Effects of Exogenous Bovine Somatotropin on Mammary Function of Late Lactating Crossbred Holstein Cows

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ABSTRACT : The objective of the present study was to determine the effect of exogenous bovine somatotropin on the mammary function in late lactating crossbred Holstein cows. Twelve 87.5% late lactating Holstein cows, approximately 30 weeks postpartum, were divided into two groups of 6 animals each. Animals in the control group were given sodium bicarbonate buffer by subcutaneous injection, while animals in the treated group were given recombinant bovine somatotropin (bST) by subcutaneous injection with 500 mg of bST (14 day prolonged-release bST). After bST injection, milk yield significantly increased from the control level on day 8 to day 20 ($p<0.05$) with a concomitant increase in mammary blood flow ($p<0.01$). An increase in mammary blood flow in response to bST treatment was greater than an increase in milk production. An increased plasma concentration of IGF-I coincided with an increase in mammary blood flow in animals treated with bST. There were no significant changes in the concentration of arterial plasma glucose concentration, the arteriovenous concentration difference (A-V difference) and mammary extraction ratio while the mammary glucose uptake increased when compared to the control group. The concentration of arterial plasma triglyceride decreased throughout the experimental period in animals give bST. The plasma concentration of acetate, and the mammary uptake for acetate significantly increased ($p<0.05$) after bST treatment. The action of bST did not affect the plasma concentration, A-V difference and extraction ratio across the mammary gland for β-hydroxybutyrate. The concentrations of milk fat and lactose tended to increase during bST treatment. Milk protein concentration initially increased in the first few days and decreased after bST injection when compared to the pretreated period. The present results indicated that bST could affect the mammary function in late lactating cows by increase in milk yield involving changes in both extra-mammary and intra-mammary mechanisms. The exogenous bST exerted its galactopoietic action through an increase in circulating IGF-I of the late lactating Crossbred Holstein cattle. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 1 : 88-95)***

Key Words : Bovine Somatotropin, Mammary Function, Late Lactation, Crossbred Holstein Cows

INTRODUCTION

The problems for dairy farming in Thailand are the low milk production and short lactating period of indigenous dairy cattle. Selecting the types of suitable crossbreeding of indiginous and exotic cattle for the tropics is practiced. Many factors can affect milk production in crossbred dairy cattle such as a high environment temperature, a lower genetic potential of dairy cattle and an inadequate supply of forage during summer. These factors can affect the physiological signals received by the mammary gland. The lactating mammary gland receives signals from the rest of body in the forms of nutrients and hormones from blood to sustain milk synthesis. Growth hormone is a major galactopoietic factor during lactation, one of its key effects being greater utilization of nutrients for milk synthesis. An increase in milk secretion in response to the treatment of bovine somatotropin (bST) requires partitioning of nutrients to accommodate an increase in the rate of milk synthesis by an increase in mammary uptake of both acetate and glucose (Fullerton, 1989). During the process of milk synthesis, mammary blood flow is a major parameter controlling milk production. Little is known on the circulating factors that involved in regulating mammary blood flow and milk synthesis. A number of studies have been carried out to elucidate the mechanism responsible for regulating mammary function during given growth hormone. Chaiyabutr et al. (2000a) reported that milk yield of 87.5% crossbred Holstein cattle decreased rapidly which coincided with the reduction of endogenous growth hormone and mammary blood flow during lactation advance to mid and late lactation. An increase in the circulating levels of growth hormone by subcutaneous injection in ruminants, milk yield has shown to rise by a mechanism which did not involve the direct action of growth hormone on the udder (Collier et al., 1984). The failure to identify specific growth hormone receptors in mammary tissue has been reported (Kazmer et al., 1986), although the presence of growth hormone receptors in mammary tissue has been noted (Glimm et al., 1990). In addition, an infusion of growth hormone into the mammary artery of sheep has also been shown not to increase milk yield (Peel and Bauman, 1987). It has been reported that growth hormone could affect mammary tissue indirectly by its action via insulin-like growth factor-I

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(IGF-I) (Peel and Bauman, 1987; Gluckman et al., 1987). An intra-arterial infusion of IGF-I into the goat mammary gland has been reported to increase both mammary blood flow and milk secretion (Prosser et al., 1990). IGF-I is a single chain peptides of 70 amino acids, synthesized mainly in the liver and other tissues (Granner, 1996) and it can be detected in milk from a number of species (Prosser et al., 1991ab). Furthermore, an increase in milk yield coincided with an increased concentrations of IGF-I in plasma and milk has been reported to occur in response to growth hormone treatment (Faulkner, 1999). However, few data are available concerning the effect of growth hormone on mammary function during late lactation in crossbred dairy cattle although mammary cell death has been demonstrated during tissue involution in the late lactating period (Walker et al., 1989). The objective of the study was, therefore, to obtain more information on whether changes in the mammary function for milk-production potentials during late lactation in crossbred dairy cattle are the direct action of growth hormone. Exogenous growth hormone administration was performed using recombinant bovine somatotropin.

MATERIALS AND METHODS

Animals and management

Twelve, non-pregnant, late lactating 87.5% crossbred Holstein cows, approximately 30 weeks postpartum were used in the experiment. They were divided into two groups of six animals each. Animals were housed in tie stall type shed, having a solid floor and open sides. The maximum temperature in the shed at noon was 34±1°C and minimum temperature at night was 26±1°C. The relative humidity was 60±4%. Animals individually received amount of concentrate mixture (7-10 kg/day) and roughage (3-5 kg/day) related to milk production and to maintain a moderate body condition score (2.5, scale=1-5). Water and mineral block were available for *ad libitum* intake. Cows were milked twice a day at (05:00 h and 16:00 h) and milk yield were recorded by weight.

Experimental procedures

Animals were divided into two groups, the control group and the experimental group. Three consecutive periods were assigned to each group, consisting of 1 week of the pre-treated period, 2 weeks of the experimental period and 1 week of the post-treated period. In the experimental period (day 1), animals in the control group were given sodium bicarbonate buffer (pH 9.4) while animals in the treated group were given recombinant bovine somatotropin (POSILAC®, Monsanto, USA) by subcutaneous injection at the tailhead depression (ischio-rectal fossa). The 14 day prolonged-release bST, contains 500 mg of sometribove zinc, which is equivalent to 36 mg of bST/cow/day. Measurements of mammary blood flow, milk yield and blood collections were performed on day -4, 4, 8, 12, 16 and 20 after bST treatment. On the day of experiment, around 1000 h mammary blood flow for half of the udder was performed either the left or right subcutaneous abdominal vein (milk vein) by using Flow meter (Transonic systems Inc., U.S.A.). After the measurement of mammary blood flow, simultaneously samples arterial and venous blood were collected from milk vein and coccygeal artery by venipuncture with a #21 needle into a heparinized tube. Blood samples were kept in crushed ice and then centrifuged at 3,000 rpm for 30 min at 4°C. Plasma samples from both coccygeal artery and milk vein were kept at -20°C until measurements of the concentration of both plasma hormone and metabolites.

Determinations of plasma and milk compositions

Plasma samples from both coccygeal artery and milk vein were used to determine the concentrations of glucose, triglyceride, β-hydroxybutyrate and acetate. The plasma glucose concentration was assayed by enzymatic oxidation in the presence of glucose oxidase (Human GmbH, Germany). The plasma triglyceride concentration was assayed by an enzymatic method (Human GmbH, Germany). The plasma β-hydroxybutyrate concentration was assayed using an enzymatic reaction in the presence of β-hydroxybutyrate dehydrogenase (Sigma Aldrich Co., USA.). The plasma acetate concentration was assayed by an enzymatic method (Boehringer Mannhelm). The arterial plasma IGF-I concentration was determined using the IGF-I kit (OCTEIA® IGF-I, ISD Ltd., UK.). The milk protein concentration was analyzed using Milkoscan. The milk lactose concentration was analyzed by the colorimetric method, as described by Tele et al. (1978). The milk fat concentration was measured using microcapillary centrifugal method (Chaiyabutr, 1994).

Calculations of parameters and statistical analysis

Arteriovenous concentration difference (A-V difference) and mammary extraction ratio of nutrients were calculated from the plasma substrate concentration in blood from both coccygeal artery and milk vein. Mammary uptake of nutrient was calculated using mammary plasma flow and nutrient A-V difference across the mammary gland. All data were presented as the means±SD. Statistical significant difference between period in the same group was determined by the paired t-test. The unpaired t-test was used to estimate the statistical significant difference between groups.

RESULTS

Effects of bST administration on the plasma concentration of IGF-I, mammary blood flow and milk yield (Table 1)

In the control group, there were no significant differences of mammary blood flow and plasma flow during the period of experiment. The daily milk yield and the ratio of blood flow to milk yield were maintained nearly at the same rate throughout the experimental period. The concentration of IGF-I in plasma decreased which was significantly different on day 8 ($p<0.05$) to day 20 ($p<0.01$) as compared to pre-treated value.

In the bST treated group, mammary blood flow increased stepwise throughout the period of study and it increased to the maximum value on day 4 ($p<0.01$) as compared to the pretreated value. Milk yield increased significantly on day 8 to day 20 ($p<0.05$) after bST injection. The ratio of mammary blood flow to milk yield increased after bST treatment and it returned to pre-treated value on day 16. The concentration of plasma IGF-I level before bST treatment was 62.67±18.50 ng/ml and it rose significantly on day 4 ($p<0.05$) and reaching maximum of 144.67±86.58 ng/ml on day 8 after bST treatment. In comparison between groups, the mean values of mammary blood flow and mammary plasma flow in animals treated with bST was higher than those of control animals throughout the experimental period. The mean concentration of plasma IGF-I in bST- treated cows was higher than those of animals in the control group and significantly higher ($p<0.05$) on day 4 of the treatment period.

Effect of bST administration on milk compositions (Table 2)

Table 2 showed the mean values of the concentration of milk protein, milk fat and milk lactose in the control group and in bST treated group. There were no significant differences of the concentration of lactose, milk protein and milk fat during the period of study in the control group. In the bST treated group, the concentrations of milk protein and milk fat concentration showed no difference in the treated period when compared to pretreated period. The concentration of milk lactose increased on day 8 and significantly increased on day 12 ($p<0.05$) after bST treatment. The concentration of milk protein in bST-treated animals was significantly lower ($p<0.05$) on day 16 than those of animals in the control group. The concentration of milk lactose in the bST-treated animals was significantly lower on day 4 ($p<0.05$) and day 12 ($p<0.01$) than those of animals in the control group.

Arterial plasma concentrations, mammary arterio-venous concentration difference, extraction ratio and mammary uptake for glucose and triglyceride during bST administration (Table 3)

In the control group, there were no significant differences of the arterial plasma glucose concentration while the concentration of arterial plasma triglyceride significantly increased on day 4 ($p<0.01$) in comparison to

Table 1. Mammary blood flow (MBF), mammary plasma flow (MPF), plasma IGF-I concentrations, milk yield, mammary blood flow/milk yield ratio and haematocrit in the control animals and animals treated with bST

	Pre-treatment	Treatment				Post-treatment
	Day -4	Day 4	Day 8	Day 12	Day 16	Day 20
MBF (ml/min)						
Control	1,889±53	1,972±195	1,942±119	1,955±97	1,931±171	1,938±178
Treatment	1,807±163	2,748±371**$^{\dagger -}$	2,715±582*†	2,464±111**$^{--}$	2,278±113**$^{--}$	2,160±65**†
MPF (ml/min)						
Control	1,336±37	1,406±178	1,398±145	1,413±116	1,398±159	1,413±162
Treatment	1,291±121	1,984±281**$^{\dagger -}$	1,946±397**$^{-}$	1,772±125**††	1,656±115**$^{--}$	1,581±60**†
IGF-I (ng/ml)						
Control	87.17±22.20	73.50± 6.98	68.33±19.11*	47.92±14.11**	44.17±12.73**	42.25±7.64**
Treatment	62.67±18.50	116.67±43.10*$^{-}$	144.67±86.58	84.25± 40.28	84.00±64.48	51.33±26.17
Milk yield (kg/day)						
Control	5.27±1.44	5.39±1.51	5.49±1.48	5.20±1.66	5.36±1.44	5.15±1.53
Treatment	4.89±0.94	5.91±1.39	5.98±1.65*	6.13±1.57*	6.37±1.17**	5.91±0.78*
MBF/ Milk yield						
Control	551±161	560±146	539±134	593±207	559±190	580±172
Treatment	556±160	710±236*	716±341	622±212	534±127	534±69
Haematocrit (%)						
Control	29.3±2.4	28.8±3.7	28.2±4.1	27.8±4.0	27.8±2.4*	27.2±3.7*
Treatment	28.6±1.3	27.8±2.0	28.1±3.7	28.2±1.9	27.3±1.8	26.8±2.8*

P-value by paired t-test with respect to the pretreated period in the same group. (** $p<0.01$, * $p<0.05$).

P-value by unpaired t-test with respect to the similar period of experiment between control and the group treated with bST, ($^{-}p<0.05$. $^{-1}p<0.01$).

Table 2. Milk compositions in the control animals and animals treated with bST

	Pre-treatment	Treatment				Post-treatment
	Day -4	Day 4	Day 8	Day 12	Day 16	Day 20
Milk composition						
Protein (gm%)						
Control	3.82±1.67	3.92±1.63	3.87±0.59	3.79±1.56	3.72±0.44	3.50±1.45
Treatment	3.42±0.43	3.67±0.73	3.37±0.34	3.38±0.38	3.04±0.47^{*-}	3.35±0.43
Fat (gm%)						
Control	4.22±1.20	3.80±0.83	3.75±0.88	4.23±1.99	4.26±1.71	3.85±1.45
Treatment	4.82±1.67	4.50±1.27	5.42±2.47	4.03±0.65	5.43±1.69	4.61±1.41
Lactose (gm%)						
Control	3.31±1.44	3.85±0.08	4.16±0.31	4.22±0.11	3.98±0.10	4.02±0.16
Treatment	3.81±0.19	3.66±0.13^{-}	4.01±0.40	4.01±0.07$^{*\dagger-}$	3.91±0.12	3.89±0.16

P-value by paired t-test with respect to the pretreated period in the same group, ($^{*}p<0.05$).
P-value by unpaired t-test with respect to the similar period of experiment between control and the group treated with bST. ($^{-}p<0.05$, $^{-\dagger}p<0.01$).

Table 3. Arterial plasma concentrations, mammary arterio-venous difference and mammary uptake for glucose and triglyceride (TG) during bST administration

	Pre-treatment	Treatment				Post-treatment
	Day -4	Day 4	Day 8	Day 12	Day 16	Day 20
Glucose						
Arterial concentration (mmol/l)						
Control	2.79±0.51	2.77±0.35	2.68±0.42	2.80±0.29	2.79±0.32	2.78±0.32
Treatment	2.70±0.41	2.56±0.28	2.37±0.15	2.35±0.23†	2.72±0.44	3.49±0.42*††
A-V dif (mmol/l):						
Control	0.59±0.36	0.58±0.20	0.67±0.34	0.70±0.14	0.72±0.22	0.58±0.24
Treatment	0.74±0.25	0.81±0.14†	0.72±0.27	0.65±0.30	0.74±0.54	0.62±0.18
Extraction ratio (%):						
Control	23.2±18.3	20.7±5.3	24.8±13.1	24.9±4.9	25.6±5.8	21.0±8.9
Treatment	28.6±12.9	31.7±5.9$^{\dagger-}$	30.0±9.8	27.2±11.9	25.2±15.0	17.6±4.2
Glucose uptake (μmole/min):						
Control	796±507	819±294	944±489	991±254	994±270	813±331
Treatment	951±355	1,581±269$^{**\dagger-}$	1,352±377	1,149±537	1,209±892	980±288
Triglyceride						
Arterial concentration (mmol/l):						
Control	0.57±0.08	0.71±0.03**	0.60±0.04	0.57±0.04	0.59±0.05	0.57±0.08
Treatment	0.54±0.08	0.41±0.03$^{*\dagger-}$	0.45±0.08*††	0.50±0.05†	0.45±0.06^{*--}	0.47±0.06†
A-V dif. (mmol/l):						
Control	0.06±0.03	0.07±0.04	0.10±0.05	0.09±0.02	0.08±0.01	0.08±0.05
Treatment	0.06±0.04	0.05±0.03	0.08±0.05	0.07±0.05	0.07±0.05	0.11±0.07
Extraction ratio (%):						
Control	11.1±5.1	9.8±6.0	16.4±9.0	15.1±4.0	12.9±1.9	13.1±8.4
Treatment	11.0±5.9	12.9±7.8	16.5±9.0	13.8±9.3	15.4±9.5	22.5±12.8
TG uptake (μmole/min):						
Control	84±38	93±50	115±45	115±31	106±24	108±72
Treatment	78±47	103±63	161±112	124±86	118±73	174±110

P-value by paired t-test with respect to the pretreated period in the same group. ($^{**}p<0.01$, $^{*}p<0.05$).
P-value by unpaired t-test with respect to the similar period of experiment between control and the group treated with bST. ($^{-}p<0.05$, $^{-\dagger}p<0.01$).

pretreatment period. There were no changes in the mammary A-V difference, the extraction ratio and the mammary uptake for both glucose and triglyceride throughout the period of study.

In animals treated with bST, there were no significant changes in the arterial plasma concentration of glucose, mammary A-V difference and the mammary extraction ratio of glucose after bST treatment. Mammary glucose uptake increased stepwise and it was significantly apparent on day 4 ($p<0.01$) after bST treatment. The concentration of arterial plasma triglyceride declined gradually during bST treatment and significantly decreased on day 4 and day 8 ($p<0.05$) after bST administration compared to the pretreated value, while there were no significant differences

of mammary A-V difference and the extraction ratio. The mammary uptake of triglyceride increased after bST treatment throughout the period of study.

In comparison between groups, the concentration of arterial plasma glucose in bST-treated animals was significant lower than those of animals in the control group on day 12. ($p<0.05$). The mammary extraction ratio of glucose was significantly higher ($p<0.01$) on day 4 in animals given bST as compared to those of animals in the control group. The mammary glucose uptake was higher on day 4 ($p<0.01$) in the bST treated animals than those of the control animals. The concentration of plasma triglyceride in animals treated with bST was lower whereas mammary triglyceride uptake was higher than those of control animals throughout the experimental period.

Arterial plasma concentrations, mammary arterio-venous difference and mammary uptake for acetate and β-hydroxybutyrate during bST administration (Table 4)

Table 4 showed that the arterial plasma concentration, mammary arteriovenous difference, extraction ratio and mammary uptake of acetate of animals in the control group did not alter throughout the period of study. In the bST treated animals, the concentration of arterial plasma acetate significantly increased on day 8 and day 12 ($p<0.05$) after bST administration. The plasma A-V difference of acetate across the mammary gland slightly increased on day 8 and day 12 whereas there were no changes in mammary extraction ratio of acetate after bST administration. The mammary acetate uptake significantly increased on day 8, day 12 and day 16 ($p<0.05$) after bST administration.

In comparison between groups, the mammary uptake of acetate in animals treated with bST was significantly higher ($p<0.05$) than those of animals in the control group on day 8 and day 12 of the study period. There were no significant differences in arterial plasma concentration, arteriovenous concentration difference and mammary extraction ratio of β-hydroxybutyrate throughout periods of study in comparison between control animals and animals given bST. The mammary uptake of β-hydroxybutyrate increased stepwise in animal treated with bST. In comparison between groups, the mammary uptake of β-hydroxybutyrate in animals given bST were higher than those of the control animals in the treatment period.

DISCUSSION

The present results showed that the lactating crossbred animals treated with bST increased milk yield throughout the experimental period with a concomitant increase in mammary blood flow. The proportion of an increase in mammary blood flow in response to bST treatment was greater than an increase in milk yield. These results provided the mechanisms that bST exerts it effect on both extra-mammary mechanisms that regulate blood flow to mammary gland and intra-mammary mechanisms that regulated utilization of nutrient for milk synthesis. In addition to the present study, Chaiyabutr et al. (2000a) provided a finding to support the role of growth hormone that during lactation advance, a decrease in milk yield of 87.5% crossbred Holstein cattle coincided with the reduction of endogenous growth hormone and mammary blood flow. The galactopoietic effect of recombinant bST given during late lactation would involve an increase in the synthetic capacity of the mammary gland (Nielson, 1988) which an involution of mammary epithelial cell would occur in this period.

In the present study, the utilization of glucose by the mammary gland was examined for intra-mammary factors during an evaluation of the effect of bST administration. It has been known that glucose is the major blood precursor for lactose synthesis. At least 85% of the carbon atom in milk lactose is derived from glucose. The linear relationship between glucose uptake by the mammary gland and milk volume reflected increased lactose synthesis along with the dilution to maintain osmolarity of milk (Linzell and Peaker, 1971). It was reported that an increase milk yield in response to growth hormone treatment requires partitioning of nutrients to accommodate an increase in the rate of milk synthesis. A decrease in glucose uptake in skeletal muscle and adipose tissues but an increase in glucose uptake in the mammary gland was noted in animals treated with growth hormone (Davis et al., 1988; Fullerton, 1989 and Zhao et al., 1996). In the present study, glucose A-V difference across the mammary gland in the animals treated with bST did not differ between the period of study. Thus, because of an increase in mammary blood flow, the uptake of glucose was about 29% higher after given bST compared to the pretreatment period. In the present study, the concentration of arterial plasma glucose slightly decreased during bST treatment which was different from the result of Peel et al. (1983), in which the plasma glucose concentration was unchanged during treatment at both early and late lactation (Miller et al., 1991). However, a significant rise in plasma glucose concentrations in goats treated with ovine growth hormone (Faulkner, 1999) and cows treated with pituitary-derived bovine growth hormone (Fleet et al., 1988) were also reported. The slight decrease in the plasma glucose level in response to bST treatment in late lactating crossbred animals might reflect a rapid enhanced mammary uptake of glucose following its mobilization from glycogen stores and hepatic gluconeogenesis (Fullerton, 1989).

The mammary A-V difference and the extraction ratio of acetate had a tendency to elevate in the bST-treated animals. Thus, the uptake of acetate by the mammary gland

Table 4. Arterial plasma concentrations, mammary arterio-venous difference and mammary uptake for acetate and β-hydroxybutyrate (β-HBA) during bST administration

	Pre-treatment	Treatment				Post-treatment
	Day -4	Day 4	Day 8	Day 12	Day 16	Day 20
Acetate						
Arterial concentration (mmol/l)						
Control	0.51±0.04	0.65±0.20	0.61±0.05	0.62±0.17	0.65±0.11	0.51±0.14
Treatment	0.53±0.09	0.71±0.14	0.71±0.09*	0.77±0.07*	0.66±0.11	0.65±0.03
A-V dif. (mmol/l)						
Control	0.29±0.07	0.34±0.07	0.32±0.07	0.31±0.10	0.33±0.12	0.30±0.06
Treatment	0.30±0.11	0.35±0.09	0.44±0.09†	0.48±0.10††	0.40±0.12	0.30±0.05
Extraction ratio (%)						
Control	57.3±18.3	54.0±7.6	53.3±13.3	50.0±8.5	49.8±10.4	60.6±17.3
Treatment	55.8±13.5	61.2±29.4	62.0±7.4	61.7±9.3†	59.8±15.4	46.5±5.5
Acetate uptake (μmole/min)						
Control	380±105	463±147	443±130	435±149	453±184	409±108
Treatment	397±158	744±264$^{-\dagger}$	943±239$^{-\dagger}$	829±168*†	682±211^{*-}	479±66
β-HBA						
Arterial concentration (mmol/l)						
Control	0.93±0.30	1.02±0.39	0.90±0.27	0.92±0.34	0.84±0.43	1.15±0.35
Treatment	1.29±0.42	1.18±0.37	1.03±0.27	1.26±0.26	1.06±0.27	1.17±0.19
A-V dif. (mmol/l)						
Control	0.57±0.42	0.62±0.38	0.50±0.30	0.68±0.35	0.52±0.49	0.68±0.30
Treatment	0.76±0.56	0.81±0.37	0.65±0.33	0.75±0.27	0.65±0.30	0.73±0.30
Extraction ratio (%)						
Control	64.9±25.9	58.7±21.5	56.2±25.4	75.5±26.0	55.6±19.3	58.9±14.6
Treatment	57.9±30.1	65.7±30.4	59.8±20.1	59.5±18.6	58.9±12.9	62.2±21.5
β-HBA uptake (μmole/min)						
Control	753±556	859±570	688±394	935±438	727±676	972±452
Treatment	1,020±761	1,620±821	1,277±642	1,335±479	1,074±507	1,155±460

P-value by paired t-test with respect to the pretreated period in the same group. (* $p<0.05$).

P-value by unpaired t-test with respect to the similar period of experiment between control and the group treated with bST, ($^{-}p<0.05$, $^{-\dagger}p<0.01$).

was resulted from increases in both mammary blood flow and the A-V difference. An increase in the rate of acetate uptake by the mammary gland was also reported in cow after 7 d of bST treatment and remained elevated for 10 d post-treatment (Fullerton et al., 1989). In the present study, animals received bST showed no effects on the plasma β-hydroxybutyrate concentration, mammary A-V difference and the extraction ratio. However mammary β-hydroxybutyrate uptake increased throughout the experimental period which was dependent on an increase in mammary blood flow. These results demonstrated that the mammary gland varies the extraction efficiency of volatile fatty acid to provide an adequate fatty acid supply for milk fat synthesis. This regulation probably involved the shifts in mammary metabolism in the mammary epithelial cell during late lactation (Chaiyabutr, 2000b), but this mechanisms in late lactation has not been well clarified.

In the present study, the concentration of milk fat showed a tendency to increase after bST treatment while the concentration of the arterial plasma triglyceride concentration decreased throughout the experimental period. Likewise the results of Miller et al. (1991), which showed a significant decrease in the plasma triglyceride level in mid-lactating cows treated with bST. These results indicated that bST has no effect on depot fat mobilization for milk fat synthesis. Changes of milk fat might involve *de novo* synthesis of short chain carbon (acetyl-CoA) and reducing equivalents in the mammary gland. In ruminant, the carbon sources used for fatty acid synthesis are acetate and β-hydroxybutyrate (β-HBA). Acetate seem to be an important carbon source for medium chain length of fatty acid. In the present study, the arterial plasma acetate concentration significantly increased on day 8 and day 12 after bST treatment. This finding was similar to the results of Fullerton et al. (1989) which found a significant increase in the level of plasma acetate in bST-treated cows. However, it did differ from results of Miller et al. (1991) and Fleet et al. (1988) that showed a decrease in the level of plasma acetate. Different results reported might be due to difference in the stages of lactation in animals used. Peel and Bauman (1987) reported that administration of bST did not change milk protein percentage when cows were in positive nitrogen balance, but the milk protein percentage of cows in negative nitrogen balance tended to decline. In the present study, milk protein tended to elevate a few day after bST injection. It slightly declined throughout the experimental period. It was

possible that animals used in the present study was late lactating cow, a low milk yield was not induced to be negative nitrogen balance. Voluntary feed intake was not affected after increases in milk yield in bST treated animals. The body condition showed to be adequate throughout the treatment period. Therefore, in late lactating animals, the nutrient partitioning response to bST treatment that supported increase in milk yield concerning substrate utilization by both peripheral tissues and the mammary gland might not in turn providing a stimulus for increasing feed intake (Bauman and Vernon, 1993).

In the present study, late lactating crossbred dairy cattle animals given bST increased the plasma concentration of IGF-I, which associated with an increase in mammary blood flow. The present results confirmed the finding in both cows and goats that the plasma IGF-I level increased in response to growth hormone treatment (Davis et al., 1988; Prosser et al., 1991b; Bauman and Vernon, 1993; McGuire et al., 1992). A number of studies indicated that bST exerted its effects indirectly on mammary blood flow by an increase in the IGF-I concentration in both blood and lactating mammary tissue during periods of bST administration (Glimm et al., 1988). Effects of IGF-I in local vessels of the mammary gland were demonstrated to relate on mammary blood flow (Prosser et al., 1990). An arterial infusion of IGF-I into the mammary gland was also shown to stimulate blood flow to the mammary gland and increase in milk production (Prosser et al., 1990). An increase in serum IGF-I paralleled with an increase in hepatic IGF-I mRNA indicating exogenous bST increases IGF-I synthesis in the liver of cows during late lactation (Sharma et al., 1994). IGF-I would play an endocrine role in mediating galactopoietic effects of exogenous bST during late lactation. Increases in concentrations of IGF-I in milk appeared to correlate with an increase in the level in mammary secretory tissue (Prosser et al., 1991b). The circulating level of IGF-I was reported to be major importance in promoting increased milk production, since an increase in milk yield correlated with changing levels of IGF-I in plasma than in milk has been noted (Faulkner, 1999).

In conclusion, exogenous bST exerted its effect on mammary function to stimulate milk secretion in late lactating crossbred Holstein cattle by involving of changes both extra-mammary mechanisms (mammary blood flow) and intra-mammary mechanisms (mammary nutrients uptake). The present study confirmed the mechanism by which growth hormone affect mammary gland function indirectly by mediated via the action of IGF-I. The action of IGF-I would cause an increase in blood flow to mammary gland and presentation of milk precursors to the gland for milk synthesis.

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