The Plasma Level of Insulin-like Growth Factor-I (IGF-I) in Relation to Mammary Circulation and Milk Yield in Two Different Types of Crossbred Holstein Cattle

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ABSTRACT : The objective of the present study was to determine the plasma level of insulin-like growth factor-I (IGF-I) in relation to mammary blood flow and milk yield including biological variables of relevance to milk synthesis in two different types of crossbred Holstein cattle at 3 different stages of lactation. Eight heifers were 87.5% HF and eight 50% HF animals were selected for the experiments. The three stages of lactation tested were: early lactation (30 days postpartum), mid-lactation (120 days postpartum) and late lactation (210 days postpartum). Animals in each group were fed a concentrate and rice straw treated with 5% urea as the source of roughage throughout the experiments. In early lactation, mammary blood flow and milk yield of 87.5% HF animals were significantly higher than those of 50% HF animals. In mid- and late lactation, both mammary blood flow and milk yield showed a proportional decrease from the early lactating period of 87.5% HF animals. The trends for persistency were observed in 50% HF animals as for udder blood flow and milk yield throughout the experimental periods. The plasma glucose level of the 50% HF animals was significantly higher than those of 87.5% HF animals in both early and mid-lactation. The concentrations of arterial plasma free fatty acids (C_{16} to C_{18}) were higher in 50% HF animals as compared with 87.5% HF animals in all periods of study. In early lactation, the concentration of plasma growth hormone (GH) of 87.5% HF animals was higher than those of the 50% HF animals, thereafter the mean level of plasma growth hormone declined in both mid- and late lactation. The concentration of plasma IGF-I of 50% HF animals was significantly higher than those of 87.5% HF animals in all stages of lactation. There were no differences among stages of lactation for the levels of plasma IGF-I, insulin and growth hormone in 50% HF animals. In 87.5% HF animals, the plasma levels of both IGF-I and insulin were lower in early lactating period while it showed an increase during mid- and late lactation. The present results indicated that the regulatory role for the higher mammary blood flow and milk yield during lactation in 87.5% HF are not mediated via the higher level of circulating IGF-I. Differences in mammary blood flow and milk yield between 50% HF and 87.5% HF animals are in part due to a higher concentration of circulating growth hormone. The lower level of circulating growth hormone in 50% HF animals would be regulated by higher levels of IGF-I, free fatty acid and glucose in plasma. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 3 : 343-348)***

Key Words : IGF-I, Growth Hormone, Mammary Blood Flow, Milk Yield, Crossbred Holstein Cattle

INTRODUCTION

Crossbred cattle between *Bos taurus* and *Bos indicus* have been exploited as an efficient tool for blending the adaptability of tropical cattle with the high milking potential of exotic breeds for increased milk production. However, there is still a need to answer the question of the type of crossbred cattle most suitable for the tropics and the management necessary for efficient dairy production in a hot climate. Genetic potential for milk production of crossbred cattle has been considered, but many factors that limit milk production of tropical dairy cattle can affect the physiological signals received by the mammary gland.

Blood flow through the ruminant mammary gland is a major parameter controlling milk production and consequently, circulating factors affecting mammary blood flow may have pronounced effects on the rate of milk secretion. Little is known about the circulating factors that are involved in regulating mammary blood flow and milk synthesis. It has been reported that milk yield of crossbred cattle containing 87.5% Holstein genetics decreased rapidly from mid to late lactation which coincided with the decrease in endogenous growth hormone and mammary blood flow during this time (Chaiyabutr et al., 2000b). An injection of exogenous bovine somatotropin (bST) showed an increase in both mammary blood flow and milk yield in late lactating 87.5% crossbred Holstein cattle (Tunwattana et al., 2003). In addition, the injection of bST elevated the level of plasma Insulin-like growth factor-I (IGF-I) and so considerable interest has been shown in the effect of IGF-I on mammary blood flow (Tunwattana et al., 2003).

IGF-I is a polypeptide, 7.5 kDa, composed of 70 amino acids and it was originally thought to be a liver derived mediator of growth hormone action (Granner, 1996). IGF-I is considered to be responsible for the mammary gland function. Infusion of IGF-I into the pudic artery of lactating goats has been shown to increase blood flow and milk production in the infused side (Prosser et al., 1990; 1994) while, an infusion of growth hormone into the mammary artery of sheep did not increase milk yield (Peel and Bauman, 1987). Peel and Bauman (1987) suggested that growth hormone could affect mammary tissue indirectly by

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its action via IGF-I. Based on these results, Tunwattana et al. (2003) reported that IGF-I may be important in controlling the mammary blood flow and efficiency of mammary substrate utilization of crossbred HF animals (at least in part by moderating growth hormone).

Although a number of studies have demonstrated differences in the effects of IGF-I and growth hormone on mammary function (Collier et al., 1984; Tunwattana et al., 2003) and an appearance of persistency for milk yield and mammary blood flow during lactating periods has been reported in 50% HF animals (Chaiyabutr et al., 2000a), but few studies have provided a quantitative description of the effects on lactating crossbred dairy cattle. The aim of this study was to determine whether differences in milk production and mammary blood flow between animals differing in their content of Holstein Friesian genetics were associated with differences in circulating levels of IGF-I and other biological variables of relevance to milk synthesis.

MATERIALS AND METHODS

Animal and management

Sixteen pregnant heifers crossbred Holstein cattle, 23-25 months old and after approximately 150 days of gestation, were selected for the experiments. These animals consisted of two groups of eight animals each of two breed types, Holstein Friesian x Red Sindhi (50:50=50% HF) and Holstein Friesian×Red Sindhi (87.5:12.5=87.5% HF). Animals in each group were fed with rice straw treated with 5% urea as the source of roughage throughout the experiments. Animals were housed in sheds. The maximum temperature in the shed at noon was 34±1°C and the minimum temperature at night was 26±1°C. Before parturition, animals were individually fed a concentrate of an average of 4.0 kg/day (DM basis) and roughage to maintain a moderate body condition score 2.5 on a 5 point scale until calving. In the lactation period, animals received an average of 4-5 kg/day of roughage in combination with 7-10 kg of the same concentrated mixture as fed before parturition. Each day, half of the food was given at between 0600-0700 h and the other half between 1600-1700 h. Animals were adequately supplied with water and a lick block of minerals throughout the experiment.

Concentrate mixture (fresh basis) consisted of soybean meal (30 kg), cotton seed (25 kg), cassava (25 kg), rice bran (15 kg), dicalcium phosphate (2 kg), $NaHCO_3$ (1.7 kg), KCl (0.7 kg) and premix (0.6 kg). The urea treated rice straw was prepared by mixing urea solution (5 kg urea dissolved in 100 litres water per 100 kg dry rice straw) with dry straw and stored under airtight conditions in a cement pit for 21 days. After 21 days, the treated rice straw with 5% urea was offered to the animals.

Experimental procedures

The experiment consisted of three consecutive periods. Period 1 was designed for early lactation (30 days postpartum), period 2 for mid-lactation (120 days postpartum) and period 3 for late lactation (210 days postpartum). Animals were fed the same ration through the completion of period 3. In all periods of experiments, the dry matter intake of each animal was recorded daily and averaged weekly. Animals were normally milked at around 0600 and 1700 h. On each sampling day at around 1100 h, a blood sample (~8 ml) was taken from the jugular vein into a heparinized tube and an arterial blood sample (~8 ml) was collected from the coccygeal artery by venipuncture with a #21 needle into another heparinized tube. Blood samples in heparinized tubes were kept in crushed ice until centrifuged at 3,000 rpm for 30 min at 4°C. Plasma from the venous blood samples were kept in aliquots (~1 ml) at -40°C until hormone concentrations were assayed. Arterial plasma samples in aliquots (~1 ml) were kept at -40°C for chemical studies. Milk yield was recorded by milking machine in each lactating period of study.

Udder blood flow measurements

On the day before sampling in each lactating period, two catheters (i.d. 1.0 mm, o.d. 1.3 mm, L 45 mm) were inserted into either the left or right subcutaneous abdominal vein (milk vein) by using an intravenous polymer catheter (Jelco, Critikon; Johnson & Johnson, U.K.) under local anesthesia. This was done in standing animals for the measurement of udder blood flow and for collection of venous blood. The tip of the first 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. Udder blood flow measurements were performed in duplicate. Blood flow through half of the udder was determined by measuring the dilution of dye T-1824 (Evans blue) by a short term continuous infusion as described by Chaiyabutr et al. (1997). In brief, a dye (T-1824) was dissolved in sterile normal saline and diluted to a concentration of 50 mg/l. On the day of study at around 1400 h, the solution was infused by a peristaltic pump (Gilson Medical electronics) at a constant rate of 85 ml/min into the milk vein for 1 min, which could produce adequate mixing of dye with blood. Before infusion, blood was drawn from downstream 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. Three consecutive plasma samples were taken during dye infusion at about 5 seconds intervals. Blood flow of half of the udder was calculated from plasma samples using the equation derived by Thompson and Thomson (1977). Quarter milking showed

Table 1. Udder blood flow and milk yield in different stages of lactation between 87.5% HF and 50% HF animals

		HF:RS (87.5:12.5) (n=8)	HF:RS (50:50) (n=8)	87.5% HF vs. 50% HF
Udder blood flow (l/min)	Early lactation	5.89±1.48	4.10±0.56	p<0.01
	Mid-lactation	4.51±0.85	4.58±0.73	ns
	Late lactation	4.29±0.80	4.66±0.72	ns
Milk yield (kg/d)	Early lactation	18.14±5.19	11.95±1.38	p<0.01
	Mid-lactation	11.36±1.27	11.43±1.90	ns
	Late lactation	9.65±0.95	11.36±1.66	p<0.05
Dry matter intake (kg/d)	Early lactation	10.67±0.77	11.43±0.58	p<0.05
	Mid-lactation	12.30±1.12	10.79±0.92	p<0.01
	Late lactation	11.75±1.54	11.68±0.89	ns
Dry matter intake/milk yield	Early lactation	0.61±0.14	0.97±0.10	p<0.001
	Mid-lactation	1.09±0.05	0.96±0.18	ns
	Late lactation	1.17±0.21	1.05±0.18	ns

P-value by unpaired t-test with respect to the similar period of lactation between 87.5% and 50% HF animals.

Table 2. Plasma concentrations of Insulin-like growth factor I (IGF-I), growth hormone (GH) and insulin in different stages of lactation between 87.5% HF and 50% HF animals

		HF:RS (87.5:12.5) (n=8)	HF:RS (50:50) (n=8)	87.5% HF vs. 50% HF
IGF-I (ng/ml)	Early lactation	34.81±22.27	75.50±30.25	p<0.01
	Mid-lactation	54.13±18.29**	70.63±19.61	ns
	Late lactation	57.50±16.32**	73.38±17.82	p<0.05
GH (ng/ml)	Early lactation	12.28±5.87	8.81±1.35	ns
	Mid-lactation	9.75±3.15	8.11±3.84	ns
	Late lactation	9.20±3.26	8.48±3.40	ns
Insulin (μu/ml)	Early lactation	18.66±9.99	22.96±11.73	ns
	Mid-lactation	23.71±8.06**	22.91±13.01	ns
	Late lactation	21.06±5.35	29.44±16.55	ns

P-value by paired t-test with respect to the early lactating period in the same group. (** p<0.01).
P-value by unpaired t-test with respect to the similar period of lactation between 87.5% and 50% HF animals.

that the yields of the two halves of the udder were similar. Udder 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.

Hormone determinations

Radio immunoassay (RIA) of growth hormone (GH) was performed on all venous plasma samples using double antibodies RIA as described by Chaiyabutr et al. (2000b). The plasma insulin concentration was quantified using a radio immunoassay (RIA) kit (Coat-a Count® Insulin, Diagnostic Products Corporation, Los Angeles, CA, USA). The plasma IGF-I concentration was determined using the IGF-I kit (OCTETA® IGF-I, ISD, Ltd., UK).

Metabolites determinations

Arterial plasma glucose concentrations were measured using enzymatic oxidation in the presence of glucose oxidase. Plasma free fatty acids (FFA, C_{16}-C_{18}) concentrations were measured by using gas chromatography (Shimazu GC-7AG Gas Chromatograph) by comparison with the internal standard heptadecanoic acid, as described by Thomson et al. (1979).

Statistics

The experimental results were evaluated among periods in each group using the paired t-test. The unpaired t-test was used to estimate the statistical significant difference between groups. Mean values are presented as mean±SD.

RESULTS

Udder blood flow, milk yield and dry matter intake

During early lactation, mammary blood flow and milk yield of 87.5% HF animals were higher (p<0.01) than that of 50% HF animals (Table 1). In mid- and late lactation, both mammary blood flow and milk yield showed a proportional decrease from early lactating period in 87.5% HF animals. In 50% HF animals, the trends for persistency of milk yield including a constant of udder blood flow through the experimental periods were observed. The total dry matter intake of 50% HF animals was significantly higher (p<0.05) in the early lactation than those of 87.5% HF animals, while in mid lactation, the total dry matter

Table 3. Plasma concentrations of glucose and free fatty acid (FFA) in different stages of lactation between 87.5% HF and 50% HF animals

		HF:RS (87.5:12.5) (n=8)	HF:RS (50:50) (n=8)	87.5% HF vs. 50% HF
Glucose (mM)	Early lactation	3.47±0.37	4.31±0.33	p<0.001
	Mid-lactation	3.28±0.49	3.88±0.40*	p<0.01
	Late lactation	3.67±0.37*	3.69±0.25***	ns
FFA (ng/ml)	Early lactation	309.75±134.10	380.40±179.25	ns
	Mid-lactation	230.70±133.60	336.90±162.92	ns
	Late lactation	210.23±70.88	319.60±119.10	p<0.05

P-value by paired t-test with respect to the early lactating period in the same group. (* p<0.05, *** p<0.001).
P-value by unpaired t-test with respect to the similar period of lactation between 87.5% and 50% HF animals.

intake of 87.5% HF animals was significantly higher (p<0.01) than those of 50% HF animals. The ratio of dry matter intake to milk production of 87.5% HF animals was significantly lower (p<0.001) than that of 50% HF animals during early lactation.

IGF-I, growth hormone and insulin

In early lactation, the concentration of plasma growth hormone (GH) showed a trend being higher in 87.5% HF animals than those of the 50% HF animals, thereafter the mean level of plasma GH declined in both mid- and late lactation (Table 2). The concentration of plasma IGF-I of 50% HF animals was significantly higher than those of 87.5% HF animals while the values of plasma insulin showed no differences between 50% HF animals and 87.5% HF animals throughout lactating periods. In 87.5% HF animals, the plasma levels of both IGF-I and insulin were lower in early lactating period while it increased during mid- and late lactation (p<0.01). There were no differences among stages of lactation in the levels of plasma IGF-I, insulin and growth hormone in 50% HF animals.

Plasma metabolites

The mean arterial plasma glucose concentration of 50% HF animals was significantly higher than those of the 87.5% HF animals in both early and mid-lactating periods (Table 3). In 50% HF animals, the plasma levels of glucose showed significant decreases in mid and late lactation as compared to the early lactating period. In 87.5% HF animals, the plasma glucose concentration was significantly higher (p<0.05) in the late lactating period as compared to the early lactating period. The mean arterial plasma FFA concentrations of 50% HF animals showed a trend being higher than those of 87.5% HF animals in all stages of lactation. There were no differences among stages of lactation in the levels of plasma FFA in either 50% HF animals or 87.5% HF animals.

DISCUSSION

Blood flow through the ruminant mammary gland is a major parameter controlling milk production; consequently, circulating factors affecting mammary blood flow may have pronounced effects on the rate of milk production. There were some interesting differences for endocrine regulation of mammary blood flow and milk yield during lactations between 87.5% HF and 50% HF animals. In the present study, circulating levels of GH have been shown to be the hormone most closely associated with milk yield and its high concentration coincided with high mammary blood flow and milk secretion in the early lactating period of 87.5% HF animals. A number of studies indicated that GH increased milk yield by a mechanism which did not involve the direct action of GH on the mammary gland (Collier et al., 1984). Injections of GH in lactating cows have been shown to elevate the plasma IGF-I concentration (Davis et al., 1987; Tunwattana et al., 2003). An infusion of IGF-I into the pudic artery of lactating goats, increased blood flow and was associated with increased milk production in the infused side (Prosser et al., 1990; Prosser et al., 1994). However, this effect of circulating IGF-I on mammary blood flow was not supported by the present study. The plasma IGF-I concentration of 87.5% HF animals was actually lower while those of mammary blood flow was higher in the early lactating period. The plasma IGF-I concentration increased while mammary blood flow and GH gradually decreased when lactation advanced to mid- and late lactating period. These findings are similar to those of other previous studies that a decrease in the plasma IGF-I levels at the beginning of lactation were associated with an increase in plasma levels of GH (Hart et al., 1989; Vicini et al., 1991). The low level of plasma IGF-I concentration was reversibly associated with the high level of GH concentration during early lactation in 87.5% HF animals which had a high genetic blood level of Holstein Friesian cattle. In contrast to 87.5% HF animals, the high concentration of IGF-I in plasma of 50% HF animals coincided with the lower levels of plasma GH concentration, mammary blood flow and milk yield. Such differences between two types of crossbred animals could be attributable to disparities in breed.

The synthesis and release of IGF-I is mainly undertaken by the liver. However, little is known about the regulation

for synthesis and secretion of IGF-I in the liver of ruminants. Mechanisms for setting the plasma IGF-I level might be different between two types of crossbred animals. It is possible from the present data, IGF-I secretion would be dependent on the availability to the liver of both GH and nutritional factors. The differences of nutritional status were apparent during early lactation between 50% HF and 87.5% HF animals. In the present study, total dry matter intake of 87.5% HF animals was significantly lower, while the milk yield was significantly higher in early lactation than those of 50% HF animals. The ratio of total dry matter intake to milk yield was lower in 87.5% HF animals. This indicates that the energy output in milk and for maintenance of 87.5% HF animals was greater than the energy consumed in the food during early lactation. Thus, the 87.5% HF animals being on lower nutritional status was reflected in lower basal level of IGF-I in comparison to 50% HF animals during early lactation (Hodgkinson et al., 1991). Negative energy balance has also been identified as a key negative regulator of hepatic IGF-I production (Weller et al., 1994; Ketelslegers et al., 1995). The low circulating insulin and glucose concentrations of 87.5% HF animals during early lactation could be another possibility in limiting IGF-I release from the liver. The crossbred cattle containing 87.5% Holstein genetic close to exotic *Bos taurus* breed may lead to different bodily adjustment to the tropical environment. No changes were apparent in 50% HF animals for either the GH levels or the IGF-I levels in all lactating periods. However, the higher plasma levels of IGF-I concomitant with lower plasma GH levels at the start of lactation of 50% HF animals appears to be a paradox. It raises a question about the importance and effect of IGF-I in the galactopoietic effect of somatotropin and the effect on nutrient partitioning in 50% HF animals. The lack of effect of higher plasma IGF-I levels in regulating mammary blood flow and milk yield in 50% HF animals may be due to changes in the pattern of IGF-I binding proteins and paracrine production and secretion of IGF-I. It probably involved plasma IGF-I binding proteins which combined with IGF-I in blood and so modulated the level of free IGF-I before it reached the mammary gland, since it has been reported that approximately 95% of the infused IGF-I was bound by the IGF binding proteins (Davis et al., 1989). However, few data are available for the synthesis and secretion of IGF binding proteins in the liver of crossbred dairy cattle. It has been reported that GH exerts its effects in peripheral tissues by stimulation of IGF-I production (Holly and Was, 1989). In addition, the role of IGF-I in regulating GH release may also involve binding proteins which combine with IGF-I in blood and so modulate the level of free IGF-I. The high circulating concentration of IGF-I in 50% HF animals probably exerts an inhibiting action on GH secretion by a negative feedback mechanism via a direct action on the pituitary gland (Fletcher et al., 1995) or by stimulating somatostatin (SS14) secretion from the hypothalamus (Bermann et al., 1994). The high basal levels of both free fatty acid and glucose in blood of 50% HF animals would be other mechanisms inhibiting GH secretion via their actions on either the hypothalamus or pituitary gland (Romo et al., 1997).

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