.004*	0.362
A-V difference (µmol/L)	Early	-4.85	-18.32	17.03	90.5	29.6	0.158	0.341	0.180
	Mid	-31.11	11.86	5.10	-11.18	18.76	0.727	0.497	0.153
	Late	-32.47	29.68	-14.86	25.09	16.89	0.539	*0.017*	0.530
Extraction (%)	Early	-20.18	-12.23	10.13	21.52	11.22	0.063	0.414	0.882
	Mid	-23.48	6.21	2.89	-4.66	13.05	0.531	0.421	0.191
	Late	-30.15	16.35	-11.77	9.20	8.20	0.266	*0.003*	0.158
Udder uptake (µmol/min)	Early	-59.65	-122.58	73.11	462.00	155.5	0.105	0.325	0.184
	Mid	-71.17	34.63	27.57	-13.93	59.63	0.643	0.604	0.252
	Late	-100.38	121.07	-57.31	98.1	67.38	0.821	*0.023*	0.637
Triacylglycerol :									
Plasma conc. (µmol/L)	Early	159.25	179.71	195.07	201.93	17.29	0.702	0.452	0.704
	Mid	209.2	230.62	182.25	202.92	24.03	0.729	0.407	0.988
	Late	199.09	210.64	321.77	249.76	48.36	0.351	0.549	0.413
A-V difference (µmol/L)	Early	42.92	32.33	58.39	70.21	6.48	0.209	0.927	0.122
	Mid	44.96	69.89	54.99	84.37	23.95	0.508	0.290	0.928
	Late	58.23	87.99	52.71	90.80	21.22	0.941	0.149	0.849
Extraction (%)	Early	36.42	23.55	32.88	37.32	4.06	0.660	0.330	0.066
	Mid	25.53	36.30	28.73	41.24	7.33	0.475	0.151	0.909
	Late	33.94	43.85	23.45	39.46	6.56	0.426	0.084	0.655
Udder uptake	Early	167.65	136.88	238.33	388.11	44.04	0.162	0.214	0.075
(µmol/min)	Mid	146.31	260.41	167.29	336.48	68.98	0.543	0.074	0.700
	Late	160.18	333.33	193.37	358.83	72.19	0.696	*0.047*	0.959

SEM = Standard error of the mean.

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

DISCUSSION

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

It is known that milk production is the result of coordination between nutrient delivery to and biosynthetic capacity of the mammary glands (Linzell and Mepham, 1974). The arterial plasma concentration of nutrients including mammary gland biosynthetic capacity and mammary blood flow would be factors affect to the mode of nutrient uptake by the gland. In the present results, the marked increases in blood flow to the mammary gland coincided with an increase in milk yield during rbST supplementation in both cooled and non-cooled cows. These results agree to previous studies by Chaiyabutr and co-worker (2005) that long-term administrations of rbST

showed a marked increase in mammary blood flow throughout lactation. Factors that might affect to increase MBF during supplemental rbST could include an increasing relative mass of many organs and tissue including mammary tissue (Moallem et al. 2004) and an increase in cardiac output (Soderholm et al., 1988) in bST treated cows.

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

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

plasma IGF-I levels on persistency of lactation in rbST treated animals was also noted. (Chaiyabutr et al., 2005).

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

It has been known that volatile fatty acid in the form of acetate are the major of energy source of normal fed ruminants. In the present study, mammary arteriovenous concentration differences, mammary extraction and mammary uptake of acetate were not affected during rbST supplementation in different stages of lactation in both cooled and non-cooled cows. Acetate uptake was not dependent upon the rate of mammary blood flow. It is known that acetate is involved in mammary gland metabolism in either de novo synthesis of short and medium-chain milk fatty acids or generation of ATP and NADPH.

The distribution of short and medium chain fatty acids in milk fat was not altered by rbST supplementation (Chaiyabutr et al., 2000[b]), indicating that acetate was partially redirected from oxidation to de novo fatty acid synthesis. In the present results, levels of A-V concentration differences and mammary extraction of β-hydroxybutyrate across the mammary gland including the arterial plasma concentration were not affected during rbST supplementation. It indicates that the utilization of β-hydroxybutyrate by the mammary tissue was not obvious during rbST administration in 87.5% HF cows. It is known that the circulating β-hydroxybutyrate arise mainly from rumen butyrate in the fed animal (Leng and West, 1969), and the principal effect of bST has been shown to increase oxidation of free fatty acids during negative energy balance in high yield lactating cows. An increase in the concentration of plasma β-hydroxybutyrate would be consistent with an increase in oxidation of free fatty acids (Bauman et al., 1988). The present study, the greater energy requirement resulting in increased hepatic ketogenesis due to greater mobilization of fat reserves (Schultz, 1974) were not apparent during rbST-supplementation in both cooled and non-cooled cows.

In the present study, the mean values for the arterial plasma concentration of free fatty acids but not for triacylglycerol increased during rbST supplementation which was more sensitive to alteration than other blood substrates. This phenomenon has been proposed as an indication of under-nutrition (Reid and Hinks, 1962). However, cows in both cooled and non-cooled cows gained weight throughout the experimental periods. A marked increase in milk yield with rbST supplementation without loss of body weight, especially during early lactation, may be due to the fact that cows were offered TMR diet to allow an adequate replacement of body reserves during lactations. Milk yield in the first lactating crossbred cows in the present study were not as great as that of multiparous cows (Sullivan et al., 1992). This is possibly related to the continued weight gain of cows during their first lactation. During early lactation, the metabolic demands of lactation during supplemental rbST in both cooled and non-cooled cows were met by dietary intake, thus not causing mobilization of body tissues as indicated by no alteration of the levels of plasma triglyceride. The marked increases in the plasma concentrations of FFA

were apparent in cows supplemental rbST in both cooled and non-cooled cows especially in mid and late stages of lactation. Thus, the lipolytic activity would be a function of rbST treatment per se in stead of the associated changes in energy balance.

The measurement of A-V differences of FFA across the mammary gland together with mammary blood flow did not provide a quantitative estimation of their total uptake by mammary tissue. The high uptake of triacylglycerol by the mammary gland especially significant increase in the late lactation in cows supplemental rbST, which is agree with the results reported by Miller et al. (1991). It is possible that the negative mammary uptakes of free fatty acids may reflect hydrolysis of triacylglycerol, since there is the release of FFA into venous blood due to triacylglycerol hydrolysis during the uptake of plasma triacylglycerol as in lactation (West et al., 1967). The releasing of FFA would be as a result of enzymatic activity of lipoprotein lipase in the mammary tissue which has been reported to be higher in the mammary tissue relative to other tissue (Shirley et al., 1973; Bauman and Griinari, 2003).

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

CHAPTER V

Effects of recombinant bovine somatotropin (rbST) administration on glucose metabolism and the utilization of glucose in the mammary gland in different stages of lactation of crossbred Holstein cattle under misty-fan cooling (MF) system.

INTRODUCTION

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

Many technologies are required to improve milk production of dairy cattle in the tropics. It has well recognized that hot environments lowered milk production in dairy cattle both directly and indirectly. Environmental modification is the most common approach to increase milk production with alleviation of severe heat stress in dairy cattle,

for example, fans and sprinklers (Fike et al., 2002), evaporative cooling system (Chan et al., 1997; Chaiyabutr et al., 2008[a]). In addition to environmental modification, the application of exogenous bovine somatotropin has been reported to minimize the effects of heat stress and potentially increased milk yields (West et al., 1991; West, 1994). Somatotropin is known to play a role in responsible for galactopoietic and contributing to homeostasis and homeorhesis in ruminants (Bauman and Currie, 1980). However, few data are available on understanding the mechanisms of milk secretion involving extra-mammary factors and intra-mammary factors during the combined effects of high environmental temperatures and bST administration.

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

designed to investigate the mechanisms of milk secretion involving extra-mammary factors and intra-mammary factors relating to body glucose metabolism and utilization of glucose by the mammary gland during rbST supplementation in 87.5% HF animals under misty-fan cooling system.

MATERIALS AND METHODS

Ten pregnant heifer crossbred 87.5% Holstein cattle were selected for the experiment. Animals were randomly divided into two groups of five animals each. Animals in the control group were housed in the normal shade (NS) in individual stall, while animals in the experimental group were housed in shade with using mister and fans cooling to reduce the environmental temperature (MF). Animals in each group were fed with the same ration of TMR twice daily throughout the experiment. Each day, the diet was given in equal portion at about 0600h and 1700h when animal were milked. Water was available at all times. All animals were weighed monthly throughout the experiment.

The experiments were carried out throughout lactating period in each group. The experiment in each group was divided into 3 stages, namely early- (Days 65-95 postpartum), mid- (Days 125-155 postpartum), and late lactating stages (Days 185-215 postpartum). The details for rbST administration procedures in each stage of lactation were described in Chapter III. Rectal temperature and respiration rate of individual animals were determined at the same time as recording ambient temperature. Ambient temperature and humidity were measured weekly throughout the experiment. The temperature humidity index (THI) was calculated according to West (1994). On each specified day of study, measurements of mammary blood flow, glucose metabolism and the utilization of glucose by the mammary gland were carried out as described in Chapter III. At around 10.00 h. both ear vein and milk vein were catheterized with the non-radiopaque intravenous catheter, gauge 18G (Surflo, Terumo Europe N.V., Belgium) under local anesthesia for infusion of solution. An arterial blood sample was collected from the coccygeal artery by venipuncture with a # 21 needle into heparinized tube. Blood samples from arterial and mammary venous blood in heparinized tube were kept in

crushed ice and then centrifuge at 3000 rpm for 30 min at 4 ºC. Plasma samples were collected and frozen at -40°C in aliquots until time of assays for measurements the concentration of metabolites.

The specific activity of labeled plasma glucose was determined by the method described by Chaiyabutr and Buranakarl (1989). The plasma glucose concentration was measured using enzymatic oxidation in the presence of glucose oxidase (Human GmBH, Germany). Plasma triacylglycerol concentration was determined by enzymatic colorimetric test (Triglyceride liquicolor, Wiesbaden, Germany). Plasma free fatty acid were determined by colorimetry after plasma extraction with chloroform, heptane and methanol and TAN solution (Wang et al., 2004).

Milk fatty acids was extracted from 1 ml of an aliquot thawed milk in 2 ml of Dole's solution (Dole, 1956), (*iso*-propanol 40: *n*-heptane 10: 1N H_2SO_4 1, v/v) shaking in water bath for 30 min. After 1 ml hexane and 1ml H_2O was added to the vial and shaking, the upper layer containing fatty acids was transferred into two vials for radioactivity assay and determination of milk fatty acids concentration. Milk extraction solution in counting vial with a scintillation cocktail was measured radioactivity of ^{14}C and ^{3}H-fat by liquid scintillation counter (Liquid scintillation analyzer, Tricarb 2300 TR, Packard instrument Co.Inc.USA.). Other portion of milk extraction was used to determine milk fatty acids concentration by colorimetry according to Wang et al. (2004) using chloroform, heptane and methanol and TAN solution. Milk fatty profiles was determined by gas chromatography (GC-2010 Gas Chromatograph, Shimazu) after extraction by chloroform and methanol (Christopherson and Glass, 1969) in comparison with the appropriate internal standard of pentadecanoic acid (C15:0). The concentration of milk lactose was determined by spectrophotometry (Teles et al., 1978). Lactose radioactivity was determined after isolation by the hydrolysis method (Wood et al., 1965). The concentration of milk citrate was determined by spectophotometry from tricarboxylic acid filtrate (White and Davies, 1963). Citrate radioactivity was determined after isolation by anion exchange chromatography (Hardwick et al., 1963).

Glucose turnover (T), recycling of glucose carbon, the metabolic glucose clearance rate (C_G), uptake of glucose by the udder (U_G), milk components output (MO), incorporation (A) of radioactivity from glucose into milk components, requirement of NADPH for fatty acid synthesis (P) in the mammary gland, net metabolism of glucose phosphorylation (G_{6p}), net metabolism of glucose (B) to the galactose or glucose moiety of lactose, metabolism of glucose via the pentose phosphate pathway (PC), net metabolism of glucose 6-phosphate via (G_{PC}), net metabolism of glucose 6-phosphate via the Embden-Meyerhof pathway (G_E) and the $^3H/^{14}C$ ratio in the plasma and related product were calculated 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. Mean values were used to evaluate the effect for all variables. Statistical significance was declared at $P<0.05$.

RESULTS

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

Mean values of measurements at experimental site during periods of studies for daily temperatures, humidities, THI, the rectal temperature and respiratory rate are shown in Table 5.1. Average values of ambient temperature in the barn during the daytime in the morning (0900 hours) between NS barn and MF barn were not significantly different, while ambient temperatures during 1400 hours at NS were significantly higher than those of MF. The high relative humidity were apparent at morning and it decreased onwards from morning to evening in both NS and MF, whereas relative humidity in MF was significantly higher than that of NS barn. THI values at the MF barn in afternoon were significantly lower in comparison with NS barn. Cows in both groups exposed to high THI values (77.8 to 85.5) in both barns. Rectal temperature recording in the morning and

afternoon (0900 to 1400 hours) of cooled and non-cooled cows were significant different whether rbST injection or not. The cooled cows showed lower rectal temperature than non-cooled cows during afternoon (1400 hours). There were significant increases in rectal temperature and respiration rate by the effect of supplemental rbST in different parts of the day. The cooled cow showed significantly lower respiratory rate than those of non-cooled cows throughout experimental periods.

Milk yield, milk compositions and its secretion

Milk yield, milk compositions and its secretion in cooled and non-cooled cows are shown in Table 5.2. It is obvious that both cooled and non-cooled cows supplemental rbST increased milk yield, which was significantly higher than that of the pretreatment period, but it decreased as lactation advances. The values of milk lactose concentration were unaltered during rbST supplementation as compared with pretreatment in both groups or among periods of lactation in the same group. The ratio of lactose output/glucose uptake were not different in comparison between cooled and non-cooled cows whether supplemental rbST or not in each stage of lactation, but it showed tendency to decrease as lactation advances. In rbST-treated cows in both NS and MF barns, the milk lactose secretion significantly increased as compared with pretreatment periods in all stages of lactation. The milk citrate concentration was significantly increased during supplemental rbST in early lactation, while its significantly decreased in late lactation in both cooled and non-cooled cows. However, during early and mid lactation, the secretions of milk citrate were significantly increased by the effect of supplemental rbST in both cooled and non-cooled cows. The concentration of milk triacylglycerol in cows supplemental rbST had tendency to increase but a significant increases were apparent in early lactation in both groups. The secretions of milk triacylglycerol were significantly increased in both cooled and non-cooled cows during rbST supplementation in all stages of lactation.

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

The utilization of glucose across the mammary gland during rbST supplementation in both cooled and non-cooled cows are shown in Table 5.3**.** Mammary plasma flow of both cooled and non-cooled cows significantly increased after rbST supplementation in all stages of lactation. During rbST supplementation mammary glucose uptake increased in each stage of lactation in both cooled and non-cooled cows. The mammary glucose uptake of both non-cooled and cooled cows significantly increased during supplemental rbST in mid and late lactations by average 37% and 34%, respectively. Plasma glucose concentration remained in constant levels throughout lactation in both groups. There were no significant changes in A-V concentration differences for glucose across the mammary gland throughout the stage of lactation. The percentage of glucose extraction was not influenced by the supplementation of rbST in both groups.

Glucose turnover and related variables

The glucose turnover rate in both cooled and non-cooled cows supplemental rbST by making simultaneous estimates of the total glucose entry rate using 3-[^{3}H]glucose infusion and the utilization rate of glucose using [U-^{14}C]glucose infusion are shown in Table 5.4. All absolute values of glucose turnover in both cooled and non-cooled cows showed no significant changes in glucose entry and utilization rate throughout the stages of lactation. Plasma glucose concentration remained at the constant level at different stage of lactation in both cooled and non-cooled cows. Supplementation of rbST did not change in glucose entry and utilization rates in compared with the pretreatment. The recycling of glucose-C was estimated by simultaneous injection of [3-^{3}H] glucose and [U-^{14}C] glucose, which showed no differences between cooled and non-cooled cows whether supplemental rbST or not. Plasma glucose clearance remained unchanged during rbST administration in both cooled and non-cooled cows. Both absolute values and percentage of utilization of glucose by tissue other than the mammary gland were

calculated from the total rate of glucose synthesis and the rate of glucose uptake by the mammary gland. The utilization of glucose of non-mammary tissues of both cooled and non-cooled cows increased as lactation advanced but it significantly decreased in cows supplemented with rbST particularly during mid lactation in comparison with the pretreatment periods. There were significant increases of body weight during the course of lactation in both groups. The body weights of both cooled and non-cooled cows whether supplemental rbST or not increased stepwise as lactation advances.

Utilization of glucose carbon in the mammary gland

Glucose uptake and incorporation into related products of lactose, citrate and triacylglycerol are shown in Table 5.5. A marked increase of the utilization of glucose carbon to milk lactose in absolute values were apparent in early and mid lactation of both cooled and non-cooled cows, while it decreased in late lactation. However, the percentage of utilization of glucose carbon for synthesis of milk lactose was not significantly different in early and mid lactation, but the significant decrease was apparent in late lactation of both cooled and non-cooled cows supplemental rbST. The absolute values and percentage of utilization of glucose carbon for synthesis of milk citrate of rbST-treated cows were significantly lower than those of the pretreatment period during mid and late lactation in both cooled and non-cooled cows. During supplementation of rbST, the utilizations of glucose carbon for synthesis of milk triacylglycerol were higher in both cooled and non-cooled cows in all stages of lactation.

Glucose metabolisms in different metabolic pathways in the udder

The effects of supplemental rbST and cooling on glucose metabolisms in different metabolic pathways in the udder are shown in Table 5.6. Data for glucose metabolism via pentose phosphate pathway have shown that the incorporation of ^{3}H from [3-^{3}H]glucose into fatty acids and the flux through the pentose phosphate pathway increased as lactation advances and during supplemental rbST in both cooled and non-cooled cows. Correction for the lower $^{3}H/^{14}C$ ratio likely to be present in intracellular glucose 6-phosphate still

gave high flux values as lactation advances and during supplemental rbST in both cooled and non-cooled cows. The results of the net metabolism of glucose 6-phosphate via the pentose phosphate pathway has been defined as glucose 6-phosphate metabolized according to the equation:

glucose 6-phosphate $\longrightarrow$ glyceraldehyde 3-phosphate + $3CO_2$

(Katz and Wood, 1963)

According to this equation , complete metabolism of one molecule of glucose 6-phosphate would require three cycles of the pentose phosphate pathway . Therefore, the flux through the pathway should be three times the net rate of glucose metabolized in the pentose phosphate pathway . From the present findings, during early and mid lactation, the intracellular glucose phosphorylated by the mammary gland were calculated to be completely metabolized via the pentose phosphate pathway in terms of absolute values and the percentages in cows supplemental rbST, which were higher as compared with pretreatment period, but these values declined in late lactation. Values of metabolism of glucose 6-phosphate via the galactose moiety of lactose decreased as lactation advanced to late lactation in both groups. Metabolism of glucose 6-phosphate via the Embden-Meyerhof pathway was calculated in term of the proportion of metabolized glucose, which was considerable variation throughout stages of lactation in cooled and non-cooled cows. The absolute rate of metabolism of glucose via the Embden- Meyerhof pathway also appeared to increase on late lactation in both cooled and non-cooled cows whether supplemental rbST or not, but the increase was not statistical significant.

Milk fatty acid concentration

The data in Table 5.7 showed the marked increases in the total milk fatty acids concentrations in all stages of lactation during supplemental rbST in both cooled and non-cooled cows. The statistical significant effects of rbST were apparent in early and mid lactation in both groups. The milk fatty acid concentrations, particularly with the long change length fatty acids (C_{16}-C_{18}) significantly increased in both cooled and non-cooled cows after supplemention of rbST in all stages of lactation.

NADPH production from glucose

Data in Table 5.8 show the requirement of NADPH for fatty acid synthesis which was calculated from milk fat ty compositions and output. The NADPH production for fatty acid synthesis significantly increased during supplementation with rbST in different stages of lactation in both cooled and non-cooled cows. The percentage of NADPH production from glucose via the pentose phosphate pathway was considerable variation throughout stages of lactation in cooled and non-cooled cows.

The 3H/14C ratio in glucose and related products

$^{3}H/^{14}C$ ratios in plasma glucose and related products at different stages of lactation of cooled and non-cooled cows during supplementation with rbST are shown in Table 5.9. The $^{3}H/^{14}C$ ratio in arterial plasma glucose was lower than that of the infusion in both groups. These values were not different among cooled and non-cooled cows supplemental rbST in different stages of lactation, indicating some recycling of glucose-C in the whole animals during periods of study. A further decrease in the $^{3}H/^{14}C$ ratio was seen in milk lactose . As the glucose moiety of lactose arises directly from plasma glucose, this decrease in the ratio was due to metabolism of glucose 6-phosphate within the udder before incorporation into lactose as galactose. The $^{3}H/^{14}C$ ratio of milk triacylglycerol was s lightly higher in both groups, indicating ^{3}H was removed and detected in milk triacylglycerol. The ^{3}H and ^{14}C from glucose were also shown to be incorporated into milk citrate . The $^{3}H/^{14}C$ ratio of milk citrate was slightly low in both groups as lactation advances.

Table 5.1. Ambient temperature, relative humidity, temperature humidity index, mean values of rectal temperature and respiratory rate measurement at 0900 and 1400h in animals supplementation with rbST housing under normal shade (NS) and misty-fan cooling (MF) at different stages of lactation.

Parameter	Stages of lactation	Time	NS		MF			[1]Effect		
			Pre	rbST	Pre	rbST	SEM	rbST	MF	rbST*MF
Ambient temperature (°c)										
	Early	0900 h	28.00	27.60	27.10	27.50	0.30	1.000	0.276	0.261
		1400h	33.90	35.10	31.60	31.80	0.74	0.386	*0.001*	0.473
	Mid	0900 h	28.00	28.50	27.60	26.90	0.30	0.749	0.090	0.082
		1400h	35.30	35.00	30.00	29.80	0.48	0.613	*0.002*	0.919
	Late	0900h	28.30	28.40	27.30	27.40	0.49	0.903	*0.040*	0.903
		1400h	33.50	34.10	29.90	29.10	0.35	0.865	*0.001*	0.098
Relative humidity (%)										
	Early	0900 h	79.80	78.80	84.00	88.80	1.65	0.299	*0.048*	0.132
		1400h	49.50	52.80	66.00	68.80	3.28	0.396	*0.001*	0.942
	Mid	0900 h	78.60	78.80	85.20	83.60	1.27	0.595	*0.009*	0.497
		1400h	52.80	50.40	78.20	74.00	3.10	0.318	*0.001*	0.779
	Late	0900h	74.50	76.30	80.80	84.00	1.77	0.206	0.054	0.686
		1400h	59.00	63.50	78.50	79.80	2.07	0.214	*0.001*	0.462
Temperature humidity index										
	Early	0900 h	78.90	78.30	78.10	79.00	0.33	0.695	0.921	0.058
		1400h	83.20	85.20	82.40	82.80	0.89	0.205	*0.003*	0.396
	Mid	0900 h	79.00	79.60	78.90	77.80	0.40	0.495	0.216	0.064
		1400h	85.50	84.80	81.50	80.80	0.39	0.116	*0.019*	0.928
	Late	0900h	78.90	79.00	78.00	78.50	0.50	0.497	0.329	0.730
		1400h	83.90	85.20	81.40	80.70	0.29	0.316	*0.004*	*0.071*
Rectal temperature (°c)										
	Early	0900 h	38.50	38.90	38.10	38.50	0.08	*0.003*	*0.032*	1.000
		1400h	39.40	40.00	39.00	39.40	0.21	0.061	*0.037*	0.817
	Mid	0900 h	38.50	39.00	38.00	38.30	0.08	*0.001*	*0.024*	0.258
		1400h	39.70	40.10	38.60	39.50	0.13	*0.002*	*0.002*	0.090
	Late	0900h	38.50	38.80	38.00	38.30	0.10	*0.015*	*0.007*	0.723
		1400h	39.20	39.90	38.40	38.80	0.16	*0.016*	*0.015*	0.309
Respiratory rate (breaths/min)										
	Early	0900 h	40.00	42.50	35.00	38.00	0.56	*0.003*	*0.003*	0.670
		1400h	73.00	82.30	55.50	68.00	4.13	*0.039*	*0.023*	0.708
	Mid	0900 h	41.20	45.80	36.40	40.40	0.66	*0.001*	*0.022*	0.663
		1400h	73.60	77.20	49.00	57.60	1.96	*0.018*	*0.001*	0.294
	Late	0900h	40.50	44.50	37.00	41.50	1.44	*0.025*	0.101	0.868
		1400h	71.50	80.00	54.30	59.30	1.09	*0.001*	*0.019*	0.159

SEM = Standard error of the mean.

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

Table 5.2 Milk yield, milk lactose, citrate and triacylglycerol secretion, milk lactose, citrate and triacylglycerol concentration and lactose output / glucose uptake in animals supplementation with rbST housing under normal shade (NS) and misty-fan cooling (MF) at different stages of lactation.

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

SEM = Standard error of the mean.

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

Table 5.3 Mammary plasma flow, arterial plasma glucose concentration, mammary glucose uptake and percentage of glucose extraction in animals supplementation with rbST housing under normal shade (NS) and misty-fan cooling (MF) at different stages of lactation.

Parameter	Stage of lactation	NS		MF		SEM	[1]Effect		
		Pre	rbST	Pre	rbST		rbST	MF	rbST*MF
Mammary plasma flow (ml/min)									
	Early	3748	4030	3923	5024	186	*0.006*	0.561	0.060
	Mid	3139	3871	3164	4141	303	*0.023*	0.822	0.696
	Late	2817	3843	3389	3792	185	*0.005*	0.676	0.131
Plasma glucose (µmol/ml)									
	Early	3.73	3.51	3.64	3.48	0.10	0.098	0.883	0.763
	Mid	3.55	3.40	3.52	3.67	0.10	0.992	0.719	0.159
	Late	3.49	3.52	3.82	3.77	0.09	0.918	0.286	0.646
A-V (µmol/ml)									
	Early	0.66	0.67	0.76	0.61	0.08	0.485	0.858	0.261
	Mid	0.62	0.58	0.74	0.72	0.07	0.810	0.480	0.605
	Late	0.78	0.86	0.81	0.80	0.08	0.552	0.650	0.352
Mammary glucose uptake (µmol/min)									
	Early	2299	2651	2438	2653	212	0.168	0.766	0.632
	Mid	1879	2437	1881	2745	355	*0.042*	0.624	0.982
	Late	2183	3235	2475	2936	253	0.051	0.530	0.203
Percentage of mammary glucose extraction (%)									
	Early	16.7	18.6	19.3	16.9	1.58	0.984	0.816	0.164
	Mid	17.1	16.7	19.6	18.8	1.57	0.696	0.461	0.398
	Late	22.2	24.4	21.5	21.5	1.62	0.530	0.901	0.373

SEM = Standard error of the mean.

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

Table 5.4 Glucose turnover rate, glucose-C-recycling, plasma glucose clearance, non-mammary glucose utilization and body weight in animals supplementation with rbST housing under normal shade (NS) and misty-fan cooling (MF) at different stages of lactation.

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

SEM = Standard error of the mean.

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

Table 5.5 Utilization of glucose carbon in the udder in animals supplementation with rbST housing under normal shade (NS) and misty-fan cooling (MF) at different stages of lactation.

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

SEM = Standard error of the mean.

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

Table 5.6 Glucose metabolism in different metabolic pathway in the udder in animals supplementation with rbST housing under normal shade (NS) and misty-fan cooling (MF) at different stages of lactation.

Parameter	Stages of lactation	NS		MF		SEM	[1]Effect		
		Pre	rbST	Pre	rbST		rbST	MF	rbST*MF
Flux through the pentose phosphate pathway calculated as ^{3}H incorporation into milk fatty acid (equivalent µmol of glucose/min):									
	Early	156.5	234.7	236.7	326.9	34.08	*0.039*	0.269	0.865
	Mid	212.7	294.2	300.0	344.1	64.36	0.358	0.25	0.779
	Late	421.3	412.9	376.0	282.9	68.33	0.479	0.415	0.552
Corrected ^{3}H incorporation into milk fatty acid (equivalent µmol of glucose/min):									
	Early	237.27	280.81	273.54	420.42	65.64	0.185	0.406	0.454
	Mid	280.22	352.92	406.02	551.58	77.09	0.195	0.096	0.649
	Late	537.57	572.53	472.91	420.03	103.24	0.933	0.501	0.682
Net metabolism of glucose 6-phosphate via the pentose phosphate pathway (µmol/min):									
	Early	70.1	102.68	97.13	143.49	18.04	0.060	0.336	0.713
	Mid	94.3	131.29	126.93	134.54	34.27	0.534	0.586	0.680
	Late	181.8	170.62	155.28	106.89	36.28	0.435	0.355	0.622
Net metabolism of glucose 6-phosphate via the pentose phosphate pathway (%):									
	Early	11.0	13.2	11.4	16.8	4.04	0.374	0.778	0.699
	Mid	13.1	14.2	16.5	14.9	4.24	0.544	0.347	0.406
	Late	12.5	8.7	9.7	6.2	2.60	0.195	0.409	0.952
Metabolism of glucose 6-phosphate via the galactose moiety of lactose (%):									
	Early	81.6	90.1	67.7	74.9	10.1	0.459	0.317	0.951
	Mid	87.1	87.4	97.2	78.3	16.3	0.583	0.972	0.571
	Late	56.0	42.7	58.9	58.3	11.2	0.553	0.515	0.588
Metabolism of glucose 6-phosphate via Embden-Meyerhof pathway (µmol/min):									
	Early	-115.8	-103.4	-197.5	-216.4	111.4	0.977	0.748	0.892
	Mid	-213.6	91.1	-345.9	-226.6	168.2	0.243	0.351	0.597
	Late	124.3	465.7	321.3	111.6	139.3	0.649	0.713	0.083
Metabolism of glucose 6-phosphate via Embden-Meyerhof pathway (%):									
	Early	-22.5	-42.1	-43.0	-25.9	11.4	0.914	0.949	0.146
	Mid	-25.6	-12.4	-61.1	-61.4	21.6	0.774	0.370	0.762
	Late	19.6	26.6	25.0	13.0	4.1	0.565	0.582	0.050

SEM = Standard error of the mean.

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

Table 5.7 Fatty acid composition of milk fat in animals supplementation with rbST housing under normal shade (NS) and misty-fan cooling (MF) at different stages of lactation.

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

SEM = Standard error of the mean.

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

Table 5.8 NADPH production for fatty acid synthesis in the udder in animals supplementation with rbST housing under normal shade (NS) and misty-fan cooling (MF) at different stages of lactation.

Parameter	Stage of	NS		MF			[1]Effect		
	lactation	Pre	rbST	Pre	rbST	SEM	rbST	MF	rbST*MF
Requirement of all NADPH for fatty acid synthesis (µmol/min)									
	Early	1134	1761	1920	2067	134	*0.020*	0.203	0.112
	Mid	1235	1698	1428	2150	181	*0.011*	0.539	0.495
	Late	1240	1460	1154	2062	150	*0.006*	0.481	0.052
Requirement of all NADPH formation from glucose via the pentose phosphate pathway (%)									
	Early	16.7	18.5	19.5	24.9	3.14	0.286	0.482	0.594
	Mid	25.6	26.5	31.6	25.6	7.36	0.744	0.667	0.651
	Late	35.4	25.6	26.5	17.8	5.16	0.112	0255	0.925

SEM = Standard error of the mean.

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

Table 5.9 3H/14C ratios in plasma glucose and related products in animals supplementation with rbST housing under normal shade (NS) and misty-fan cooling (MF) at different stages of lactation.

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

SEM = Standard error of the mean.

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

DISCUSSION

In the present study, the values of THI in NS and MF barns in either morning or afternoon, were always higher than critical value (THI 72) for lactating dairy cows in both barns (Smith et al., 2006). Animals were therefore always subjected to moderate heat stress throughout experimental periods (i.e. THI = 77.8 to 85.5). Thus the effect of misters and fans for cooling animals in the present study was not sufficient to completely eliminate heat stress. The values of THI might not accurately reflect heat stress when using a mister and fan system for evaporative cooling that result in higher humidity but also cause cooling. Although the cooling effect using the misty-fan system was not sufficient to adequately reduce THI in the barn, there is a beneficial effect as indicated by a lower RR and RT in cooled cows and also higher milk yield throughout lactation. These results support the study of Fike et al.(2002) that housing cows during the day with fans and sprinklers effectively reduced heat stress as indicated by lower body temperature and respiration rate. In the present study, an increase in milk yield of rbST-supplemented cows was accompanied by an increase in both RT and RR in comparison with cows without rbST supplementation in both cooled and non-cooled cows throughout the experimental periods. The observation for an increase in heat production during rbST supplementation agrees with the reports of West et al.(1991) and West (1994) that rbST-treated cows in a hot environment may increase heat production in higher and lower milk producing cows.

It is known that dairy cattle adapt to high temperatures with variety of hormonal and metabolic responses, one of which may involve changes in the process of milk synthesis in the mammary gland. Milk yield, milk compositions and its secretion during rbST administration in the experiment is shown in Table 5.2. It is obvious that administration of rbST to cooled and non-cooled cows increased milk yield, which was significantly higher than that of the pretreatment period. Milk yield initially showed significant increases in early lactation of cooled and non-cooled cows either supplemental rbST or not and it decreased as lactation advances. These findings confirm that an increase in

milk yield in response to rbST administration will not be sustained indefinitely (Bauman, 1992), and it is influenced by the stage of lactation (Phipps et al., 1991). The low potential for extended persistency of lactation in rbST treated animals appears similar to that which occurs in higher yielding cows (Chase, 1993). However, it has been reported that the response to somatotropin for whole lactation might be reduced if treatment begins very early in lactation (Bauman and Vernon, 1993; Burton et al., 1994).

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

Effects of supplemental rbST and cooling on glucose kinetics are shown in Table 5.3. Plasma glucose concentrations in both cooled and non-cooled cows maintained over a wide range at different stages of lactation. It indicates that steady state conditions between the rate of gluconeogenesis and the rate of utilization of glucose existed in the body pool of glucose in both groups. However, it has been reported that the plasma glucose concentration would increase during injection of bovine somatotropin in cows

with low milk yield but not in cows with high milk yields (Bines et al, 1980). The difference in response in terms of changes in plasma glucose level may reflect the differences of utilizations for lactose synthesis between high yielding and low yielding cows.

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

The utilization of glucose across the mammary gland during supplemental rbST in both cooled and non-cooled cows at different stages of lactation are complex regulatory mechanisms, It would depend both on the partitioning of blood flow between extra-mammary tissues and local regulation. The present results for the mammary uptake of

plasma glucose in both groups are not based on changes in A-V concentration differences and extraction ratio of glucose. An increase in the rate of blood flow to the mammary gland during supplemental rbST in both cooled and non-cooled cows, would be a major determinant of the rate of glucose uptake by the mammary gland. In all stages of lactation, the net mammary glucose uptake increased approximately 8-48% during supplemental rbST as compared with the pretreatment period in both groups. Glucose extracted by the mammary gland has several possible metabolic fates in mammary epithelial cells that may occur at another level than transmembrane transport (Xiao and Cant, 2003). The glucose uptake by the mammary gland during supplemental rbST and cooling was rate limiting for the transport of glucose to the mammary cell. The high blood flow to the mammary gland during supplemental rbST would decrease the transit time of glucose, thereby reduction for prolonging the contact time between glucose in blood and glucose transporter in mammary epithelial cell (Chaiyabutr et al., 2007[b]).

It is known that glucose is an important intermediary of metabolism for the biosynthesis of lactose, triacylglycerol and citrate by the mammary gland. The bovine mammary gland cannot systhesize its own glucose because of lacking of glucose-6-phosphatase (Scott et al., 1976). Glucose plays a crucial role in their metabolism and lactose synthesis, which is formed in Golgi vesicles from a combination of glucose either directly or after phosphorylation to glucose 6-phosphate and conversion to UDP-galactose (Ebner and Schanbacher, 1974). The calculated amount of metabolism of glucose 6-phosphate to the galactose moiety of lactose during supplemental rbST in both cooled and non-cooled cows in different stages of lactation would be sufficient to account for the cytosolic lactose synthesis .The utilization of glucose carbon incorporation to lactose in the udder increased in both early and mid lactation but not for late lactation during supplemental rbST in both cooled and non-cooled cows. The decrease in the metabolism of glucose 6-phosphate to the galactose moiety of lactose as lactation advanced to late lactation in both cooled and non-cooled cows would affect to the lactose synthesis and milk production in both groups. A low enzymatic activity for lactose synthesis might be expected to appear as lactation advances. According to Davis and

Bauman (1974), 50 to 60% of the glucose in the glucose-6-phosphate pool is converted into galactose. Major part of the galactose has been shown to derive from mammary extracted glucose, as well as from glycerol and other metabolic pathways. However, glucose is not the sole carbon source for lactose synthesis but remains the main one. An increase in the glucose concentration in milk representing an increase in glucose concentration in the mammary epithelial cell during prolonged treatment of rbST has been noted (Chaiyabutr et al., 2008[c]).

It is known that 80–85% of lactose carbon atoms arise from glucose (Faulkner and Peaker, 1987; Bickerstaffe et al., 1974). The quantitative utilization of the glucose taken up by the mammary gland is used directly in the synthesis of lactose, while the remaining of extracted glucose can participate in the supply of ATP (Embden-Meyerhof pathway and the tricarboxylic acid cycle), other portions would be metabolized via the pentose phosphate pathway, In the present studies , glucose 6-phosphate metabolized via the pentose phosphate pathway by average 10-17% throughout lactation in both cooled and non-cooled cows without rbST, while it increased from 13 % in pretreatment to 15 % on early and mid-lactation but it decreased in the late lactation after supplemental rbST (Table 5.6). These results also agree with prolonged treatment of rbST in crossbred HF cows showing that percentage values of glucose 6-phosphate metabolized via the pentose phosphate pathway were variable in different stages of lactation (Chaiyabutr et al., 2008[c]). These values are different in comparable to those obtained previously in the isolated perfused udder of cow by Wood and co-workers (1965), in which about 23- 30% of the glucose was metabolized via the pen tose phosphate pathway. It is probable that no consideration of the recycling of glucose 6-phosphate metabolized via the pentose cycle in the udder with the consequent loss of ^{3}H from glucose 6-phosphate (Davis and Bauman 1974). However, the net proportion of the metabolism of glucose 6-phosphate via the pentose cycle pathway was increased during supplemental rbST at early stage of lactation of cooled and non-cooled cows. Metabolism of glucose via the pentose phosphate pathway yields 2 molecules of NADPH per molecule of glucose, only one of which could be labelled with ^{3}H in the present experiments . In the present studies ,

estimates of the contribution of the pentose phosphate pathway in providing NADPH for fatty acid synthesis *in vivo* have been estimated by based on the assumption that all the glucose that was oxidized to CO_2 was metabolized via the pentose phosphate pathway . High metabolism of glucose 6-phosphate in early lactation of rbST treated cows appeared to be due primarily to a high flux through the lactose synthesis and to pentose phosphate pathway, probably reflecting the high milk production during rbST supplementation.

The utilization of glucose carbon by the mammary epithelial cell for the synthesis of lactose, citrate and triacylglycerol are shown in Table 5.5. Absolute amount of glucose carbon incorporation to milk lactose were increased during supplemental rbST in early and mid lactation in both cooled and non-cooled cows but it decreased in late lactation. These findings would parallel to its effects on milk yield. It indicates that during supplemental rbST in late lactation, the metabolism of glucose-6-phosphate declines the flux towards the pentose phosphate pathway and in lactose synthesis. In parallel, a higher proportion of glucose-6- phosphate would be metabolized via the Embden-Meyerhof pathway and was oxidized in the tricarboxylic acid cycle . During supplemental rbST in each stage of lactation, both the proportion and absolute amount of glucose carbon incorporation to milk triacylglycerol were increased, while both the proportion and absolute amount of glucose carbon incorporation to milk citrate were decreased. These changes can be interpreted in terms of metabolic shifts that are occurring within the mammary epithelial cell, and one might speculate that such changes reflect the high flux of the utilization of glucose carbon by the mammary epithelial cell through the rate of lactose synthesis and milk production during supplemental rbST. In addition to the use of glucose carbon for milk fat synthesis, the hydrogen from glucose has been shown to be incorporated into milk fat in early and mid lactation in both cooled and non-cooled cows supplemental rbST (Table5.6), although studies *in vitro* have shown that fatty acid synthesis could occur from the utilization of acetate in the perfused goat udder (Hardwick et al., 1963). It has been known that milk fat is synthesized from fatty acids of both blood lipids and from de novo synthesis within the mammary epithelial cells. However, an increase in milk fat after rbST supplementation was associated with the increased yield of

long-chain fatty acids characteristic of plasma free fatty acids and body fat. Significant increases in plasma free fatty acids in rbST-treated cows have been published elsewhere (Chaiyabutr et al., 2007[b]). Thus, the lipolytic activity would be a function of bST treatment per se in stead of the associated changes in energy balance.

Glucose can also participate in the milk fat formation, by supplying the glycerol (triose phosphate pathway) and the NADPH essential to elongating milk fatty acids (pentose phosphate and isocitrate dehydrogenase pathways). However,very marginally, less than 11% of glucose could supply carbon atoms for the synthesis of milk triacylglycerol in either supplemental rbST or cooling. Data findings in Table 5.8 provide evidence that 17% to 35 % of the NADPH required for fatty acid synthesis *de novo* in the udder of cooled and non-cooled cows without rbST arose from glucose metabolism , while 18% to 27% of the NADPH was required during rbST supplementation. If there is a common pool of glucose 6-phosphate which is available for both lactose synthesis and pentose phosphate metabolism , then the recycling of glucose 6-phosphate within the udder would result in too low a value for NADPH production from glucose . The net metabolism of glucose in the pentose phosphate pathway can be calculated from the incorporation of ^{3}H from [3-^{3}H]glucose in fatty acids assuming that the NADPH is used exclusively for bi osynthesis of fatty acids (Katz et al ., 1974). This technique has been used to study the *in vitro* metabolism of rat mammary and adipose tissue (Katz and Wals, 1970,1972 ; Katz et al., 1966) and it was also used for the study of the *in vivo* metabolism of goat mammary tissue (Chaiyabutr et al., 1980).

Metabolism of glucose 6-phosphate via the pentose phosphate pathway usually loss of all ^{3}H from [3-^{3}H]glucose in lactating cows . During lactation, a higher level of $^{3}H/^{14}C$ ratio in milk triacyglycerol (Table 5.9) was due to an increase in disequilibrium of the triose phosphate isomerase reaction occur ing in the udder of crossbred animals which needs to be further investigated . Tritium and carbon-14 in glucose molecule were also shown to be incorporated into m ilk citrate which provided by averaged 22 μmol/min (16.5-.25.5) in cooled and non-cooled cows without rbST and provided by averaged 16

μmol/min (8.8-21.2) for the carbon skeleton of citrate during rbST supplementation in both groups. Milk citrate could be synthesized from 2-oxoglutarate via the NADP-dependent isocitrate dehydrogenase reaction (Hardwick, 1965). In addition ^{3}H is lost to NADPH or water in metabolism via the pentose phosphate pathway or glycolytic pathway, so it is likely that ^{3}H incorporation into milk citrate was also via NADP^{3}H. It is possible that the incorporation of ^{3}H into milk citrate may occur in different manners in the exchange reaction of the cytosolic NADP- dependent isocitrate dehydrogenase . Both fatty acid synthesis and the NADP-dependent isocitrate dehydrogenase reaction may have different mechanisms with a common pool of cytosolic NADPH between cows without rbST and cows supplemental rbST. Significant increases in the concentration of FFA in milk were apparent in cooled and non-cooled cows supplemental rbST as compared with the pretreatment period in each stage of lactation (Table 5.7). A similar result for an increase in milk fat content due to prolonged administration of rbST has also been observed previously (West et al., 1991 ; Chaiyabutr et al., 2008[b]). It has been known that milk fat is synthesized from fatty acids of both blood lipids and from de novo synthesis within the mammary epithelial cells.

In conclusion , the data presented here represent the estimation *in vivo* of glucose metabolism in the mammary gland and its distribution to lactose synthesis , the pentose phosphate pathway and the Embden-Meyerhof pathway by the effects of supplemental rbST and cooling in 87.5% HF animals. The rbST exerts its galactopoietic action, in part, association with an increase in mammary blood flow, which partitions the distribution of glucose to the mammary gland. The stimulant effect for milk yield by supplemental rbST was transiently and the glucose turnover rate was not significantly increased as compared with pre-treatment period in all stages of lactation. It indicates that rbST induced enhancement of milk yield in all stages of lactation, which would be compensated by mobilization of body energy reserves (i.e. plasma free fatty acids) to the extent of the elevated energy requirements for supporting the increased milk production. During early and mid lactation, the glucose taken up by the udder of both cooled and non-cooled cows

without supplemental rbST, an average 14% and 12% were metabolized in the pentose phosphate pathway and contributed to NADPH production , respectively. During supplemental rbST, the glucose taken up by the udder of both cooled and non-cooled cows, an average 13% and 12% were metabolized in the pentose ph osphate pathway and contributed to NADPH production , respectively. An increased flux of the sufficient pool of intracellular glucose 6-phosphate during early and mid lactation came across through the lactose synthesis and pentose cycle pathway during rbST supplementation. On late lactation of cooled and non-cooled cows, the glucose taken up by the udder were metabolized in the pentose phosphate pathway by averaged from 11% to 7.4% and contributed to NADPH production from 30% to 22% after supplemental rbST. In the present study, mammary plasma flow was significantly increased after rbST supplementation, while milk yield of rbST-treated cows was not significantly greater than that of pretreatment in late lactation. It would appear that a larger proportion of the glucose 6-phosphate is metabolized via Embden-Meyerhof pathway in late lactation. The present results indicate that the regulation of biosynthetic capacity within the mammary gland would be influenced more by local than by systemic factors in identification of the utilization of substrates in the rate of decline in milk yield with advanced lactation.

CHAPTER VI

Responses of insulin like growth factor-I, insulin and plasma metabolites to recombinant bovine somatotropin (rbST) administration in different stages of lactation in crossbred Holstein cattle under misty-fan cooling (MF) system.

INTRODUCTION

Bovine somatotropin (bST) is a peptide hormone which is well known to be responsible for galactopoiesis in ruminants, not prolactin or adrenocorticotropin. It has been reported that the concentration of plasma bST in 87.5% crossbred Holstein cattle decreased rapidly as lactation progressed to mid- and late lactation. This decrease could contribute to a reduction in milk yield and mammary blood flow (Chaiyabutr et al. 2000[d]). However, little is known about the other circulating factors that are involved in regulating mammary blood flow, a major parameter controlling milk production (Davis & Collier, 1985). The bST is known as a homeorrhetic hormone concerned with both growth and lactation, but the mechanism of action of bST in crossbred dairy cattle on milk production is a controversial area. Receptors for growth hormone(GH) have not been demonstrated on secretory epithelial cells of mammary tissue (Akers, 1985). The effects of GH on milk production are thought to be indirectly mediated *via* nutrient partitioning effects or *via* insulin-like growth factor-I (IGF-I) (Bauman, 1992). There has been discussion as to whether IGF-I mediates the galactopoietic effects of growth hormone. Some studies support this role with an infusion of IGF-I into the pudic artery of lactating goats, which has been shown to increase blood flow and milk production on the infused side (Prosser et al. 1990; Prosser et al. 1994). Infusion of GH into the mammary artery of sheep did not increase milk yield (Peel & Bauman, 1987). Several other reports, refuting the role of IGF-I as mediators of GH action, have been published (Barber et al. 1992; Flint et al. 1992; Plaut et al.1993). It has been reported that GH can stimulate milk production under circumstances in which IGF-I does not (Prosser & Davis, 1992). Chaiyabutr et al. (2000) reported that the galactopoietic effect of GH was not associated

with the plasma level of IGF-I as lactation advances in 87.5% HF animals. The plasma level of IGF-I has been shown to remain at the same level as lactation advances, despite declining circulating bST, mammary blood flow and milk yield (Chaiyabutr et al. 2004). These data did not support a role for IGF-I in mediating the action of GH on milk production. However, an increase in plasma IGF-I level with a concomitant increase in both mammary blood flow and milk yield in late lactation, was seen after exogenous administration of rbST in 87.5%HF animals (Tanwattana et al., 2003).

Despite a number of studies looking at these differences, there have been few observations about the mechanism of short persistency of lactation in 87.5% HF dairy cattle whether it relates to the role of GH or a mechanism other than the circulating level of GH. High environmental temperature would be another factor which affects milk production in dairy cows in the tropic (Smith et al. 2006; Bohmanova et al. 2007). The interaction effects between thermal stress and the role of GH on lactation performance in crossbred lactating cattle is not yet clear. A number of studies in lactating cows showed that the high ambient heat load would reduce the response to bST treatment and low milk yields (Kronfeld, 1988 ; Molett et al., 1986), while other studies showed no changes in milk yields (Cole and Hansen, 1993). Attempts have been made to minimize the impact of thermal stress on milk production in dairy cattle with different types of environmental modifications, such as water spray and fans, evaporative cooling system (Armstrong et al., 1988 ; Armstrong et al., 1993; Ryan et al., 1992; Chaiyabutr et al., 2008). During lactation, mammary gland function is dependent on hormonal stimuli and the provision of nutrients from the blood to sustain milk synthesis. To understand this apparent paradox, more data are required for the knowledge concerning link between bST supplementation in high ambient temperature exposure. The objective of the present study was to determine the relationship between housing under shade with or without misters and fans and supplementation with rbST or not during three period of lactation (early, mid and late lactation) of crossbred 87.5% HF animals. Measurements for evaluation the effectiveness of these treatments were circulating levels of IGF-I, insulin, mammary blood flow and biological variables relevant to milk production. It might lead to better understanding

adaptability in crossbred cattle and choosing suitable crossbred dairy cattle for increased milk production in the tropics.

MATERIALS AND METHODS

Ten crossbred 87.5% HF cows were devided into the control (n=5) and experimental (n=5) groups. The control animals were kept under shaded house (NS), while the experimental animals were kept under shaded house with misty-fan cooling (MF) system. Three consecutive periods of study were used for each group. These studies consisted of a periods of 65-95 days postpartum (early-lactation), 125-155 days postpartum (mid-lactation), 185-215 days postpartum (late-lactation).

Each animal was performed by the pretreatment without recombinant bovine somatotropin (rbST) and treatment with rbST. Thus, the combinations were control animals in shade without rbST injection (NS), shade with rbST injection (NS + rbST); in the experimental group, animals in shade plus MF without rbST injection (MF) and shade plus MF with rbST injection (MF + rbST). Pretreatment were performed on day 65, 125, and 185 of early, mid, and late lactation, respectively. The study at treatment period was carried out at week 4 after pretreatment in each stage of lactation by subcutaneous injection with 500 mg of recombinant bovine somatotropin (rbST, POSILAC, Monsanto, USA) every 2 weeks. The dry matter intake of each animal was measured by weighing the TMR offered and refused each day. Animals were milked twice daily by using a milking machine and milk production were recorded daily.

In each specified day of each lactation period, measurement of mammary blood flow were carried out as described previously. An arterial blood sample was collected from the coccygeal vessel by venipuncture with a # 21 needle into heparinized tube. Blood sample in heparinized tube were kept in crushed ice and then centrifuge at 3000 rpm for 30 min at 4 ºC. Plasma samples were collected and frozen at -40 ºC in aliquots until time of assays for measurements the level of hormones and metabolites.

The plasma IGF-I concentration was determined by Chemiluminescence immunoassay using an IMMULITE® Analyzer (IMMULITE IGF-1, Diagnostic Products Corporation, Los Angeles, CA). The plasma insulin concentration was quantified for bovine insulin using ELISA technique (Mercodia Bovine Insulin, Mercodia AB, Sylveninsgatan 8, Uppsala, Sweden). Plasma glucose concentrations were measured using enzymatic oxidation in the presence of glucose oxidase. The the plasma concentration for triglyceride was determined by enzymatic colorimetric test (Triglyceride liquicolor^{mono} Su-Trimr, Germany). The plasma protein concentrations A) were conducted with an automatic clinical chemistry analyzer (Operator Manual BT 2000 Plus, Biotecnica Instruments S.P.A. Via Licenza, Rome, Italy).

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.

RESULTS

Changes in dietary dry matter intake, milk yield, mammary blood flow and body weight.

The dietary dry matter intake, milk yield, mammary blood flow and body weight in cooled and non-cooled cows are shown in Table 6.1. The enhancement of milk yield in both cooled and non-cooled cows supplemental rbST was significantly higher than that of the pretreatment period, but it decreased as lactation advances. The peak milk yield in both group declined from the early period of lactation as lactation advanced to mid and late lactation whether supplemental rbST or not. The body weight of both cooled and non-cooled cows increased stepwise of lactation advances whether supplemental rbST or not. It is obvious that both cooled and non-cooled cows supplemental rbST increased mammary plasma flow and mammary blood flow, which were significantly higher than those of the pretreatment periods. The ratio of mammary blood flow to the rate of milk yield was not influenced by the supplementation of rbST in both cooled and non-cooled cows. However, cows in both groups among treatment gained in weight as lactation

advances. DMI of cooled cows were significantly higher than those of non-cooled cows during early and mid lactation, but no difference was occurred in late lactation. The values of DMI in rbST-treated cows were higher than those values in the pretreatment period in all stage of lactation.

Changes in plasma concentrations of IGF-I, insulin and plasma metabolites.

Changes in plasma concentration of IGF-I, insulin and plasma metabolites are shown in Table 6.2. The plasma IGF-I concentration were not affected by the effect of misty-fans cooling system alone in each stage of lactation. The concentration of plasma IGF-I in rbST treated animals was significantly higher than that of the pretreatment animals throughout all lactation periods in both cooled and non-cooled cows. The plasma insulin concentration were not significantly different between cooled and non-cooled cows whether supplemental rbST or not throughout the stage of lactation. Concentration of plasma glucose, triglyceride and protein concentration were not influenced by the supplemental of rbST in both cooled and non-cooled cows.

Table 6.1 The changes in dietary dry matter intake (DMI) , milk yield, mammary blood flow (MBF), mammary plasma flow (MPF)and body weight in animals supplentation with rbST housing under normal shade (NS) and misty-fan cooling (MF) at different stages of lactation.

Parameters	Stages of	NS		MF			[1]Effect		
	lactation	Pre	rbST	Pre	rbST	SEM	MF	rbST	MF* rbST
DMI	Early	6.14	7.05	7.22	8.49	0.312	*0.049*	0.088	0.575
(kg/day)	Mid	6.18	7.49	8.72	10.00	0.450	*0.013*	*0.020*	0.973
	Late	7.57	7.87	8.26	9.32	0.151	0.362	*0.002*	*0.037*
Milk yield	Early	13.39	15.43	14.82	15.84	0.31	0.684	*0.001*	0.140
(kg/day)	Mid	11.13	13.10	13.79	15.73	0.54	0.269	*0.003*	0.549
	Late	10.31	11.77	11.29	15.00	0.61	0.372	*0.003*	0.101
MBF	Early	4969	5222	5242	6555	265	0.524	*0.018*	0.081
(ml /min)	Mid	4142	5053	4133	5434	388	0.821	*0.021*	0.629
	Late	3751	5096	4435	4968	249	0.735	*0.005*	0.141
MPF	Early	3748	4030	3923	5024	186	0.561	*0.006*	0.060
(ml/min)	Mid	3139	3871	3164	4141	303	0.822	*0.023*	0.696
	Late	2817	3843	3389	3792	185	0.676	*0.005*	0.131
MBF/milk yield	Early	535.0	491.6	554.5	583.0	19.29	0.685	0.701	0.100
(L/kg)	Mid	615.8	612.7	473.9	534.9	49.30	0.568	0.573	0.534
	Late	561.9	672.2	589.7	597.5	63.51	0.888	0.391	0.430
Body	Early	358.8	380.8	360.2	373.8	6.48	0.893	*0.025*	0.535
weight	Mid	382.4	383.2	381.8	411.4	4.17	0.586	*0.007*	*0.009*
(kg)	Late	398.2	393.0	425.0	423.0	4.89	0.268	0.483	0.752

SEM = Standard error of the mean.

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

Table 6.2 The plasma concentration of the insulin like growth factor I (IGF-I), insulin, glucose, triglyceride and protein in animals supplementation with rbST housing under normal shade (NS) and misty-fan cooling (MF) at different stages of lactation.

Parameters	Stages of lactation	NS		MF		SEM	[1]Effect		
		Pre	rbST	Pre	rbST		MF	rbST	MF* rbST
Plasma IGF-I	Early	118.2	196.0	87.4	114.8	18.8	0.301	*0.023*	0.216
(ng/ml)	Mid	115.6	183.2	121.8	208.4	36.1	0.644	0.065	0.798
	Late	128.2	350.9	124.1	220.4	41.5	0.221	*0.005*	0.166
Plasma insulin	Early	0.72	0.92	0.51	1.38	0.36	0.821	0.173	0.382
(L/kg)	Mid	1.19	1.01	0.97	1.23	0.27	0.996	0.887	0.436
	Late	0.57	1.24	1.76	1.21	0.29	0.102	0.830	0.071
Plasma glucose	Early	67.19	63.19	65.44	62.58	1.834	0.883	0.098	0.763
(mg/dl)	Mid	63.93	61.12	63.27	66.04	1.931	0.739	0.154	0.591
	Late	62.81	63.41	68.75	67.80	1.570	0.686	0.256	0.464
Plasma triglyceride	Early	11.88	13.41	14.55	15.06	1.39	0.243	0.510	0.760
(mg/dl)	Mid	15.61	17.20	13.60	15.14	1.79	0.729	0.407	0.998
	Late	14.85	15.71	24.00	18.63	3.607	0.351	0.549	0..413
Plasma protein	Early	8.52	8.79	9.23	8.82	0.18	0.428	0.718	0.092
(g/dl)	Mid	9.11	8.49	8.57	8.97	0.29	0.956	0.706	0.114
	Late	8.70	8.13	8.98	8.88	0.22	0.460	0.179	0.327

SEM = Standard error of the mean.

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

DISCUSSION

Diary herds in tropical countries are mixed exotic breeds and cross-bred animals. The potential for milk production is governed by a variety of factors, for example, environmental temperature, stage of lactation, energy balance and nutrition including functional of the endocrine gland (Chaiyabutr et al., 2007[b]). Bovine Somatotropin (bST) , is one of hormone which is known to be responsible for galactopietic action in part through increase in TBW and ECF in association with an increase in mammary blood flow (MBF), which contribute nutrients partitioning to the mammary gland for milk synthesis (Maksiri et al., 2005; Chaiyabutr et al., 2007[a]). Animals treated with rbST showed increased milk yield and circulating levels of IGF-I throughout lactation. These finding were similar to those of previous studies on lactating cows showing that the injection of GH, elevated plasma IGF-I concentrations (Davis et al., 1987 ; Tunwattana et al., 2003). A number of studies indicated that GH increased milk yield by a mechanism which did not involve the direct action of GH on the mammary gland (Collier et al., 1984). The indirect effects of GH on milk production are thought to be mediated either via IGF-I or nutrient partitioning effects (Bauman, 1992).

The synthesis and release of IGF-I is mainly by the liver (Granner, 1996). However, little is known about the regulation of synthesis and secretion of IGF-I in the liver of ruminant. Mechanisms for regulating the plasma IGF-I level are known to be dependent on the availability in the liver of both GH and some nutritional factors (Clemmous and Underwood, 1991). The results from this study suggested that, the increase in IGF-I secretion throughout the experiment would appear to be maintain by the availability of exogenous rbST in the liver. Exogeneous rbST administration in the present study was sufficient to achieve a satisfactory stimulation of IGF-I (Collier et al., 1988). GH is key regulator of the hepatic expression of circulating IGF-I, and circulating concentrations of IGF-I are sensitive to nutritional factors in many species of animals. No differences in the nutritional status between cooled and non-cooled cows were apparent in all lactating periods. Animal in both groups were equally well-fed. Animals with a lower nutritional state having a lower basal level of IGF-I (Hodginson et al., 1991) or a

negative energy balance, have reduced hepatic IGF-I production (Weller et al.,1994; Ketteslegers et al., 1995) would not be expected to occur in the present study.

The rbST was not affected on plasma level of triglyceride glucose and protein throughout lactation periods in both cooled and non-cooled cows, although GH has been known to elevate concentrations of fat (free fatty acids) and glucose in the blood (Vernon and Finley, 1988). These results could not be a factor in limiting IGF-I release from the liver (McGuire et al., 1991). However, an increase in extracellular water compartments including the plasma volume in animals treated with exogenous rbST was showed in Chapter IV. These responses could be attributed to an increase in the plasma pool of circulating substrates (plasma volume x concentration), facilitating the portioning of nutrients for milk synthesis and IGF-I secretion.

However, crossbred HF animals with either short-term or long-term rbST treatment in different stages of lactation have also been shown to increase in MBF which were concomitant with an increase in circulating levels of IGF-I (Chaiyabutr et al., 2005; Maksiri et al., 2005; Tanwattana et al., 2003). It indicates that bST plays a role for an increase in MBF, requiring IGF-I as a mediator increasing MBF directly (Forsyth, 1996). In the present study an increases in IGF-I and MBF during supplemental rbST in both cooled and non-cooled cows in different stages of lactation were similar to those of previous studies. In the mammary gland, the role played by insulin seems to be different. This experiments demonstrate that an increase in mammary glucose uptake in rbST in both cooled and non-cooled cows would not be attributed to the changes in plasma insulin concentration. The results in this study have provided evidence that the mammary glucose uptake, lactose secretion and milk yield of cooled and non-cooled cows were not affected by both circulating insulin levels and plasma glucose concentration during rbST supplementation. These results support the view that the mammary gland of the ruminant appears to be relatively insensitve to insulin.

Milk yield initially showed significant increases in early lactation of cooled and non-cooled cows either supplemental rbST or not and it decreased as lactation advances.

These findings confirm that an increase in milk yield in response to rbST administration will not be sustained indefinitely (Bauman, 1992), and it is influenced by the stage of lactation (Phipps et al., 1991). The low potential for extended persistency of lactation in rbST treated animals appears similar to that which occurs in higher yielding cows (Chase, 1993).

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

During lactation, the blood flow to the mammary gland is the major parameter controlling milk production. In the present study, an increased mammary blood flow was concomitant with an increase in IGF-I in the rbST treated cows in both cooled and non-cooled. This study focused on the effect of IGF-I on mammary blood flow and whether it increased the availability of substrates to the mammary gland. There were indications that GH plays a role, requiring IGF-I as a mediator, which in turn stimulates milk yield. The present results support previous studies on goats (Hart et al., 1980) and cows (Davis et al., 1988),which also reported an increase in mammary blood flow during administration of exogenous growth hormone at different periods of lactation. The ratio of udder blood flow to the rate of milk yield remain at the steady level as lactation advanced in both cooled and non-cooled cows whether supplemental rbST or not. A decrease in milk secretion concomittant with a decrease in mammary blood flow, caused no difference in a

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

In the present study, during long-term administrations of rbST in both cooled and non-cooled cows, milk yield rose to a peak in early lactation and then gradually declined as lactation advances, while the plasma concentration of IGF-I did not decrease in the rbST treated animals in both cooled and non-cooled cows. These findings suggest that the stimulatory effect of recombinant bovine GH on milk production is not mediated solely by IGF-I. Changes in milk production during the progress of lactation in rbST treated animals might not be controlled systemically but also locally within the mammary gland. There are a number of possible explanations for this apparent finding. It probably involves greater synthesis of plasma IGF-I binding proteins as lactation advances which combines with IGF-I in the blood and so modulates the level of free IGF-I before it reached the mammary gland. It has been reported that approximately 95% of the infused IGF-I is bound by IGF binding proteins (Davis et al., 1988). Mammary tissue is itself capable of synthesizing an IGF-I binding protein (e.g. IGFBP-5) during mammary gland involution in late lactation and this could inhibit IGF-mediated cell survival (Tonner et al., 1997; Flint & Knight, 1997) and initiate involution and a decrease in milk yield.

CHAPTER VII

GENERAL DISCUSSION

The studies in Chapter IV-VI for the effects of cooling using misty-fan cooling and recombinant bovine somatotropin on milk production relating to body and mammary gland glucose metabolism in crossbred Holstein cattle are involved changes in both extra-mammary factors and intra-mammary factors. The results presented in this study indicate that an application of misty-fans cooling (MF) to 87.5% crossbred Holsteins under hot environment could reduce the adverse effects of heat stress in animals and improve milk production. The environmental temperatures measured in NS and MF barns in the present study showed differences in AT and THI, especially in the afternoon throughout the experimental periods. However, the THI in both barns ranged from 77.8-85.5. Cows in both groups would be subjected to moderate heat stress (Fuquay, 1981). MF barn was not sufficient to completely eliminate heat stress in cows, because the range for THI measured at daytime under misters and fans throughout the experimental periods, were always higher than the critical value of comfortable zone (72 for THI) (Smith et al., 2006). THI values 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. However, a partial alleviation of heat stress under MF was significantly lower in both respiratory rate and rectal temperature and also higher milk yield in comparison with those of non-cooled cows throughout lactation. However, the respiratory rate and rectal temperature were increased during rbST supplementation in both cooled and non-cooled cows. These results agree with previous reports (Sullivan et al., 1992; Tarazon et al., 1999) in cows treated with rbST. Although rbST-treated cows increases heat production associated with high milk yield, it also increases heat dissipation (Johnson et al., 1991; West, 1994). Cows in both groups gained in weight as lactation progress.

It is known that body water is used for make up the largest portion of milk about 87 percent water and for evaporative cooling during heat dissipation mechanism. Body water is also used for the vehicle in blood distribution to mammary glands. Thus, water is the most important nutrient for lactating cows subjected to heat stress. Water intake is highly correlated with milk yield and DMI. Water turnover values in ruminants have been shown to be related to the food and water intake and metabolism of animal (Murphy, 1992) including exposure to high ambient temperature (Chaiyabutr et al., 1987). The present study was concerned with determining the effectiveness of misty-fan cooling and supplemental rbST on body fluids, mammary blood flow, milk production and nutrients uptake by the mammary gland in different stages of lactation of crossbred Holstein cattle (Chapter IV). Milk production is the result of coordination between nutrient delivery to and biosynthetic capacity of the mammary glands (Linzell and Mepham, 1974). The arterial plasma concentration of nutrients and mammary blood flow would be factors affect to the mode of nutrient uptake by the mammary gland for biosynthetic capacity. In the present results, the marked increases in blood flow to the mammary gland would be the determinant for milk synthesis which has been shown to coincide with an increase in milk yield during rbST supplementation in both cooled and non-cooled cows. These results were similar in all series of experiment (Chapter IV to VI). These results agree to previous studies by Chaiyabutr and co-worker (2005) that long-term administrations of rbST showed a marked increase in mammary blood flow throughout lactation. Factors that might affect to increase MBF during supplemental rbST could include an increasing relative mass of many organs and tissue including mammary tissue (Moallem et al. 2004) and an increase in cardiac output (Soderholm et al., 1988). However, crossbred HF animals with either short-term or long-term rbST treatment in different stages of lactation have also been shown to increase in MBF which were concomitant with an increase in circulating levels of IGF-I (Chaiyabutr et al., 2005; Maksiri et al., 2005; Tanwattana et al., 2003). It indicates that bST plays a role for an increase in MBF, requiring IGF-I as a mediator increasing MBF directly (Forsyth, 1996). The results in Chapter VI for increases in IGF-I and MBF during supplemental rbST in

both cooled and non-cooled cows in different stages of lactation were similar to those of previous studies. In the mammary gland, the role played by insulin seems to be different. The present experiments demonstrate that an increase in mammary glucose uptake in rbST in both cooled and non-cooled cows would not be attributed to the changes in plasma insulin concentration. The results in ChapterVI have provided evidence that the mammary glucose uptake, lactose secretion and milk yield of cooled and non-cooled cows were not affected by both circulating insulin levels and plasma glucose concentration during rbST supplementation. These results support the view that in the ruminant, the mammary gland appears to be relatively insensitve to insulin.

The marked increases in both the absolute values of plasma volume and blood volume, ECF and TBW in both cooled and non-cooled cows were apparent during the administration of rbST when compared with the pre-treatment period in each stage of lactation (Chapter IV). An increase in ECF leads to an increase in MBF as secondary responses, thereby the increase in MBF drives nutrients supply per se to the mammary gland and increase in milk production in rbST treated cows. However, during lactation advanced to late lactation in both cooled and non-cooled cows, the decline in milk yields were still apparent, although MBF, ECF TBW were still in high levels during supplemental rbST. These results suggest that an increase in milk yield of crossbred dairy cattle in response to rbST administration will not be sustained indefinitely (Bauman, 1992), and it is influenced by the stage of lactation (Phipps et al., 1991). These data suggest that changes in milk production during the progress of lactation in rbST treated cows might not be controlled systematically but also locally within the mammary gland (Chaiyabutr et al., 2005).

The supply of glucose is a principal determinant of the milk yield. Milk production requires glucose for synthesis of lactose which is essential for milk secretion and glucose moiety of lactose arises directly from plasma glucose (Ebner and Schanbacher, 1974). The studies in Chapter IV demonstrated that an increase in milk yield without an alteration of the plasma glucose concentration and A-V concentration

differences during supplemental rbST in both cooled and non-cooled cows indicates that this requires a substantial increase in supply of glucose to the mammary gland. An increase in mammary blood flow of cows supplemental rbST would be the mediation of nutrient delivery for glucose uptake by the mammary gland (Linzell, 1973). However, in the present study (Chapter IV), an increase in mammary plasma flow during rbST supplementation in each stage of lactation would not be a major factor for increase in milk production throughout lactation. Other investigations have shown that mammary glucose uptake was depended on an increase in the arterial plasma glucose concentration during bST administration (Sandles et al., 1988; Fullerton et al., 1989), whereas other works have demonstrated no differences (McDowell et al., 1987; Mepham, 1993). The present results support the latter observations during rbST supplementation. It is possible that an increase in a number of specific glucose transporters at the mammary cell membrane was related with an increase in body protein synthesis during rbST administration,which might proportion to an increase in MBF (Prosser, 1988; Madon et al., 1990). Therefore, the limited transport of glucose into mammary cell would not apparent by these means.

The major of energy source of normal fed ruminants are the volatile fatty acids in the form of acetate and β-hydroxybutyrate. In the present study, mammary arteriovenous concentration differences, mammary extraction and mammary uptake of acetate and β-hydroxybutyrate were not affected during rbST supplementation in different stages of lactation in both cooled and non-cooled cows (Chapter IV). Both acetate and β-hydroxybutyrate uptake were not dependent upon the rate of mammary blood flow. Acetate is known to involve in mammary gland metabolism in either de novo synthesis of short and medium-chain milk fatty acids or generation of ATP and NADPH. It is known that the circulating β-hydroxybutyrate arise mainly from rumen butyrate in the fed animal (Leng and West, 1969), and the principal effect of bST has been shown to increase oxidation of free fatty acids during negative energy balance in high yield lactating cows. An increase in the concentration of plasma β-hydroxybutyrate would be consistent with an increase in oxidation of free fatty acids (Bauman et al., 1988) e.g. during hepatic

ketogenesis due to greater mobilization of fat reserves in starved animals (Schultz, 1974), which were not apparent during rbST-supplementation in both cooled and non-cooled cows

During rbST supplementation the mean values for the arterial plasma concentration of free fatty acids but not for triacylglycerol increased which was more sensitive to alteration than other blood substrates. This phenomenon has been proposed as an indication of under-nutrition (Reid and Hinks, 1962). However, cows in both cooled and non-cooled cows gained weight throughout the experimental periods. A marked increase in milk yield with rbST supplementation without loss of body weight, especially during early lactation, may be due to the fact that cows were offered TMR diet to allow an adequate replacement of body reserves during lactations. Milk yield in the first lactating crossbred cows in the present study were not as great as that of multiparous cows (Sullivan et al., 1992). This is possibly related to the continued weight gain of cows during their first lactation. During early lactation, the metabolic demands of lactation during supplemental rbST in both cooled and non-cooled cows were met by dietary intake, thus no apparent mobilization of body tissues as indicated by unchanged levels of plasma triglyceride. The marked increases in the plasma concentrations of FFA were apparent in cows supplemental rbST in both cooled and non-cooled cows especially in mid and late stages of lactation are reported in Chapter IV. Thus, the lipolytic activity would be a function of rbST treatment per se in stead of the associated changes in energy balance. The measurement of A-V differences of FFA across the mammary gland together with mammary blood flow did not provide a quantitative estimation of their total uptake by mammary tissue. The high uptake of triacylglycerol by the mammary gland especially significant increase in the late lactation in cows supplemental rbST, which is agree with the results reported by Miller et al. (1991). It is possible that the negative mammary uptakes of free fatty acids may reflect hydrolysis of triacylglycerol, since there is the release of FFA into venous blood due to triacylglycerol hydrolysis during the uptake of plasma triacylglycerol as in lactation (West et al., 1967). The releasing of FFA would be as a result of enzymatic activity of lipoprotein lipase in the mammary tissue which has

been reported to be higher in the mammary tissue relative to other tissue (Shirley et al., 1973; Bauman and Griinari, 2003). Local changes for biosynthetic capacity within the mammary gland would be a factor in identification of the utilization of substrates in the rate of decline in milk yield with advancing lactation.

In the present study, the balance data for the utilization of both short chain and long chain fatty acids were performed by calculating their likely contribution to milk free fatty acids knowing its composition and substracting these values from the measured uptake of the substrates (Figure 7.1-7.6). Acetate and β-hydroxybutyrate were grouped together because it is known that they both contribute to the synthesis of milk fatty acids including C_{16} (Annison et al., 1968). It is clear that uptake of milk fat precursors confirm the theory that acetate and β- hydroxybutyrate are major precursor of milk fatty acids. The present studies confirmed the previoius report that there was a negligible oxidation of free fatty acid by the mammary gland in normal fed cows (Chaiyabutr et al., 2007b).

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

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

possible that both cooled and non-cooled cows were in positive energy balance, since irreversible losses of glucose has been shown to increase in cows with negative energy balance (McDowell et al.,1987). The reversible turnover rate of [3-^{3}H]glucose represents the total glucose turnover rate as the ^{3}H is not recycled from products of partial glucose degradation (Katz et al ., 1965). Thus, simultaneous injection of [3-^{3}H]glucose and [U-^{14}C]glucose was used to estimate the recycling of glucose-C as in the present studies in cooled and non-cooled cows, which showed no differences between the pretreatment and rbST treated period in all stages of lactation (Chapter V). These findings suggest that a constant level of tricarbon units originally derived from glucose being again reincorporated into glucose, which was not affected by either cooling or the supplemental rbST.

The utilization of glucose across the mammary gland during supplemental rbST in both cooled and non-cooled cows at different stages of lactation are complex regulatory mechanisms, it would depend on the partitioning of blood flow between extra-mammary tissues and local regulation. The results from Chapter V show that the mammary uptake of plasma glucose in both groups are not based on changes in A-V concentration differences and extraction ratio of glucose. In all stages of lactation, the net mammary glucose uptake increased approximately 8-48% during supplemental rbST as compared with the pretreatment period in both groups. Glucose extracted by the mammary gland has several possible metabolic fates in mammary epithelial cells that may occur at another level than transmembrane transport (Xiao and Cant, 2003). The glucose uptake by the mammary gland was rate limiting for the transport of glucose to the mammary cell during supplemental rbST and cooling. The high blood flow to the mammary gland would decrease the transit time of glucose during supplemental rbST, thereby reduction for prolonging the contact time between glucose in blood and glucose transporter in mammary epithelial cell (Chaiyabutr et al., 2007[b]).

Since glucose cannot synthesize by the bovine mammary gland, which lacking glucose-6-phosphatase (Scott et al., 1976). Glucose plays a crucial role in their

metabolism and lactose synthesis, which is formed in Golgi vesicles from a combination of glucose either directly or after phosphorylation to glucose 6-phosphate and conversion to UDP-galactose (Ebner and Schanbacher, 1974). The calculated amount of metabolism of glucose 6-phosphate to the galactose moiety of lactose during supplemental rbST in both cooled and non-cooled cows in different stages of lactation would be sufficient to account for the cytosolic lactose synthesis.The utilization of glucose carbon incorporation to lactose in the udder increased in both early and mid lactation but not for late lactation during supplemental rbST in both cooled and non-cooled cows. The decrease in the metabolism of glucose 6-phosphate to the galactose moiety of lactose as lactation advanced to late lactation in both cooled and non-cooled cows would affect to the lactose synthesis and milk production in both groups (Chapter V). A low enzymatic activity for lactose synthesis might be expected to appear as lactation advances. According to Davis and Bauman (1974), 50 to 60% of the glucose in the glucose-6-phosphate pool is converted into galactose. Major part of the galactose has been shown to derive from mammary extracted glucose, as well as from glycerol and other metabolic pathways. However, glucose is not the sole carbon source for lactose synthesis but remains the main one. An increase in the glucose concentration in milk representing an increase in glucose concentration in the mammary epithelial cell during prolonged treatment of rbST has been noted (Chaiyabutr et al., 2008[c]).

It is known that 80–85% of lactose carbon atoms arise from glucose (Faulkner and Peaker, 1987; Bickerstaffe et al., 1974). The quantitative utilization of the glucose taken up by the mammary gland is used directly in the synthesis of lactose , while the remaining of extracted glucose can participate in the supply of ATP (Embden-Meyerhof pathway and the tricarboxylic acid cycle), other portions would be metabolized via the pentose phosphate pathway, The studies in Chapter V are reported that glucose 6-phosphate metabolized via the pentose phosphate pathway by average 10-17% throughout lactation in both cooled and non-cooled cows without rbST, while it increased from 13 % in pretreatment to 15 % on early and mid-lactation but it decreased in the late lactation after supplemental rbST. These results also agree with prolonged treatment of rbST in

crossbred HF cows showing that percentage values of glucose 6-phosphate metabolized via the pentose phosphate pathway were variable in different stages of lactation (Chaiyabutr et al., 2008[c]). These values are different in comparable to those obtained previously in the isolated perfused udder of cow by Wood and co- workers (1965), in which about 23- 30% of the glucose was metabolized via the pentose phosphate pathway . It is probable that no consideration of the recycling of glucose 6-phosphate metabolized via the pentose cycle in the udder with the consequent loss of ^{3}H from glucose 6-phosphate (Davis and Bauman 1974). However, the net proportion of the metabolism of glucose 6-phosphate via the pentose cycle pathway was increased during supplemental rbST at early stage of lactation of cooled and non-cooled cows. Metabolism of glucose via the pentose phosphate pathway yields 2 molecules of NADPH per molecule of glucose, only one of which could be labelled with ^{3}H in the present experiments . In the present studies , estimates of the contribution of the pentose phosphate pathway in providing NADPH for fatty acid synthesis *in vivo* have been estimated by based on the assumption that all the glucose that was oxidized to CO_2 was metabolized via the pentose phosphate pathway. High metabolism of glucose 6-phosphate in early lactation of rbST treated cows appeared to be due primarily to a high flux through the lactose synthesis and to pentose phosphate pathway , probably reflecting the high milk production during rbST supplementation.

Absolute amount of glucose carbon incorporation to milk lactose was significantly higher during supplemental rbST in early and mid lactation in both cooled and non-cooled cows but, it decreased in late lactation. These finding would parallel to it effects on milk yield. It indicates that during rbST supplementation in late lactation, the metabolism of glucose-6-phosphate declines the flux towards the pentose phosphate pathway and in lactose synthesis. In parallel, a higher proportion of glucose-6-phosphate would be metabolized via the Embden-Meyerhof pathway and was oxidized in the tricarboxylic acid cycle. During supplemental rbST in each stage of lactation, both the proportion and absolute amount of glucose carbon incorporation to milk triacylglycerol

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

Glucose can also participate in the milk fat formation, by supplying the glycerol (triose phosphate pathway) and the NADPH essential to elongating milk fatty acids (pentose phosphate and isocitrate dehydrogenase pathways). Data findings in Chapter V provide evidence that 17% to 35 % of the NADPH required for fatty acid synthesis *de novo* in the udder of cooled and non-cooled cows without rbST arose from glucose metabolism, while 18% to 27% of the NADPH was required during rbST supplementation. If there is a common pool of glucose 6-phosphate which is available for both lactose synthesis and pentose phosphate metabolism, then the recycling of glucose 6-phosphate within the udder would result in too low a value for NADPH production from glucose. The net metabolism of glucose in the pentose phosphate pathway can be calculated from the incorporation of ^{3}H from [3-^{3}H]glucose in fatty acids assuming that the NADPH is used exclusively for biosynthesis of fatty acids (Katz et al., 1974). This

technique has been used to study the *in vitro* metabolism of rat mammary and adipose tissue (Katz and Wals, 1970,1972; Katz et al., 1966) and it was also used for the study of the *in vivo* metabolism of goat mammary tissue (Chaiyabutr et al., 1980).

Metabolism of glucose 6-phosphate via the pentose phosphate pathway usually loss of all ^{3}H from [3-^{3}H]glucose in lactating cows. During lactation, a higher level of $^{3}H/^{14}C$ ratio in milk triacyglycerol (Chapter V) was due to an increase in disequilibrium of the triose phosphate isomerase reaction occuring in the udder of crossbred animals which needs to be further investigated. Tritium and carbon-14 in glucose molecule were also shown to be incorporated into milk citrate which provided by averaged 22 μmol/min (16.5-.25.5) in cooled and non-cooled cows without rbST and provided by averaged 16 μmol/min (8.8-21.2) for the carbon skeleton of citrate during rbST supplementation in both groups. Milk citrate could be synthesized from 2-oxoglutarate via the NADP-dependent isocitrate dehydrogenase reaction (Hardwick, 1965). In addition ^{3}H is lost to NADPH or water in metabolism via the pentose phosphate pathway or glycolytic pathway, so it is likely that ^{3}H incorporation into milk citrate was also via NADP^{3}H. It is possible that the incorporation of ^{3}H into milk citrate may occur in different manners in the exchange reaction of the cytosolic NADP- dependent isocitrate dehydrogenase. Both fatty acid synthesis and the NADP-dependent isocitrate dehydrogenase reaction may have different mechanisms with a common pool of cytosolic NADPH between cows without rbST and cows supplemental rbST. Significant increases in the concentration of FFA in milk were apparent in cooled and non-cooled cows supplemental rbST as compared with the pretreatment period in each stage of lactation (Chapter V). A similar result for an increase in milk fat content due to prolonged administration of rbST has also been observed previously (West et al., 1991; Chaiyabutr et al., 2008[b]). It has been known that milk fat is synthesized from fatty acids of both blood lipids and from de novo synthesis within the mammary epithelial cells.

In conclusion, the results in this study demonstrates that an increase in MBF during rbST supplementation would be a major determinant in the mediation of nutrient

delivery and uptake by the mammary glands for increase in milk production. Local changes for biosynthetic capacity within the mammary gland would be a factor in identification of the utilization of substrates in the rate of decline in milk yield with advancing lactation in both cooled and non-cooled cows whether supplemental rbST or not. The esti mation *in vivo* of glucose metabolism in the mammary gland and its distribution to lactose synthesis , the pentose phosphate pathway and the Embden-Meyerhof pathway by the effects of supplemental rbST and cooling in 87.5% HF animals. The rbST exerts its galactopoietic action, in part, association with an increase in mammary blood flow, which partitions the distribution of glucose to the mammary gland. The stimulant effect for milk yield by supplemental rbST was transiently and the glucose turnover rate was not significantly increased as compared with pre-treatment period in all stages of lactation. It indicates that rbST induced enhancement of milk yield in all stages of lactation, which would be compensated by mobilization of body energy reserves (i.e. plasma free fatty acids) to the extent of the elevated energy requirements for supporting the increased milk production. During early and mid lactation, the glucose taken up by the udder of both cooled and non-cooled cows without supplemental rbST, an average 14% and 12% were metabolized in the pentose phosphate pathway and contributed to NADPH production, respectively. During supplemental rbST, the glucose taken up by the udder of both cooled and non-cooled cows, an average 13% and 12% were metabolized in the pentose phosphate pathway and contributed to NADPH production , respectively. An increased flux of the sufficient pool of intracellular glucose 6-phosphate during early and mid lactation came across through the lactose synthesis and pentose cycle pathway during rbST supplementation. On late lactation of cooled and non-cooled cows, the glucose taken up by the udder were metabolized in the pentose phosphate pathway by averaged from 11% to 7.4% and contributed to NADPH production from 30% to 22% after supplemental rbST. In the present study, mammary plasma flow was significantly increased after rbST supplementation, while milk yield of rbST-treated cows was not significantly greater than that of pretreatment in late lactation. It would appear that a larger proportion of the glucose 6-phosphate is metabolized via Embden-Meyerhof

pathway in late lactation. The present study indicate that the regulation of biosynthetic capacity within the mammary gland would be influenced more by local than by systemic factors in identification of the utilization of substrates in the rate of decline in milk yield with advanced lactation. During long-term administrations of rbST in both cooled and non-cooled cows, milk yield rose to a peak in early lactation and then gradually declined as lactation advances, while the plasma concentration of IGF-I did not decrease in the rbST treated animals in both cooled and non-cooled cows. These findings suggest that the stimulatory effect of recombinant bovine GH on milk production is not mediated solely by IGF-I. Changes in milk production during the progress of lactation in rbST treated animals might not be controlled systemically but also locally within the mammary gland.

Figure 7.1 The metabolic pathway involved in the metabolism of the precursor of milk in early lactation period during rbST administration of Holstein cows housing in normal shade (NS). (The value shown are in micromole/min.)

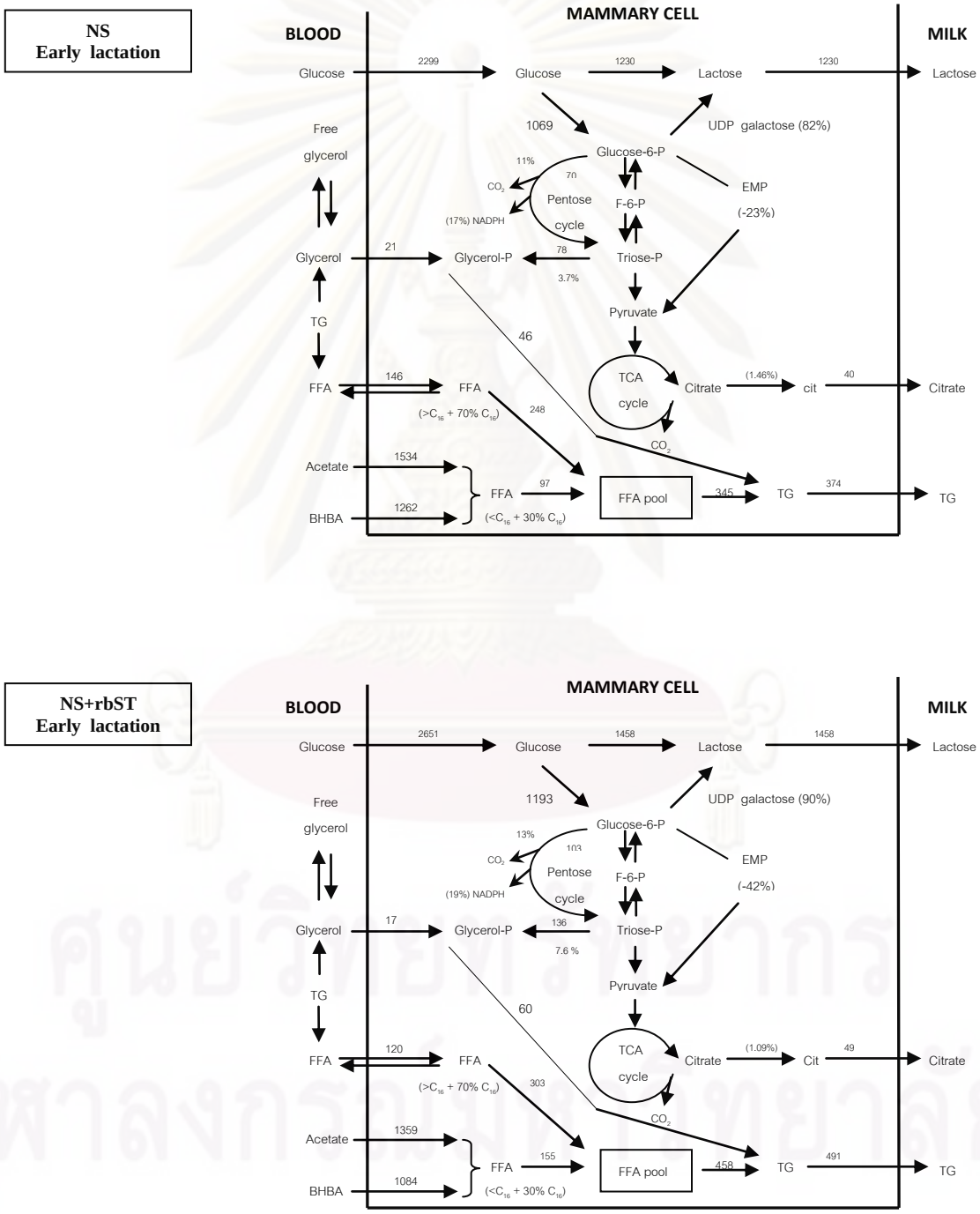


Figure 7.2 The metabolic pathway involved in the metabolism of the precursor of milk in mid lactation period during rbST administration of Holstein cows housing in normal shade (NS). (The value shown are in micromole/min.)

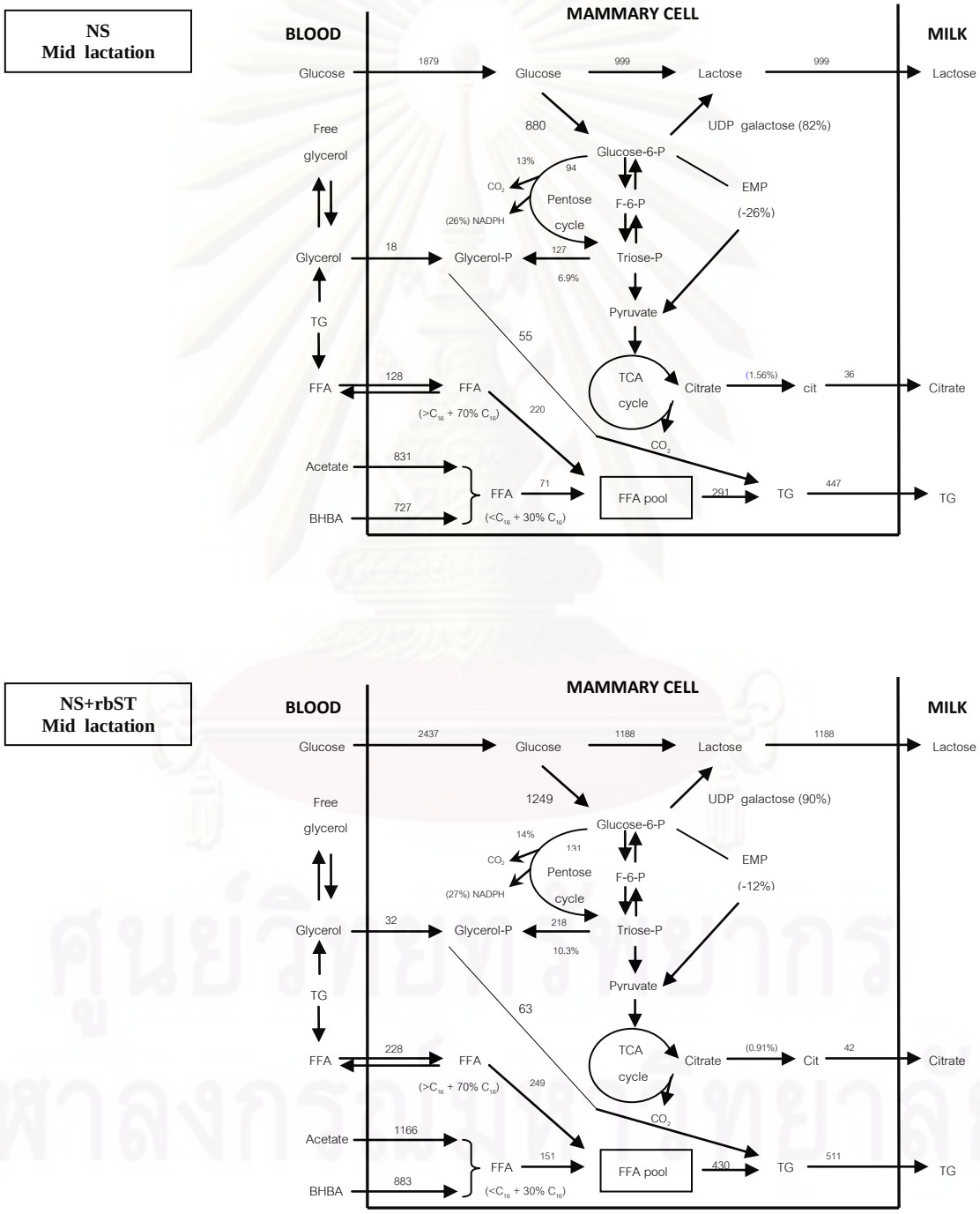


Figure 7.3 The metabolic pathway involved in the metabolism of the precursor of milk in late lactation period during rbST administration of Holstein cows housing in normal shade (NS). (The value shown are in micromole/min.)

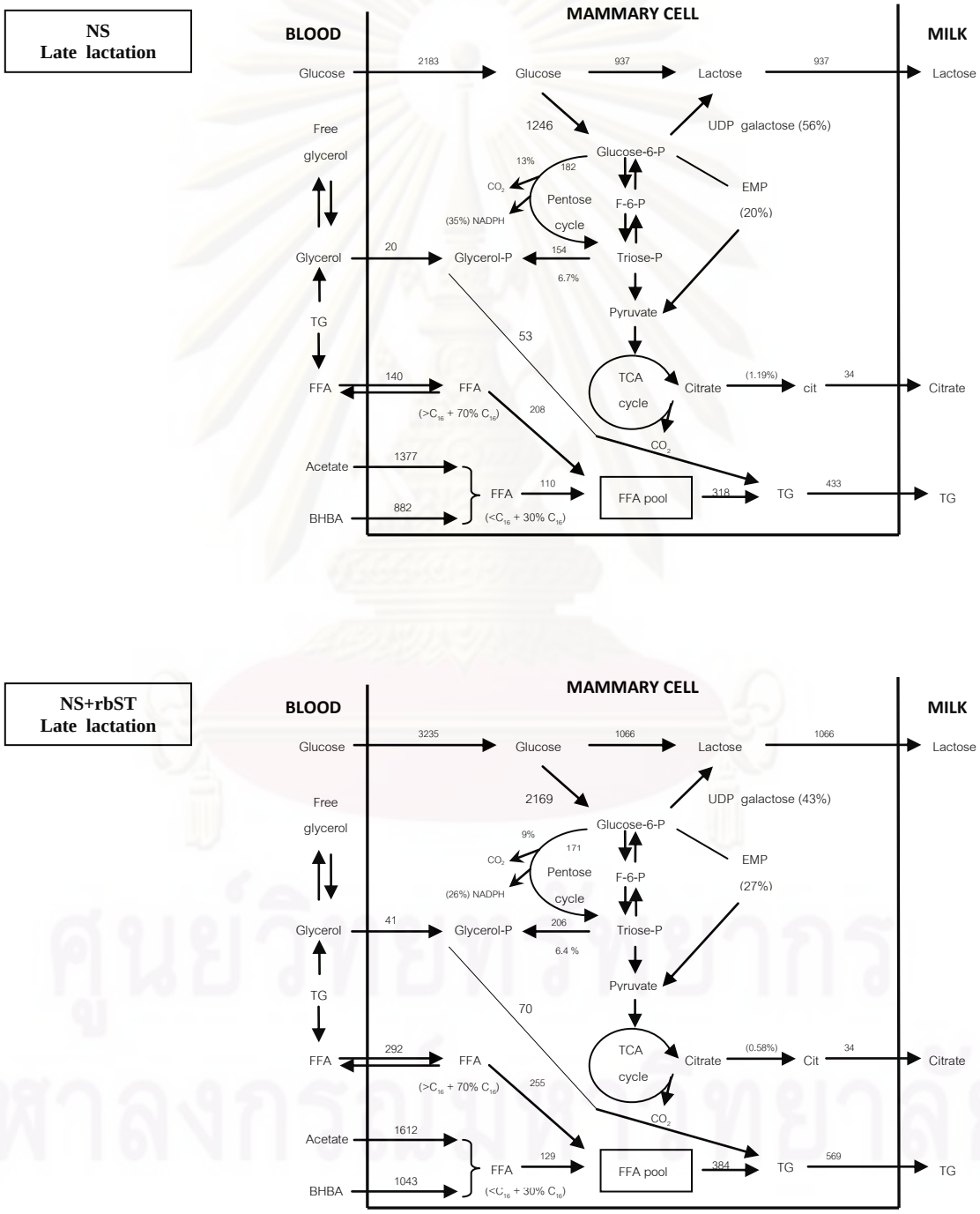


Figure 7.4 The metabolic pathway involved in the metabolism of the precursor of milk in early lactation period during rbST administration of Holstein cows housing in misty-fan cooling system (MF). (The value shown are in micromole/min.)

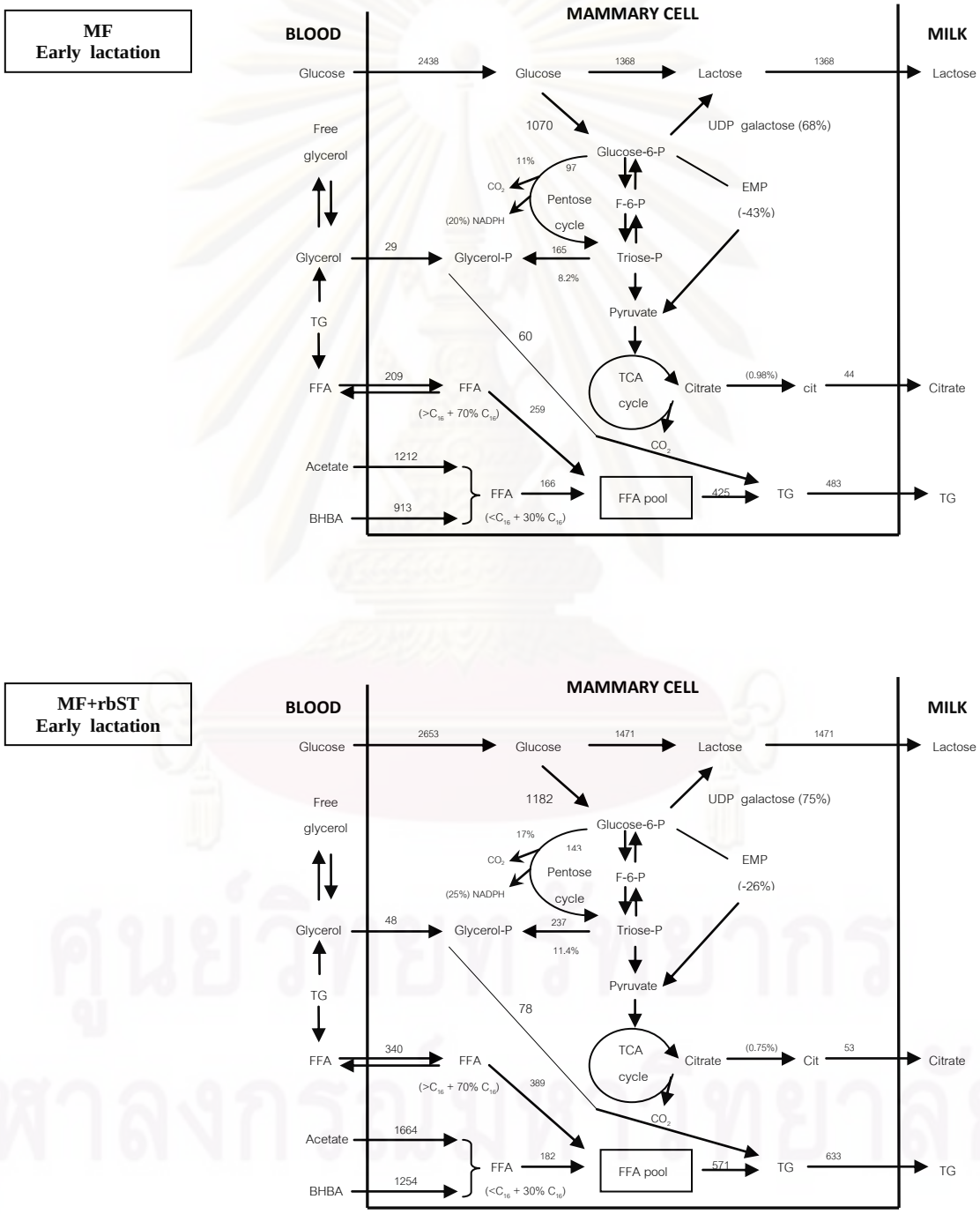


Figure 7.5 The metabolic pathway involved in the metabolism of the precursor of milk in mid lactation period during rbST administration of Holstein cows housing in misty-fan cooling system (MF). (The value shown are in micromole/min.)

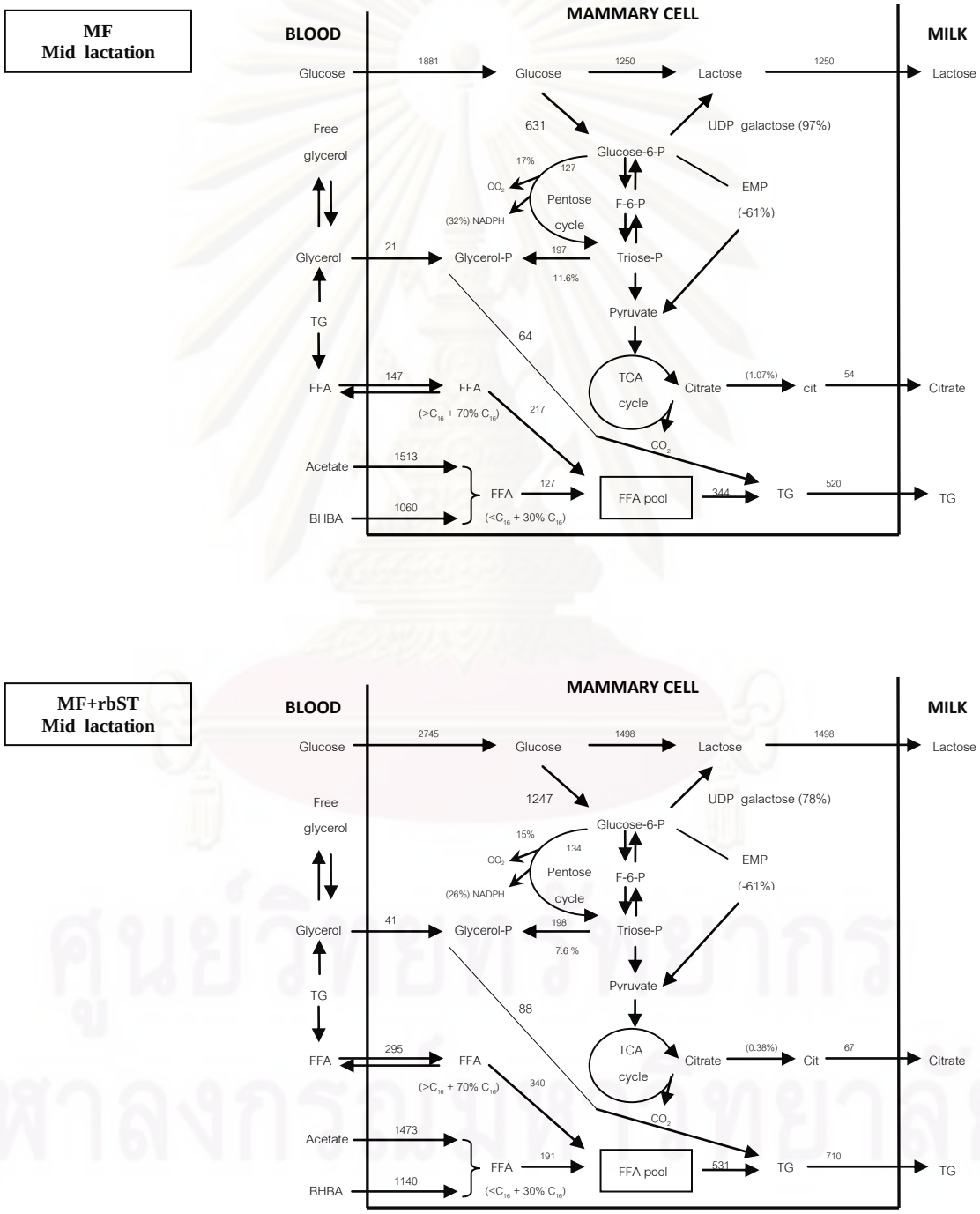
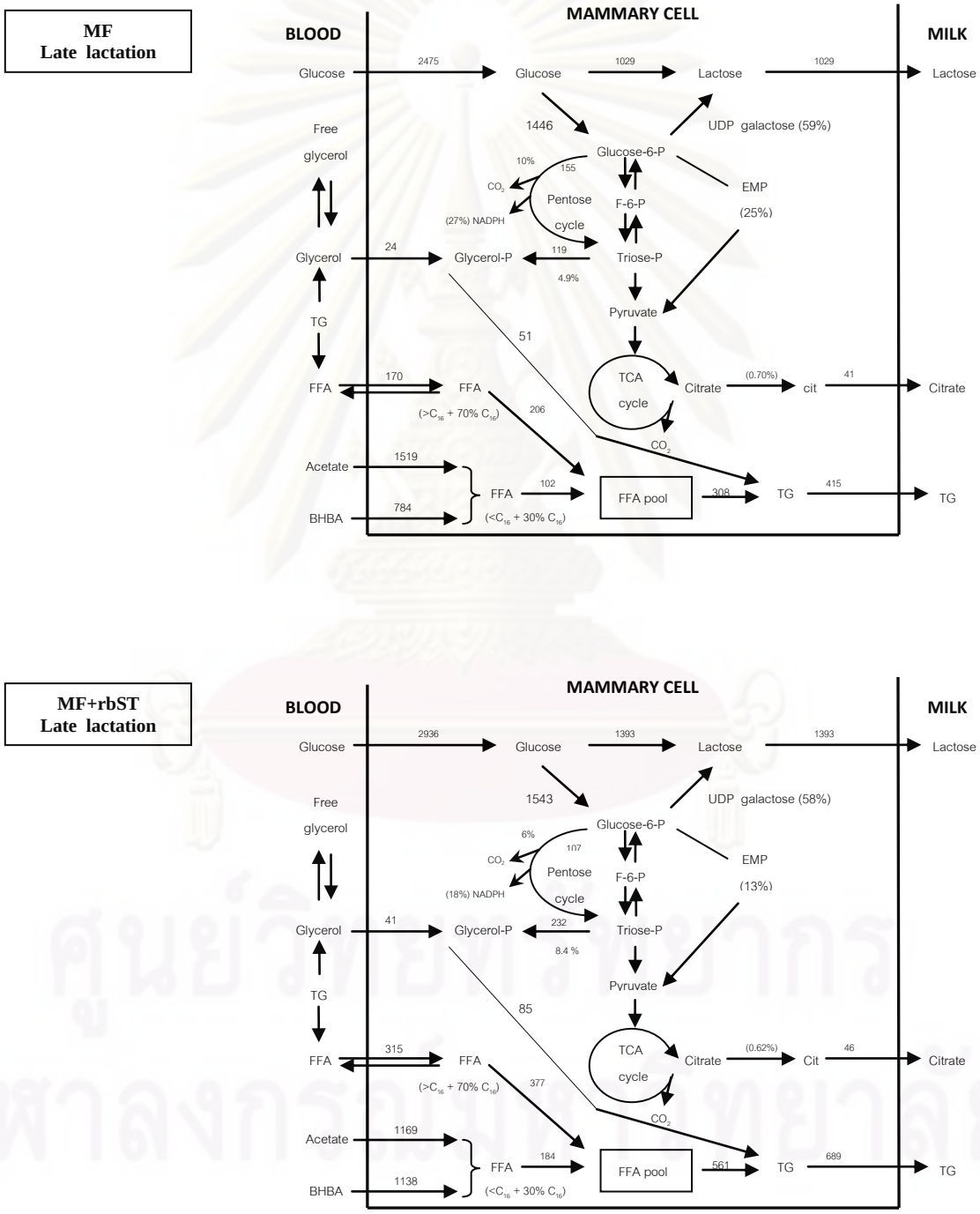


Figure 7.6 The metabolic pathway involved in the metabolism of the precursor of milk in late lactation period during rbST administration of Holstein cows housing in misty-fan cooling system (MF). (The value shown are in micromole/min.)



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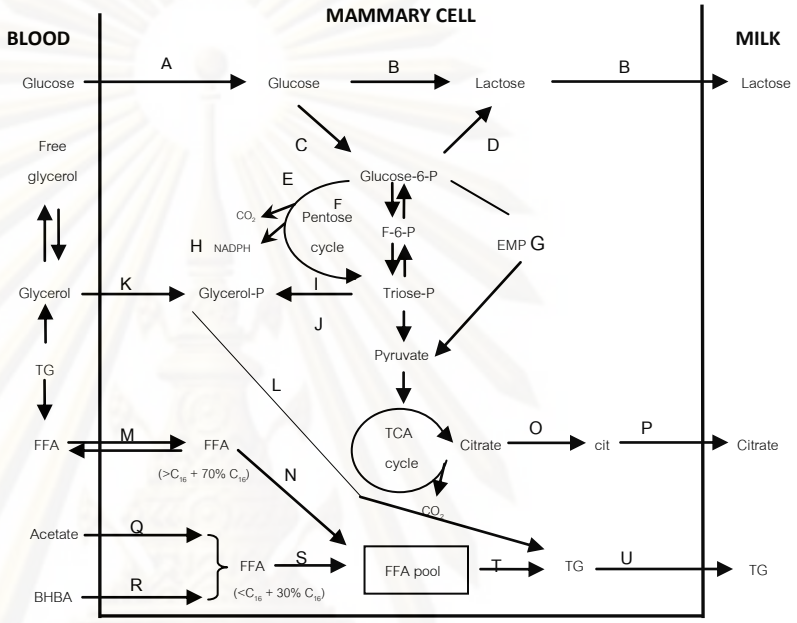
APPENDIX

The metabolic pathway involved in the metabolism of the precursor of milk at different stage of lactation during rbST administration of Holstein cows housing in misty-fan cooling system (MF). (The value shown are in micromole/min.)



A = Mammary glucose uptake

B = Milk lactose secretion

C = A – B

D = Percentage of metabolism of glucose – 6 – phosphate via the galactose moiety of lactose

E = Percentage of net metabolism of glucose – 6 – phosphate via the pentose phosphate pathway

F = Net metabolism of glucose – 6 – phosphate via the pentose phosphate pathway

G = Percentage of metabolism of glucose – 6 – phosphate via the EMP

H = Percentage of requirement of all NADPH formation from glucose via the pentose phosphate pathway

I = [14-C] - Glucose incorporation into milk triacylglycerol (TG)

J = Percentage of glucose carbon appearing as milk TG

K = Udder uptake of plasma TG concentration x MW of glycerol / MW of TG

L = Milk TG secretion x MW of glycerol / MW of TG

M = Udder uptake of plasma TG concentration $\times \frac{\text{MW of TG} - \text{MW of glycerol}}{\text{MW of TG}}$

N = Concentration of ($>C_{16}$ + 70% C_{16}) in milk fat x Milk yield

O = Percentage of glucose carbon appearing as milk citrate
P = Milk citrate secretion
Q = Udder uptake of plasma acetate concentration
R = Udder uptake of β- hydroxybutyrate concentration
S = Concentration of ($<C_{16}$ + 30% C_{16}) in milk fat x Milk yield
T = N + S
U = Milk TG secretion

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