Relationship of Early Lactation and Bovine Somatotropin to Water Metabolism and Mammary Circulation of Crossbred Holstein Cattle

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ABSTRACT : The study was carried out to evaluate the effect of exogenous bovine somatotropin on water metabolism in relation to mammary function in early lactation of crossbred Holstein cattle. Ten, 87.5% crossbred Holstein cattle were divided into two groups of 5 animals each. At day 60 of lactation, the control group was given placebo while animals in the experimental group were given recombinant bovine somatotropin (rbST) by subcutaneous injection with 500 mg of rbST (14-days prolonged-release rbST). In rbST-treated animals, milk yield increased 19.8%, which coincided with a significant increase in water intake ($p<0.01$), while DM daily intake was not different when compared to the control animals. Water turnover rate as absolute values significantly increased ($p<0.05$), while the biological half-life of water did not change in rbST-treated animals. Total body water (TBW) and total body water space (TOH) as absolute values significantly increased ($p<0.01$) in rbST-treated animals, while it was decreased in the control animals. Absolute values of empty body water (EBW) markedly increased ($p<0.05$), which was associated with an increase in the extracellular fluid (ECF) volume. Absolute values of plasma volume and blood volume were also significantly increased ($p<0.05$) in rbST-treated animals. The increase in mammary blood flow in rbST-treated animals was proportionally higher than an increase in milk production. The plasma IGF-1 concentration was significantly increased ($p<0.01$) in rbST-treated animals when compared with those of control animals during the treatment period. Milk fat concentration increased during rbST treatment, while the concentrations of both protein and lactose in milk were not affected. The present results indicate that rbST exerts its effect on an increase in both TBW and EBW. An increased ECF compartment in rbST-treated animals might partly result from the decrease in fat mass during early lactation. The action of rbST on mammary blood flow might not be mediated solely by the action of IGF-1 for increase in blood flow to mammary gland. An elevation of body fluid during rbST treatment in early lactation may be partly a result of an increase in mammary blood flow in distribution of milk precursors to the gland. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 11 : 1600-1608)***

Key Words : rbST, Mammary Blood Flow, Total Body Water, Early Lactation, Cross Bred Holstein Cattle

INTRODUCTION

It is known that lactating dairy cows metabolize large amounts of water and are affected rapidly by water deprivation. An increase in water intake during lactation closely matched to increase in water secreted in milk (Woodford et al., 1984), which milk composition has about 87% of water (Murphy, 1992). An alteration in bodily function during lactation is apparent; for example, blood volume (Chaiyabutr et al., 1997) and cardiac output (Hanwell and Peaker, 1977), are increased. These changes may effectively alter body fluid and thus circulatory distribution including the blood supply to the mammary gland. The lactating mammary gland receives signals from the rest of body in forms of nutrient and hormones from blood. Mammary blood flow is thus a major parameter controlling milk production in a way to carry milk precursors to the mammary gland at the process of milk synthesis.

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During lactation, coordination between nutrient delivery and biosynthetic capacity are thought to be under endocrine control with homeorhetic mechanism. However, the mechanism of action for growth hormone on milk production remains unclear. Several lines of evidence indicate that administration of growth hormone does not act directly on mammary gland. The receptors for growth hormone have not been demonstrated on epithelial cells of mammary tissue (Akers, 1985). It has been reported that mammary blood flow and milk secretion of crossbred cattle containing 87.5% Holstein (HF) genes were significantly higher during early lactation and markedly declined when lactation advanced. The levels of plasma growth hormone of 87.5% HF cows also rose in early period of lactation and markedly reduced in mid and late lactation (Chaiyabutr et al., 2000). Water turnover rate and total body water as percentage of body weight of 87.5% HF animals showed a poor adjustment to the tropical environment (Chaiyabutr et al., 1999). During early lactation, high producing cows cannot consume enough dry matter to meet nutrient requirements. Negative energy balance and body fat mobilization is usually apparent. A few studies in crossbred dairy cattle have been conducted to study effects of bovine somatotropin on the interaction between body fluid, fat

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

Particulars	Urea-treated rice straw	Concentrate
Dry matter	57.7	89.4
Crude protein	7.0	17.2
Acid detergent fibre	43.5	20.7
Neutral detergent fibre	70.2	28.4

mass and mammary function during early to mid lactation.A decrease in fat-free mass coinciding with a decrease in ECF, but not TBW, has been reported in growth hormone deficient humans, and it appears to increase fat-free mass and decreased fat mass during GH therapy (Janssen et al., 1997). An appearance of a shorter lactation persistency of crossbred cattle containing 87.5% Holstein genes during transition period from early to mid-lactation has also been reported to be due to the reduction of the growth hormone level (Chaiyabutr et al., 2000). During early lactation, nutrient partitioning relating to circulatory distribution are known to make a contribution of resources to the mammary gland for a high milk synthesis (Linzell, 1974) Changes in body fluid have not been evaluated for responsibility of the rapid decreasing milk secretion during lactation advances in 87.5% HF animals. Therefore, the objective of this study is to determine the physiological mechanism behind the result of the decrease in milk secretion, with concomitant decreases in both the level of circulating growth hormone and mammary blood flow during advanced lactation in 87.5% HF animals (Chaiyabutr et al., 2000), may be related in part to changes in body fluid. The present experiment was conducted with respect to the effect of administration of rbST on body water metabolism, mammary function, mammary blood flow and other physiological parameters during peak yield in early lactation.

MATERIALS AND METHODS

Animals and management

Ten, late pregnancy 87.5% crossbred Holstein cattle were used in the experiment. They were divided into two groups of five animals each. All animals were housed in tie stall type sheds, having a solid floor and open sides. The maximum temperature in the shed at noon was 34±1°C and the minimum temperature at night was 26±1°C. The relative humidity was 68±12%. Animals in each group were fed with rice straw treated with 5% urea as the source of roughage throughout the experiment. Animals individually received an average of a 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. Concentrate formulation was prepared in fresh weight (kg/100 kg) which consisted of soy bean meal 26.3 kg, cotton seed 37 kg, cassava 28.5 kg, rice bran 3.3 kg, limestone 1.3, dicalcium phosphate 1.5 kg, sodium bicarbonate 1.1 kg, potassium chloride 0.8 kg and premix 0.2 kg. The urea treated rice straw was prepared by rice straw sprayed with urea solution was mixed thoroughly and stored under airtight conditions in a cement pit for 21 days (5 kg urea dissolved in 100 litres water per 100 kg dry rice straw). After 21 days, the rice straw treated with 5% urea was offered to the animals. A continuous supply of treated rice straw was made available by using a two pit×21 day system of urea treatment. Food was given in equal portions at about 06.00 h and 17.00 h when animals were milked. All samples of urea treated rice straw and concentrate were analyzed for dry matter, crude protein and ash using procedures described by AOAC (1984). ADF and NDF were analyzed according to Van Soest and Robertson (1980). Water was available for *ad libitum* intake.

Experimental procedures

Animals were divided into control (n = 5) and experimental (n = 5) groups. Two consecutive periods of experiments were carried out in each group, consisting of the pretreatment period (45 days postpartum), and treatment periods of 105 days postpartum (early lactation). At the start of treatment period at day 60 of lactation, rbST-treated animals were injected subcutaneously every 14 days until the end of experiment with 500 mg of recombinant bovine somatotropin (rbST) suspended in 792 mg of a prolonged-release formulation in sesame oil (POSILAC, Monsato, USA), while animals in the control group were injected subcutaneously every 14 days with 800 mg of sterile sesame oil without rbST as placebo. An injection was administered at the post scapular site.

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 experiment. The dry matter intake of each animal was determined by measuring both the concentrate and roughage offered and refused each day. On the day of experiment in each period, measurements of the total body water, water turnover rate, mammary blood flow, plasma volume and extracellular fluid were carried out. The rate of milk secretion was recorded by hand milking in the afternoon and measurement of mammary blood flow was carried out. In each period of study animals were weighed after collecting the milk sample.

Animal preparation

On the day before the experiment in each period, two catheters (i.d. 1.0 mm, o.d. 1.3 mm, length 45 mm) were inserted into either the left or right milk vein by using an intravenous polymer catheter (Jelco, Critikon; Johnson &

Johnson, U.K.) under local anesthesia. This was carried out on the standing animal for 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. The catheter for both isotope and dye injection was inserted into an ear vein under local anesthesia. All catheters were flushed with sterile heparinized normal saline and were left in place during the experiment.

Determinations of water intake, total body water, water turnover and empty body water

Estimation of the rate of water intake of each animal in each period of experiments was recorded by an average over seven days from weighing daily water consumption from the water bowl. The water turnover rate and total body water were determined in each animal by tritiated water dilution techniques. The animal was injected intravenously via the ear vein with carrier free tritiated water in normal saline at a single dose of 2,500 μCi per animal. The equilibration time was determined by taking blood samples for 3 days after the injection. Blood samples were collected 4, 8, 20, 26, 32, 44, 50, 56, 68 and 74 h subsequent to the injection for water turnover measurements (Chaiyabutr et al., 1997). The preparation for sample counting was achieved by the internal standardization technique as described by Vaughan and Boling (1961). The corrected activity of the samples, in disintegrations per minute (d.p.m.), were plotted on semi-logarithmic paper against time, in hours after dosing, and the extrapolated activity at theoretical zero time of complete mixing of radio-isotope was used to determine the total body water space (TOH). The TOH space was calculated:

$$\text{TOH space (ml)} = [\text{standard count (dis/min)} \times \text{dose (ml)}] / [\text{radio activity counts at zero time (dis/min)}]$$

The biological half-life of tritium labelled water (T1/2) was determined from the slope of the linear regression line obtained from plot on semi-logarithmic paper of the activity of the samples taken over the period of 3 days against time.

The water turnover rate was calculated from the equation: Water turnover rate (l/d) = 0.693×TOH space/ biological half-life. The total body water was calculated by using the corrected factor (1-fraction of plasma solids)× TOH space (Chaiyabutr et al., 1997).

Empty body water (EBW) did not include water associated with gastrointestinal contents or the water in the fetus. The EBW was estimated from the disappearance curve of tritium in blood plasma for each animal. A two compartment open system model was used to estimate the EBW (Shipley and Clark, 1972). The exponential equation describing the two compartment model was calculated from the equation:

$$Y = Ae^{-k1t} + Be^{-k2t}$$

Where, Y = concentration of tritium in plasma at time t

A = plasma concentration intercept of the fast phase of the plasma curve

B = plasma concentration intercept of the slow phase of the plasma curve

k1 = first order rate constant of the fast phase

k2 = first order rate constant of the slow phase

t = time in minutes

Determinations of plasma volume, extracellular fluid and intracellular fluid

In each period of study, plasma volume was measured by a dye dilution technique using Evans blue dye (T-1824) (E. Merck, Darmstadt, Germany) and extracellular fluid volume (ECF) was measured using sodium thiocyanate (NaSCN). The injection of 20 ml of the 0.5% T-1824 (0.5 g/100 ml normal saline), and 20 ml of the 10% NaSCN solution (10 g/100 ml normal saline) were given into the ear vein catheter. Venous blood samples from the jugular vein were taken at 20, 30, 40 and 50 min after dye injection. The dilution of dye at zero time was determined by using semi logarithmic concentration on time extrapolation. Blood volume was calculated from the plasma volume and packed cell volume (PCV). Intracellular fluid (ICF) was calculated by subtracting ECF from TBW. Plasma osmolality was measured using the freezing point depression method (Advance Osmometer model 3, Massachusetts, USA). The plasma solids concentration was determined by a refractometer.

Determination of mammary blood flow

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).

Milk collection and determinations of milk compositions

Milk was collected by hand milking and kept in formaldehyde. The formalinized milk sample (300 μl of 40% formalin in 30 ml of fresh milk) was kept at 4°C for determinations of lactose (Tele et al., 1978); fat and protein concentrations by the colorimetric method using Gerber methods (Clunie Harvey and Hill, 1967) and Milkoscan (Milkoscan 4,000, Foss Electrique), respectively.

Determination of the plasma IGF-1 concentration

The arterial plasma IGF-1 concentration was determined

Table 2. Dietary dry matter intake, water intake and milk yield in the control animals and animals treated with rbST (Values are means±SD (n = 5))

	Period of experiments	Control group	rbST group	Control vs. rbSTgroup[1]
Dry matter intake:				
Total DM intake (kg/d)	Pretreatment	11.41±0.66	12.30±0.76	NS
	Treatment	11.64±1.11	13.01±1.67	NS
Total DM intake:	Pretreatment	3.41±0.38	3.40±0.36	NS
(kg/100 kg)	Treatment	3.26±0.10	3.32±0.27	NS
Water intake (l/d):	Pretreatment	58.66±13.16	65.20±10.57	NS
	Treatment	60.22±12.31	70.89±12.43**	NS
Milk yield (kg/d):	Pretreatment	12.98±1.53	13.37±2.66	NS
	Treatment	13.11±1.85	16.02±3.99*	NS
DM intake/milk yield:	Pretreatment	0.89±0.12	0.96±0.25	NS
	Treatment	0.90±0.12	0.85±0.22*	NS
Body weight (kg):	Pretreatment	336.9±31.1	363.6±27.1	NS
	Treatment	357.1±34.0**	391.2±35.6*	NS

P-values by paired t-test: * $p<0.05$, ** $p<0.01$, with respect to the pretreated period in the same group.
[1] Statistical analysis of treatment differences, NS = Nonsignificant ($p>0.05$).

Table 3. Plasma volume, blood volume and packed cell volume in the control animals and animals treated with rbST (Values are means±SD (n = 5))

	Period of experiments	Control group	rbST group	Control vs. rbSTgroup[1]
Plasma volume:				
(L)	Pretreatment	16.0±1.3	16.6±1.4	NS
(L/100 kg)	Treatment	17.4±1.9	19.4±3.2*	NS
	Pretreatment	4.7±0.4	4.6±0.2	NS
	Treatment	4.8±0.2	4.9±0.6	NS
Blood volume:				
(L)	Pretreatment	22.3±2.0	23.3±2.0	NS
	Treatment	24.6±3.2	26.9±4.7*	NS
(L/100 kg)	Pretreatment	6.6±0.5	6.4±0.3	NS
	Treatment	6.9±0.3	6.9±0.8	NS
Hct (%):	Pretreatment	28.1±1.5	28.6±0.9	NS
	Treatment	29.2±2.5	27.9±1.4	NS
Plasma osmolality:	Pretreatment	280±4	274±6	NS
(mOsm/kg)	Treatment	280±5	276±3	NS

P-values by paired t-test: * $p<0.05$, with respect to the pretreated period in the same group.
[1] Statistical analysis of treatment differences, NS = Nonsignificant ($p>0.05$).

using the automated chemiluminescent immunoassays with alkaline phosphatase conjugated polyclonal rabbit anti-IGF-1 antibody in an IMMULITE® Analyzer (IMMULITE IGF-1, Diagnostic Products Corporation, Los Angeles, CA). The arterial plasma samples were processed in duplicate. All samples were included in the same assay to eliminate interassay variation. Intraassay variation for CV of samples was 6.7%.

Statistical analysis

All the data obtained were presented as the means±SD. Statistical significant differences between periods in the same group were determined by the student's paired t-test. The student's unpaired t-test was used to estimate the statistical significant differences between groups (Snedecor and Cochran, 1989).

RESULTS

Daily dry matter intake, water intake and milk yield (Table 2)

No significant differences in the total daily dry matter intake (DM) or DM as a percent body weight were apparent between control and rbST-treated animals. Daily water intake of rbST-treated animals significantly increased ($p<0.01$), which coincided with an increase in milk production. In animals given rbST in early lactation, milk yield increased from 13.4 to 16.0 kg/d/animal ($p<0.05$). In contrast to rbST-treated animals, milk yield of the control animals were not significantly different between pretreatment and treatment periods although the peak of milk yield occurred at week 10 in both groups. An evaluation of the dry matter intake and milk yield revealed

that during the treatment period the mean ratios of total DM intake to milk yield of rbST-treated animals were lower than those of control animals. The mean ratios of dry matter intake to milk yield decreased significantly ($p<0.05$) in the treatment period of rbST-treated animals. The body weight significantly increased ($p<0.05$ and $p<0.01$) in treatment period as compared with those of the pretreatment period in both control animals and rbST-treated animals.

Plasma volume, blood volume, plasma osmolality and packed cell volume (Table 3)

In the pretreatment period, there were no significant differences of the plasma volume and blood volume as absolute values or as percentages of body weight between control animals and rbST-treated animals. Plasma volume and blood volume as absolute values significantly increased ($p<0.05$) during treated with rbST when compared with the pretreatment period. There were no significantly different of the packed cell volume and plasma osmolality throughout period of studies in both groups.

The water turnover rate, biological half-life and total body water (Table 4)

In the pretreatment period, there were no significant differences for the water turnover rate between control and rbST- treated animals. In the treatment period, water turnover rate as absolute values significantly increased ($p<0.05$) in animals given rbST when compared with the control animals or with the pretreatment period in the same group. An average of water turnover rate as a percent of body weight and the water turnover rate per body fat free wet weight ($kg^{0.82}$) were not significantly different between controls and rbST-treated animals throughout the study period. There were no changes in the biological half-life of tritiated water between control animals and animals given rbST. The TOH space and total body water vary with the size of animals. The TOH space and total body water as absolute values was not significantly different between control animals and rbST-treated animals in the pretreatment period. The TOH space and total body water as absolute values of rbST-treated animals significantly increased ($p<0.01$) than those of control animals in the treatment period. The TOH space and total body water as absolute values in the treatment period were significantly increased ($p<0.01$) over that of pretreatment period in rbST-treated animals. In the control animals, total body water and TOH space period were significantly decreased compared with those in the pretreatment period. There were no significant differences in TOH space and total body water as a percentage of body weight between control animals and animals given rbST.

Empty body water, gut water, extracellular fluid and intracellular fluid (Table 5)

There were no significant differences of the EBW as absolute values or as percentages of body weight between

Table 4. Changes in water turnover rate and total body water in the control animals and animals with rbST (Values are means±SD (n = 5))

	Period of experiments	Control group	rbST group	Control vs. rbSTgroup[1]
Water turnover rate:				
(L/d)	Pretreatment	60.00±13.57	69.63±18.53	NS
	Treatment	60.65±10.06	85.20±19.35*	p<0.05
(L/100 kg/d)	Pretreatment	17.52±3.31	19.56±5.50	NS
	Treatment	17.03±2.70	22.11±5.90	NS
($ml/kg^{0.82}/d$)	Pretreatment	499.7±97.7	564.4±156	NS
	Treatment	490.0±75.9	645.50±168	NS
Biological half-life (d)	Pretreatment	3.25±0.60	2.94±0.78	NS
	Treatment	3.03±0.44	2.60±0.71	NS
TOH space:				
(L)	Pretreatment	268.7±16.42	283.0±8.70	NS
	Treatment	260.7±14.75*	304.6±12.23**	p<0.01
(L/100 kg)	Pretreatment	79.95±3.04	78.13±5.37	NS
	Treatment	73.23±3.09**	78.22±5.91	NS
Total body water:				
(L)	Pretreatment	246.6±13.2	259.3±7.5	NS
	Treatment	238.7±11.6**	278.3±11.3**	p<0.01
(L/100 kg)	Pretreatment	73.42±3.2	71.57±5.01	NS
	Treatment	67.11±3.4**	71.48±5.49	NS

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

[1] Statistical analysis of treatment differences, NS = Nonsignificant ($p>0.05$).

Table 5. Empty body water, gut water, extracellular fluid and intracellular fluid in the control animals and animals treated with rbST (Values are means±SD (n = 5))

	Period of experiments	Control group	rbST group	Control vs. rbST group[1]
Empty body water:				
(L)	Pretreatment	145.84±12.3	148.25±11.21	NS
	Treatment	147.05±7.02	163.81±12.65	p<0.05
(L/100 kg)	Pretreatment	43.34±1.74	40.82±2.41	NS
	Treatment	41.37±2.75	42.09±4.54	NS
Gut water:				
(L)	Pretreatment	100.76±7.88	111.0±7.52	NS
	Treatment	91.69±6.77	114.5±6.50	p<0.01
(L/100 kg)	Pretreatment	30.08±3.15	30.75±4.10	NS
	Treatment	25.74±1.44*	29.39±2.25	p<0.05
Extracellular fluid:				
(L)	Pretreatment	76.55±7.53	77.74±9.25	NS
	Treatment	82.92± 11.18	88.61±10.95*	NS
(L/100 kg)	Pretreatment	22.87±3.08	21.37±1.79	NS
	Treatment	23.38±3.96	22.65±1.95	NS
Intracellular fluid:				
(L)	Pretreatment	170.1±14.96	181.5±9.26	NS
	Treatment	155.8±16.06**	189.7±4.61	p<0.01
(L/100 kg)	Pretreatment	50.55±2.95	50.20±5.22	NS
	Treatment	43.73±3.71	48.83±4.87	NS

P-values by paired t-test: * p<0.05, ** p<0.01, with respect to the pretreated period in the same group.
[1] Statistical analysis of treatment differences, NS = Nonsignificant (p>0.05).

Table 6. Changes in mammary circulation and the plasma concentration of IGF-1 in the control animals and animals treated with rbST (Values are means±SD (n = 5))

	Period of experiments	Control group	rbSTgroup	Control vs. rbSTgroup[1]
Mammary plasma flow	Pretreatment	2,438±331	2,549±342	NS
(ml/min)	Treatment	2,730±357	3,927±1,203*	NS
Mammary blood flow	Pretreatment	3,286±461	3,548±463	NS
(ml/min)	Treatment	3,817±616	5,310±1,620*	NS
Mammary blood flow/	Pretreatment	364±25	397±111	NS
milk yield	Treatment	420±32*	491±152	NS
IGF-1 (ng/ml)	Pretreatment	40±15	50±29	NS
	Treatment	48±16	209±42**	p<0.01

P-values by paired t-test: * p<0.05,** p<0.01, with respect to the pretreated period in the same group.
[1] Statistical analysis of treatment differences, NS = Nonsignificant (p>0.05).

control animals and rbST-treated animals in the pretreatment period. In the treatment period, the EBW as absolute values of rbST-treated animals was higher (p<0.05) when compared with control animals. The value of gut water of rbST-treated animals significantly increased (p<0.01), while it significantly decreased (p<0.05) in control animals in the treatment period.

The ECF volume of rbST-treated animals was significantly higher (p<0.05) in the treatment period than in the pretreatment period. An absolute value of ICF volume was significantly increased (p<0.01) in animals given rbST when compared with control animals in the treatment period. Absolute values of the ICF volume of the control animals showed a significant decrease (p<0.01) in treatment period when compared with pretreatment period. No significant differences were seen in intracellular fluid volume as percentage of body weight in either group in comparisons between the treatment period and the pretreatment period.

Mammary circulation and the plasma concentration of IGF-1 (Table 6)

Mammary plasma flow and mammary blood flow significantly increased (p<0.05) in rbST-treated animals over those of control animals. An increase in mammary blood flow coincided with an increase in milk yield in rbST-treated animals. The ratio of mammary blood flow to milk yield showed no significant differences between control animals and rbST-treated animals. The plasma IGF-1 concentration was significantly increased (p<0.01) in rbST-treated animals when compared with those of control animals during the treatment period.

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

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

P-values by paired t-test: * $p<0.05$, with respect to the pretreated period in the same group.
[1] Statistical analysis of treatment differences, NS = Nonsignificant ($p>0.05$).

Effects of rbST administration on milk composition (Table 7)

There were no significant differences in the concentration of protein and lactose in milk between the control and rbST-treated animals. Milk fat concentration significantly ($p<0.05$) increased in rbST-treated animals when compared with the control animals. The concentration of milk fat of rbST-treated animals was significantly increased ($p<0.05$) in the treatment period when compared with the pretreatment period.

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 a 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 weeks 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 60 days 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 the experimental period. However, the effect of rbST administration significantly influenced 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 water to make up the largest portion of milk and for evaporative cooling for heat dissipation. The rbST-treated animals increased water intake in the early period of lactation from 65 to 71 kg/day/animal, which was about 9%, and accounted for 19.8% of an increased 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 humans 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 would be 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 change in water turnover rate per body fat free wet weight ($kg^{0.82}$) or 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 occur in rbST-treated animals during the transition period from early to mid lactation. 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 goats (Mepham et al., 1984; Davis et al., 1988). 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, increased 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 workers 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 could 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 the 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 may partly result 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%), occurred despite 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 could be achieved in part by local vasodilatation (Linzell, 1974), causing changed distribution of milk precursors to the gland.

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