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Effects of Long Term Exogenous Bovine Somatotropin on Nutrients Uptake by the Mammary Gland of Crossbred Holstein Cattle in the Tropics

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ABSTRACT : Ten, first lactation, 87.5%HF dairy cattle were used to investigate effects of long-term administration of recombinant bovine somatotropin (rbST) on nutrient uptake by the mammary gland at different stages of lactation. Measurements of arterial plasma concentrations and arterial-venous differences of metabolites across the mammary gland were performed in combination with measurment of mammary blood flow to estimate the mammary uptake. Animals in experimental groups were injected subcutaneously every 14 days from day 60 of lactation with a prolonged-release formulation of 500 mg of rbST (POSILAC, Monsanto, USA) or with sterile sesame oil without rbST in the control group. During early lactation, the milk yield of rbST-treated animals was higher than that of the control animals ($p<0.05$). The peak milk yield in both groups of animals declined from the early period of lactation with progression to mid- and late-lactation. No significant changes were observed in the concentration of milk lactose, while the concentrations of milk protein significantly increased as lactation advanced to mid- and late-lactation in both groups. Milk fat concentrations were significantly higher in rbST-treated animals than in control animals, particularly in early lactation ($p<0.05$). Mammary blood flow (MBF) markedly increased during rbST administration and was maintained at a high level throughout lactation. The mean arterial plasma concentrations for glucose and acetate of rbST-treated animals were unchanged. The net mammary glucose uptake of rbST-treated animals increased approximately 20% during early lactation, while it significantly decreased ($p<0.05$), including the arteriovenous differences (A-V differences) and extraction ratio across the mammary gland, as lactation advanced to mid- and late-lactation. A-V differences, mammary extraction and mammary uptake for acetate increased during rbST administration and were significantly higher ($p<0.05$) than in the control animals in early and mid-lactation. Mean arterial plasma concentrations for β-hydroxybutyrate and free glycerol were unchanged throughout the experimental periods in both groups. A-V differences and extraction ratio of β-hydroxybutyrate across the mammary gland did not alter during rbST administration. Mean arterial plasma concentrations for free fatty acids (C_{16} to C_{18}), but not for triacylglycerol, increased in rbST-treated animals and were significantly higher than in control animals during early lactation ($p<0.01$). These findings suggest that an increase in MBF during rbST administration would not be a major determinant in the mediation of nutrient delivery and uptake by the mammary gland for increased milk production. Local changes in biosynthetic capacity within the mammary gland would be a factor in the utilization of substrates resulting in the rate of decline in milk yield with advancing lactation. (**Key Words** : rbST, Nutrients, Mammary Gland Uptake, Crossbred Holstein Cattle)

INTRODUCTION

Milk production is the result of coordination between nutrient delivery to and biosynthetic capacity of the mammary glands. The rate of supplying to mammary gland is determined by the substrate concentration in the arterial plasma and mammary blood flow (MBF) providing substrates at appropriate rates to sustain milk synthesis. There is evident that substrate supply to the mammary gland is often inadequate to maintain the maximum rate of milk synthesis (Linzell and Mepham, 1974). The mammary gland may be producing milk at a rate below its potential. However, the rate of milk production depends on function of number of secretory cells and their metabolic activity. The delivery of nutrients to the mammary gland is dependent on the physiological state of the animal by homeostatic and homeorhetic mechanisms (Bauman and Currie, 1980). During early lactation, nutrients are partitioned from peripheral tissues to the mammary gland to support the requirements for milk synthesis during peak lactation. Bovine somatotropin (bST) is known as a homeorhetic hormone connected with both growth and lactation, but the mechanism of action of bST on milk

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Table 1. Chemical composition of experimental diet and nutrient analysis as a percentage of dry matter

	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 formation: ingredients by fresh weight (100 kg $^{-1}$) 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 premix (0.6 kg).

production is a controversial area, as receptors for bST have not been demonstrated on secretory epithelial cells of mammary tissue (Akers, 1985). Although a number of reviews have been published on the relationship between the plasma bST concentration and milk yield in ruminant (Bauman, 1992) in both normal and hot environments (Johnson et al., 1991; West et al., 1991), the role of bST in relationship to persistent lactation in dairy cattle in the tropics is not yet clear. Genetic selection has intensified the physiological demand on peripheral tissues to provide nutrients to the mammary gland partially because of the limitation of energy intake during peak lactation. It has been reported that in the crossbred cattle containing 87.5% Holstein Friesian (HF) genes decreased in milk yield, which was related to reductions in mammary blood flow and circulating bST as lactation advances to mid- and late lactation (Chaiyabutr et al., 2000a, 2000b). It is not known which factors are the cause and which factors are the effect for the short persistency of lactation in crossbred Holstein cattle about the function of mammary tissue and the utilization of substrate in the mammary gland. Factors other than arterial plasma concentration including mammary gland biosynthetic capacity (intra-mammary factor) and blood flow (extra-mammary factor) would also affect to the mode of nutrient uptake by the glands of 87.5% HF animals (Chaiyabutr et al., 2002). An investigation of how key blood metabolites are delivered to and extracted by the mammary glands of crossbred cattle treated with bST which have not been determined, although quantification and examination of mammary gland uptake of blood metabolites by measuring arterial-venous difference across the mammary glands without recording of blood flow, have been described in Bos taurus (McDowell et al., 1987; Miller et al., 1991). However, in spite of the large number of studies that have been shown the relationship between MBF and milk production during bST administration, there is still not complete agreement for this relationship by which experimental studies in goats showed no support to the increasing MBF could enhance milk production (Lacasse and Prosser, 2003). Therefore, the present experiment was conducted to investigate patterns of nutrient uptake by measuring mammary blood flow and combining these with measurements of arterial-venous differences across the mammary glands for the net mammary uptake of nutrients during long-term administration of rbST throughout lactation in crossbred, 87.5% Holstein dairy cattle in the tropics.

MATERIALS AND METHODS

Animals and management

Ten, first lactation, non-pregnant, crossbred, 87.5% Holstein 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. Animals received urea treated rice straw (58% dry matter) as roughage by averaged 4 kg/d in combination with a concentrated mixture (89% dry matter) 7 kg/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 20.00 h. 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. Details of the preparation of diets was described previously (Chaiyabutr et al., 2005) Briefly, the urea treated rice straw was prepared by mixing urea solution with dry straw (5 kg urea dissolved in 100 litres 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×21 day system of urea treatment. After 21 days, the treated rice straw with 5% urea was offered to the animals.

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 subcutaneously

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 (ischiorectal fossa). Animals of both groups were fed the same ration. from before parturition and throughout the study. 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.

On the day of the experiment at around 1100 h, mammary blood flow (MBF) through half of the udder was determined and blood samples were taken from the milk vein and from the coccygeal artery by venipuncture with a #21 needle into a heparinized tubes. Blood samples were kept in crushed ice and then centrifuged at 3,000 rpm for 30 min at 4°C. Plasma from both venous and arterial blood samples in aliquots at -40°C until nutrient concentrations were assayed.

Mammary blood flow measurements

Mammary 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). Briefly. a dye (T-1824) was dissolved in sterile normal saline and diluted to a concentration of 100 mg/L. The solution was infused by a peristaltic pump (Gilson Medical electronics) at a constant rate of 85 ml/min into either the left or right milk vein via an intravenous polymer catheter (#18). which could produce adequate mixing of dye with blood. Before infusion. blood was drawn from the other catheter about 20 cm. downstream from the first one in the milk vein as a pre-infusion sample. About 10 seconds after starting the infusion. 10 ml of blood was drawn from downstream in the milk vein at a constant rate into a heparinized tube. Two consecutive measurements were taken during each dye infusion at about 5 min intervals. Plasma flow of half of the udder was calculated from plasma samples using the equation derived by Thompson and Thomson (1977). Udder blood flow was calculated from plasma flow and packed cell volume. Quarter milking showed that the yields of the two halves of the udder were similar. In the present study. primiparous animals were used. This was done to ensure that an accurate estimation of the mammary venous outflow from the mammary gland was possible without blood draining from the abdominal side due to valvular incompetence of external pudic vein which non-mammary blood may dilute the mammary blood in the milk vein of multiparous animals (Linzell. 1974). The present study using first calf heifers. the valves in the external pudic vein would be competent. The non-mammary blood would not suspect to be entering the external pudic vein. The venous blood drainage would leave the mammary glands via both veins (Linzell. 1960). 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 was leaving via the milk vein in the standing animals. Since. no detectable effect on MBF was observed when external pudic vein was clamped during MBF measurement. Mammary blood flow was therefore calculated by doubling the flow measured in one milk vein (Bickerstaffe et al.. 1974). Packed cell volume was measured after centrifugation of the blood in a microcapillary tube.

Milk sampling and determinations of milk compositions

Milk was collected in the afternoon of specified day. 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.

Metabolite determinations

Plasma glucose concentrations were measured using enzymatic oxidation in the presence of glucose oxidase. Plasma free fatty acid (FFA. C_{16}-C_{18}) concentrations were measured by using gas chromatography (Shimazu GC-7AG Gas Chromatograph) in comparison with the internal standard. The internal standard of triheptadecanoate and heptadecanoic acid was used for estimation of plasma triacylglycerol and FFA respectively as described by Thompson et al. (1975). Plasma β-hydroxybutyrate concentrations were assayed using an enzymatic reaction in the presence of β-hydroxybutyrate dehydrogenase (Sigma Chemical Co.). Plasma acetate concentrations were determined by chromatographic method. Plasma glycerol concentrations were determined by enzymatic method.

Mammary uptake of metabolites and extraction of metabolites by the mammary gland were calculated as follows:

Mammary uptake
= mammary plasma flow
×arteriovenous differences (A-V);

Extraction ratio = (A-V)/A

Statistical analysis

All mean values are presented as mean±SD. The experimental results were evaluated by analysis of variance; the significant differences among periods of measurements

Table 2. Means±SD for dry matter intake, milk yields and milk compositions during prolonged rbST administration

Parameters	Measurements	Control group	rbST group	Control vs. rbST group
DMI (kg/d)	Pretreated	11.4±0.7[a]	12.3±0.8[a]	NS
	Early	11.6±1.1[a]	13.0±1.7[a]	NS
	Mid	12.2±1.8[a]	13.9±1.3[a]	NS
	Late	12.3±1.8[a]	13.4±1.7[a]	NS
Milk yield (kg/d)	Pretreated	13.0±1.5[a]	13.3±2.7[a]	NS
	Early	13.1±1.9[a]	16.0±2.1[a]	p<0.05
	Mid	12.9±1.5[a]	14.6±1.9[a]	NS
	Late	11.5±1.0[a]	13.0±1.3[a]	NS
Protein (gm %)	Pretreated	3.2±0.21[c]	3.2±0.16[b]	NS
	Early	3.3±0.15[bc]	3.2±0.25[b]	NS
	Mid	3.4±0.19[b]	3.5±0.17[a]	NS
	Late	3.7±0.05[a]	3.6±0.12[a]	NS
Fat (gm %)	Pretreated	3.6±0.76[a]	3.9±0.60[a]	NS
	Early	3.6±0.25[a]	4.7±0.78[a]	p<0.05
	Mid	3.9±0.66[a]	4.2±0.63[a]	NS
	Late	3.9±0.71[a]	4.7±0.79[a]	NS
Lactose (gm %)	Pretreated	4.5±1.02[a]	4.9±0.24[a]	NS
	Early	4.5±0.55[a]	4.8±0.49[a]	NS
	Mid	4.9±0.40[a]	4.6±0.56[a]	NS
	Late	4.8±0.29[a]	4.8±0.38[a]	NS

[a,b] Means within a column with different superscripts between measurements differ significantly (p<0.05).
Comparison of p-values of control group vs. rbST-treated group using unpaired t-test. NS = Not significant.

Table 3. Means±SD for mammary plasma flow (MPF), mammary blood flow (MBF), and body weight during prolonged rbST administration

Parameters	Measurements	Control group	rbST group	Control vs. rbST group
MPF (ml/min)	Pretreated	2,438±331[a]	2,594±342[b]	NS
	Early	2,730±357[a]	3,927±1203[ab]	p<0.05
	Mid	2,698±319[a]	3,983±1183[a]	p<0.05
	Late	2,692±290[a]	3,533±1055[ab]	NS
MBF (ml/min)	Pretreated	3,286±461[a]	3,548±463[b]	NS
	Early	3,817±616[a]	5,310±1620[ab]	p<0.05
	Mid	3,821±533[a]	5,458±1627[a]	p<0.05
	Late	3,750±476[a]	4,814±1464[ab]	NS
MBF/milk yield (L/kg)	Pretreated	364±25[a]	397±111[a]	NS
	Early	420±32[a]	491±152[a]	NS
	Mid	433±89[a]	539±156[a]	NS
	Late	471±69[a]	539±168[a]	NS
Body weight (kg)	Pretreated	337±31.1[a]	364±27.1[b]	NS
	Early	357±34.0[a]	391±35.6[ab]	NS
	Mid	370±33.8[a]	412±35.5[ab]	NS
	Late	379±29.8[a]	421±43.5[a]	NS

[a,b] Means within a column with different superscripts between measurements differ significantly (p<0.05).
Comparison of p-values of control group vs. rbST-treated group using unpaired t-test, NS = Not significant.

were compared by Duncan's multiple range test. Values were compared between control and rbST-treated groups, mean differences were examined statistically by an unpaired t-test.

RESULTS

Changes in DMI milk yield and milk compositions

Milk yield and milk compositions are shown in Table 2. The enhancement of milk yield in animals given rbST were higher than those of the control animals given placebo and the persistency of production were higher in these animals throughout their lactation. The peak milk yield in both groups of animals declined from the early period of lactation as lactation advanced to mid and late lactation. Compared with the pre-treatment period, 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. During early lactation, the milk yield of rbST treated animals was higher than those of the control animals ($p<0.05$). Both the control animals and rbST-treated animals showed no significant changes in the concentration of milk lactose throughout the course of lactation. The concentrations of milk protein significantly increased during lactation advances to mid and late lactation in both groups. Milk fat concentrations of rbST-treated animals were higher than those of control animals throughout lactation and significantly greater in the early lactation ($p<0.05$).

Changes in mammary blood flow and body weight

The rate of mammary blood flow and plasma flow markedly increased during rbST administration (Table 3). The udder blood flow of rbST treated animals increased from 3,548 to 5,310 and 5,458 ml/min ($p<0.05$) in both early and mid lactation, respectively, as compared with pretreatment period., while there were no significant changes in the control animals. The values of both udder plasma flow and udder blood flow of rbST treated animals were significantly higher than those of the controls during early and mid lactation ($p<0.05$). 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. The body weights of both control animals and rbST treated animals increased stepwise as compared with the pretreated period. while rbST treated animals had a significant higher weight gain in late lactation ($p<0.05$) as compared with pretreatment period.

Table 4. Means±SD for plasma concentrations, arteriovenous differences and mammary uptake for glucose and acetate during prolonged rbST administration

Parameters	Measurements	Control group	rbST Group	Control vs. rbST Group
Glucose :				
Arterial concentrate	Pretreated	3.78±0.23 ^{a}	3.72±0.14 ^{a}	NS
(μmol/ml)	Early	3.83±0.37 ^{a}	3.80±0.24 ^{a}	NS
	Mid	3.60±0.14 ^{a}	3.77±0.15 ^{a}	NS
	Late	3.67±0.05 ^{a}	3.62±0.19 ^{a}	NS
A-V (μmol/ml)	Pretreated	0.93±0.30 ^{a}	0.98±0.15 ^{a}	NS
	Early	0.90±0.12 ^{a}	0.76±0.07 ^{ab}	p<0.05
	Mid	0.78±0.12 ^{a}	0.59±0.21 ^{b}	NS
	Late	0.73±0.21 ^{a}	0.62±0.21 ^{b}	NS
Extraction (%)	Pretreated	25±7 ^{a}	26±3 ^{a}	NS
	Early	24±4 ^{a}	20±3 ^{ab}	NS
	Mid	22±3 ^{a}	15±6 ^{b}	p<0.05
	Late	20±6 ^{a}	17±6 ^{b}	NS
Udder uptake (μmol/min)	Pretreated	2,323±963 ^{a}	2,502±496 ^{ab}	NS
	Early	2,459±472 ^{a}	3,010±941 ^{a}	NS
	Mid	2,087±284 ^{a}	2,201±433 ^{ab}	NS
	Late	1,979±625 ^{a}	2,018±369 ^{b}	NS
Acetate :				
Arterial concentrate	Pretreated	1,102±160 ^{a}	1,171±109 ^{a}	NS
(μmol/L)	Early	1,180±212 ^{a}	1,238±101 ^{a}	NS
	Mid	1,009±157 ^{a}	1,129±130 ^{a}	NS
	Late	1,251±124 ^{a}	1,199±154 ^{a}	NS
A-V (μmol/L)	Pretreated	479±305 ^{a}	575±146 ^{a}	NS
	Early	458±160 ^{a}	624±57 ^{a}	p<0.05
	Mid	357±109 ^{a}	628±223 ^{a}	p<0.05
	Late	477±200 ^{a}	552±172 ^{a}	NS
Extraction (%)	Pretreated	42±24 ^{a}	49±16 ^{a}	NS
	Early	38±8 ^{a}	51±7 ^{a}	p<0.05
	Mid	35±8 ^{a}	55±14 ^{a}	p<0.05
	Late	37±13 ^{a}	46±13 ^{a}	NS
Udder uptake (μmol/min)	Pretreated	1,111±617 ^{a}	1,448±372 ^{b}	NS
	Early	1,281±543 ^{a}	2,464±873 ^{a}	p<0.05
	Mid	964±340 ^{a}	2,317±389 ^{a}	p<0.001
	Late	1,310±616 ^{a}	1,858±620 ^{ab}	NS

^{a,b} Means within a column with different superscripts between measurements differ significantly ($p<0.05$).
Comparison of p-values of control group vs. rbST-treated group using unpaired t-test. NS = Not significant.

Arterial plasma concentration, A-V concentration differences and mammary uptakes of glucose and acetate

The mean arterial plasma concentrations for glucose were largely unchanged throughout periods of study in both controls and rbST-treated animals (Table 4). However, as lactation advances, the A-Vdifferences and extraction ratio of glucose across the mammary gland decreased as compared with the pretreatment period in both groups. The large extent of decreases were apparent in mid-lactation and late lactation when compared with the pretreatment period ($p<0.05$) in rbST-treated animals. The A-V differences of glucose during early lactation and the extraction ratio during mid-lactation for rbST-treated animals significantly decreased ($p<0.05$) when compared with control animals. The net mammary glucose uptake of rbST-treated animals increased approximately 20% in early lactation as compared with the pretreatment period and it decreased as lactation advanced to midlactation and late lactation ($p<0.05$). The arterial plasma acetate concentrations were largely unchanged throughout periods of study in both controls and rbST-treated animals. However, the A-V concentration differences and extraction ratio for acetate of rbST-treated animals showed non-significant increases, but the mammary uptake for acetate significantly increased during early and midlactation as compared with the pretreatment period ($p<0.05$). These changes were significantly higher ($p<0.05$) than those of the controls during early and mid-lactation.

Table 5. Means±SD for plasma concentrations, arteriovenous differences and mammary uptake for β-hydroxybutyrate and glycerol during prolonged rbST administration

Parameters	Measurements	Control group	rbST group	Control vs. rbST group
β-Hydroxybutyrate :				
Arterial concentrate	Pretreated	1,604±735[a]	1,136±303[a]	NS
(μmol/L)	Early	1,777±563[a]	1,248±384[a]	NS
	Mid	1,608±447[a]	1,420±408[a]	NS
	Late	1,510±258[a]	1,172±213[a]	p<0.05
A-V (μmol/L)	Pretreated	688±293[a]	458±116[a]	NS
	Early	761±239[a]	462±220[a]	NS
	Mid	636±191[a]	582±175[a]	NS
	Late	528±165[a]	441±238[a]	NS
Extraction (%)	Pretreated	44±7[a]	42±11[a]	NS
	Early	44±11[a]	37±12[a]	NS
	Mid	40±5[a]	41±9[a]	NS
	Late	36±15[a]	37±17[a]	NS
Udder uptake (μmol/min)	Pretreated	1,679±736[a]	1,156±269[a]	NS
	Early	2,116±835[a]	1,986±1344[a]	NS
	Mid	1,710±572[a]	2,403±1272[a]	NS
	Late	1,421±488[a]	1,370±500[a]	NS
Glycerol :				
Arterial concentrate	Pretreated	37±6[a]	40±7[a]	NS
(μmol/L)	Early	39±14[a]	39±5[a]	NS
	Mid	36±3[a]	31±4[b]	p<0.05
	Late	33±5[a]	31±5[b]	NS
A-V (μmol/L)	Pretreated	4±6[a]	-1±6[a]	NS
	Early	3±26[a]	-4±4[a]	NS
	Mid	6±5[a]	0.5±9[a]	NS
	Late	2±5	-6±7[a]	NS
Extraction (%)	Pretreated	10±15[a]	-5±16[a]	NS
	Early	-2±64[a]	-12±12[a]	NS
	Mid	17±12[a]	0.2±28[a]	NS
	Late	6±14[a]	-24±30[a]	NS
Udder uptake (μmol/min)	Pretreated	11±14[a]	-2±16[a]	NS
	Early	9±61[a]	-16±16[a]	NS
	Mid	17±12[a]	-0.2±40[a]	NS
	Late	7±12[a]	-19±18[a]	p<0.05

[a,b] Means within a column with different superscripts between measurements differ significantly ($p<0.05$).
Comparison of p-values of control group vs. rbST-treated group using unpaired t-test, NS = Not significant.

Arterial plasma concentration, A-V concentration differences and mammary uptakes of β-hydroxybutyrate and glycerol

The mean arterial plasma concentrations for β-hydroxybutyrate and free glycerol were unchanged throughout experimental periods in both groups (Table 5). The A-V differences and extraction ratio of β-hydroxybutyrate were not affected during treatment periods in either the controls or rbST-treatment. The mammary uptake for β-hydroxybutyrate of rbST-treated animals trended to increase as lactation advances but no significant differences in comparison with pretreatment period, while it remained constant through the course of lactation in the control animals. The A-Vdifferences and extraction ratio of free glycerol and net mammary uptake across the mammary gland in both groups showed variable.

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

The mean arterial plasma concentrations for free fatty acid (C_{16} to C_{18}) of rbST-treated animals were increased after rbST administration as compared with the pretreatment period (Table 6). The significant differences of the free fatty acid concentration were apparent in the early lactation ($p<0.01$) between control animals and rbST-treated animals. The values of A-Vdifferences and the negative uptake by the mammary gland for FFA were variable during lactating periods in both groups. The mean arterial plasma concentrations for triacylglycerol (C_{16} to C_{18}) showed no significant differences after rbST administration throughout lactation. The A-V differences and the net uptake of triacylglycerol across the mammary gland showed no

Table 6. Means±SD for plasma concentrations, arteriovenous differences and mammary uptake for free fatty acid and triacylglycerol during prolonged rbST administration

Parameters	Measurements	Control group	rbST group	Control vs. rbST group
Free fatty acid (C16-18) :				
Arterial concentrate	Pretreated	437.3±109.9 [a]	402.7±83.4 [a]	NS
(μmol/L)	Early	375.3±34.0 [a]	546.1±104.0 [b]	p<0.01
	Mid	394.3±62.2 [a]	485.4±185.5 [a]	NS
	Late	371.5±57.3 [a]	430.3±58.8 [a]	NS
A-V (μmol/L)	Pretreated	-27.6±70.9 [a]	-12.5±9.1 [a]	NS
	Early	-71.9±87.3 [a]	-16.4±81.8 [a]	NS
	Mid	-21.3±14.7 [a]	-27.5±63.6 [a]	NS
	Late	-31.8±38.9 [a]	-12.1±18.7 [a]	NS
Extraction (%)	Pretreated	-6.4±14.3 [a]	-3.4±2.4 [a]	NS
	Early	-20.0±23.7 [a]	-3.7±16.7 [a]	NS
	Mid	-6.0±4.6 [a]	-8.0±14.6 [a]	NS
	Late	-8.1±10.1 [a]	-2.8±4.0 [a]	NS
Udder uptake (μmol/min)	Pretreated	-70.5±186.5 [a]	-30.5±22.5 [a]	NS
	Early	-210.9±274.1 [a]	-87.2±280.2 [a]	NS
	Mid	-54.1±33.6 [a]	-88.1±240.4 [a]	NS
	Late	-90.1±109.6 [a]	-28.2±50.1 [a]	NS
Triacylglycerol (C16-18) :				
Arterial concentrate	Pretreated	148±50 [a]	143±49 [a]	NS
(μmol/L)	Early	128±50 [a]	128±14 [a]	NS
	Mid	145±45 [a]	178±72 [a]	NS
	Late	155±79 [a]	166±45 [a]	NS
A-V (μmol/L)	Pretreated	54±49 [a]	53±34 [a]	NS
	Early	52±53 [a]	36±24 [a]	NS
	Mid	65±48 [a]	98±64 [a]	NS
	Late	74±62 [a]	82±52 [a]	NS
Extraction (%)	Pretreated	33±24 [a]	33±16 [ab]	NS
	Early	35±22 [a]	27±15 [b]	NS
	Mid	42±18 [a]	49±17 [a]	NS
	Late	41±23 [a]	45±21 [ab]	NS
Udder uptake (μmol/min)	Pretreated	138±135 [a]	136±87 [a]	NS
	Early	130±121 [a]	164±137 [a]	NS
	Mid	166±105 [a]	348±222 [a]	NS
	Late	188±150 [a]	263±151 [a]	NS

[a,b] Means within a column with different superscripts between measurements differ significantly ($p<0.05$).
Comparison of P-values of control group vs. rbST-treated group using unpaired t-test, NS = not significant.

significant increases in rbST-treated animals in comparison with pretreatment, but mammary extraction ratio of triacylglycerol was significantly lower in early than that of mid-lactation after rbST treatment. There were no significant differences of A-V differences, extraction ratio and net uptake of triacylglycerol during lactation advances in control animals.

DISCUSSION

In the present results, there were marked increases in both plasma flow and blood flow to the mammary gland during long-term rbST administration in crossbred Holstein cattle. An increase in MBF coincided with an increase in milk yield during early lactation in rbST-treated animals. The relationship between MBF and milk yield showed an increase in the ratio of MBF: milk yield as lactation advances during administration of rbST. Similar results for an increase in this ratio during administration of growth hormone were also noted in dairy cows (McDowell et al., 1987). It was assumed that the mass of mammary tissue was not affected by the exogenous somatotropin. The increase in blood flow to the mammary gland more than milk yield would be partially attributable to an increase in cardiac output which has been reported in lactating cows injected daily with bST (Soderholm et al., 1988). However, crossbred HF animals with either short-term or long-term rbST treatment in different stages of lactation have also been shown to increase in MBF which were concomitant with an increase in circulating levels of IGF-I (Tanwattana et al., 2003; Chaiyabutr et al., 2005; Maksiri et al., 2005). It indicates that bST plays a role an increase in MBF, requiring IGF-I as a mediator increasing MBF directly (Forsyth, 1996). The present results for the mammary uptake of key plasma substrates are not based on changes in A-V concentration differences and extraction ratio in both the controls and rbST-treated animals. During early lactation in rbST-treated animals, an increase in the rate of blood flow to the mammary gland would be a major determinant of the rate of glucose uptake by the mammary gland. Several investigations have shown that mammary gland glucose uptake was depended on an increase in the arterial plasma glucose concentration during bST administration (Sandles et al., 1988; Fullerton et al., 1989) whereas other works have demonstrated no differences (McDowell et al., 1987; Mepham, 1993). Results of the present study support the latter observations during rbST administration. However, the decreases in both A-V concentration differences and extraction ratio for glucose occurred as lactation advances in either controls or rbST-treated animals, whereas arterial plasma glucose concentrations were unchanged, indicating that glucose uptake by the mammary gland was affected by stage of lactation and the activity of the mammary epithelial cell. The great extent of reduction in A-V concentration differences and the extraction ratio of glucose as lactation advances in rbST-treated animals indicate that glucose transport by the mammary cell was rate limiting step. The high blood flow to the mammary gland during rbST administration would decrease the transit time of glucose, thereby reduction for prolonging the contact time between glucose in blood and mammary epithelial cell. It would affect to the specific glucose transporter at the mammary cell membrane (Prosser, 1988; Madon et al., 1990) for the transport of glucose into mammary cell. Therefore, the utilization of glucose by the mammary gland would be limited by this means. It is possible that a promoting the utilization of glucose by the mammary gland would depend on a necessary combination of these means.

It has been known that volatile fatty acid in the form of acetate are the major of energy source of normal fed ruminants. In the present study, mammary arteriovenous concentration differences, extraction ratio and mammary uptake of acetate increased in different stages of lactation as compared with pretreatment period in rbST-treated animals. An elevation of mammary acetate uptake during rbST administration is explainable in light of the high energy and substrate demands for milk synthesis, because acetate is involved in mammary gland metabolism in either de novo synthesis of short and medium-chain milk fatty acids or generation of ATP and NADPH. The distribution of short and medium chain milk fatty acids in milk fat was not altered by rbST treatment (data not presented), indicating that acetate was partially redirected from oxidation to de novo fatty acid synthesis. Acetate uptake was also critically dependent upon rate of mammary blood flow. Circulating β-hydroxybutyrate arise mainly from rumen butyrate in the fed animal (Leng and West, 1969). In the present result, levels of A-V concentration differences and extraction ratio of β-hydroxybutyrate across the mammary gland including the arterial plasma concentration, were not affected during rbST administration. It indicates that the utilization by the mammary tissue was not obvious during rbST administration in 87.5% HF feeding on urea treated rice straw as a roughage. Although the principal effect of bST has been shown to increase oxidation of free fatty acids during negative energy balance in lactating cows. An increased concentrations of plasama β-hydroxybutyrate would be consistent with an increase in oxidation of free fatty acids (Bauman et al., 1988). The greater energy requirement resulting in increased hepatic ketogenesis due to greater mobilization of fat reserves (Schultz, 1974) were not apparent in rbST-treated animals.

In the present experiment, the mean values for the arterial plasma concentration of free fatty acid but not for triacylglycerol increased in rbST-treated animals which

were more sensitive to alteration than other blood substrates. This phenomenon has been proposed as an indication of under-nutrition (Reid and Hinks, 1962). However, animals in both groups showed no differences of DMI and 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. A significant increase in the concentration of FFA was apparent in rbST-treated animals as compared with the control animals during early lactation. Thus, the lipolytic activity would be a function of rbST treatment per se in stead of the associated changes in energy balance. The measurement of A-V differences of FFA across the mammary gland together with mammary blood flow did not provide a quantitative estimation of their total uptake by mammary tissue. The pattern uptake of triacylglycerol by the mammary gland did not significantly alter in different stages of lactation in comparison to the pretreatment period in rbST-treated animals, which is agree with the results reported by Miller et al. (1991). It is possible that the negative mammary uptakes of both free fatty acids and glycerol may reflect hydrolysis of triacylglycerol, since there is the release of FFA into venous blood due to triacylglycerol hydrolysis during the uptake of plasma triacylglycerol as in lactation (West et al., 1967). The releasing of FFA would be as a result of enzymatic activity of lipoprotein lipase in the mammary tissue which has been reported to be higher in mammary tissue relative to other tissue (Shirley et al., 1973; Bauman and Griinari, 2003). Milk fat concentrations increased in rbST-treated animals which coincided with an increase in the plama FFA concentration. The increase in arterial plasma FFA concentration during rbST administration may be a determinant of FFA uptake by the mammary gland for synthesis of milk fat, since prediction of nearly fourfold increase in FFA uptake in bST-treated cows was noted (Miller et al., 1991).

In conclusion, these experiments demonstrated that the rbST exerts its galactopoietic action, in part, association with an increase in MBF, which partitions the distribution of nutrients to the mammary gland. Long term administration of a slow-release formulation of rbST to crossbred dairy cattle could not improve lactation persistency by slowing down the post-peak rate of decline as the lactation advances. The stimulant effect for milk yield was less in late lactation despite a high level of MBF during long term administration of rbST. An increase in MBF during rbST administration would not be a major determinant in mediation of nutrient uptake by the mammary glands for increase in milk production. Local changes for biosynthetic capacity within the mammary gland would be a factor in identification of the utilization of substrates in the rate of decline in milk yield with advancing lactation. These results show no coordination between mammary gland metabolism and MBF at different stages of lactation, although an increase in metabolism of the mammary gland leading to increase in MBF has been noted (Lacasse and Prosser, 2003). Further research is needed to determine the mechanisms by which bovine somatotropin influences mammary gland metabolism during lactation advance in crossbred cattle in the tropics.

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