Journal of Agricultural Science (2007), **145**, 173–184. © 2007 *Cambridge University Press*
doi:10.1017/S002185960700679X *Printed in the United Kingdom*

Effects of long-term exogenous bovine somatotropin on water metabolism and milk yield in crossbred Holstein cattle

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(*Revised MS received 13 November 2006; First published online 12 February 2007*)

SUMMARY

The present study was conducted to evaluate the effects of long-term administration of recombinant bovine somatotropin (rbST) on the regulation of body fluids, mammary blood flow (MBF) and other variables relevant to milk synthesis, in crossbred dairy cattle (0·875 of Holstein–Friesian (HF) genes and 0·125 Red Shindi (RS) genes. Ten first lactation, non-pregnant, animals were chosen and divided into the control and experimental groups of five animals each. Four consecutive measurements were carried out in each group beginning on days 45 (pre-treatment), 105, 165 and 225 of lactation. Animals that had completed 60 days of lactation were injected subcutaneously every 14 days with 500 mg of 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 control. All animals were fed with rice straw treated with 50 g urea/l as the source of roughage in combination with a similar concentrate throughout the experiments. During the treatment periods, the daily dry matter intake (DMI) was numerically greater for rbST-treated animals than for control animals, while the relative values of DMI per kg body weight and water intake showed no differences.

Animals in both groups gained weight throughout the experiment with no significant differences between the groups. Animals receiving rbST for 45 days increased their peak milk yield from 13·4 kg/day per animal during pre-treatment to 15·9 kg/day per animal (18·7% increase) on day 105 of measurement and this peak yield was higher (19·5%) than those of control animals in the same period. Milk yields on days 225 in late lactation of both groups significantly decreased ($P<0·05$) in comparison with the early and mid-lactating periods. Over the course of the experiment, milk yield of the rbST-treated animals was significantly higher than those of the control animals ($P<0·01$). The administration of rbST significantly increased MBF ($P<0·05$) and mammary plasma flow (MPF) ($P<0·01$). The ratio of MBF to milk yield slightly increased as lactation advanced in both controls and rbST-treated animals. The administration of rbST significantly increased the absolute values of both plasma volume ($P<0·01$) and blood volume ($P<0·05$) when compared with the control animals. The control animals showed no significant changes in values of extracellular water (ECW) throughout the course of treatment periods. The rbST-treated animals increased in both the absolute values and the relative (proportion of body weight) values of ECW throughout the experiment ($P<0·05$). The estimated values of intracellular water (ICW) in both groups showed no significant changes during the course of treatments. There were no significant changes in the water turnover rate (WTO) and the biological half-life of tritiated water in different periods of lactation in both groups. The absolute values of total body water space (TOH) and total body water (TBW) were significantly greater in rbST-treated than control animals ($P<0·05$). The relative values of both TOH and TBW as a proportion of body weight of control animals decreased, while no alteration was apparent in

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rbST-treated animals during the course of treatments. These differences were statistically significant ($P<0{\cdot}05$). The absolute values of empty body water (EBW) of the control animals showed no significant changes, while animals treated with rbST tended to increase in absolute values of EBW throughout the course of treatments. The effects of these differences were statistically significant ($P<0{\cdot}05$). There were no significant changes in relative values of EBW during the course of treatments in both controls and rbST-treated animals. These data demonstrated that the rbST exerts its galactopoietic action in part through increases in TBW, EBW and ECW in association with an increase in MBF, which partitions the distribution of nutrients to the mammary gland for milk synthesis. During this long-term administration of rbST, the stimulant effect for milk yield was less in late lactation despite a higher MBF. The decline in milk yield during rbST treatment without fall in MBF in late lactation must be attributed to a local change within the mammary gland.

INTRODUCTION

Many factors can affect milk production in dairy cattle in the hot-humid tropics. These include high environmental temperature, 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 adaptability of tropical cattle with the high milking potential of exotic breeds. There is still a need to identify those crossbred cattle that are most suitable for the tropics. During studies on the regulation of body fluids and mammary blood flow (MBF) in different types of crossbred Holstein Friesian cattle (Chaiyabutr *et al.* 1997, 2000*a*), it was noted that 0·50 Holstein–Friesian (HF):0·50 Red Shindi (RS) animals showed differences in the distribution of their body fluids and MBF from 0·875 HF:0·125 RS animals during late pregnancy and different stages of lactation. The 0·875 HF animals had lower efficiency in water retention mechanism and poor adaptation to tropical environment in comparison to 0·50 HF (Chaiyabutr *et al.* 2000*a*). A low persistent lactation yield, with a decrease in MBF during the transition period from early to mid-lactation, was noted in the 0·875 HF animals. MBF has been known to be a major determinant for the rate of substrate supply for milk synthesis (Davis & 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 ruminants 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 (Johnson *et al.* 1991; West *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.

A number of studies indicate that injection of bST increases milk production as a result of both direct and indirect effect of somatotropin via physiological changes including partitioning of nutrients to the mammary gland and on mammary cell numbers and activity (Bauman & Vernon 1993; Bauman 1999). However, there are differences in experimental results on the role of bST with regard to feed intake and energy utilization. In the short-term studies over the period of 10 days of bST administration, milk yield increased with unchanged feed intake (Machlin 1973), while in studies in which bST has been administered over prolonged periods of lactation, milk yield and feed intakes increased (Soderholm *et al.* 1988; Oldenbroek *et al.* 1989). These results indicate that somatotropin may stimulate milk synthesis via a mechanism other than nutrient partitioning. In lactating dairy cows, large amounts of water are normally consumed and are closely related to feed intake and energy metabolism. During lactation, body water is used as a source of milk as well as for evaporative cooling during heat dissipation and as a vehicle in distribution of blood to the mammary glands. During feed withdrawal, a reduction in body fluids has been noted which is accompanied by decreases in milk yield and MBF (Chaiyabutr *et al.* 1980). Increase in MBF relating to an increase in cardiac output has been shown in cows during treatment with bST (Davis *et al.* 1988) but whether these changes follow changes in body fluids during bST administration is a matter of debate. During lactation, an increase in both metabolic activity and heat production has been reported in bST-treated cows (West *et al.* 1991). Such an effect would make thermoregulation in high-producing cows more difficult in the tropics, since dairy cattle in high ambient temperatures require more maintenance energy and appropriate body fluid for loss of body heat by evaporative cooling. Chaiyabutr *et al.* (2000*a*, *b*) reported that in 0·875 HF:0·125 RS animals, a rapid decrease in milk yield was related to reductions in MBF and circulating bST

as lactation advances to mid- and late lactation. These changes were not apparent in crossbred dairy cattle containing 0·50 Holstein genes (Chaiyabutr *et al*. 2000*b*). It is not known which factors are the cause and which factors are the effects for such reductions. Therefore, the role of bST is complex and may not be the only factor. The interactions between genetic potential for lactation performance and nutritional status or other environmental limitations remain to be clarified. Little is known about how somatotropin modifies water metabolism of crossbred cattle although an elevation of total body water (TBW) and extracellular water (ECW) has been noted in humans deficient in growth hormone following injections of human somatotropin (Janssen *et al*. 1997).

The present experiment was therefore conducted to investigate whether the long-term administration of recombinant bsT (rbST) would change body fluids, MBF and other variables relevant to milk synthesis and thus whether restoring body fluids would maintain milk production in different stages of lactation in crossbred cattle under tropical conditions.

MATERIALS AND METHODS

Animals and management

Ten first lactation, non-pregnant, 0·875 HF:0·125 RS dairy cattle were selected for the experiment. They were divided into two groups with five animals in each. They were fed with rice straw treated with 50 g urea/l 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 using a dry bulb thermometer. The relative humidity was calculated from the readings of dry and wet bulb thermometers. The maximum temperature in the shed at noon was 34 ± 1 (S.D.) °C and the minimum temperature at night was 26 ± 1 (S.D.) °C. The relative humidity was 68 ± 12 (S.D.) %. Animals received an average of 4 kg/day of roughage in combination with a concentrated mixture (7 kg/day) to maintain a moderate body condition score of 2·5 during the experiment (scale = 1–5) (Wildman *et al*. 1982). Urea-treated rice straw was offered four times a day at 08.00, 12.00, 16.00 and 20.00 h. Concentrate was fed twice daily at 08.00 and 14.00 h. 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. Each day samples of both feeds were collected and kept at −20 °C for dry matter determination and chemical analysis. Samples of urea-treated rice straw and concentrate were analysed for dry matter, crude protein and ash using procedures described by AOAC (1984). ADF and NDF were analysed according to Van Soest & Robertson (1980). Animals had free access to water and were fed their respective rations throughout the experimental period. The chemical composition of feeds is presented in Table 1. Concentrate formulation was prepared in fresh weight (kg/l00 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 kg, dicalcium phosphate 1·5 kg, sodium bicarbonate 1·1 kg, potassium chloride 0·8 kg and a vitamin–mineral premix 0·2 kg. (One kg of vitamin–mineral premix supplied 2400 000 i.u. of vitamin A, 500 000 i.u. of vitamin D_3, 500 i.u. of vitamin E, 2 mg of vitamin B_{12} and minerals consisted of 24 g of magnesium, 10 g of ferrous, 8 g of manganese, 8 g of zinc, 2 g of copper and met NRC (1989) requirements for all trace elements.) The urea-treated rice straw was prepared by spraying rice straw with urea solution, mixing thoroughly and storing under airtight conditions in a cement pit for 21 days (5 kg urea dissolved in 100 litres of water/100 kg dry rice straw). After 21 days, the rice straw treated with 50 g urea/l was offered to the animals.

Table 1. *Chemical composition of feeds used in the experiment* (*g/kg on dry matter basis*)

Particulars	Urea-treated rice straw	Concentrate
Dry matter	580	894
Crude protein	89	178
Acid detergent fibre	612	215
Neutral detergent fibre	672	288
Lignin	88	70
Ash	168	56

Experimental procedures

Animals were divided into control ($n = 5$) and experimental ($n = 5$) groups. Four consecutive measurements were carried out in each group. These consisted of a pre-treatment measurement on day 45 of lactation (pre-peak lactation) and three measurements on days 105 (early lactation), 165 (mid-lactation) and 225 (late lactation). After 60 days of lactation, treated animals were injected subcutaneously at biweekly intervals until the end of the study with 500 mg of rbST suspended in 792 mg of a prolonged release formulation of sesame oil (POSILAC, Monsanto, USA). Animals in the control group were injected subcutaneously at biweekly intervals with 800 mg of sterile sesame oil without rbST. Injections were administered at the tail head depression (ischiorectal 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

https://doi.org/10.1017/S002185960700679X Published online by Cambridge University Press

individual water bowl of each animal. The daily water intake per animal in each period of lactation was recorded by averaging over 7 days. Animals were normally milked at around 06.00 and 17.00 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 on 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 an intravenous polymer catheter (Jelco, Critikon; Johnson & Johnson, UK), under local anaesthesia. 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 200 mm from the first one. The catheter for isotope infusion and dye injection was inserted into an ear vein, under local anaesthesia. 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

On the day before the experiment began in each period, blood flow 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). In the present study, primiparous animals were used. This was to ensure that an accurate estimation of mammary venous outflow from the mammary gland was possible without blood draining from the abdominal side due to valvular incompetence which may occur if blood from the external pudic vein dilutes mammary blood in the mammary milk vein of multiparous animals (Linzell 1974). In the present study, using first-calf heifers, the valves in the external pudic vein would be competent. Non-mammary blood should not enter the external pudic vein. The venous blood drainage would leave the mammary glands via both veins (Linzell 1960). If it occurred as such in dairy cattle, an estimation of MBF in the milk vein might underestimate total MBF. However, in the present study, the rate of blood flow was measured in the milk vein in the standing animal. It has been demonstrated in dairy cattle by Bickerstaffe *et al.* (1974) that most of the mammary venous blood would leave via the milk vein in the standing animals. No detectable effect on MBF was observed when the external pudic vein was clamped during MBF measurement.

The water turnover rate (WTO), TBW and empty body water (EBW) were determined in each animal by tritiated water dilution techniques. On the specified day, the animal was injected intravenously via the ear vein with carrier-free tritiated water in normal saline at a single dose of 3000 μCi per animal. The equilibration time was determined by taking blood samples for 3 days after the injection. Blood samples were collected at 20, 30, 40, 50 and 60 min and 4, 8, 20, 26, 32, 44, 50, 56, 68 and 74 h subsequent to the injection. Preparation of samples for counting was achieved by the internal standardization technique as described by Vaughan & Boling (1961). The corrected activity of samples, in disintegration per minute (d.p.m.), was plotted on semi-logarithmic paper against time. The dilution curve of tritiated water in plasma was described by an exponential equation using a compartmental model system (Shipley & Clark 1972) for determinations of the WTO, TBW and EBW. The exponential equation describing the one-compartment model was calculated:

$$Y = A e^{-k1t}$$

where Y is concentration of tritium in plasma at time t (nanocurie/ml, nci/ml); A is plasma concentration intercept 1 in nci/ml.

The extrapolated activity at theoretical zero time of complete mixing of radioisotope was used to determine the total body water space (TOH). The TOH was calculated:

$$\text{TOH (ml)} = [\text{standard count (d.p.m.)} \times \text{dose (ml)}] / [\text{radioactivity counts at zero time (d.p.m.)}]$$

The biological half-life of tritium-labelled water (Tl/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 WTO was calculated from the equation:

$$\text{WTO (litre/day)} = 0.693 \times \text{TOH space/Tl/2}$$

TBW was calculated by using the corrected factor (1 − fraction of plasma solids) × TOH space (Chaiyabutr *et al.* 1997). The plasma solids concentration was determined by a refractometer.

EBW does not include water associated with gastrointestinal contents or the water in the fetus (Andrew *et al.* 1995), which was estimated from the disappearance curve of tritium in blood plasma for each animal. Blood samples were taken at various time intervals after injection for each animal. The corrected activity of samples, in d.p.m., was plotted on semi-logarithmic paper against time. The dilution curve of tritiated water in plasma was described by an exponential equation using the two-compartment open system model (Shipley & Clark 1972) for calculation of the EBW. The exponential equation

describing the two-compartment model was calculated from the equation:

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

where Y is the concentration of tritium in plasma at time t (nci/ml). A is plasma concentration intercept 1 of the fast phase of the plasma curve in nci/ml; B is plasma concentration intercept 2 of the slower phase in nci/ml; $k1$ and $k2$ are first order rate constants of the fast phase and the slow phase of each pool, respectively; and t is time in minutes. The intercept 2 (B) was obtained by extrapolation to zero time from the plasma curve of the activity of samples which decreased at a constant (linear) rate. The intercept 1 (A) was estimated to find the early rapid phase of the plasma curve activity from a series of values along the extrapolated line of the slow phase and the original composite curve which fell rapidly at first after injection of tritiated water. Point by point arithmetic subtraction of the linear extrapolation of slow phase curve from the original composite curve gave fast phase of the plasma curve. Additional subtractions up to 240 min define points on a line which extrapolated to give intercept 1 (A). The sum of the two intercepts, A and B, equals the concentration of tritiated water in plasma at 0 time, and this concentration was used to estimate EBW in the equation:

$$\text{EBW (ml)} = [\text{standard count (d.p.m.)} \times \text{dose (ml)}] / [\text{radioactivity counts of } A + B \text{ (d.p.m.)}]$$

Determinations of plasma volume, extracellular fluid and intracellular fluid

In each animal per measurement, the injection of 20 ml of sodium thiocyanate solution (10 g/100 ml normal saline) and 20 ml of the Evans blue dye (T-1824) (0·5 g/1000 ml normal saline) were given via an 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 & Kare (1959). Intracellular water (ICW) was calculated by subtracting ECW from EBW. Plasma osmolality was measured using the freezing point depression method (Advance Osmometer model 3, USA).

Statistical analyses

Statistical analysis was conducted using SPSS 14.0 for Windows. In order to control the experimental error relating to the effects of treatment and stages of lactation, the pre-treatment data were used as a covariate in the analysis of covariance (ANCOVA) using the statistical model:

$$\text{Yijk} = \mu + b(\text{Xij} - X) + \text{ti} + \text{mj} + (\text{ti} \times \text{mj}) + \text{eijk}$$

where Yijk = observation, μ = overall mean, b = regression coefficient, ti = number of treatments ($i = 1, 2$), mj = number of measurements ($j = 1, 2, 3$) and eijk = residual error. Mean values were assessed by least square means (LSM) for specified days of measurements during the treatment in different stages of lactation.

RESULTS

Changes in DM, water intake, milk yield and body weight

The daily DMI was numerically greater for rbST-treated than for control animals throughout the lactation (on average 0·7 *v.* 0·2 kg/day, 1·6 *v.* 0·8 kg/day and 1·1 *v.* 0·9 kg/day for days 105, 165 and 225 of lactation, respectively) (Table 2). These differences were not statistically significant. Moreover, the relative values of DMI per kg body weight showed no differences between controls and rbST-treated animals during experimental periods. The mean values of daily water intake in both the control animals and rbST-treated animals showed no significant increases during the experimental periods. Animals receiving rbST for 45 and 105 days increased their milk yield from 13·4 kg/day per animal during pre-treatment to 15·9 kg/day per animal (18·7%) on day 105 (early lactation) and 14·5 kg/day per animal (8·2%) on day 165 (mid-lactation) of lactation, respectively. For treated or control animals, milk yields on day 225 in late lactation significantly decreased ($P < 0·05$) in comparison with the early and mid-lactating periods. The peak yield of the rbST-treated animals was 19·5% greater than that of comparable controls. Moreover, over the whole course of the experiment, milk yield of the rbST-treated animals was significantly higher than that of the controls ($P < 0·01$).

A DMI:milk yield ratio was calculated and used as an indicator of the efficiency of conversion of nutrients to milk. The mean ratio of DMI to milk yield for rbST-treated animals on day 105 (early lactation) was significantly lower than that for day 225 in late lactation. The control animals showed no significant changes in the ratio throughout lactation as lactation advances. Animals in both groups gained weight throughout the experiment. Body weight gains in mid- and late lactation for both control and rbST-treated animals were significantly greater ($P < 0·05$) than in early lactation. Throughout the experiment, body weight gains were not

https://doi.org/10.1017/S002185960700679X Published online by Cambridge University Press

Table 2. *Least square means and standard errors for DMI, water intake, milk yield and body weight of the control animals and rbST-treated animals in different stages of lactation*

	Days of lactation during control treatment				Days of lactation during rbST treatment					
Parameters	Day 45 (non-treated)	Day 105	Day 165	Day 225	Day 45 (non-treated)	Day 105	Day 165	Day 225	S.E.M.	*P*-values
DMI (kg/day)	(11·4)	11·9	12·5	12·5	(12·3)	12·8	13·7	13·1	0·69	NS
DMI (kg/day/100 kg BW)	(3·3)	3·8	3·3	3·2	(3·3)	3·3	3·4	3·2	0·09	NS
Water intake (kg/day)	(59)	63	77	77	(65)	68	71	72	3·6	NS
Milk yield (kg)	(13·1)	$13·3^{a}$	$13·0^{a}$	$11·7^{b}$	(13·4)	$15·9^{a}$	$14·5^{a}$	$12·9^{b}$	0·53	$P<0·01$
DMI/milk yield (kg/kg)	(0·9)	$0·9^{b}$	$1·0^{ab}$	$1·1^{a}$	(1·0)	$0·8^{b}$	$1·0^{ab}$	$1·0^{a}$	0·05	NS
Body weight (kg)	(337)	373^{a}	385^{b}	394^{b}	(363)	377^{a}	398^{b}	406^{b}	6·2	NS

Least squares means were adjusted using data collected from specified days of measurements during long-term treatments of either control or rbST commencing at 60 days postpartum; the pre-treatment (non-treated) data were used as a covariate in the analysis of covariance.
P-values for statistical significance of interaction effects between control group and rbST-treated group by F-tests, NS = not significant.
LSM values within the same row with different superscripts in each group are significantly different ($P<0·05$).

significantly different between the rbST-treated and control animals.

Changes in plasma volume, blood volume, ECW and ICW

Plasma volume or blood volumes both in absolute values or the relative values (percentage of body weight) throughout the course of their lactation are shown in Table 3. The administration of rbST significantly increased the absolute values of both plasma volume ($P<0·01$) and blood volume ($P<0·05$) when compared with the control animals throughout the course of treatment. Relative values of plasma volume and blood volume were not significantly changed during the course of treatment for both groups, but the magnitude of response was larger in rbST-treated than in control animals (8·7 *v.* 1·8 % for plasma volume and 7·8 *v.* 3·3 % for blood volume in the early period of lactation). The packed cell volume and plasma osmolality of both controls and rbST-treated animals were unchanged throughout treatment periods. The control animals showed no significant changes in ECW either in absolute values or the relative values (percentage of bodyweight) throughout the course of the treatment period. The rbST-treated animals increased in both the absolute values and the relative values of ECW throughout the experiment; for the absolute value, the difference was significantly greater than in control animals. The increase in ECW was larger in rbST-treated than control animals (13·3 *v.* 9·0 %, 24·7 *v.* 7·6 % and 35·8 *v.* 13·8 % for measurements in early, middle and late lactation periods, respectively). The estimated values of ICW in both control animals and rbST-treated animals showed no significant changes throughout the course of treatments.

Changes in the WTO, TBW, TOH and EBW

No obvious changes were seen for the absolute value of WTO, the value of WTO per fat-free, wet, body weight ($kg^{0·82}$) (MacFarlane & Howard 1972) and the biological half-life of tritiated water over the course of treatments between the controls and rbST-treated animals (Table 4). Significant differences were observed in the absolute values of both TOH and TBW between controls and rbST-treated animals during the course of treatment periods ($P<0·05$). The magnitude of response to the effect of treatment (increase from pre-treatment value) for TBW was larger in rbST-treated than control animals (10·8 *v.* 0·4 L, 22·7 *v.* 7·1 L and 22·6 *v.* 21·7 L on days 105, 165 and 225 of measurements, respectively). The relative values of both TOH and TBW (percentage of body weight) for control animals decreased as lactation advanced, while animals treated with rbST showed no significant changes during the course of treatments. The effects of these differences were statistically significant ($P<0·05$). The absolute values of EBW of the control animals showed no significant changes, while for animals treated with rbST there was a trend to increased absolute values of EBW throughout the course of treatments. The effect of these differences was statistically significant ($P<0·05$). There were no significant changes in relative values of EBW during the course of treatments in both controls and rbST-treated animals.

Changes in mammary plasma flow (MPF), MBF and the ratio MBF:milk yield

The effects of rbST treatment on MBF and MPF are shown in Table 5. Administration of rbST resulted in increased MBF and MPF. The increase in response

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Table 3. *Least square means and standard errors for plasma volume, blood volume, packed cell volume, plasma osmolality (Posm), extracellular water (ECW) and intracellular water (ICW) of the control animals and rbST-treated animals in different stages of lactation*

	Days of lactation during placebo treatment				Days of lactation during rbST treatment					
Parameters	Day 45 (non-treated)	Day 105	Day 165	Day 225	Day 45 (non-treated)	Day 105	Day 165	Day 225	S.E.M.	*P*-values
Plasma volume (litre)	(15·9)	17·8	17·6	17·4	(16·6)	19·0	19·8	20·0	0·65	$P<0·01$
Plasma volume (litre/100 kg)	(4·7)	4·8	4·6	4·5	(4·6)	5·0	5·0	4·9	0·37	NS
Blood volume (litre)	(22·2)	25·3	25·0	24·7	(23·3)	26·2	27·6	27·7	1·01	$P<0·05$
Blood volume (litre/100 kg)	(6·6)	6·8	6·5	6·3	(6·4)	6·9	7·0	6·8	0·23	NS
Packed cell volume (%)	(28·1)	29·4	29·7	29·1	(28·7)	27·7	28·4	27·4	0·53	NS
Posm (mOsm/kg)	(280)	279	280	286	(274)	277	278	279	1·7	NS
ECW (litre)	(77)	83	82	87	(78)	88	97	106	4·9	$P<0·05$
ECW (litre/100 kg)	(23)	23	22	22	(21)	23	24	25	1·1	NS
ICW (litre)	(103)	96	107	114	(117)	130	114	124	10·2	NS
ICW (litre/100 kg)	(30)	27	29	30	(32)	34	28	29	2·4	NS

Least squares means were adjusted using data collected from specified days of measurements during long-term treatments of either placebo (control) or rbST commencing at 60 days postpartum; the pre-treatment (non-treated) data were used as a covariate in the analysis of covariance.
P-values for statistical significance of interaction effects between control group and rbST-treated group by F-tests, NS = not significant.

to stage of lactation (increase from pre-treatment value) was larger in rbST-treated than control animals (47 *v.* 18 %, 51 *v.* 19 % and 33 *v.* 8 % for days 105, 165 and 225 of MBF measurements, respectively). These differences were statistically significant for MBF ($P<0·05$) and MPF ($P<0·01$). The ratio of MBF to milk yield slightly increased as lactation advanced in both controls and rbST-treated animals.

DISCUSSION

In previous studies it has been observed that crossbred cattle containing 0·875 Holstein genes showed a shorter persistency of lactation as compared to 0·5 HF animals. A rapid decline in the peak of milk yield coincided with decreases in MBF and the concentration of plasma growth hormone (Chaiyabutr *et al.* 2000*a*, *b*). The present study was designed to investigate if long-term administration of rbST would increase body fluids and thereby increase MBF and other variables relevant to milk synthesis. Long-term treatment with 500 mg of rbST suspended in a prolonged-release formulation, administered every 14 days as in the present study, is the dose rate recommended for *Bos taurus* cows. The treatment of rbST was initiated at the earlier stage of lactation. Milk yield increased 18·7 % on day 105 in early lactation and *c.* 8·2 % on day 165 in mid-lactation. It decreased *c.* 3·7 % on day 225 in late lactation when compared with the pre-treatment period. Low responses in milk yield during rbST treatment in different stages of lactation are similar to previous reports in dairy crossbred cattle (Phipps *et al.* 1991). A rapid decline of milk yield in rbST-treated animals seems to be similar to that which occurs in higher yield cows (Chase 1993). These results indicated that an increase in milk yield of dairy crossbred cattle, in response to rbST administration, would not be sustained for long and is influenced by the stage of lactation. Animals in both groups were fed *ad libitum* and total DMI were not significantly different between controls and rbST-treated animals throughout the experimental periods. However, the daily DMI were 0·6–1·2 kg/day more for rbST-treated animals than the control animals during the treatment periods. Although not statistically significant, the difference may be biologically significant. This increased DMI resulted in energy intake by rbST-treated animals of a magnitude sufficient to account for 1·2–2·6 kg/day of the extra milk secretion. However, the ratio of DMI to milk yield for rbST-treated animals was lower on day 105 in early lactation when compared with the pre-treatment value and it was statistically significantly lower than that on day 225 of treatment. However both controls and rbST-treated animals still gained weight throughout the experiment. It is known

https://doi.org/10.1017/S002185960700679X Published online by Cambridge University Press

Table 4. *Least square means and standard errors for the WTO, TBW, TOH, EBW and the biological half-life of tritiated water of the control animals and rbST-treated animals in different stages of lactation*

Parameters	Days of lactation during placebo treatment				Days of lactation during rbST treatment				S.E.M.	*P*-values
	Day 45 (non-treated)	Day 105	Day 165	Day 225	Day 45 (non-treated)	Day 105	Day 165	Day 225		
WTO (litre/day)	(59)	67	78	80	(71)	79	90	86	6·2	NS
WTO (ml/kg$^{0·82}$/day)	(499)	519	588	590	(564)	615	660	617	43·2	NS
Biological half-life (day)	(3·3)	2·9	2·6	2·7	(2·9)	2·7	2·7	2·7	0·18	NS
TOH space (litre)	(269)	269	277	295	(283)	296	313	310	9·7	$P<0·05$
TOH space (litre/100 kg)	(80)	73	72	75	(78)	79	79	76	1·8	$P<0·05$
TBW (litre)	(247)	247	254	268	(259)	270	282	282	9·4	$P<0·05$
TBW (litre/100 kg)	(73)	67	66	68	(72)	72	72	70	1·6	$P<0·05$
EBW (litre)	(179)	182	193	204	(194)	215	208	227	10·1	$P<0·05$
EBW (litre/100 kg)	(53)	51	52	53	(54)	56	52	54	2·4	NS

Least squares means were adjusted using data collected from specified days of measurements during long-term treatments of either placebo (control) or rbST commencing at 60 days postpartum; the pre-treatment (non-treated) data were used as a covariate in the analysis of covariance.
P-values for statistical significance of interaction effects between control group and rbST-treated group by F-tests, NS = not significant.

that support of milk secretion comes through provision of substrates to the mammary gland. The increased milk yield with rbST treatment in the present study is more 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 on day 105 in early lactation, may be due to the fact that animals were well fed to allow an adequate replacement of body reserves. Crossbred animals used in the present study were first-calf heifers where milk yields would be lower than in multiparous cows (Sullivan *et al*. 1992) and they would still be growing. These low producing animals would be in a state of energy balance. Their metabolic demands during their first lactation were being adequately met by dietary intake as is apparent in continued weight gain. Triglycerides are known to restore body weight during periods of excess energy availability and are mobilized during periods of energy deprivation. In a previous report (Chaiyabutr *et al*. 2005), the absence of altered plasma triglyceride and glucose concentrations in rbST-treated crossbred HF animals was shown as an indication they were less responsive to the partitioning effect of rbST – a lack of mobilization of body tissues during administration of rbST. The other possible explanation is that crossbred HF animals in the present study containing 0·125 *Bos indicus* (RS) genes may be considered to be intermediate type (between dairy and beef types), which may account for the greater weight gain and fat deposition coupled with an increase in milk yield during rbST administration. During the course of the studies the greater weight gain was apparent in rbST-treated animals. Thus the rbST increased lean tissue growth in these crossbred animals. It is known that a Holstein–Friesian receiving adequate nutrition should be much larger weight than this, suggesting that nutrition is limiting the expression of the genetics of the animals in terms of both growth and lactation. However, the mode of action of somatotropin in mediating the interactions between genetic potential for lactational and nutritional performances in crossbred dairy cattle remains to be further studied.

The rbST-treated animals increased milk yield by an average 2·65 kg/day/animal (2·2 litres/day of water) during early lactation. This would account for 0·79 of the increase in water intake (from 65·2 kg/day to *c*. 68·0 kg/day). During early to late lactation, water intake increased while milk production decreased. It might suggest that the water deficit between intake and milk production is due to water loss in other routes especially various types of evaporation during heat dissipation in this tropical climate. The rbST-treated animals increased body fluid compartments (TOH, TBW, EBW, ECW and blood volume) throughout all periods of study, while control animals markedly decreased relative values (in comparison to pre-treatment values) for both TOH and TBW on both days 105 and 165 of lactation. An increase in ECW would be influenced by an increase in voluntary intake (MacFarlane *et al*. 1959). This has been reported to occur after a few weeks of rbST administration (Coghlan *et al*. 1977). However, the ECW

Table 5. *Least square means and standard errors for MPF, MBF, and the ratio MBF: Milk yield of the control animals and rbST-treated animals in different stages of lactation*

	Days of lactation during placebo treatment				Days of lactation during rbST treatment					
Parameters	Day 45 (non-treated)	Day 105	Day 165	Day 225	Day 45 (non-treated)	Day 105	Day 165	Day 225	S.E.M.	*P*-values
MPF (ml/min)	(2438)	2770	2738	2732	(2549)	3887	3943	3493	306·4	$P<0·01$
MBF (ml/min)	(3286)	3901	3905	3833	(3548)	5226	5375	4731	445·6	$P<0·05$
MBF/milk yield (litre/kg)	(364)	431	453	513	(397)	485	532	533	46·7	NS

Least squares means were adjusted using data collected from specified days of measurements during long-term treatments of either placebo (control) or rbST commencing at 60 days postpartum; the pre-treatment (non-treated) data were used as a covariate in the analysis of covariance.
P-values for statistical significance of interaction effects between control group and rbST-treated group by F-tests, NS = not significant.

compartment did not include rumen water; thus any changes of ruminal fluid volume should not affect the determination of the extracellular fluid volume. These results indicate that somatotropin plays an important role in water regulation and probably relates 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 advanced, animals gained more weight in both controls and the rbST-treated animals. However, the greater percentage increase in live weight of rbST-treated animals could be due, at least in part, to the direct effect of somatotropin on the increased body cell mass. The mean body weight of rbST-treated animals increased from pre-treated value by 13, 34, and 43 kg, which would be accounted for by increased TBW averaged 11 litres (84%), 22 litres (65%) and 23 litres (53%) on days 105, 165 and 225 during treatments, respectively. This suggests that accumulation of body water was less as lactation advances and a portion of the remainder of the increase was required for other bST-sensitive body cell mass. Sodium retention due to the effect of somatotropin on renal tubular reabsorption of sodium (Wyse *et al.* 1993) while retaining constant plasma osmolality as in the present result could be another explanation for 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 (Tyrrell *et al.* 1988; Johnson *et al.* 1991; West *et al.* 1994). In the present study, the higher values of ECW were apparent after rbST administration throughout the course of lactation, and for ICF in early lactation only on day 105. They were significantly higher than those of controls. These findings suggest that the expansion of body fluid in rbST-treated animals 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, the WTO of both groups of crossbred cattle would not be influenced by the effect of pregnancy (Chaiyabutr *et al.* 1997) or changes in environmental conditions (Ranjhan *et al.* 1982). However, the WTO was slightly higher in the rbST-treated animals than controls in any periods measured throughout the experiment. These findings relate to the fact that lactation requires more water and more loss of water due to secretion in milk (which is about 0·87 water) and would account for these phenomena. Water loss with the increase in milk yield of the rbST-treated animals might be compensated by a larger body water pool (TBW or EBW) which restores body fluids to equilibrium. No obvious changes of WTO and half-life of tritiated water were apparent during periods of study in both controls and rbST-treated animals. These results indicate that maintenance of the normal balance of distribution of water within the various body fluid compartments occurred during the experiment. In domestic ruminants, there is a diffusion of water across the ruminal mucosa, but the mechanism is still unclear for the net movement of water between rumen and plasma. Some studies of body composition of dairy cows indicate that an estimation of body water from the two-compartmental analysis of disappearance of D_2O in blood plasma greatly over-predicted the gut water (Andrew *et al.*

https://doi.org/10.1017/S002185960700679X Published online by Cambridge University Press

1995), while Arnold & Trenkle (1986) indicated that compartmental analysis of D_2O dilution in blood plasma did not distinguish gut water from EBW, because equilibrium time for D_2O within gut contents was variable throughout the gastrointestinal (GI) tract. However, it has been reported that the net movement of water across the rumen is small and will be affected by changes in an osmotic gradient in the rumen (Engelhardt 1970; Faichney & Boston 1985). Animals in both groups in the present study were fed with the same diet which presumably maintained a similar level of osmolality of ruminal fluid throughout periods of lactation. It seems likely that the rate of distribution of water between gut and plasma at the time of sampling would not vary in the present study. An estimation of EBW in the present study was calculated from the two-compartment analysis of disappearance of ^3H_2O in the blood. Any change in plasma ^3H_2O concentration with some penetration of water from gut to blood plasma due to change in nutritional status would be small. Therefore, overestimation of EBW would not be expected. As lactation advanced the marked reduction in relative values for both TOH and TBW in the control animals was apparent. The animals used in the present study being 0·875 HF, they had genetic potential close to the exotic *B. taurus* breed which might be responsible for poor adjustment in a tropical environment (Nakamura *et al.* 1993; Chaiyabutr *et al.* 2000*a*). One may suppose that these changes are the factors influencing the process of lactation. Animals in the control group could not maintain a high level of their body fluids as lactation advanced resulting in shorter lactation. An increase in ECW induced by rbST leads to an increase in MBF as a secondary response, facilitating increased milk production. The marked increases in MBF were apparent in rbST-treated animals throughout lactation. These results support other findings showing increases in MBF and milk secretion in both goats and cows given exogenous growth hormone (Mepham *et al.* 1984; Davis *et al.* 1988). An increase in MBF has been shown to result from the effect of an increase in cardiac output without any alteration in heart rate during growth hormone treatment (Davis *et al.* 1988). In the present study, an increase in both blood volume and plasma volume in rbST-treated animals would provide a greater venous return and stroke volume and increase cardiac output, resulting in increased blood supply to the mammary gland. Thus, the increase in milk yield after the peak period (compared with control animals) could have been due to an increased availability of substrates for the mammary gland. However, observations in both controls and rbST-treated animals showed an increase in a ratio of MBF : milk yield as lactation progressed. The decline in milk yield over the 32 weeks of the experiment to 0·81 and 0·88 of the peak lactation values in rbST-treated animals and control animals, respectively, was not proportional to MBF during treatment. A decrease in mammary cell activity would occur as lactation progressed and this could partially explain the increase in MBF per unit milk yield observed as lactation advanced in both groups. However, the progressive decline in milk yield of rbST-treated animals, despite a higher level of either MBF or ECW, implies that the mass of mammary tissue was not affected by the rbST 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). It seems that the effect of rbST on mammary circulation is indirect and mediated via insulin-like growth factor-I (IGF-I), although a number of studies have demonstrated that similar increases in milk secretion and MBF occurred during growth hormone treatment in goats and cows (Mepham *et al.* 1984; Davis *et al.* 1988). Injection of rbST in different stages of lactation in crossbred cows elevated both plasma IGF-I concentrations and udder blood flow (Tanwattana *et al.* 2003; Chaiyabutr *et al.* 2005).

In conclusion, these experiments demonstrated that the rbST exerts its galactopoietic action, in part, through increases in TBW, EBW and ECW in association with an increase in MBF, which partitions the distribution of nutrients to the mammary gland for milk synthesis. The stimulant effect for milk yield was less in late lactation even though a high level of MBF was maintained during long-term administration of rbST. The decline in milk yield during rbST treatment without facilitating of MBF in late lactation must be attributed to a local change within the mammary gland. Further studies are needed to determine the mechanisms by which bovine somatotropin influences mammary gland metabolism as lactation develops in crossbred cattle in the tropics.

This study was supported by the Thailand Research Fund, Grant No. BRG 2/02/2545.

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