

Table 7: Milk compositions in the control animals and animals treated with rbST.Values are means $\pm$ SD. (n= 5).

		Period of			
		experiments	Control group	rbSTgroup	Control vs rbSTgroup ¹
Milk composition:					
Protein (gm%)	Pretreatment		3.15 $\pm$ 0.21	3.16 $\pm$ 0.16	NS
	Treatment		3.27 $\pm$ 0.15	3.16 $\pm$ 0.25	NS
Fat (gm%)	Pretreatment		3.60 $\pm$ 0.76	3.90 $\pm$ 0.60	NS
	Treatment		3.60 $\pm$ 0.25	4.70 $\pm$ 0.77*	P<0.05
Lactose (gm%)	Pretreatment		4.49 $\pm$ 1.02	4.90 $\pm$ 0.24	NS
	Treatment		4.52 $\pm$ 0.55	4.79 $\pm$ 0.49	NS

P-values by paired t-test: * P<0.05, with respect to the pretreated period in the same group.

¹Statistical analysis of treatment differences, NS = Nonsignificant (P>0.05).

DISCUSSION

The present study was designed to clarify whether a shorter lactation persistency of crossbred cattle containing 87.5% Holstein genes during lactation advance was due to the reduction of the growth hormone level (Chaiyabutr et al., 2000) or associated with some other mechanisms. We found that in the rbST-treated animal, milk yield over the 6 wk of the experiment significantly increased (by 19.8%) in the rbST - treated animals. Milk yield of the control animals receiving placebo slightly increased in the early period of lactation. Mishra and Shkla (2004) also reported higher milk yield of 25 % due to exogenous administration of rbST after 60 days of postpartum in lactating buffalo. It is recognized that an increase in milk production is closely correlated to dry matter intake and dry matter intake to water consumption (Murphy, 1992). In the present study, total DM intake was not significantly different between control animals and rbST-treated animals throughout experimental period. However, the effect of rbST administration significantly influenced the milk production efficiency. The ratio of dry matter intake to milk production was lower in rbST-treated animals as compared to those of control animals at treatment period of lactation. It indicates that the energy output in milk and for maintenance was greater than energy consumed in the food for the rbST-treated animals. The control animals were approximately in energy equilibrium, there being no change in the ratio of total DM intake to milk yield during period of study.

During lactation, dairy cattle consume more of water to make up the largest portion of milk and for evaporative cooling for heat dissipation mechanism. The rbST-treated animals increased water intake in the early period of lactation from 65 to 71 kg/day/animal, which was about 9% accounted for 19.8% of an increase milk yield from the pretreatment period. This result shows that milk production affects

water intake including body water turnover rate. The rbST-treated animals increased body fluid compartments i.e. TBW, EBW and plasma volume, while the control animals decreased TBW with that of a higher milk secretion in the early period of lactation. An increase in the EBW in rbST-treated animals would be due to an increase in ECF compartment, while ICF compartment did not change through the period of study. Thiocyanate space does not include rumen water; therefore changes of ruminal fluid volume would not affect an estimation of extracellular volume (Woodford et al., 1984). An increase in water intake with rbST treatment in early lactation would contribute to an elevation of gut water content.

An increase in both absolute TBW and ECF of rbST-treated animals agrees with the report in GH deficient human treated with GH (Janssen et al., 1997). An elevation of body weight with rbST treatment would be the direct effect of somatotropin on increases in body cell mass and fat free mass. High milk yield during early lactation usually occurs with negative energy balance with body fat mobilization causing a decrease in fat mass. This may be attributed to an increase in body water with rbST treatment. Further evidence has shown that GH (or IGF-1) may act directly on renal function relating to receptors of both GH and IGF-1 on the renal proximal tubular cell (Janssen et al., 1997). The sodium retaining by the effect of somatotropin on the renal tubular reabsorption of sodium would be another explanation for an expansion of both TBW and ECF.

A higher water reserve in animals given rbST would not only provide a higher reservoir of soluble metabolites for biosynthesis of milk but was also useful in slowing down the elevation in body temperature during lactation in hot conditions (Chaiyabutr et al., 1997). The decrease in TBW of the control animals from early to mid-lactation occurred rather rapidly which may be attributed to a relatively lower efficiency in the water retention mechanism in crossbred cattle containing

87.5%Holstein genes although the estimated water intake was slightly higher (Chaiyabutr et al., 1997). In the present study, the rbST-treated animals showed no significant changes of the water turnover rate per body fat free wet weight ($\text{kg}^{0.82}$) and the biological half-life of tritium in all periods of experiment in comparison to control animals. This indicates that increased losses of water with increase in milk yield with rbST treatment might be compensated by a larger body water pool, which animals could restore their body fluids to equilibrium in lactating period with no significant change of body water turnover rate and water half-life. Short persistency of lactation may not be occurred in rbST-treated animals during transition period from early to midlactation. This is a case in which a response pattern in milk yield of rbST-treated animals differed from that in early lactation of crossbred cattle containing 87.5%Holstein genes (Chaiyabutr et al 1999).

In the present study, increases in mammary blood flow to the udder of rbST-treated animals agree with several reports in both cows and goat (Davis et al., 1988; Mephram et al; 1984). A marked increase in mammary blood flow of rbST-treated animals could not be attributed to a change in blood volume and plasma volume, which remained nearly constant as a percent of body weight. In lactating dairy cows, increase blood flow to the mammary gland may allow plasma volume to remain nearly constant despite loss of body weight (Woodford et al., 1984).

The present results confirm the study in both cows and goats that the plasma IGF-1 level increased in response to growth hormone treatment. (Davis et al., 1988; Gulay et al., 2004). Several investigations show the effect of rbST on mammary circulation was indirect, mediated via IGF-1 (Capuco et al., 2001), whereas other works have demonstrated the direct effect of IGF-1 on an increase in the mammary blood flow and increase in milk production (Etherton and Bauman, 1998). An elevation of both plasma IGF-1 concentration and udder blood flow was also noted in

late lactating crossbred cows treated with rbST (Tanwattana et al., 2003). The present study confirms that mammary blood flow is a major determining factor for supply of nutrients for milk synthesis and follows the pattern of changes of milk yield.

Milk fat content of rbST-treated animals was increased, while milk protein and milk lactose were not changed by rbST treatment. Milk fat was synthesized in the mammary epithelial cells. The fatty acids used to synthesize the milk fat arise from both blood lipids and from de novo synthesis within the mammary epithelial cells. An increased fat content in milk due to rbST injection has been observed previously (West et al., 1990). Milk fat content of cows in positive energy balance is not influenced by rbST treatment, and milk fat yield follows the trend of milk production (West et al., 1990). However, an increase in milk fat after rbST injection would relate to an increase in the mobilization of long-chain fatty acids from body reserves when cows are in negative energy balance (McDowell et al., 1987). Peel and Bauman (1987) reported that administration of rbST 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 conclusion, rbST exerts its effect on an increase in both TBW and EBW. An increase in ECF compartment would be due to the increase in water intake during early lactation which correlated with an increase in water secretion in milk. Increased ECF in rbST-treated animals might be partly resulted from the decrease in fat mass during early lactation. The present results indicate that growth hormone affecting mammary gland function might not be mediated solely by the action of IGF-1 on an increase in blood flow to mammary gland. The lack of effect of higher plasma IGF-1 levels in regulating mammary blood flow and milk yield in crossbred dairy cattle has also been noted (Chaiyabutr et al., 2003). An elevation of body fluid particularly blood volume (+15 %) despised large increases in mammary blood flow (+50 %)

during rbST treatment. These observations could suggest that a marked increase in blood flow through the mammary glands resulting from rbST administration would be achieved in part by local vasodilatation (Linzell, 1974), causing in distribution of milk precursors to the gland.

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CHAPTER IV

Effects of Long-term Exogenous Bovine Somatotropin on Water Metabolism and Milk Yield in Crossbred Holstein Cattle

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Effects of Long-term Exogenous Bovine Somatotropin on Water Metabolism and Milk Yield in Crossbred Holstein Cattle

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ABSTRACT

This study was designed to clarify whether the short lactation persistency occurring in the crossbred cattle in the tropics would be affected by a reduction in circulating growth hormone in association with changes of body fluid and mammary blood flow. Ten, first lactation, 87.5% Holstein Friesian (HF) animals were chosen and divided into two groups of 5 animals each. Four consecutive study periods were carried out in each group. These consisted of a pretreatment period (45 days post-partum)(pre-peak lactation) and three treatment periods during early lactation(105 days post-partum, mid-lactation (165 days post-partum) and late lactation (225 days post-partum). After 60 days of lactation, animals were injected sub-cutaneously biweekly intervals until the end of study with 500 mg of recombinant bovine somatotropin (rbST). The milk yield per day of rbST-treated animals increased in early lactation (19.8 %), mid-lactation (9.5%) and decreased in late lactation (-2.7%) when compared with the pretreatment period. Absolute values of total body water (TBW), extracellular water (ECW), plasma volume and blood volume were significantly increased during rbST treatment. The estimated value of intracellular water (ICW) of the rbST-treated animals showed no differences, whereas it significantly decreased in the control animals during early and mid-lactation. The water turnover rate (WTO) of rbST-treated animals significantly increased in early and mid-lactation. Mammary blood flow (MBF) significantly increased during rbST administration in all stages of lactation. These data demonstrated that the rbST exerts its galactopoietic action, in part, through increases in both the TBW and ECW in association with an increase in MBF for milk production.

(**Key words:** exogenous bovine somatotropin, water metabolism, crossbred Holstein cattle, milk yield)

Abbreviation key: **HF** = Holstein Friesian, **MBF** = mammary blood flow, **TBW** = total body water, **WTO** = water turnover rate, **ECW** = extracellular water, **ICW** = intracellular water, **rbST** = recombinant bovine somatotropin.

INTRODUCTION

Many factors can affect milk production in dairy cattle in the hot-humid tropics including high environmental temperatures, lower genetic potential for milk production in indigenous cattle and inadequate supply of food during the dry summer months. Several approaches have been attempted to try to improve dairy productivity in the tropics. Crossbreeding of indigenous and exotic cattle for tropical use has been exploited as an efficient tool for blending the adaptability of tropical cattle with the high milking potential of exotic breeds, resulting in increased milk production. There is still a need to identify the types of crossbred cattle that are the most suitable for the tropics. During one study on the regulation of body fluids and mammary blood flow (**MBF**) in different types of crossbred Holstein Friesians (**HF**) cattle (Chaiyabutr et al., 1997; 2000a), it was noted that 50%**HF** animals showed differences in the distribution of their body fluids and **MBF** from 87.5%**HF** animals during late pregnancy and different stages of lactation. The 87.5%**HF** animals had lower efficiency in water retention mechanism and poor adaptation to tropical environment, in comparison to 50%**HF** animals (Chaiyabutr et al., 2000a). A low persistent lactation yield, with a decrease in **MBF** during the transition period from early to mid-lactation, was noted in the 87.5%**HF** animals. **MBF** has been known to be a

major determinant for the rate of substrate supply for milk synthesis (Davis and Collier, 1985). The control mechanism for MBF in different stages of lactation in crossbred dairy cattle has not been fully elucidated. Differences 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 somatotropin (bST) is known as a homeorrhetic hormone connected with both growth and lactation. The importance of bST for maintaining milk output in ruminant is well established (Bauman, 1992). 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; Johnson et al., 1991), the role of bST in body water regulation, in relationship to persistent lactation in crossbred dairy cattle in the tropics is not yet clear.

During lactation, an alteration in many bodily functions is apparent; for example, blood volume and cardiac output are increased (Chaiyabutr et al., 1997; Hanwell and Peaker, 1977) and blood flow in many parts of body is increased including MBF. It was reported that a decrease in milk yield was related to reductions in MBF and circulating bST as lactation advances to mid- and late lactation in 87.5%HF animals (Chaiyabutr et al., 2000a, 2000b). It is not known which factors are the cause and which factors are the effect 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., 2000b). 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, although an elevation

of total body water (TBW) and extracellular water (ECW) was noted in humans deficient in growth hormone, after taking injections of human somatotropin (Janssen et al., 1997).

To provide some of this information, the present experiment was carried out to determine whether recombinant bovine somatotropin (rbST) played an important role in maintaining milk yield in association with changes of body fluids and MBF, in crossbred dairy cattle containing 87.5%Holstein genes. Long-term treatment with rbST at different stages lactation were carried out to obtain a more complete picture of the role of somatotropin on lactation persistency, in crossbred dairy cattle in the tropics.

MATERIALS AND METHODS

Animals and Management

Ten, first lactation, non-pregnant, 87.5%HF dairy cattle were selected for the experiment. They were divided into two groups, five animals in each. Animals in each group were fed with rice straw treated with 5% urea, as the source of roughage throughout the experiments. All animals were housed in sheds, tethered in individual stalls and fed twice daily. The ambient temperature was recorded by a dry bulb thermometer. The relative humidity was calculated from the reading of dry and wet bulb thermometers. The maximum temperature in the shed at noon was $34\pm 1^{\circ}\text{C}$ and the minimum temperature at night was $26\pm 1^{\circ}\text{C}$. The relative humidity was $68\pm 12\%$. Animals received an average of 4 kg/d of roughage in combination with a concentrated mixture (7kg/d), to maintain a moderate body condition score 2.5 during the experiment, (scale = 1 to 5)(Wildman et al.,1982). The chemical composition of the feed is presented in Table 1. The dry matter intake (DMI) of each animal was determined by measuring both the concentrate and roughage offered and subtracting the amount refused each day. Urea

treated rice straw was offered four times a day at 08.00, 12.00, 16.00 and 20.00h. Concentration was fed two times at 0800 and 1400h. Each day, during feeding trial, sub-sample of both feed was collected for dry matter determination. Feed sample was collected every day and kept at -20 C for chemical analysis. Animals had free access to water and were fed their respective rations throughout the experimental period.

Experimental Procedures

Animals were divided into control (n=5) and experimental (n=5) groups. Four consecutive study periods were carried out in each group. These consisted of a pretreatment period (45 days post-partum)(pre-peak lactation) and three treatment periods during early lactation(105 days post-partum), mid-lactation (165 days post-partum) and late lactation (225 days post-partum). After 60 days of lactation, animals were injected sub-cutaneously biweekly intervals until the end of study with 500 mg of recombinant bovine somatotropin (rbST) suspended in 792 mg of a prolonged-release formulation of sesame oil (POSILAC, Monsanto, USA). Animals in the control group were injected subcutaneously biweekly intervals with 800 mg of sterile sesame oil without rbST, as a placebo. Injections were administered at the tail head depression (ischio-rectal fossa). Animals of both groups were fed the same ration, from before parturition and throughout the study. The measurement of daily water consumption of each animal was calculated by weighing the individual water bowl of each animal. The daily water intake per animal in each period of lactation was recorded by averaging over seven days. Animals were normally milked at around 0600 h and 1700 h using a milking machine and milk production was recorded daily. Milk yield per day per animal was recorded at each period

of lactation. Animals were weighed after collecting the milk sample in each specified day.

To measure MBF and to collect venous blood, cows were cannulated on the specified day before the experiment began at each period. While the cow was standing, two catheters (i.d. 1.0 mm, o.d. 1.3 mm, L 45 mm) were inserted into either the left or right milk vein using a intravenous polymer catheter (Jelco, Critikon; Johnson & Johnson, U.K.), under local anesthesia. The tip of the catheter was positioned near the sigmoid flexure anterior to the point at which the vein leaves the udder. The other catheter was positioned downstream about 20 cm from the first one. The catheter for isotope infusion and dye injection was inserted into an ear vein, under local anesthesia. All catheters were flushed with sterile, heparinized, normal saline (heparin 25 i.u./ml normal saline) and were left in place during the experiment.

Determinations of MBF and Water Metabolism

MBF through half of the udder was determined by measuring the dilution of dye T-1824 (Evans blue) using short term continuous infusion and adapted from the method of measuring blood flow in the milk veins of cattle as previously described (Chaiyabutr et al., 1997).

The water turnover rate (WTO) and TBW were determined by tritiated water dilution techniques using a single dose intravenous injection of 3,000 μ ci per animal, of carrier free tritiated water in normal saline. The equilibrium time was determined by taking blood samples for 3 days after the injection. Blood samples for measurements of WTO, biological half-life of tritiated water, TBW and the total body water space (TOH), using a correction factor from the plasma solids concentration, were performed as previously

described (Chaiyabutr et al., 1997). The plasma solids concentration was determined by a refractometer.

In each animal per period, the injection of 20 ml of sodium thiocyanate solution (10 g/100 ml normal saline) and 20 ml of the Evans blue dye (T-1824) (0.5 g/100 ml normal saline) was given via a ear vein catheter to estimate ECW volume and the plasma volume, respectively. Venous blood samples from the jugular vein were taken at 20, 30, 40 and 50 min after dye injection. Dilution of dye at zero time was determined by using a semi logarithmic concentration on time extrapolation. Blood volume was calculated from the plasma volume and packed cell volume (Chaiyabutr et al., 1980). The measurement method for ECW was modified from the method used by Medway and Kare (1959). Intracellular water (ICW) was calculated by subtracting ECW from TBW. Plasma osmolality was measured using the freezing point depression method (Advance Osmometer model 3, U.S.A.).

Statistical Analyses

The experimental results were examined statistically by a paired t-test for variables within a treatment which were compared against the pretreatment values in the same group. Mean values of variables within a period were compared across treatments between group by an unpaired t-test. Mean values are presented as mean $\pm$ S.D.

RESULTS

DM intake, water intake, milk yield and body weight in the controls and rbST-treated animals are shown in Table2. The total, daily, DM intakes were not significantly different between the controls and rbST-treated animals, during the experimental periods. No significant increases occurred in the daily water intake in both early and mid-lactation of

the control animals but rose in late lactation, when compared with the pre-treatment period. In contrast, the daily water intake increased significantly stepwise as lactation advanced in the rbST-treated animals. Animals receiving rbST for 45 days, significantly increased ($P<0.01$) their milk yield from 13.4 ± 2.6 kg/d during the pre-treatment period to 16.0 ± 2.1 kg/d (19.8 %) over early lactation and for the 105 days period, milk yield increased by 9.5% in mid-lactation, while animals received rbST for 165 days, milk yield decreased by 2.7%, all in comparison with the pre-treatment period. Milk yield of the rbST-treated animals increased significantly ($P<0.05$) above the milk yield of the control animals in the early period of lactation and continued at a high level throughout lactation. However, peak yields occurred during the early period of lactation; thereafter yields declined in both groups as lactation advanced. A DMI : Milk yield ratio was calculated and used as indicator of the efficiency of conversion of nutrients to milk. The mean ratio of total DMI to milk yield of rbST-treated animals were significantly decreased ($P<0.05$) after rbST administration in the early period of lactation. The mean ratio of DMI to milk yield of the control animals showed no significant changes throughout lactation. Body weights of both control animals and rbST-treated animals significantly rose ($P<0.01$) in a stepwise fashion above their initial weights in the pretreatment period but rbST-treated animals had a greater percentage change than those of the control animals throughout the lactation (by average 7.8 vs. 5.9%, 13.3 vs. 9.6% and 15.6 vs. 11.6 % for early, mid- and late lactation, respectively).

The control animals showed no significant changes in plasma volume or blood volume either in terms of absolute values or the relative values as a percentage of body weight, throughout the course of their lactation (Table 3). The absolute value of plasma volume and blood volume of rbST-treated animals significantly increased ($P<0.05$) but the

relative values as a percentage of body weight were unchanged during the course of treatment. The packed cell volume and plasma osmolality of both the control animals and rbST-treated animals were unchanged throughout lactation. An increase ($P<0.05$) in the absolute values of ECW was observed in rbST-treated animals, while there were no significant changes in the control animals throughout lactation. The value of ECW as a percentage of body weight in both the control and the rbST-treated animals was unchanged throughout periods of study. The estimated value of ICW in the control animals significantly decreased ($P<0.05$) in the early and mid lactation periods, while the rbST-treated animals showed no differences during the course of all treatments. The ICW of the rbST-treated animals increased significantly ($P<0.001$) above the ICW of the control animals in the early period of lactation and continued at a high level throughout lactation.

The average WTO and the WTO per fat free, wet, body weight ($\text{kg}^{0.82}$) (MacFarlane and Howard, 1972) were significantly higher, while the biological half-life of tritiated water was significantly shorter in mid and late lactation as compared with the pre-treatment period in the control animals (Table 4). The rbST-treated animals showed significantly increased ($P<0.05$) WTO during mid- and late lactation. In the treated animals, receiving rbST for 45 days, the WTO was significantly higher ($P<0.05$) when compared with the control animals over a similar period. The WTO per fat free, wet, body weight ($\text{kg}^{0.82}$) and the biological half-life of tritiated water in rbST-treated animals were unchanged throughout lactation. In both early and mid-lactation periods, the control animals showed significant reductions ($P<0.05$) in both TOH and TBW as the percentage of body weight. In contrast to the control animals, absolute values of TOH and TBW of rbST-treated animals were significantly higher ($P<0.01$) than those of the control animals over similar

periods during early and mid lactation. The values of TOH and TBW as a percentage of body weight of rbST-treated animals showed no significant differences during all periods of lactation.

MBF increased significantly ($P < 0.05$) during rbST administration in both early and mid lactation, while there were no significant changes in all periods of lactation in the control animals (Table 5). The ratio of MBF to milk yield slightly increased as lactation advance in both the control animals and the rbST-treated animals.

Table 1. Chemical composition of feeds used in the experiment (% on dry matter basis)

Particulars	Urea-treated rice straw	Concentrate
Dry matter	58.0	89.4
Crude protein	8.9	17.8
Acid detergent fibre	61.2	21.5
Neutral detergent fibre	67.2	28.8
Lignin	8.8	7.0
Ash	16.8	5.6

Concentrate formulation: fresh weight (kg/100 kg) consisted of soy bean meal 30 kg, cotton seed 25 kg, cassava 25 kg, rice bran 15 kg, di-calcium phosphate 2 kg, sodium bicarbonate 1.7 kg, potassium chloride 0.7 kg and vitamin/mineral premix 0.6 kg.

The urea treated rice straw was prepared by mixing a urea solution (5 kg urea dissolved in 100 litres water per 100 kg dry rice straw) with dry straw and stored in airtight conditions in a cement pit for 21 days before being offered to the animals.

Table 2. Means $\pm$ SD of DMI, water intake, milk yield and body weight in different stages of lactation of 87.5%HF animals for the control group¹ and rbST-treated group¹.

Measurement	Lactation period ²	Control Group	rbST Group	Contrasts ³
DMI, kg/d	Pretreated	11.4 $\pm$ 0.7	12.3 $\pm$ 0.8	NS
	Early	11.6 $\pm$ 1.1	13.0 $\pm$ 1.7	NS
	Mid	12.2 $\pm$ 1.8	13.9 $\pm$ 1.3	NS
	Late	12.3 $\pm$ 1.8	13.4 $\pm$ 1.7	NS
Water intake, kg/d	Pretreated	58.7 $\pm$ 13.2	65.2 $\pm$ 10.6	NS
	Early	60.2 $\pm$ 12.3	70.9 $\pm$ 12.4**	NS
	Mid	73.7 $\pm$ 16.2	74.2 $\pm$ 8.9*	NS
	Late	74.1 $\pm$ 19.4*	75.3 $\pm$ 12.4**	NS
Milk yield, kg/d	Pretreated	13.0 $\pm$ 1.5	13.4 $\pm$ 2.7	NS
	Early	13.1 $\pm$ 1.8	16.0 $\pm$ 2.1**	P<0.05
	Mid	12.9 $\pm$ 1.5	14.6 $\pm$ 1.9	NS
	Late	11.5 $\pm$ 1.0	13.0 $\pm$ 1.3	NS
DMI/Milk yield, kg/kg	Pretreated	0.9 $\pm$ 0.12	1.0 $\pm$ 0.25	NS
	Early	0.9 $\pm$ 0.12	0.9 $\pm$ 0.22*	NS
	Mid	1.0 $\pm$ 0.13	1.0 $\pm$ 0.06	NS
	Late	1.1 $\pm$ 0.19	1.0 $\pm$ 0.12	NS
Body weight, kg	Pretreated	337 $\pm$ 31	364 $\pm$ 27	NS
	Early	357 $\pm$ 34**	391 $\pm$ 36**	NS
	Mid	370 $\pm$ 34**	412 $\pm$ 36***	NS
	Late	379 $\pm$ 29**	420 $\pm$ 43**	NS

¹For the control group, n =5; for rbST- treated group, n=5.

²Statistical test of P-values between periods of lactation in each group using paired t-test of * (P<0.05), ** (P<0.01), *** (P<0.001), with respect to pretreated period.

³Contrasts : comparison of P-values of control group vs. rbST-treated group using unpaired t-test, NS=not significant.

Table 3. Means $\pm$ SD of plasma volume, blood volume, packed cell volume, plasma osmolality (Posm), extracellular water (ECW) and intracellular water (ICW) in different stages of lactation of 87.5%HF animals for the control group¹ and rbST-treated group¹.

Measurement	Lactation period ²	Control Group	rbST Group	Contrasts ³
Plasma volume, L	Pretreated	16.0 $\pm$ 1.3	16.6 $\pm$ 1.4	NS
	Early	17.4 $\pm$ 1.9	19.4 $\pm$ 3.2*	NS
	Mid	17.1 $\pm$ 2.0	20.2 $\pm$ 2.0**	P<0.05
	Late	17.0 $\pm$ 0.8	20.5 $\pm$ 3.4**	P<0.05
Plasma volume, L/100 kg	Pretreated	4.7 $\pm$ 0.4	4.6 $\pm$ 0.2	NS
	Early	4.8 $\pm$ 0.2	4.9 $\pm$ 0.5	NS
	Mid	4.6 $\pm$ 0.3	4.9 $\pm$ 0.6	NS
	Late	4.5 $\pm$ 0.3	4.8 $\pm$ 0.6	NS
Blood volume, L	Pretreated	22.2 $\pm$ 2.0	23.3 $\pm$ 2.0	NS
	Early	24.6 $\pm$ 3.2	26.9 $\pm$ 4.7*	NS
	Mid	24.3 $\pm$ 3.0	28.3 $\pm$ 2.8**	NS
	Late	24.0 $\pm$ 1.0	28.4 $\pm$ 5.1*	NS
Blood volume, L/100 kg	Pretreated	6.6 $\pm$ 0.5	6.4 $\pm$ 0.3	NS
	Early	6.8 $\pm$ 0.3	6.8 $\pm$ 0.7	NS
	Mid	6.6 $\pm$ 0.6	6.9 $\pm$ 0.8	NS
	Late	6.3 $\pm$ 0.4	6.8 $\pm$ 0.8	NS
Packed cell volume, %	Pretreated	28.1 $\pm$ 1.5	28.6 $\pm$ 0.9	NS
	Early	29.2 $\pm$ 2.5	27.9 $\pm$ 1.4	NS
	Mid	29.5 $\pm$ 2.3	28.6 $\pm$ 0.5	NS
	Late	28.9 $\pm$ 1.1	27.6 $\pm$ 0.9	NS
Posm, mOsm/kg	Pretreated	280 $\pm$ 4	274 $\pm$ 6	NS
	Early	280 $\pm$ 5	276 $\pm$ 3	NS
	Mid	280 $\pm$ 2	277 $\pm$ 6	NS
	Late	286 $\pm$ 1	279 $\pm$ 5	P<0.01
ECW, L	Pretreated	76.5 $\pm$ 7.5	77.7 $\pm$ 9.3	NS
	Early	82.9 $\pm$ 11.2	88.6 $\pm$ 10.9*	NS
	Mid	81.8 $\pm$ 12.4	97.5 $\pm$ 18.5*	NS
	Late	86.6 $\pm$ 11.5*	106.1 $\pm$ 22.2*	NS
ECW, L/100 kg	Pretreated	22.8 $\pm$ 3.1	21.4 $\pm$ 1.8	NS
	Early	23.4 $\pm$ 3.9	22.6 $\pm$ 2.0	NS
	Mid	22.2 $\pm$ 3.5	23.5 $\pm$ 3.0	NS
	Late	22.8 $\pm$ 2.1	25.1 $\pm$ 3.7	NS
ICW, L	Pretreated	170.0 $\pm$ 14.9	181.5 $\pm$ 9.3	NS
	Early	155.8 $\pm$ 16.1**	189.7 $\pm$ 4.6	P<0.001
	Mid	163.6 $\pm$ 19.2	194.4 $\pm$ 27.9	NS
	Late	173.5 $\pm$ 29.2	183.9 $\pm$ 31.7	NS
ICW, L/100 kg	Pretreated	50.6 $\pm$ 3.0	50.2 $\pm$ 5.2	NS
	Early	43.7 $\pm$ 3.7**	48.8 $\pm$ 4.9	NS
	Mid	44.4 $\pm$ 5.4*	47.4 $\pm$ 7.7	NS
	Late	45.7 $\pm$ 6.1	43.8 $\pm$ 6.9	NS

¹For the control group, n=5; for rbST-treated group, n=5.

²Statistical test of P-values between periods of lactation in each group using paired t-test of * (P<0.05), ** (P<0.01), with respect to pretreated period.

³Comparison of P-values of control group vs. rbST-treated group using unpaired t-test, NS= not significant.

Table 4. Means $\pm$ SD of the water turnover rate(WTO), total body water (TBW), total body water space (TOH) and the biological half-life of tritiated water in different stages of lactation of 87.5%HF animals for the control group¹ and rbST-treated group¹.

Measurement	Lactation period ²	Control Group	rbST Group	Contrasts ³
WTO, L/d	Pretreated	60.0 $\pm$ 13.5	70.6 $\pm$ 18.5	NS
	Early	60.6 $\pm$ 10.1	85.2 $\pm$ 19.4*	P<0.05
	Mid	71.5 $\pm$ 11.5***	95.7 $\pm$ 36.7*	NS
	Late	73.4 $\pm$ 16.3*	92.6 $\pm$ 32.9	NS
WTO, L/100kg/d	Pretreated	17.5 $\pm$ 3.3	19.6 $\pm$ 5.5	NS
	Early	17.0 $\pm$ 2.7	22.1 $\pm$ 5.9	NS
	Mid	19.3 $\pm$ 2.1	23.4 $\pm$ 9.0	NS
	Late	19.2 $\pm$ 3.4	21.8 $\pm$ 6.8	NS
WTO, ml/kg ^{0.82} /d	Pretreated	499.7 $\pm$ 97.7	564.4 $\pm$ 156.0	NS
	Early	490.0 $\pm$ 75.9	645.5 $\pm$ 168.1	NS
	Mid	558.6 $\pm$ 64.0*	690.5 $\pm$ 265.1	NS
	Late	560.9 $\pm$ 103.6*	647.4 $\pm$ 205.9	NS
Biological half-life, d	Pretreated	3.2 $\pm$ 0.6	2.9 $\pm$ 0.8	NS
	Early	3.0 $\pm$ 0.4	2.6 $\pm$ 0.7	NS
	Mid	2.6 $\pm$ 0.4**	2.6 $\pm$ 0.9	NS
	Late	2.7 $\pm$ 0.5*	2.6 $\pm$ 0.7	NS
TOH space, L	Pretreated	268.7 $\pm$ 16.4	283.0 $\pm$ 8.7	NS
	Early	260.7 $\pm$ 14.7*	304.6 $\pm$ 12.2**	P<0.001
	Mid	268.6 $\pm$ 12.4	320.9 $\pm$ 34.6*	P<0.01
	Late	286.2 $\pm$ 33.9*	318.0 $\pm$ 38.8	NS
TOH space, L/100 kg	Pretreated	79.9 $\pm$ 3.0	78.13 $\pm$ 5.4	NS
	Early	73.2 $\pm$ 3.1**	78.22 $\pm$ 5.1	NS
	Mid	72.9 $\pm$ 4.7*	78.0 $\pm$ 7.5	NS
	Late	75.4 $\pm$ 4.6	75.6 $\pm$ 5.4	NS
TBW, L	Pretreated	246.6 $\pm$ 13.2	259.2 $\pm$ 7.5	NS
	Early	238.7 $\pm$ 11.6**	278.3 $\pm$ 11.3**	P<0.001
	Mid	245.4 $\pm$ 12.4	291.9 $\pm$ 32.7*	P<0.01
	Late	260.1 $\pm$ 31.7	290.0 $\pm$ 35.1	NS
TBW, L/100 kg	Pretreated	73.4 $\pm$ 3.2	71.6 $\pm$ 5.0	NS
	Early	67.1 $\pm$ 3.4***	71.5 $\pm$ 5.5	NS
	Mid	66.6 $\pm$ 4.1**	70.9 $\pm$ 7.0	NS
	Late	68.5 $\pm$ 4.5	68.9 $\pm$ 4.9	NS

¹For the control group, n=5; for rbST-treated group, n=5

²Statistical test of P-values between periods of lactation in each group using paired t-test of * (P<0.05), ** (P<0.01), *** (P<0.001) with respect to pretreated period.

³Comparison of P-values of control group vs. rbST-treated group using unpaired t-test, NS= not significant.

Table 5. Means $\pm$ SD of mammary plasma flow(MPF), mammary blood flow(MBF), and the ratio MBF/ Milk yield in different stages of lactation of 87.5%HF animals for the control group¹ and rbST-treated group¹.

Measurement	Lactation period ²	Control Group	rbST Group	Contrasts ³
MPF, ml/min	Pretreated	2438 $\pm$ 331	2594 $\pm$ 342	NS
	Early	2730 $\pm$ 357	3927 $\pm$ 1203*	NS
	Mid	2698 $\pm$ 319	3983 $\pm$ 1183*	NS
	Late	2692 $\pm$ 290	3533 $\pm$ 1055	NS
MBF, ml/min	Pretreated	3286 $\pm$ 461	3548 $\pm$ 463	NS
	Early	3817 $\pm$ 616	5310 $\pm$ 1620*	NS
	Mid	3821 $\pm$ 533	5458 $\pm$ 1627*	NS
	Late	3750 $\pm$ 476	4814 $\pm$ 1464	NS
MBF/ Milk yield, L/kg	Pretreated	364 $\pm$ 25	397 $\pm$ 111	NS
	Early	420 $\pm$ 32	491 $\pm$ 152	NS
	Mid	433 $\pm$ 89	539 $\pm$ 156	NS
	Late	471 $\pm$ 69	539 $\pm$ 168	NS

¹For the control group, n=5; for rbST-treated group, n=5.

²Statistical test of P-values between periods of lactation in each group using paired t-test of * (P<0.05), with respect to pretreated period.

³Comparison of P-values of control group vs. rbST-treated group using unpaired t-test, NS= not significant.

DISCUSSION

The present study was designed to clarify whether poorer lactation persistency in crossbred cattle containing 87.5%Holstein genes was affected by a reduction in circulating growth hormone in association with changes of body fluid and mammary circulation. Long-term treatment with rbST was administered to 87.5%HF animals that had completed 60 days of lactation prior to the experiment. The 500 mg of rbST used biweekly intervals in the present study is the dose rate recommended for *Bos taurus* cows. This treatment of rbST was initiated at the earlier stage of lactation, milk yield increased in early lactation (+19.8 %) and in mid-lactation (+9.5%), but it decreased by 2.7% during late lactation in comparison with the pretreatment period. Low responses in milk yield during rbST treatment in the later stage of lactation are similar as previously reported in dairy crossbred cattle (Phipps et al., 1991). A rapid decline of yield resulting the shorter persistency of lactation of rbST-treated animals seems to be similar to those which occur in higher yielding cows (Chase, 1993). These results indicated that an increase in milk yield of dairy crossbred cattle, in response to rbST administration, will not be sustained for long, being influenced by stage of lactation.

Animals in both groups were fed ad libitum and total DMI were not significantly different between control animals and rbST-treated animals, throughout the experimental periods. However, the ratio of DMI to milk yield of rbST-treated animals, was lower in early lactation when compared with the pretreatment period but animals still gained weight throughout the experiment in both groups. It has been known that the support of milk secretion would come through provision of substrate and stimulation of mammary cell activity. Unfortunately, the present studies on the mammary cell activity were not available. The rbST increased milk yield relating to mammary cell activity appears

contradictory. Whereas some studies show no mammogenic effect of bST (Binelli et al., 1995), other studies show a possible mammogenic effect when cattle are administered bST (Knight et al., 1992). It indicates that the increased milk yield with rbST treatment in the present study is rather dependent upon the adequacy of the nutritional provision than the mobilization of body stores. A marked increase in milk yield with rbST treatment without loss of body weight, especially during early lactation, may be due to the fact that the animals were well fed to allow an adequate replacement of body reserves. Milk yield in the first lactation of crossbred animals in the present study would be lesser than those of multiparous cows (Sullivan et al. 1992), which is possibly related to the continued weight increase observed in animals during their first lactation. These results provide the physiological differences between crossbred animals and exotic breeds in partitioning ability, which would be inherited and capacity for milk production. Thus, the metabolic demands of lactation of the crossbred HF animals would be met by dietary intake during early lactation. In our previous report of the same line experiment (Chaiyabutr et al., 2005), no mobilization of body tissues as indicated by no alteration of the plasma levels of both triglyceride and glucose was noted in crossbred HF animals treated with rbST. Triglyceride has been known to restore during period of excess energy availability and are mobilized during periods of energy deprivation. No significant change in the plasma triglyceride concentration could be attributed to the higher milk production in the rbST- treated animals as diversion of surplus nutrient from diet for milk synthesis.

The rbST-treated animals increased water intake in the early period of lactation, from 65.2 kg/d to 70.9 kg/d, which is about 9%, accounting for 19.8% of their pre-treatment milk yield. An increase in milk yield which general contain 87% of water would account

for most of the increased water intake as lactation advanced in the rbST-treated animals. This result shows that milk production affects water intake, including the body water turnover rate. The rbST-treated animals increased body fluid compartments throughout all periods of study i.e. TBW, ECW and blood volume, while the control animals decreased TBW in comparison to pretreatment values in the early period of lactation. An increase in ECW would be influenced by an increase in voluntary intake (MacFarlane et al., 1959), which has been reported to occur after a few weeks of rbST administration (Coghlan et al., 1977). However, the ECW compartment did not include rumen water; thus any changes of ruminal fluid volume should not affect the determination of extracellular fluid volume. These results indicate that somatotropin plays an important role in water regulation and probably relating to the galactopoietic effect. Although the mechanisms responsible for water regulation are not yet fully known in ruminants, the expansion of ECW and TBW after growth hormone administration has been noted in growth hormone deficient humans (Janssen et al., 1997). As lactation advances, animals gained more live weight in both the control and the rbST-treated animals. However, a greater percentage increase in live weight of rbST-treated animals could be considered, at least in part, to be the direct effect of somatotropin on the increased body cell mass. This would be attributable to an accumulation of body water. The sodium retention effect of somatotropin on the renal tubular reabsorption of sodium (Wyse et al., 1993) while retaining constant plasma osmolality in the present result, would be another explanation for explaining water retention in the ECW compartment.

The high body water content of rbST-treated animals seems to be related to the adaptation of the animals to a tropical environment. An increase in both metabolic activity and heat production has been reported in bST-treated cows (West et al., 1991).

However, it was suggested that even though bST increases heat production, it also increases heat dissipation (Johnson et al., 1991, West et al., 1994). In the present study, the higher TBW and ECW of animals receiving rbST would not only provide a higher reservoir of soluble metabolites for biosynthesis of milk but also slow down any elevation of body temperature during lactation in hot conditions. In the present study, animals in both groups were not pregnant and were housed in the same shed in the same environment. Thus, a change in the water turnover rate of both groups of crossbred cattle was not influenced by the effect of pregnancy (Chaiyabutr et al., 1997) or changes in environmental conditions (Ranjhan et al., 1982). However, the rbST-treated animals showed no significant changes in the water turnover rate per fat free, wet, body weight ($\text{kg}^{0.82}$) and the biological half-life of tritiated water, in any periods measured in the current experiment, in comparison to the control animals. This indicates that water loss with the increase in milk yield of the rbST-treated animals might be compensated by a larger body water pool, which restores their body fluids to equilibrium, with no significant changes of body water turnover rate and water half-life. In contrast to the rbST-treated animals, the biological half-life of tritiated water in the control animals was significantly shorter, while the water turnover rate was significantly higher as lactation advanced to mid and late lactation. These changes would be due to the process of lactation requiring more water and more loss of water secretion in milk, which is generally known to be about 87% and would account for these phenomena. The control animals being 87.5%HF were genetically similar to the exotic *bos taurus* breed which might lead to poor adjustment in a tropical environment (Chaiyabutr et al., 2000a; Nakamura et al., 1993). The TBW and ICW of the control animals showed to be decreased during advanced lactation; it should be assumed that these changes are the

factors influencing lactation persistency. Animals could not maintain their body fluids which resulted in the rapid approach of the end of their normal short lactation.

The marked increase in the MBF was apparent in rbST-treated animals throughout lactation. This result supported other findings showing increases in MBF and milk secretion in both goats and cows given exogenous growth hormone (Hart et al., 1980; Davis et al., 1988). An increase in MBF has been shown to be the effect of an increase in cardiac output perfusing to the udder without any alteration in heart rate during growth hormone treatment (Davis et al., 1988). In the present results, an increase in both blood volume and plasma volume in rbST-treated animals would provide a greater venous return and stroke volume for increase in cardiac output, resulting in increased the blood supply to the mammary gland. Thus, the rate at which the milk yield elevated after the peak period when compared with the control animals, could have been due primarily to an increased availability of substrates for the mammary gland. However, observations in both the control animals and rbST-treated animals showed an increase in a ratio of MBF/milk yield as lactation advanced. The resultant progressive decline in milk yield of rbST-treated animals with still a higher level of either MBF or ECW, could be accounted for by changes in intra-mammary factors. 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 udder (Collier et al., 1984). In addition, study in vitro suggests that bST does not directly stimulate mammary secretory function (Gertler et al., 1983). The indirect action of rbST on mammary function may occur through some other agent e.g. insulin like growth factor-I, as administration of rbST in late, lactating, crossbred cows elevated milk yield, which coincided with increased plasma IGF-I concentration and udder blood flow (Tanwattana et al., 2003).

CONCLUSIONS

These experiments demonstrated that the rbST exerts its galactopoietic action through increases in both the TBW and ECW in association with an increase in MBF, which partitions the distribution of nutrients to the mammary gland for milk production. The data also suggest that as the lactation advances, the action of rbST does not prevent the decrease in the mammary function which still had a progressive decline in milk yield. Further studies are needed to determine the mechanisms by which bovine somatotropin influence mammary gland metabolism during lactation advance in crossbred cattle in the tropics.

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CHAPTER V

**Effects of long-term administration of recombinant bovine somatotropin
on milk production and insulin like growth factor-I, plasma levels of
insulin and it's metabolites in crossbred Holstein cattle**

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Effects of long-term administration of recombinant bovine somatotropin on milk production and insulin like growth factor-I, plasma levels of insulin and it's metabolites in crossbred Holstein cattle

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SUMMARY

The objective of this study was to determine the in vivo relationship between the long-term administration of recombinant bovine somatotropin (rbST), circulating levels of IGF-I and insulin, mammary blood flow and other variables relevant to milk synthesis, in crossbred, Holstein cattle. Ten, first lactating, non-pregnant, crossbred, Holstein dairy cattle were divided into two groups of five animals each; an experimental group and a control group. Animals in each group were fed with rice straw, treated with 5% urea, as the source of roughage. Four consecutive study periods were carried out in each group. These consisted of a pretreatment period (45 days postpartum before lactation peak) and three treatment periods during early lactation (105 days postpartum), mid-lactation(165 days postpartum) and late lactation (225 days postpartum). During the treatment periods, animals that had completed 60 days of lactation were injected subcutaneously every 14 days with 500 mg of recombinant bovine somatotropin (rbST) (POSILAC, Monsanto, USA) in the experimental group, while animals in the control group were injected subcutaneously every 14 days, with 800 mg of sterile sesame oil, without rbST, as a placebo. During the pre-treatment period, there were no significant differences in plasma concentrations of IGF-I, insulin and other parameters between the control group and the experimental group. During the treatment periods, the increase in the concentration of plasma IGF-I in rbST treated animals was significantly higher than in the control animals throughout the lactating period. Plasma glucose, protein and triglyceride concentrations in each group remained stable throughout the study. The total daily dry matter intakes were not significantly different between the groups. Milk yield increased by 20% with rbST

treatment and it was 22% greater than that of the control animals receiving placebo in early lactation. Milk yield of rbST treated animals rose to a peak in early lactation and then gradually declined. In late lactation, milk yield of rbST treated animals was decreased to 19 % as compared with early lactation. Udder plasma flow and udder blood flow markedly increased with rbST treatment and there were no significant changes in the control animals. The ratio of udder blood flow to the rate of milk production increased to mid and late lactation in controls and the rbST treated animals. These findings suggest that a short persistency of lactation in rbST treated animals was similar to the control animals receiving placebo. 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. 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.

INTRODUCTION

It is known that crossbreeding *Bos taurus* and *Bos indicus* has been an efficient tool for blending the adaptability of tropical cattle with the high milk potential of exotic breeds and thus increasing milk production. There is still a need to discover which crossbred cattle are most suitable for the tropics. It is not only the genetics that have to be considered but many other factors which affect the signals received by the mammary gland. Many factors including the concentration of plasma growth hormone (GH), have pronounced effects on the rate of milk secretion. It has been reported that the concentration of GH 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. 2000a). 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).

Bovine GH is known as a homeorhetic hormone concerned with both growth and lactation, but the mechanism of action of bovine GH on milk production is a controversial area. Receptors for 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. 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). 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, Igeda & Vonderhaar, 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. (2000b) reported that the galactopoietic effect of GH 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 GH, 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 (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. This could relate to the role of GH or a mechanism other than the circulating level of GH. To understand this apparent paradox we studied primarily the short persistency of lactation. Although GH has been known to be a major stimulus for the production of IGF-I and IGF-I is believed to play a role both in mammary development and milk production by mediating the effects of GH (Bauman,1992). Circulating concentrations of IGF-I are also sensitive to the nutritional status in many animal species (see reviews Clemmons & Underwood, 1991). The objective of the present study was to determine the *in vivo* relationship between long-term exogenous administration of bST, circulating levels of IGF-I, mammary blood flow and biological variables relevant to milk synthesis in 87.5% HF animals. Long-term administration of rbST, throughout lactation, might lead to better understanding adaptability in crossbred cattle. This could provide information about choosing suitable crossbred dairy cattle for increased milk production in the tropics.

MATERIALS AND METHODS

Animals and managements

Ten, first lactating, non-pregnant, crossbred, 87.5% Holstein dairy cattle were selected for the experiment. They were divided into two groups of five animals each. Animals in each group were fed with rice straw treated with 5% urea as the source of roughage. All animals were housed in sheds and tethered in individual stalls and fed twice daily. The maximum temperature in the shed at noon was $34\pm1^{\circ}\text{C}$ and the minimum temperature at night was $26\pm1^{\circ}\text{C}$. The relative humidity was $68\pm12\%$. Animals received an average of 4 kg/day of roughage in combination with the same concentrated mixture (7 kg/day) to maintain a moderate body condition score (2.5, scale = 1 to 5). The chemical composition of the feed is presented in Table 1. Each day, the food was given in equal portions at about 06.00 h and 17.00 h when the animals were milked. Animals had free access to water and were fed their respective rations throughout the experimental period.

The urea treated rice straw was prepared by mixing the urea solution with dry straw (5 kg urea dissolved in 100 liter water per 100 kg dry rice straw). Rice straw sprayed with urea solution was mixed thoroughly and stored under airtight conditions in a cement pit for 21 days. A continuous supply of treated rice straw was made available by using a 2 pit x 21 day system of urea treatment. After 21 days, the rice straw treated with 5% urea was offered to the animals.

Experimental procedures

Animals were divided into the control (n=5) and experimental (n=5) groups. Four consecutive periods of study were used for each group. These consisted of a pretreatment period (45 days postpartum before lactation peak) and three treatment periods of 105 days postpartum (early lactation), 165 days postpartum (mid- lactation) and 225 days postpartum (late lactation). During the treatment periods, animals in the experimental group, which had completed 60 days of lactation, were injected subcutaneously every 14 days until the end of study with 500 mg of recombinant bovine somatotropin (rbST). It was suspended in 792 mg of a prolonged-release formulation of sesame oil (POSILAC, Monsanto, USA). Animals in the control group were injected subcutaneously every 14 days with 800 mg of sterile sesame oil without rbST. Injection in each animal was administered at the tail head depression

(ischiorectal fossa). From the pretreatment to the end of the treatment periods, animals of both groups were fed the same ration starting before parturition until the completion of the study. The dry matter intake of each animal was measured by weighing the concentrate and roughage offered and refused each day. Animals were normally milked at around 0600 h and 1700 h using a milking machine and milk production was recorded daily. Measurements of the udder blood flow were carried out in the afternoon. At around 1100 h, an arterial blood sample was collected from the coccygeal artery, by venipuncture with a #21 needle and into a heparinized tube. Blood samples were kept in crushed ice and then centrifuged at 3000 rpm for 30 min at 4°C. Arterial plasma samples were collected for measurements of the level of hormones and metabolites. Plasma samples in aliquots were collected and frozen at -40°C until the time of the assays. Animals were weighed after collecting a milk sample in each period.

Mammary blood flow measurements

On the day before the experiment began and in each period of the experiment, two catheters (i.d. 1.0 mm, o.d. 1.3 mm, L 45 mm) were inserted into either the left or right milk vein using a intravenous polymer catheter (Jelco, Critikon; Johnson & Johnson, U.K.), under local anesthesia. This was done on the standing animal for the measurement of mammary blood flow. The tip of the catheter was positioned near the sigmoid flexure, anterior to the point at which the vein leaves the udder. The other catheter was positioned downstream, about 20 cm from the first one. All catheters were flushed with sterile heparinized normal saline and were left in place during the experiment. Blood flow through half of the udder was determined by measuring the dilution of dye T-1824 (Evans blue) after a short term, continuous infusion, adapted from a method of measuring blood flow in the milk veins of cattle as previously described (Chaiyabutr et al. 1997).

Determination of plasma hormones and metabolite concentration

The plasma IGF-I concentration was determined using Automated Chemiluminescent Immunoassays of IGF-I in an IMMULITE® Analyzer (IMMULITE IGF-1, Diagnostic Products Corporation, Los Angeles, CA). The plasma insulin concentration was quantified using a radio immunoassay (RIA) kit

(Coat a Count[®] Insulin, Diagnostic Products Corporation, Los Angeles, CA). Arterial plasma glucose concentrations were measured using enzymatic oxidation in the presence of glucose oxidase. Plasma triglyceride and total protein concentrations were measured by using an enzymatic colorimetric test and the Biuret test, respectively (Biotechnica Instruments, s.p.a., Italy).

Statistical analysis

Data were compared between the periods of lactation in each group using a paired t-test. Between group trials and mean differences were examined statistically by an un-paired t-test. Mean values are presented as mean $\pm$ S.D.

RESULTS

Changes in plasma concentrations of IGF-I and insulin and plasma metabolites (Table 2)

There was no significant difference in plasma IGF-I concentrations during the pre-treatment period between control animals and the rbST treated animals. The concentration of plasma IGF-I in rbST treated animals was significantly higher ($P<0.001$) than that of the control animals throughout all lactating periods. As this advanced to mid- and late lactation, the mean levels of both plasma IGF-I and insulin in the control animals remained constant and similar to the pre-treatment period. During mid- and late lactation, the plasma insulin levels significantly increased ($P<0.05$) over that seen during the pre-treatment period in rbST treated animals. Plasma glucose and protein concentrations remained stable throughout all periods of study, while the plasma triglyceride concentration slightly increased during mid- and late lactation in both groups.

Changes in dietary dry matter intake, milk yield, udder blood flow and body weight (Table 3)

The total daily dry matter intakes were not significantly different between the control and the rbST treated animals. Studies during the pre-treatment period of both groups were started 45 days post partum. The enhancement of milk yield in animals given rbST was higher than that of the control animals receiving placebo throughout their lactation. The peak milk yield in both groups declined from the early period of lactation as lactation advanced to mid and late lactation. Compared with the pre-

treatment value, the actual increases in milk yield during the different lactating periods was 20%, 10% and -2% for animals receiving the rbST over 45, 105 and 165 days, respectively. In early lactation, milk yield of rbST treated animals was 22% greater ($P<0.05$) than that of the control animals receiving placebo. An evaluation of the dry matter intake and milk yield revealed that the mean ratios of total dry matter intake to milk yield in rbST treated animals was significantly less ($P<0.05$) in the early period of lactation. The mean ratio of total dry matter intake to milk yield showed no significant changes throughout lactation in the control animals. The body weights of both control animals and rbST treated animals significantly increased stepwise as compared with the pre-treatment period, while rbST treated animals had a higher weight gain than control animals receiving placebo throughout the lactation. The rate of udder blood flow markedly increased during rbST administration. The udder blood flow of rbST treated animals increased from 3548 to 5310 and 5458 ml/min ($P<0.05$) in early and mid lactation, respectively, while there were no significant changes in the control animals receiving placebo. The ratio of udder blood flow to the rate of milk yield increased as lactation advanced in both the control and the rbST treated animals.

Table 1. *Chemical composition of feed components (% on dry matter basis)*

Particulars	Urea-treated rice straw	Concentrate
Dry matter	58.0	89.4
Crude protein	8.9	17.8
Acid detergent fibre	61.2	21.5
Neutral detergent fibre	67.2	28.8
Lignin	8.8	7.0
Ash	16.8	5.6

Concentrate formulation: fresh weight (kg/100 kg) consisted of soy bean meal 30 kg, cotton seed 25 kg, cassava 25 kg, rice bran 15 kg, dicalcium phosphate 2 kg, sodium bicarbonate 1.7 kg, potassium chloride 0.7 kg and vitamin/mineral premix 0.6 kg. .

Table 2. *The Plasma concentrations of the insulin like growth factor 1 (IGF-1), insulin, glucose, protein and triglyceride in different stages of lactation in the control and rbST treated animals (n = 5 in each group)*

	Period of lactation	Control VS		
		Control Group	rbST Group	rbST Group
Plasma IGF-1 (ng/ml)	Pretreated	40±15	50±29	ns
	Early	48 ±16	209±42***	P<0.001
	Mid	47 ±13	202±55***	P<0.001
	Late	55±16	151±63***	P<0.01
Plasma insulin (µg/l)	Pretreated	0.32±0.23	0.34±0.31	NS
	Early	0.33±0.29	0.64±0.37	NS
	Mid	0.29±0.14	1.04±0.53*	P<0.01
	Late	0.42±0.32	0.83±0.89*	NS
Plasma glucose (mg/dl)	Pretreated	69±4	68±2	NS
	Early	70±7	69±4	NS
	Mid	65±3	69±3	NS
	Late	67±1	66±3	NS
Plasma triglyceride (mg/dl)	Pretreated	11.4±3.4	13.7±4.7	NS
	Early	11.3±4.4	11.2±1.2	NS
	Mid	12.7±3.9	15.7±6.3	NS
	Late	13.6±6.9	14.5±3.9	NS
Plasma protein (g/dl)	Pretreated	8.14±0.64	8.29±0.46	NS
	Early	8.05±0.91	7.72±0.42	NS
	Mid	7.75±0.95	7.99±0.41	NS
	Late	8.39±0.69	7.75±0.46	NS

P-values by paired t-test: * P<0.05, *** P<0.001 with respect to the pretreated period in each group.

P-values by unpaired t-test between the control animals and rbST treated animals.

Table 3. *The changes in dietary dry matter (DM) intake, milk yield, udder blood flow and body weight in different stages of lactation in the control and rbST treated animals (n = 5 in each group).*

	Period of lactation	Control VS		
		Control Group	rbST Group	rbST Group
Dry matter intake (kg/d)	Pretreated	11.41±0.66	12.30±0.76	NS
	Early	11.64±1.11	13.01±1.67	NS
	Mid	12.22±1.76	13.91±1.28	NS
	Late	12.29±1.80	13.37±1.70	NS
Milk yield (kg/d)	Pretreated	13.0±1.5	13.3±2.7	NS
	Early	13.1±1.9	16.0±2.1**	P<0.05
	Mid	12.9±1.5	14.6±1.9	NS
	Late	11.5±1.0	13.0±1.3	NS
DM intake/Milk yield	Pretreated	0.89±0.12	0.96±0.25	NS
	Early	0.90±0.12	0.85±0.22*	NS
	Mid	0.95±0.13	0.95±0.06	NS
	Late	1.07±0.19	1.03±0.12	NS
Udder blood flow (ml/min)	Pretreated	3286±461	3548±463	NS
	Early	3817±616	5310±1620*	NS
	Mid	3821±533	5458±1627*	NS
	Late	3750±476	4814±1464	NS
Udder blood flow/ Milk yield	Pretreated	364±25	397±111	NS
	Early	420±32*	491±152	NS
	Mid	433±89	539±156	NS
	Late	471±69	539±168	NS
Body weight (kg)	Pretreated	336.9±31.1	363.6±27.1	NS
	Early	357.1±34.0**	391.2±35.6**	NS
	Mid	369.8±33.8**	412.4±35.5***	NS
	Late	379.2±29.8**	420.9±43.5**	NS

P-values by paired t-test: * P<0.05, ** P<0.01, *** P<0.001 with respect to the pretreated period in each group.

P-values by unpaired t-test between control animals and rbST treated animals.

DISCUSSION

Dairy herds in tropical countries are of mixed exotic breeds and crossbreeds. The potential for milk production of most indigenous cattle in the tropics is less than that of dairy cattle in temperate countries, while indigenous cattle have resistance to tropical diseases and a high level of heat tolerance (Nakamura et al., 1993). Exotic *Bos taurus* breeds have higher milk production but they also have inherent disadvantageous traits. Crossbreeding has been exploited as an efficient tool for blending the adaptability of tropical cattle with the high milking production of exotic breeds. We found that different types of crossbred Holstein Friesians (HF) showed differences in persistency of lactation and mammary circulation. Crossbred cattle containing 87.5% Holstein genes as compared to 50%HF animals, had a low persistency of lactation. We noted a quick decrease in the peak rate of decline with rapid decreases in both mammary blood flow and the concentration of plasma growth hormone (GH)(Chaiyabutr et al. 2000a). However, milk synthesis in ruminant is complex and dynamic depending on several factors including stage of lactation, energy balance and nutrition management. The present study was designed to clarify whether short lactation, occurring in crossbred cattle containing 87.5% Holstein genes (Chaiyabutr et al. 2000b), could be attributed to a decrease in the circulating level of GH or some other mechanism. Long-term administration of recombinant bovine somatotropin (rbST) was undertaken in 87.5%HF animals that had completed 60 days of lactation prior to treatment. The 500 mg of rbST used in the present study and given twice weekly was the dose recommended for *Bos taurus* cows. Animals treated with rbST showed increased milk yields and circulating levels of IGF-I throughout lactation. These findings 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 (Clemmons and Underwood, 1991). From the present data, the increase in IGF-I

secretion throughout the study would appear to be maintained by the availability of exogenous rbST in the liver. Exogenous rbST administration in the present study was sufficient to achieve a satisfactory stimulation of IGF-I (Collier et al., 1988). GH is a 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 the controls and the rbST treated animals were apparent in all lactating periods. Animals in both groups were equally well-fed. Animals with a lower nutritional state having a lower basal level of IGF-I (Hodgkinson, Bass & Gluckman, 1991) or a negative energy balance, have reduced hepatic IGF-I production (Weller et al. 1994; Ketelslegers et al. 1995), would not be expected to occur in the present study.

The rbST had no effect on plasma levels of triglyceride, glucose and protein throughout lactation, although GH has been known to elevate concentrations of fat (free fatty acids) and glucose in the blood (Vernon & Finley, 1988). These results could not be a factor in limiting IGF-I release from the liver (McGuire et al. 1995). However, an increase in extracellular water compartments including the plasma volume in animals treated with exogenous rbST was observed (Chaiyabutr et al., unpublished data). These responses could be attributed to an increase in the plasma pool of circulating substrates (plasma volume x concentration), facilitating the partitioning of nutrients for milk synthesis and IGF-I secretion.

During mid- and late lactation, plasma insulin levels have been shown to increase over those seen during the pre-treatment period in rbST treated animals. This suggests that an increase in IGF-I secretion would be dependent on the availability to the liver of both GH and insulin (Luo & Murphy, 1991). The relationship between GH and insulin was not apparent for rbST treated animals in early lactation. However, maintaining the plasma concentration of glucose with high concentrations of insulin was apparent in rbST treated animals. This indicates that in later lactation, elevated plasma concentrations of exogenous GH decreased the responsiveness of peripheral tissues to high concentrations of insulin. This would spare glucose for insulin insensitive tissues, particularly the mammary gland.

The milk yield of the control animals receiving placebo, slightly increased after treatment started. Peak yield were smaller in the controls than in rbST treated animals and decreased as lactation advanced to mid and late lactation. Milk yield responses at 45 days of rbST treatment in early lactation were significantly greater (+20%

compared with pre-treatment) than to the 105 days of rbST treatment in mid lactation(+9.5%) and to 165 days of rbST treatment in late lactation (-2.%) in late ; thus, rbST affects the shape of the lactation curve. These results confirm the finding that an increase in milk yield in response to rbST administration will not be sustained indefinitely (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, the effect of rbST administration significantly influenced milk production efficiency. The ratio of total dry matter intake to milk production was lower in rbST treated animals when compared to that of the control animals consuming similar DM at a similar period of lactation. It indicated that rbST is one of the factors capable of stimulating mammary gland synthetic capacity in crossbred lactating animals.

However, animals in both groups gained weight throughout the experiment. A marked increase in milk yield 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. These results provide the physiological differences between crossbred animals and exotic breeds in partitioning ability, which would be inherited. During early lactation, the metabolic demands of lactation in both groups of the crossbred HF animals 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 animals. We 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, Lawrence & Mepham 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 increased as lactation advanced to

mid and late lactation in both the control and the rbST treated animals. A greater decrease in milk secretion, with minimal changes in mammary blood flow, caused a high 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 is 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, milk yield rose to a peak in early lactation and then gradually declined over 32 weeks of the experiment to 19 % as compared with early lactation, whilst the plasma concentration of IGF-I and the mammary blood flow did not decrease in the rbST treated animals. 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. 1989). Mammary tissue is itself capable of synthesizing an IGF-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.

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CHAPTER VI

Effects of Long-term Administration with Recombinant Bovine Somatotropin on the Plasminogen-Plasmin System and Milk Compositions in Crossbred Holstein Cattle

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Effects of Long-term Administration with Recombinant Bovine Somatotropin on the Plasminogen-Plasmin System and Milk Compositions in Crossbred Holstein Cattle

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ABSTRACT

The present study was designed to clarify whether long-term administration of recombinant bovine somatotropin (rbST) suppresses milk plasmin-plsminogen activity within the mammary gland and allow a persistence of milk production during different stages of lactation in crossbred Holstein cattle. Ten, first lactation, 87.5%HF animals were divided into two groups of 5 animals each. Four consecutive periods of study were carried out in each group, a pretreatment period (45 days postpartum) and three consecutive treatment periods. In the treatment periods, the rbST-treated animals, which had completed 60 days of lactation, were injected subcutaneously every 14 days with 500 mg of rbST (POSILAC, Monsanto, USA) until the end of the study, while the control animals were injected subcutaneously every 14 days with 800 mg of sterile sesame oil, as a placebo. The treatment periods were carried out in early lactation (105 days postpartum), mid-lactation (165 days postpartum) and late lactation (225 days postpartum). Animals receiving rbST gave greater milk yields than control animals in all stages of lactation. The milk yield of rbST-treated animals significantly increased in early lactation ($P<0.01$), when compared with the initial pretreatment period. The peak milk yield in both groups declined from the early period of lactation as lactation advanced to mid and late lactation. Udder blood flow significantly increased during rbST administration, while there were no significant changes throughout lactation in the control animals. The concentration of milk lactose of both controls and rbST treated animals showed no significant changes throughout lactation, while the concentrations of milk protein and milk fat of rbST-treated animals increased during advanced lactation. The milk fat concentration of rbST-treated animals had a significantly greater ($P<0.05$) than that of control animals in the early lactation. No significant changes for the

concentration of milk Na and K including Na/K ratio in comparison with control animals at different stages of lactation. The concentration of milk Cl significantly increased during advanced lactation in the control animals, while the concentration of milk Cl of rbST-treated animals significantly decreased ($P<0.05$) in the early lactation. The plasminogen and plasmin activities increased during lactation advances in both groups. The concentration of plasmin in milk gradual increased, while milk plasminogen concentration significantly increased as lactation advances in both the controls and rbST-treated animals. The plasminogen : plasmin ratio decreased in the control animals while it increased in rbST-treated animals as lactation advances. These findings demonstrate that administration of rbST cause animals to maintain milk plasmin at low concentration throughout lactation. The decrease in milk secretion during the progress of lactation might not be controlled by changes in extra-mammary factors but, in part, through changes within the mammary gland relating to the activity of the plasmin-plasminogen system.

Key words : rbST, Milk Yield, Plasminogen - Plasmin activity, Crossbred Holstein Cattle

INTRODUCTION

Bovine growth hormone or somatotropin (bST) is known as a homeorrhetic hormone connected with both growth and lactation. The relationship between plasma ST concentration and milk yield has been defined. An advance of lactation is characterized by decrease in milk yield and concomitant decrease in blood ST concentration (Hart et al., 1980). The importance of bST for enhancing and maintaining milk production in dairy ruminants is well established (Bauman, 1999). Administration of a slow-release formulation of bST to dairy ruminants improves lactation persistency by slowing down the post peak rate of decline (Gallo et al 1997). The milk production above peak using sustained release of bST in cows beginning at 60 days postpartum by Bauman et al.(1989) showed no increase in milk production and controversial. A few data are available for the role of bST for short persistency of lactation in crossbred dairy cattle in the tropics, although Chaiyabutr et al.(2000) reported that the concentration of bST of lactating crossbred cattle containing 87.5%Holstein genes markedly decreased as lactation advances to mid- and late lactation; this decrease could attribute to decreases in milk yield and mammary blood flow.

A number of studies indicate that bST can delay involution of the mammary gland by reducing the activity of the plasmin-plasminogen system, an important initiator of tissue remodeling during lactation advance in dairy ruminants (Baldi et al., 1997; Politis et al., 1990). There is evident that the progressive loss of milk synthesis capacity by mammary epithelial cells occurs during mammary involution, although substrate supply to the mammary gland is often adequate to maintain the maximum rate of milk synthesis. As lactation advances, a leaky of cell tight junctions is also apparent during involution of the secretory tissue. In the process of the proteolysis, the proteinase responsible is plasmin, which is transferred from blood into milk as an inactive precursor (plasminogen) and then converted to active plasmin by plasminogen activators, which are produced in quantity within the mammary gland especially in the late lactation. Increases in plasmin production in milk are important in determining milk production and initiate the onset of involution within the mammary gland (Ossowski et al., 1979). Little is known about responsible for this proteolysis relating to the role of growth hormone on the persistency of lactation in crossbred cattle. Thus, the objective of the present study was to determine the relationship between milk plasmin-plasminogen and milk yield including milk compositions during long-term administration of rbST in different stages of lactation in 87.5% HF animals.

MATERIALS AND METHODS

Animals and managements

Ten, first lactating, non-pregnant crossbred 87.5%Holstein dairy cattle were selected for the experiment. They were divided into two groups of five animals each. Animals in each group were fed with rice straw treated with 5% urea as the source of roughage throughout the experiments. All animals were housed in sheds and tethered in individual stalls and fed twice daily. The maximum temperature in the shed at noon was $34\pm1^{\circ}\text{C}$ and the minimum temperature at night was $26\pm1^{\circ}\text{C}$. The relative humidity was $68\pm12\%$. Animals individually received an average of 4 kg/day of roughage in combination with the same concentrated mixture (7 kg/day) to maintain a moderate body condition score (2.5, scale = 1 to 5). The chemical composition of feeds is presented in Table 1. Each day, the food was given in equal portions at about 06.00 h

and 17.00 h when animals were milked. Animals had free access to water and animals were fed their respective rations throughout the experimental period.

The urea treated rice straw was prepared by mixing the urea solution with dry straw (5 kg urea dissolved in 100 liter water per 100 kg dry rice straw). Rice straw sprayed with urea solution was mixed thoroughly and stored under airtight conditions in a cement pit for 21 days. A continuous supply of treated rice straw was made available by using a 2 pit x 21 day system of urea treatment. After 21 days, the rice straw treated with 5% urea was offered to the animals.

Experimental procedures

Animals were divided into control (n=5) and experimental (n=5) groups. Four consecutive periods of study were carried out in each group, consisting of the pretreatment period (45 days postpartum), and treatment periods of 105 days postpartum (early lactation), 165 days postpartum (mid- lactation) and 225 days postpartum (late lactation). In the treatment period, animals in the experimental group which had completed 60 days of lactation, were injected subcutaneously every 14 days until the end of study with 500 mg of recombinant bovine somatotropin (rbST) suspended in 792 mg of a prolonged-release formulation in sesame oil (POSILAC, Monsanto, USA), while animals in the control group were injected subcutaneously every 14 days with 800 mg of sterile sesame oil without rbST as placebo. Injection in each animal was administered at the tail head depression (ischio-rectal fossa). From the beginning of pretreatment to the end of treatment period, animals of both groups were fed the same ration from before parturition through the completion of study. The dry matter intake of each animal was determined by measuring both the concentrate and roughage offered and refused each day.

Milk sampling and determinations of milk compositions

Animals were normally milked at around 0600 h and 1700 h by a milking machine and daily milk yield (kg/day) was recorded and weekly average of each animal was calculated. Milk was collected in the afternoon of specified day and divided to two portions. One was kept in fresh milk for determination of the plasmin-plasminogen concentration and other portion was kept in formalinized milk. The formalinized milk sample (300 µl of 40% formaldehyde in 30 ml of fresh milk) was kept at 4°C for lactose, fat and protein concentrations by the colorimetric method (Tele et al.,1978), Gerber method and infrared method using Milkoscan, respectively. The concentrations

of electrolytes in aqueous phase of milk were estimated for sodium (Na) and Potassium (K) using Flame photometry, Chloride (Cl) concentration by Chloridometer (Corning).

Plasmin and plasminogen determination

The concentrations of plasmin and plasminogen in milk or casein fractions were determined the method of Korycka-Dahl et al (1983) with a slight modification. Briefly, The plasmin activity was performed by measuring the rate of hydrolysis of the chromogen substrate (H-D-valyl-L-leucyl-L-lysine-p-nitroanilide dihydrochloride, S-2251, Chromogenix Instrumentation Laboratory, Italy). Formation of p-nitroanilide resulting from substrate cleavage by plasmin was measured spectrophotometrically at 405nm. 1 unit of activity of plasmin and plasminogen was defined as the amount of enzyme that produced a change in absorbance at 405 nm of 0.001 in 1 min at pH 7.4, 37 C when p-nitroanilide was produced from S-2251 substrate.

Udder blood flow measurements

On the specified day in each period, measurements of the udder blood flow were carried out in the afternoon. Udder blood flow measurements were performed in duplicate. Blood flow through half of the udder was determined by measuring the dilution of dye T-1824 (Evans blue) by a short term continuous infusion as described by Chaiyabutr et al. (1997). Udder blood flow was calculated by doubling the flow measured in one milk vein (Bickerstaffe et al., 1974). Packed cell volume was measured after centrifugation of the blood in a microcapillary tube.

Statistical analysis

Values were compared between the periods of lactation in each group using a paired t-test. Between group trials and mean differences were examined statistically by an unpaired t-test. Mean values are presented as mean $\pm$ SD.

RESULTS

Udder blood flow and milk yield during different stages of lactation