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Interactions of Circulating Metabolic Hormones and Metabolites of Crossbred Holstein Cattle in Response to Supplemental Recombinant Bovine Somatotropin (rbST) and Cooling Management with Misters and Fans at Different Stages of Lactation in the Tropics

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Abstract: The low milk production of both exotic and crossbred cattle is still the main problem in dairy farming in the tropics. A shorter persistency of lactation of crossbred cattle has been shown to relate to a rapid decrease in the plasma bovine somatotropin as lactation progressed to mid and late lactation. However, the mechanisms relating to high environmental temperature and the role of endocrine system in regulating milk production remain to be investigated. The purpose of this study focused on interactions of metabolic hormones, feed intake and other variables relevant to milk synthesis during exogenous rbST in crossbred 87.5% Holstein Friesian (HF) cattle housed under cooling management with Misters and Fans (MFC). Ten primiparous cattle were divided into two groups of five cows each housing in Normal Shaded (NS) as a non-cooled group and in shaded with MFC as a cooled group. Each cow was administered of recombinant bovine somatotropin (rbST) with three consecutive treatment with 500 mg of rbST in every 14 days in early, mid and late stages of lactation. Ambient temperatures and THI at MFC barn were significantly lower. Both MFC and rbST treatment had significantly effect on rectal temperature and respiratory rate. The cooled cows significantly increased in DMI and milk yields and more responses to the effect of rbST administration. The positive energy balances were seen throughout experimental periods. Both plasma concentrations of Free Fatty Acids (FFA) and milk fat were significantly elevated by rbST administrations in both groups. Plasma thyroxin (T4), cortisol and insulin levels remained unchanged. Cooled cows showed significant low in the concentration of plasma leptin while plasma IGF-1 were significantly elevated and plasma leptin were significantly decreased during administrations of rbST. These findings demonstrate that both IGF-1 and leptin responses were the prominent mediators of rbST in stimulating the galactopoiesis in both groups but were regulated differently. A significant response to rbST with the high concentrations of plasma IGF-1 would share a role in stimulating milk production direct to the mammary gland function. The appearances of low plasma leptin levels due to the effect of cooling management and the lipolytic effect of rbST administration in either group, resulting subsequently increased DMI in the supply of nutrients to the mammary gland for facilitating milk synthesis.

Key words: Misters and fans, rbST, IGF-1, leptin, crossbred holstein cattle

INTRODUCTION

A low milk yield and shorter persistency of lactation of crossbred dairy cattle is still the main problem for dairy farming in tropical countries. High environmental temperature and relative humidity is known to be one of several factors influencing to lactation performance of dairy cattle. Longed term exposure to high environmental temperature reduces both voluntary food intake and milk yield of cows (Fuquay, 1981; Huber *et al*., 1994;

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Shibata and Mukai, 1979). Normally, a sufficient feed intake relating to milk production is required for energy balance between energy intake and energy expenditure for lactation and maintenance. The reduction in nutrient intake during sexposure to high temperatures is a major cause which will inevitably reduce source of nutrients in distribution to the mammary gland for milk synthesis. The mechanisms responsible for food intake and lactation performance as part of regulatory responses during exposure to high temperature have not fully described, although many of the profound metabolic and hormonal alterations in dairy cattle have been reported by either lower plasma bovine somatotropin (bST) (Mitra *et al*., 1972) and thyroxin concentrations (Magdub *et al*., 1982) or higher serum cortisol concentration (Wise *et al*., 1988). The interaction between the metabolic hormones and the metabolic status of the animal is complex and not completely explained. Bovine somatotropin is known to be responsible for galactopoiesis and persistency of lactation (Bell, 1995; Svennersten-Sjaunja and Olsson, 2005). It has been previously reported that the short lactation persistency of 87.5% Holstein Friesian (HF) cattle under tropical environment was attributable to a rapid decline of the plasma bST concentration and the mammary blood flow as lactation progressed to mid and late lactation (Chaiyabutr *et al*., 2000a, b). The decrease in the plasma bST level was not associated with other metabolic hormones either IGF-1 or insulin as well as the total Dry Matter Intake (DMI) which remained unchanged at different stages of lactation (Chaiyabutr *et al*., 2004). These physiological phenomena do not point to the regulatory role of IGF-1 as a local mediator of the galactopoietic effects of bST. However, the mechanisms of bST-stimulated lactational performance are complex, since an administration of exogenous recombinant bovine somatotropin (rbST) increased the level of plasma IGF-1 accompanying with increases in milk yield and mammary blood flow while the level of plasma insulin and DMI remained unchanged (Chaiyabutr *et al*., 2005). A number of studies indicate that bST can increase milk yield by increasing the mammary blood flow in the provision of nutrients to sustain milk synthesis via an action of IGF-1 (Prosser *et al*., 1990, 1994).

The effect of bST on feed intake is known to be one of the mechanisms that can increase milk yield. However, the interaction between the role of bST and the metabolic status of the animal under high environmental temperature can not be concluded to be the only trigger in alteration in feed intakes and milk production. Exogenous rbST induced not only the secretion of IGF-1 in crossbred HF cattle but also increased mobilization of Free Fatty Acids (FFA) from adipose tissue (Chaiyabutr *et al*., 2007). It led to raise the question of whether the lipolytic effect during administration of rbST would affect to the secretion of leptin which is adipocyte-derived hormone. Leptin has shown to play a role in alteration of body mass via its control on feed intakes and energy balance (Chilliard *et al*., 2000; Inoue *et al*., 2005; Thomas *et al*., 2001). The role of leptin in regulating complex and dynamic changes in energy balance has received limited attention in ruminant. It has been reported that high-energy intake in heifer cattle would increase the serum IGF-1 concentration and body fatness which in turn, increases the serum leptin concentration (Delavaud *et al*., 2000). In addition, the effect of high environmental temperature has been reported to stimulate secretion of leptin from adipose tissue (Accorsi *et al*., 2005). Taken data together indicate a complex mechanism of interactions of circulating metabolic hormones and metabolites in the regulation of both feed intake and milk production in dairy cattle in the tropics. An increase in milk yield of crossbred dairy cattle in the tropics can be performed by variety of means, for examples improvement in genetic potential of indigenous cattle, reduced the effect of high environmental temperature and/or combination with exogenous hormonal treatment are likely involved in producing high milk production responses (Chaiyabutr *et al*., 2007). However, the mechanisms for interactive effects of metabolic hormones and metabolites under the high environment temperature in conjunction with exogenous rbST in regulating milk production and feed intake in crossbred cattle remain to be investigated.

Therefore, to better understand the mechanisms by which rbST acts on process of milk production under high environmental temperature. It was designed to draw together information that could explain the mechanisms by which exogenous rbST under misty fan cooling management regulated metabolism and thereby enabled treated cattle to increase milk production. The studies were focused on interactions of circulating hormonal profiles for leptin, insulin, IGF-1, T4 and cortisol including the plasma metabolites, energy balance, feed intake and milk production in 87.5% HF animals at different stages of lactation.

MATERIALS AND METHODS

All procedures were conducted in accordance with the guidelines for Animal Experimentation of the Faculty of Veterinary Science, Chulalongkorn University and approved by the Experimental Animal Ethics Committee of the Faculty of Veterinary Science, Chulalongkorn University (No. 0831029).

Animals, housing and managements: Ten primiparous, non-pregnant crossbred cattle, containing 87.5% Holstein (HF) genes, age 36±2 months, average body weight 366±33 kg and BCS 2.5±0.14 were selected for the present study and were devided randomly into two groups of five animals each. Cows in both groups were housed in open-sided with a tiled-roof and tie-stall barn which was separated into two parts by a metal sheet wall. Cows were managed similarly to the previous experiment by Chaiyabutr *et al*. (2011). In brief, cows in the first group were housed in a Normal Shaded barn (NS) as the non-cooled animals and cows in the second group were housed in a normal shaded barn with two sets of Misty Fan Cooling (MFC) as cooled animals. Each system consisted of a 65 cm. Diameter blade fan circulating 81 m^3/min of air with oscillation coverage of 180°. The amount of water discharged from 4 spray heads was 7.5 L/h and mist droplet was 0.01 mm. Animals were exposed to MFC for 45 min at 15 min intervals from 06:00-18:00 h. At night, animals were exposed to MFC for 15 min at 45 min intervals from 18:00-06:00 h. The ambient temperature and the relative humidity at NS and MFC barns were recorded using a wet and dry bulb thermometer. The relative humidity was read by psychrometric chart depending on wet and dry bulb temperature. Averages of ambient temperatures and humidity were recorded during the daytime (13:00 h) for 3 days before start of the first injection of rbST (pre-treated period) and for 3 days after the end of the 3rd injection of rbST (treated period) of each stage of lactation. A Temperature-Humidity Index (THI) was calculated from the average ambient temperature of dry and wet bulb temperatures using the following equation; THI = 0.72 (wb+db)+40.6 where wb is the wet bulb temperature and db is the dry bulb temperature expressed in °C (McDowell *et al*., 1976).

Cows in both groups were fed with the same diet as Total Mixed Ration (TMR) twice daily for *ad libitum* intake at 06:00 and 17:00 h throughout the experimental period which was formulated according to NRC requirements (National Research Council, 2001) 10-15 kg/cow/day of a TMR diet for producing cows. The 100 kg of feed ingredients of TMR diet composed of 50 kg of pine apple waste, 23 kg of soybean meal, 20 kg of cotton seed, 3 kg of rice bran, 1.4 kg of lime stone, 1.4 kg of di-calcium phosphate, 300 g of sodium bicarbonate, 100 g of potassium chloride and 800 g of mineral and vitamin premix. Chemical compositions of TMR diet had 39.1% of Dry Matter (DM) which composed of organic matter 92.7% of DM, crude protein 18% of DM, acid detergent fiber 20.1% of DM and neutral detergent fiber 33.9% of DM. Dry Matter Intake (DMI) of each cow was measured daily by calculating the amount of TMR intake and weighing back the refusals. Water was given to cows in ***ad libitum.*** Cows with averaged 60±1 days in milk at start of trial. Cows were milked twice daily at around 06:00 and 17:00 h using a milking machine and milk production was recorded daily of both the pre-treatment and the treatment periods at each stage of lactation. At 13:00 h on each specify day during measurements of ambient temperature, rectal temperature of each cow was measured by electronic thermometer and respiratory rates was measured on standing animal by counting flank movements. Body weight of all animals were recorded by weighing monthly throughout experimental periods.

Experimental design: The protocol of the present study in cooled and non-cooled cows were designed similarly to that by Chaiyabutr *et al.* (2011). Cows housing under either in NS or MFC barn were studied in three consecutive stages of lactation: early lactation (60-90 days postpartum), mid lactation (120-150 days postpartum) and late lactation (180-210 days postpartum) (Fig. 1). On each stage of

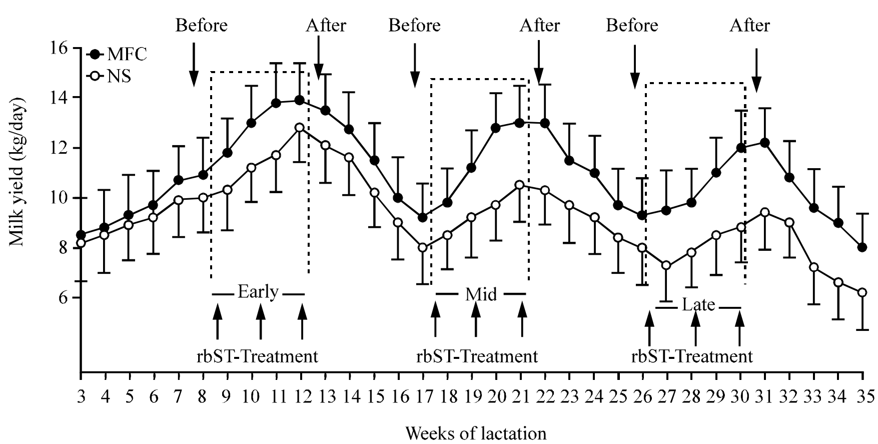

Fig. 1: Mean values of weekly milk yield on time course of rbST treatment at early, mid and late stages of lactation in cows housing under mist-fan cooling (MFC, solid circles) barn or under normal shade (NS, open circles) barn. Before = timed study for pre-treatment; After = timed study for after treatment

lactation, two periods of study were performed for pre-treatment and treatment of rbST. Measures were made 1 day before start of rbST treatment (pre-treated period) and 1 day after the end of rbST treatment (treated period). At the beginning of treatment, each cow was received the first dose subcutaneous injection of 500 mg rbST (Posilac, Monsanto, USA). Subsequently, two additional injections of rbST were given at 2 weeks intervals. Thereafter, measures were made 1 day after the end of the 3rd rbST injection (rbST treated period). In comparison, the effect of rbST administration were performed among different stages of lactation. During the last 30 days of each stage of lactation, no rbST injections were conducted in order to allow the milk yield from the effect of rbST treatment to return to the control level within the next 3 weeks (Kirchgessner *et al*., 1991) before the start of the next stage of lactation.

Sample collection and chemical analysis: At around 1300 h on the specified day of the experiment in either pre-treatment or treatment period of each stage of lactation, blood sample from each cow was collected from the coccygeal vessel by venopuncture into the tube containing heparin 25 i.u./mL blood which was placed on ice. Plasma was harvested by centrifugation at 3,000 rpm for 30 min and stored at -20°C for hormones and metabolites analyses. Plasma glucose concentrations were measured using enzymatic oxidation in the presence of glucose oxidase (glucose liquicolor, Wiesbaden, Germany). The plasma concentration of acetate was assayed by the acetic acid UV-method (R-Biopharm, Darmstadt, Germany). Plasma free fatty acids were determined by colorimetry after plasma extraction with chloroform, heptane and methanol and TAN solution (Wang *et al*., 2004). Plasma β-hydroxybutyrate concentrations were assayed using an enzymatic reaction in the presence of β-hydroxybutyrate dehydrogenase (R-Biopharm, Darmstadt, Germany) and plasma triglyceride concentration was determined by enzymatic colorimetric test (Triglyceride liquicolor, Wiesbaden, Germany). The plasma Insulin like Growth Factor-1 (IGF-1) concentration was determined by Chemiluminescence immunoassay using an IMMULITE® Analyzer (IMMULITE IGF-1, Diagnostic Products Corporation, Los Angeles, CA). Plasma insulin concentrations were determined using a commercially available insulin kit (Diagnostic Products Corporation, Los Angeles, CA, USA). Plasma leptin concentrations were determined using a radioimmunoassay kit specific for multi-species hormone (Linco Research, Inc., USA). The concentrations of plasma thyroxin (T4) were determined by using an Electrochemiluminescence Immunoassay (ECLIA) (Roche Diagnostics GmbH, USA) by Elecsys and cobas e immunoassay analyzers (Indianapolis, IN, USA). Plasma cortisol concentrations were measured by using a chemiluminescence immunoassay in an immulite analyzer (DPC, Los Angeles, CA). Milk samples from morning milking were used to determine concentrations of milk fat, lactose and milk protein using Milkoscan (Milko-Scan 133B, A/S N. Foss Electric, Hillerod, Denmark).

Calculation: The Net Energy intake (NEi), Net Energy for maintenance (NEm) and net energy for lactation (NE_l) were calculated according to the formula suggested by National Research Council (2001). Then, energy balance was calculated from energy intake subtracts by energy for maintenance and energy for lactation using the following equation:

$$NE_i \text{ (Mcal/day)} = \text{Feed energy} \times \text{DMI}$$

$$NE_m \text{ (Mcal/day)} = 0.08 \times BW^{0.75}$$

$$NE_l \text{ (Mcal/kg)} = [(0.0929 \times \text{Fat \%}) + (0.0547 \times \text{Protein \%}) + (0.039 \times \text{Lactose \%})] \times MY$$

$$\text{Energy balance (Mcal)} = NE_i - (NE_m + NE_l)$$

Where:
DMI = The Dry Matter Intake (kg DM/day)
$BW^{0.75}$ = The metabolic body weight (kg)

Statistical analysis: Data of individual cow were adjusted for covariate effects with data from the pre-treatment period before the start of the treatment period at each stage of lactation. Data were analyzed using the General Linear Model (GLM) procedure (SPSS for windows, V14.0; SPSS Inc., Chicago, IL, USA) to study either main effects or interaction of treatment and housing. Differences were considered significant at p<0.05. The statistical model used was:

$$Y_{ijk} = \mu + A_l + H_i + A(H)_{il} + B_j + (HB)_{ij} + A(HB)_{ijl} + Cov_k + e_{ijkl}$$

Where:
Y_{ijk} = The observation
μ = The overall mean
A_l = The animal effect
H_i = The house effect as main plot (i is NS, MFC)
$A(H)_{il}$ = The main plot error (animal l in house i)
B_j = The treatment effect (rbST) as a split plot (j is with and without rbST administration)
$(HB)_{ij}$ = The interaction effect between treatment and house
$A(HB)_{ijl}$ = The split plot error (animal l in house i and treatment j)
Cov_k = The covariate effect
e_{ijk} = The residual error

The differences of environmental parameters between NS and MFC barn were determined by unpaired t-test. Statistical significance was declared at $p<0.05$.

RESULTS

Ambient temperature, relative humidity, THI, rectal temperature and respiratory rate: Average values of all measurements taken at 1300 h for ambient temperature, relative humidity, THI, rectal temperature and respiratory rate during rbST administration at different stages of lactation of cows housing in NS and MFC barns are shown in Fig. 2 and 3. Ambient temperature in NS barn (range from 33.6-34.3°C) was higher than that of MFC barn (range from 29.1-30.9°C) throughout experimental periods. The relative humidity in MFC barn (range 73.8-81.2%) was significantly higher than that of NS barn (range 49.6-58.0%). Mean THI values of MFC barn in both the pre-treatment and treatment periods at 13:00 h (range 80.2-82.9) were significantly lower ($p<0.05$) in comparison with that of NS barn (range 82.94-85.02). Cows in MFC barn showed significantly lower rectal temperature and respiratory rate than those of cows housed in NS barn. During administrations of rbST in both groups both rectal temperature and respiratory rate showed tendency to increase in comparison with pre-treatment period at different stages of lactation.

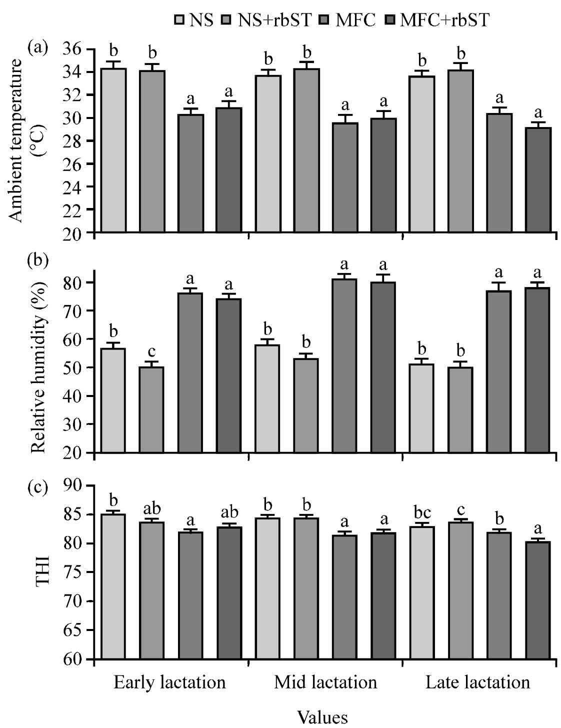

Fig. 2: Measurements of environmental condition for ambient temperature, relative humidity and THI were recorded at around 13:00 h during pre-treated and rbST-treated periods under Mist-Fan Cooling (MFC) and Normal Shade (NS) barns at different stages of lactation. Means with different superscripts (a, b, c) within each stage of lactation are significantly different ($p<0.05$). Values are the means and vertical lines represent the SD

DMI, milk yield, milk compositions and body weight: The results in Table 1 show that there were no significant differences in body weight between cooled and non-cooled cows at different stages of lactation. The DMI of cooled cows were statistically higher than those of non-cooled cows in early and mid stages of lactation but was not apparent in late stage of lactation. However, the interaction effect between MFC and rbST treatment was significantly apparent ($p<0.05$) for an increment in DMI in late stage of lactation. After administrations of rbST, DMI significantly increased when compared to pre-treated values at different stages of lactation in either group. Milk yield and 40 g kg^{-1} Fat Collected Milk (FCM) of cooled cows were higher than those of non cooled cows for the pre-treated period by averaged 18.9% but these results were not statistically significant at any stage of lactation. The more significant increases in milk yields and 40 g kg^{-1}

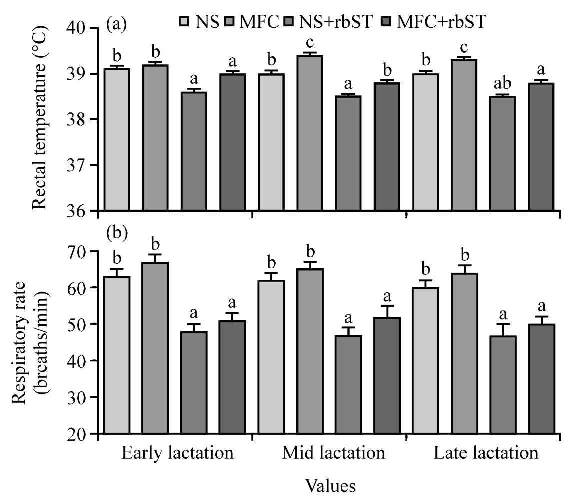

Fig. 3: Measurements of the respiratory rate and rectal temperature were recorded at around 13:00 h in cows housing under Mist-Fan Cooling (MFC) barn and Normal Shade (NS) barn during pre-treated and rbST-treated periods at different stages of lactation. Means with different superscripts (a, b, c) within each stage of lactation are significantly different ($p<0.05$). Values are the means and vertical lines represent the SD

Table 1: Effects of rbST administration on BW, DMI, milk yield and milk compositions of cross-bred Holstein cows housed under MFC and NS barns

Parameters	Lactation periods	NS		MFC			Effect[2, 3]		
		Pre-treated[1]	rbST[1]	Pre-treated	rbST	SEM	MFC	rbST	MFC×rbST
Body weight	Early	358.00	380.00	373.00	376.00	7.30	NS	NS	NS
(kg)	Mid	383.00	379.00	383.00	408.00	4.40	NS	NS	NS
	Late	396.00	395.00	426.00	422.00	4.40	NS	NS	NS
DMI	Early	6.14	7.05	7.22	8.49	0.31	*	**	NS
(kg/day)	Mid	6.18	7.49	8.75	10.00	0.45	*	*	NS
	Late	7.57	7.88	8.26	9.32	0.15	NS	**	*
Milk yield	Early	9.99	11.82	11.51	13.55	0.28	NS	**	NS
(kg/day)	Mid	9.42	10.03	11.37	13.05	0.33	NS	**	NS
	Late	8.02	9.63	9.69	12.17	0.43	NS	**	NS
4% FCM	Early	8.62	10.21	10.49	13.51	0.65	NS	**	NS
(kg/d)	Mid	9.05	10.76	11.08	14.72	0.77	NS	*	NS
	Late	8.63	11.69	9.67	13.75	1.33	NS	**	NS
Milk fat	Early	3.20	3.78	3.63	4.51	0.33	NS	**	NS
(g%)	Mid	3.68	4.34	4.02	4.68	0.43	NS	*	NS
	Late	4.39	4.70	4.13	4.99	0.64	NS	NS	NS
Lactose	Early	4.62	4.34	4.82	4.79	0.10	NS	NS	NS
(g%)	Mid	4.27	4.47	4.79	4.84	0.12	NS	NS	NS
	Late	4.29	4.01	4.58	4.68	0.11	NS	NS	NS
Milk protein	Early	3.17	3.61	3.48	3.63	0.13	NS	NS	NS
(g%)	Mid	3.79	3.84	4.09	4.26	0.15	NS	NS	NS
	Late	4.25	4.02	4.30	4.32	0.17	NS	NS	NS

[1]Pre = Pre-treatment of rbST, rbST = Treatment of rbST; [2]Effect; MFC = Misty-Fan Cooling effect, rbST = rbST effect, MFC×rbST = Interaction effect of MFC and rbST; [3]Significant; NS = p>0.05, *p<0.05 and **p<0.01

Table 2: Effects of rbST administration on plasma metabolites of cross-bred Holstein cows housed under MFC and NS barns

Parameters	Lactation periods	NS		MFC			Effect[2, 3]		
		Pre-treated[1]	rbST[1]	Pre-treated	rbST	SEM	MFC	rbST	MFC×rbST
Acetate	Early	3.67	3.05	2.35	2.61	0.318	NS	NS	NS
(mg/dL)	Mid	2.60	2.92	3.59	3.06	0.296	NS	NS	NS
	Late	4.42	3.93	3.28	2.47	0.457	**	NS	NS
B-OH-butyrate	Early	11.90	12.09	8.48	8.95	0.886	NS	NS	NS
(mg/dL)	Mid	10.53	11.40	11.68	9.69	0.743	NS	NS	NS
	Late	12.25	13.06	9.94	9.75	1.568	NS	NS	NS
Triglyceride	Early	11.88	13.41	14.55	15.06	1.290	NS	NS	NS
(mg/dL)	Mid	15.61	17.20	13.60	15.14	1.793	NS	NS	NS
	Late	14.85	15.71	24.00	18.00	3.607	NS	NS	NS
FFA	Early	3.05	3.70	2.35	2.61	0.318	NS	NS	NS
(mg/dL)	Mid	3.14	4.63	3.32	3.91	0.351	NS	*	NS
	Late	2.42	3.63	3.01	4.66	0.409	NS	**	NS
Albumin	Early	4.27	4.22	4.18	4.21	0.058	NS	NS	NS
(g/dL)	Mid	4.18	4.08	4.14	4.20	0.071	NS	NS	NS
	Late	4.27	4.31	4.18	4.19	0.063	NS	NS	NS
Glucose	Early	59.02	60.88	68.72	62.22	4.767	NS	NS	NS
(mg/dL)	Mid	62.26	61.12	63.27	66.07	1.831	NS	NS	NS
	Late	69.55	61.12	65.88	66.13	3.098	NS	NS	NS

[1]Pre = Pre-treatment of rbST, rbST = Treatment of rbST; [2]Effect; MFC = Misty-Fan Cooling effect, rbST = rbST effect, MFC×rbST = Interaction effect of MFC and rbST; [3]Significant; NS = p>0.05, *p<0.05 and **p<0.01

FCM were apparent after administrations of rbST and returned to the control level within the next 3-4 weeks at each stage of lactation in either group (Fig. 1). A significant increases in milk yield by averaged 15.7% for the effect of rbST at early and mid stage of lactation of both groups while a significant increase in milk yield by averaged 22.9% for the effect of rbST at late stage of lactation. No significant changes in milk compositions in terms of percentages of protein and lactose were apparent. The concentration of milk fat significantly increased during rbST administrations particularly in early and mid stages of lactation in both groups but there was no statistically significant in the late stage of lactation.

The concentration of plasma metabolites: The results concerning changes in the concentrations of plasma metabolites are shown in Table 2. The mean arterial plasma concentrations for acetate of cooled cows at late stage of lactation were significantly lower than those of non-cooled cows. Plasma concentrations of β-hydroxybutyrate, glucose, triglyceride and albumin were not significantly affected by rbST administrations at different stages of lactation in both groups. The effect of rbST administrations significantly increased the concentrations of plasma FFA at mid and late stages of lactation as compared to pre-treated periods but it showed no significant increases in early stage of lactation in both groups.

Energy balance: The results in Table 3 show the studies of energy balance during rbST administration in all stages of lactation in both groups of cross-bred Holstein cows. Cooled cows housing in MFC barn significantly increased ($p<0.05$) in NE_l especially in early and mid lactation as compared to those of cows housing in NS barn and more significant increases after rbST administrations at different stages of lactation in both groups. However, the interaction effect between MFC and rbST treatment was significantly apparent ($p<0.05$) for an increment in NE_l in late stage of lactation. The NE_l increased in cooled cows at different stages of lactation especially the significant increase in early lactation ($p<0.05$) were apparent as compared to those of non-cooled cows. After administrations of rbST at different stages of lactation, the NE_l significantly increased ($p<0.05$) in both groups. Positive values of net energy balance were apparent in cooled cows and more significant increases during mid stage of lactation. Administration of rbST in crossbred cows showed no alterations of values of net energy balance in all stages of lactation in both groups. Cooling cows showed no statistical changes in the efficiency of feed utilization in all stages of lactation. Administration of rbST significantly increased the efficiency of feed utilization ($p<0.05$) at late stage of lactation of both groups.

Concentrations of plasma leptin, IGF-1, insulin, T4 and cortisol: The results in Table 4 show that no significant

Table 3: Effects of rbST administration on energy balance of cross-bred Holstein cows housed under MFC and NS barns

		NS		MFC			Effect[2,3]		
Parameters	Lactation periods	Pre-treated[1]	rbST[1]	Pre-treated	rbST	SEM	MFC	rbST	MFC×rbST
NE-intake	Early	16.40	18.81	19.27	22.66	0.830	*	**	NS
(Mcal/day)	Mid	16.50	20.00	23.28	26.71	1.200	*	*	NS
	Late	20.22	21.04	22.05	24.89	0.400	NS	**	*
NE-maintenance	Early	7.89	8.26	8.15	8.19	0.120	NS	NS	NS
(Mcal/day)	Mid	8.31	8.42	8.31	8.70	0.070	NS	NS	NS
Late	8.52	8.51	8.99	8.93	0.07	NS	NS	NS	
NE-lactation	Early	3.22	3.76	4.02	5.52	0.370	*	*	NS
(Mcal/day)	Mid	3.65	4.57	4.46	6.16	0.410	NS	*	NS
	Late	3.76	5.23	4.01	5.92	0.380	NS	*	NS
Energy balance	Early	5.29	6.79	7.10	8.95	0.960	NS	NS	NS
(Mcal)	Mid	4.54	7.20	10.50	11.85	1.350	*	NS	NS
	Late	7.93	7.30	9.06	10.04	0.650	NS	NS	NS
Feed efficiency	Early	1.44	1.46	1.46	1.60	0.113	NS	NS	NS
(kg milk/kg feed)	Mid	1.45	1.51	1.28	1.45	0.149	NS	NS	NS
	Late	1.14	1.49	1.18	1.46	0.127	NS	*	NS

[1]Pre-treated = Pre-treatment of rbST, rbST = Treatment of rbST; [2]Effect; MFC = Misty-Fan Cooling effect, rbST = rbST effect, MFC×rbST = Interaction effect of MFC and rbST; [3]Significant; NS = $p>0.05$, *$p<0.05$ and **$p<0.01$

Table 4: Effects Effects of rbST administration on plasma concentrations of leptin, IGF-1 insulin, T4 and cortisol of crossbred Holstein cows housed under MFC and NS barns

		NS		MFC			Effect[2,3]		
Parameters	Lactation periods	Pre-treated[1]	rbST[1]	Pre-treated	rbST	SEM	MFC	rbST	MFC×rbST
Leptin	Early	2.59	2.38	2.26	2.05	0.080	NS	*	NS
(ng/mL)	Mid	2.53	2.17	2.05	1.88	0.050	*	***	NS
	Late	2.48	2.20	2.03	1.84	0.080	**	**	NS
IGF-1	Early	118.20	196.50	87.40	114.80	18.76	NS	*	NS
(ng/mL)	Mid	115.60	183.20	112.20	218.00	32.72	NS	*	NS
	Late	128.20	350.90	124.10	220.40	41.53	NS	**	NS
INSulin	Early	0.72	0.92	0.91	1.38	0.360	NS	NS	NS
(ng/mL)	Mid	1.19	1.01	0.97	1.23	0.270	NS	NS	NS
	Late	0.75	1.24	1.76	1.21	0.290	NS	NS	NS
T4	Early	8.08	7.26	10.64	8.52	0.510	NS	NS	NS
(μg%)	Mid	9.96	10.82	13.87	13.04	1.900	NS	NS	NS
	Late	7.95	7.99	10.89	12.15	2.040	NS	NS	NS
Cortisol	Early	2.15	2.24	1.87	1.74	0.650	NS	NS	NS
(μg%)	Mid	3.20	1.37	2.56	2.01	0.730	NS	NS	NS
	Late	1.54	2.22	2.14	1.63	0.680	NS	NS	NS

[1]Pre-treated = Pre-treatment of rbST, rbST = Treatment of rbST; [2]Effect; MFC = Misty-Fan Cooling effect, rbST = rbST effect, MFC×rbST = Interaction effect of MFC and rbST; [3]Significant; NS = $p>0.05$, *$p<0.05$, **$p<0.01$ and ***$p<0.001$

changes in the concentrations of plasma insulin, cortisol and T4 were apparent after administrations of rbST at different stages of lactation in both groups. At the pre-treated period of each stage of lactation, there were no differences in the concentrations of plasma IGF-1 between cooled cows and non-cooled cows while the marked increases in the concentrations of plasma IGF-1 were apparent after administrations of rbST at different stages of lactation in both groups. The declines in the concentrations of plasma leptin of cooled cows were significantly apparent in mid and late stages of lactation but were not significantly apparent in early lactation. The significant reductions of the concentrations of plasma leptin were more pronounced after administrations of rbST at different stages of lactation in either group.

DISCUSSION

The present study shows that environmental modifications using a fan and high-pressure mist cooling system were effective in decreasing the air temperature in a free stall barn by average 3.5°C. It can reduce both dry bulb ambient temperature and Temperature Humidity Index (THI) especially in the afternoon as compared to that of normal shade barn throughout the experimental periods, although relative humidity measured in MFC barn were relative high. In the present study, MFC barn was not sufficient to completely eliminate the level of heat stress in cows because the minimum THI measured during daytime under misters and fans also rose to values that were higher than the threshold level of comfortable zone, 72 for THI (Armstrong, 1994). Crossbred HF in both groups would be subjected to moderate heat stress (Fuquay, 1981). However, cows under MFC system especially in the afternoon of the warmest part of the day could be partially mitigate heat load which lower both respiratory rate and rectal temperature in comparison with those of non-cooled cows. These changes were consistent with the findings of previous studies on dairy cows which also reported partial alleviation of heat stress under several methods of evaporative cooling system (Armstrong *et al*., 1993; Chaiyabutr *et al*., 2008; Fike *et al*., 2002). However, THI might not accurately reflect of heat stress in crossbred lactating cows under MFC cooling system that deliver a pressurized spray with considerable fan air movement in the barn, resulting both high humidity and a cooling effect.

The effect of rbST administrations not only increased in milk yield of either cooled or non-cooled cows but also increased in both the respiratory rate and rectal temperature. These findings for the consequent effect of rbST administrations in dairy cattle have been documented (Sullivan *et al*., 1992; Tarazon *et al*., 1999). It indicates that the role rbST would provide for both calorigenic and galactopoietic effects. An increase in body temperature in rbST-treated cows may be independent of a lactational effect (Cole and Hansen, 1993; Elvinger *et al*., 1992; West *et al*., 1990). Stages of lactation of cows in different environmental conditions had no influence on the rbST effect. However, increased heat production associated with increased milk yield by administration of rbST was less pronounced by a cause of heat stress in the present study, since during administration of rbST, cows could also increase heat dissipation in reliance upon evaporative cooling (respiration and sweating) (Johnson *et al*., 1991; West, 1994). The present study provide evidence that cooled cows in MFC barn showed higher milk yield than those of non-cooled cows in either pre-treatment or rbST treatment period. The proportion of the reduction in milk yield as the lactation progress to the late stage of lactation of cooled cows was still smaller than that of non-cooled cows. However, rbST effect on milk yield in late stage of lactation was higher than those of early and mid lactation in either group. It is probably that the effect of rbST can maintain mammary parenchymal tissue during the involution period of the late stage of lactation (Baldi *et al*., 2002). In the present study, body weights were not significant different between cooled and non-cooled cows. The body weights of cows in both groups increased as lactation progress which would be attributed to the growing effect of primiparous cows used in this experiment.

It is known that the role of the endocrine system is a complex process in regulating milk production during changes in environmental temperature in ruminant. The present studies show that the plasma concentrations of cortisol and T4 of cows housing in MFC were not different from those of cows in NS barns at all stages of lactation, even though circulating levels of both cortisol and T4 have been used as indicators warning signals of environmental heat stress (Pusta *et al*., 2003) and the plasma T4 level has been shown to be inversely related to environmental temperature (Mohammed and Johnson, 1985). The present results differed from our previous studies in crossbred HF cows housing in the close-sided barn under an evaporative cooling system in which both plasma T4 and cortisol levels of cooled cows were lower than those of non-cooled cows housing in open-sided barn in all stages of lactation (Chaiyabutr *et al*., 2008). It indicates that changes of the activities of both adrenal gland and thyroid gland are homeorhetic regulators involving in acclimatory responses to changes of environmental temperature in either the degree or duration of heat exposure. An elevation of body temperature during administration of rbST especially in non-cooled cow was probably not enough to affect to the thermoregulartory capabilities and capacity of cows to reach a new condition of climatic stress. An increased heat production during somatotropin treatment would be within the range that could be dissipated by the cows (Manalu *et al*., 1991). In addition, it is probable that crossbred HF cows in both groups have been acclimatized to tropical environmental temperatures already which were able to restore thermal balance throughout periods of studies, resulting in unchanged plasma levels of T4 and cortisol. Therefore, both T4 and cortisol levels might not play a role in the regulation of milk yield and food intake during rbST administration in cows housing under either MFC or NS barn, although injections of T4 are well known to be galactopoietic in cows that have established lactation.

The present results showed marked increases in the plasma levels of IGF-1 after rbST administration at different stages of lactation of both cooled and non-cooled cows. The direct effect of exogenous rbST on increase in milk yield and high plasma IGF-1 level was similar to the previous report in lactating crossbred Holstein cattle by Chaiyabutr *et al*. (2005). The synthesis and release of IGF-1 is mainly by the liver which is dependent on the availability to the liver of bST, insulin and some nutritional factors (Clemmons and Underwood, 1991; Luo and Murphy, 1991). The low circulating insulin concentration and the negative energy balance have demonstrated to be a key negative regulatory of hepatic IGF-1 production causing an uncoupling of the plasma IGF-1/bST axis (Ketelslegers *et al*., 1995; Weller *et al*., 1994). The present results showed no changes in the level of plasma insulin and positive energy balance during administrations of rbST in all stages of lactation in both groups. Cows in both groups were fed in a similar diet throughout lactation. Thus, it is possible from the present study that a marked increase in IGF-1 secretion would be dependent on the availability to the liver of both exogenous rbST and energy status especially positive energy balance for hepatic IGF-1 production. A remarkable feature of the responses to administration of rbST was the elevation of the plasma IGF-1 concentrations after the end of the 3rd rbST injection in each stage of lactation which has returned to the pretreatment values within the next 4 weeks. These results suggest that a mediator of the high IGF-1 level would be involved. Therefore, the galactopoietics action of exogenous rbST on an increase in milk yield would be partly mediated by the rise in circulating IGF-1. The effect of the high level of plasma IGF-1 has been shown to associate with an increase in mammary blood flow in supplying nutrient for milk synthesis as shown by the experiments in both cow and goat (Chaiyabutr *et al*., 2005; Prosser *et al*., 1990, 1994). However, at the pre-treated period of the present experiment, there were no differences in the plasma levels of IGF-1 between cooled and non-cooled cows which indicate that the effect of cooling on increased DMI and milk yield was not involved on the action of IGF-1. The mechanism of action of rbST on increases in milk yield and DMI would be mediated by other mediators other than IGF-1.

In the present results, the decreases in the plasma leptin levels were apparent during administrations of rbST at different stages of lactation in both cooled and non-cooled cows. In general, leptin production and secretion by adipocytes has been well-known to be under complex regulation. Leptin plays a role not only regulate feed intake by acting on the hypothalamus to diminish appetite but also increase energy expenditure in both ruminants and monogastric animals (Chelikani *et al*., 2004; Inoue *et al*., 2005). Therefore, an increase in food intake and subsequent increase in milk yield by the effect of exogenouse rbST would be partly due to the low leptin secretion. In the present study, plasma leptin levels were lower in cooled cows than those of non-cooled cows housing in NS barn. These results supported the findings that the secretion of leptin from adipose tissue were decreased during acute cold exposure (Ricci *et al*., 2000) and increased in high environmental temperature (Accorsi *et al*., 2005). The low plasma leptin level of cooled cows housing under MFC barn could be a factor to promote DMI throughout experimental studies. A decrease in plasma leptin concentration in cooled cows did not involve to the levels of plasma insulin and plasma glucose concentrations which remained unchanged throughout the studies. Although, the mechanism of depressed leptin secretion would be partly due to the depressed insulin action which has been demonstrated in sheep exposed to the cold environment (Asakuma *et al*., 2003). The action of insulin to stimulate leptin secretion from adipose tissue has been reported in both vivo and *in vitro* in rats and humans (Boden *et al*., 1997; Cheng *et al*., 2000). However, the role of leptin relating to insulin action is still unclear since ruminants are less sensitive to insulin as compared with those of mono-gastric animals (Sasaki and Takahashi, 1983). The present studies show that rbST decreased plasma leptin to a greater extent in all stages of lactation and that insulin was unlikely to play a role. The low plasma leptin levels could be ascribed partly by the action of exogenous rbST on lipolysis (Goodman and Grichting, 1983) which would reduce the secretion of leptin from adipose tissue.

It is known that energy for milk production is derived from feed intake and body tissue reserves. The calculated positive energy balance during administrations of rbST at different stages of lactation in both cooled and non-cooled cows indicate an adequate energy intake occurred for milk production in crossbred Holstein cattle. An increase in net energy intake in cooled cows accompanying with administration of rbST would be attributed to an increase in DMI in respond to the low plasma leptin level. Cooled cows with rbST administration did not improve both plasma albumin and total plasma protein concentrations, although responses in voluntary intake have been observed after some weeks of rbST administration. In the present study, mean milk protein concentrations remained constant during rbST administration which might be a further evidence of no energy deficit (Heinonen *et al*., 1988), although lower milk protein percentage has been reported in the cows administered with bST (West *et al*., 1990).

In the present results, no alterations in the plasma triglyceride concentrations were apparent after administrations of rbST in both groups while increases in the plasma FFA concentrations were noted. These results are in agreement with previous reports in cows treated with bST (Lough *et al*., 1988). The metabolic effect of bST is known to have direct action on adipose tissue to promote lipolysis with antagonistic to insulin action (Goodman and Gricting, 1983). Thus, an increase in lipolytic sensitivity with increase in the plasma FFA concentrations in rbST-treated cows in the present study would be a function of rbST treatment per se without involvement in either negative energy balance or the plasma insulin level. The magnitude of the effect of mobilization of depot fat after administration of rbST was probably too small to compromise the weight gain and body condition of primiparous crossbred cows throughout lactation. An increase in plasma FFA concentration after rbST administration may indicate that nutrition partitioning was mobilized from adipose tissue to supply as energy and substrate for milk fat synthesis. Nevertheless, an increase in voluntary intake in cooled cows and rbST treated cows clearly represent a coordinated response of voluntary intake to rbST action and the effect of cooling. The data in the present results showed no significant alterations of the plasma concentrations of triglycerides and other plasma metabolites, i.e., β-hydroxybutyrate and glucose, reflected no negative energy balance resulting from the higher milk production.

CONCLUSION

The compelling studies with exogenous administration of rbST on milk production in crossbred HF cows housing either in NS or MFC barns at different stages of lactation demonstrate that the role of rbST alone was not sufficient to contribute directly to increase milk yield and DMI. A combination of administration of rbST in cows kept under conditions of low environmental temperature with mister and fan showed greater milk yields and DMI than those of non-cooled cows with administration of rbST. These changes are likely demanded the involvement of endocrine mediators to explain the effect of administration of rbST on increases in milk yield and DMI. Among of several hormonal interactions, IGF-1 and leptin were the prominent mediators of rbST in stimulating galactopoiesis. It was shown that IGF-1 and leptin mediated the interactions between lactational performance of crossbred HF cows and housing in different environmental temperature but were regulated differently. A significant response to rbST with the high concentrations of plasma IGF-1 would be an endocrine mediator of rbST in stimulating milk production in both cooled and non-cooled cows. The appearances of low plasma leptin levels were occurred via the effect of cooling management and more responses to the lipolytic effect of rbST administration in either group. Changes in low plasma leptin levels subsequently increased DMI in the supply of nutrients to the mammary gland for facilitating milk synthesis.

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