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Effects of Supplemental Recombinant Bovine Somatotropin (rbST) and Cooling with Misters and Fans on Renal Function in Relation to Regulation of Body Fluids in Different Stages of Lactation in Crossbred Holstein Cattle

D. Boonsanit, S. Chanpongsang[1] and N. Chaiyabutr*

Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand

ABSTRACT: The aim of this study was to investigate the effect of supplemental recombinant bovine somatotropin (rbST) and cooling with misters and fans on renal function in relation to regulation of body fluids in different stages of lactation in crossbred Holstein cattle. Ten, 87.5% crossbred Holstein cattle were divided into two groups of 5 animals each, housing in a normal shaded barn (NS) and in a shaded barn with a mister-fans cooling system (MF). The experiment in each group was divided into 3 phases, early- (Day 75 postpartum), mid- (Day 135 postpartum), and late stage of lactation (Day 195 postpartum). The pre-treatment study was conducted on the starting day of each stage of lactation and the treatment study was performed after the end of the pre-treatment, during which the animal was injected with 500 mg of rbST (POSILAC) every 14 days for three times. During the study, ambient temperature at the hottest period daily in the MF barn was significantly lower, while relative humidity was higher than that of the NS barn. The temperature humidity index (THI) in both barns ranged from 79-85 throughout the periods of study. Cows in the MF barn showed a lower rectal temperature and respiration rate as compared with cows in the NS barn. The effect of rbST administration increased both rectal temperature and respiration rates of cows housed in either the NS or MF barn. Milk yield significantly increased in cows treated with rbST in all stages of lactation. Increases in mammary blood flow, accompanied by increases of total body water (TBW), extracellular fluid (ECF), blood volume (BV) and plasma volume (PV), were observed in both groups of cows receiving rbST in all stages of lactation. No alterations of renal blood flow and glomerular filtration rate were observed in cows receiving rbST, but decreases in urinary excretion and fractional excretion of sodium, potassium and chloride ions appeared to correlate with reduction in the rate of urine flow and osmolar clearance during rbST administration. These results suggest that the effect of rbST supplementation to cows housed either in NS or MF barns on body fluid volume expansion is attributable to changes in the rate of electrolyte excretion by the kidney. The increased availability of renal tubular reabsorption of sodium, potassium and chloride ions during rbST treatment was a major factor in retaining body water through its colligative properties in exerting formation of an osmotic force mechanism.
(**Key Words :** Renal Function**,** rbST, Mister-fans Cooling, Body Fluids, Crossbred Holstein Cattle)

INTRODUCTION

The low milk production of both exotic and crossbred cattle is still the main problem in dairy farming in the tropics. The regulation of milk secretion in different types of crossbred cattle has been shown to be inherited and is thought to be among the causes of differences in bodily functions. The lower efficiency of water retention and poor adaptation in a tropical environment have been reported in 87.5% HF animals in comparison with 50% HF (Chaiyabutr et al., 1997; 2000). There is a rapid reduction of milk yield as lactation advances to mid- and late-lactation in 87.5% HF animals. The reduction of milk yield is attributed to a decrease in mammary blood flow (MBF) coinciding with the decline of plasma bovine somatotropin (bST) concentration. These changes would account for the short lactation persistency (Chaiyabutr et al., 2000). In addition to animal genetics, other factors may affect milk production in dairy cattle in the tropics, such as high environmental temperature. Animals in high ambient temperatures will suffer excessive heat load and impairment of physiological function, including body fluids (Hahn et al., 1999).

Many technologies are required to improve milk

* Corresponding Author: N. Chaiyabutr.
E-mail: Narongsak.C@Chula.ac.th
[1] Department of Animal Husbandry, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand.
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production of dairy cattle in the tropics. Environmental modification is the most common approach to increase milk production with alleviation of severe heat stress in dairy cattle, for example, with fans and sprinklers (Fike et al., 2002) or an evaporative cooling system (Chan et al., 1997; Chaiyabutr et al., 2008). Other technologies can increase milk production in dairy cattle in hot weather, for example, by the application of exogenous bovine somatotropin (West, 1994). There are inconsistent results as regards the relationship between effects of high environmental temperature and the application of exogenous bST on milk production. Some reports showed that bST-treatment of lactating cows could increase heat production (Tyrrell et al., 1988; Elvinger et al., 1992; Cole and Hansen, 1993) and caused a decrease in milk production during thermal imbalance. Other studies showed that dairy cows treated with exogenous bST increased milk production in both a normal thermal zone and a hot environment (Staples et al., 1988; Johnson et al., 1991). The increase in heat production in bST-treated cows in high temperature may not be great enough to alter the cow's ability to maintain homeothermy; since it has been reported that exogenous rbST in crossbred dairy cows increased milk yield accompanying by increases in total body water (TBW) and extra-cellular fluid(ECF) (Maksiri et al., 2005; Chaiyabutr et al., 2007). These observations suggest that higher total body water may be useful in slowing down the elevation in body temperature in hot conditions through evaporative cooling during heat dissipation. These parameters are considered to be factors that may be involved in shorter persistency of lactation if animals cannot maintain their body fluids.

The kidneys are known to be an important organ in regulating both volume and composition of body fluids. The regulation of fluid volume and body composition depends on the coordinated action of multiple mechanisms of various hormones in regulating water intake and excretion. There are no published data concerning the effects of bST on controlling kidney function in regulation of body fluids in dairy cows, although it was previously reported that administration of growth hormone resulted in increases in ECF and total body water in crossbred dairy cattle (Chaiyabutr et al., 2007) and volume expansion with sodium retention in humans with severe growth hormone deficiency (Johannsson et al., 2002). The present study therefore aimed to investigate the control mechanism for body fluid expansion after rbST supplementation in crossbred dairy cows; the study focused exclusively on renal events during rbST supplementation under high ambient temperature with or without mister and fan cooling in different periods of lactation. This information may contribute to better insight into the physiological basis for the regulation of milk secretion under high temperature and supplemental rbST.

MATERIALS AND METHODS

Animal management

Ten, first lactation, non-pregnant, 87.5% crossbred Holstein cattle were randomly selected and divided into two groups of five animals each. Animals in both groups were housed in an open-sided barn with a tiled-roof. Animals in group 1 were housed in a normal shaded barn (NS) and animals in group 2 were housed in a shaded barn with cooling by misters and fans (MF). The barn (16 m long×7 m wide×3.5 m high) was separated into two parts. The first part (8 m long×7 m wide×3.5 m high) was arranged for animals in normal shade and the second part of the barn was equipped with two mister and fan systems for cooling the 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 mister spray heads (mounted relative to the fan) was 7.5 L/h and the size of mist droplet was 0.01 mm. Animals were exposed to MFC for 45 minutes at 15-minute intervals from 06:00 h to 18:00 h. At night, animals were exposed to MFC for 15 minutes at 45-minute intervals from 18:00 h to 06:00 h. All animals were fed with a total mixed ration (TMR) throughout the experiments. Samples of TMR were analyzed for dry matter (DM) and chemical composition (Table 1). Individual feed intake was recorded daily and dry matter intake (DMI) determined. Samples of TMR were analyzed for crude protein and ash using procedures described by AOAC (1990). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed according to Van Soest et al. (1991).

The ambient temperature was recorded by a dry bulb thermometer. The relative humidity was calculated from the

Table 1. Feed ingredients and chemical composition of the TMR diet

Ingredients	Kg
Pine apple waste	50
Soybean meal	23
Rice bran	3.0
Cotton seed	20
Lime stone	1.4
Di-calcium phosphate	1.4
Sodium bicarbonate	0.3
Potassium chloride	0.1
Mineral and vitamin premix	0.8
Total	100
Chemical composition	
Dry matter (DM)	39.1
Organic matter (% DM)	92.7
Crude protein (% DM)	18.0
Acid detergent fiber (% DM)	20.1
Neutral detergent fiber (% DM)	33.9

reading of a dry and wet bulb thermometer. Ambient temperature and humidity were measured once weekly during the period of hottest daily temperature (13.00 to 14.00 h). The temperature humidity index (THI) was calculated according to West (1994) where: THI = td-(0.55-0.55RH) (td-58) with td = dry bulb temperature (°F), and RH = relative humidity. Animals were normally milked at around 0600 h and 1700 h using a milking machine and milk production was recorded daily. Cows were weighed before treatment and at weekly intervals during treatment.

Experimental procedures

The experiment in each group was divided into 3 phases, namely early- (Day 75 postpartum), mid- (Day 135 postpartum), and late-lactating periods (Day 195 postpartum). The pre-treatment study was conducted on the starting day of each phase. At the end of the pre-treatment, within the same day, the animal was injected with the first subcutaneous dose of 500 mg of recombinant bovine somatotropin (rbST) (POSILAC, Monsanto, USA). Subsequently, the animal was injected with two consecutive doses of rbST every 2 weeks. Thereafter, within 2 days after the third injection, the treatment study was conducted. The pre-treatment, 3 doses of rbST, and the treatment periods were performed during the first 30 days and the same procedures were followed for each phase. During the last 30 days of each phase, no experiments were conducted in order to allow the milk yield from the effect of rbST treatment to return to the control level (Kirchgessner et al., 1991).

On each specified day of both pre-treatment and treatment period, measurements of mammary blood flow, renal function and total body fluids were performed. The measurement of mammary blood flow (MBF) was performed in the morning (9.00-10.00 h). Two polymer catheters (i.d. 1.0 mm, o.d. 1.3 mm, L 45 mm; Jelco, Critikon; Johnson & Johnson, UK) were inserted into either the left or right milk vein under local anesthesia for determination of MBF as previously described (Chaiyabutr et al., 2007).

After determination of MBF, the study of renal function and body fluids were performed subsequently. A catheter for isotope injection, dye injection and para-aminohippurate (PAH) solution infusion was inserted into an ear vein, under local anesthesia. The catheter was flushed with heparinized, normal saline (heparin 25 i.u./ml normal saline) and was left in place during the experiment. The bladder was fitted with an indwelling catheter (Foley catheter, no 22) for urine collection. The free end of the balloon catheter was introduced into the bladder and secured with the inflated retaining cuff (60 ml. bulb capacity) during urine collection.

The procedures used in the present study were performed in accordance with the principles and guidelines of the Faculty of Veterinary Science, Chulalongkorn University. These guidelines were formulated to comply with international standards and are in accordance with the principles and guidelines of the National Research Council of Thailand.

Determinations of renal hemodynamics and excretion of electrolytes

Measurement of renal hemodynamics was started by injection of 20 ml priming dose solution (2.5% PAH solution) via the ear vein and followed immediately by a sustaining infusion of 0.5% PAH in normal saline at the rate of 2 ml/min. The solution was infused at a constant rate throughout the experimental study using a peristaltic pump (Eyela, MP-3, Tokyo Rikakikai, Japan). After a 2 h equilibration period of infusion, the experiments were carried out on duplicate urine samples collected over an accurately timed period about 15-20 min. To ensure each collection was accurate, the urine sampling was started after the bladder was voided. Coccygeal blood sampling was performed at the midpoint of urine collection. Plasma and urine samples were kept at -20°C for determinations of endogenous creatinine, PAH, electrolytes and osmolarity.

The clearance (C) of endogenous creatinine and PAH were used to measure GFR and ERPF, respectively, based on the Fick Principle as previously described by Chaiyabutr et al. (1992). The effective renal plasma flow (ERPF) was measured by PAH clearances using standard techniques (Smith, 1962). Renal blood flow (RBF) was obtained by dividing ERPF by 1-packed cell volume. Filtration fraction (FF) was obtained by dividing GFR by ERPF. Plasma and urine samples were analyzed for concentrations of sodium and potassium ions by flame photometer (Flame photometer 410C, Ciba Corning Inc., USA), chloride ion by Chloridometer (Chloride analyzer 925, Ciba Corning Inc., USA) and osmolality by osmometer (Osmometer 3D3, Advance Instrument Inc., USA). Fractional excretion of electrolyte (% FE) was obtained by dividing clearance of electrolyte by GFR. Tubular solute-free water clearance (C_{H2O}) was calculated by subtraction of the rate of urine flow (V) from osmolar clearance (C_{osm}).

Determinations of water intake, total body water, extracellular fluid, plasma volume and blood volume

Estimation of the rate of water intake of each animal in each experimental period was recorded as an average over three days from weighing daily water consumption by water meter. On each specified day, in the afternoon (13.00-14.00 h), the measurements of total body water (TBW), extracellular fluid (ECF) and plasma volume (PV) were performed. The injections of 1 ml of tritiated water (2,500 μci per animal), 20 ml of sodium thiocyanate solution (10 g/100 ml normal saline) and 20 ml of Evans blue dye (T-1824) (0.5 g/100 ml normal saline) were performed via an ear vein catheter for estimation of TBW, ECF and PV, respectively. After dye injection, blood samples from the jugular vein were taken at 20, 30, 40, 50 and 60 min for ECF and PV determinations. Plasma samples were collected at 4, 8, 20, 26, 32, 44, 50, 56, 68 and 72 h subsequent to the injection for determination of TBW.

Total body water (TBW) was determined in each animal by dilution techniques using tritiated water as previously described (Chaiyabutr et al., 1997). TBW = (standard count (dis/min)×dose (ml))/(radio activity counts at zero time (dis/min)).

The concentration of sodium thiocyanate in plasma was performed by the method of Medway and Kare (1959) for estimation of ECF volume. Blood volume was calculated from the plasma volume and packed cell volume (Chaiyabutr et al., 1980).

Determination of mammary blood flow

Blood flow through half of the udder for MBF was determined by measuring the dilution of dye T-1824 (Evan blue) given by short term continuous infusion as previously described (Chaiyabutr et al., 1997).

Statistical analysis

Data for milk yield, DMI and water intake in each lactating period were adjusted for covariate effects using mean values at 14 d before the pretreatment study. The stages of lactation (early, mid and late) were analyzed separately. The statistical analyses were performed using the general linear models procedure of statistical software package SPSS (SPSS for windows, V13.0; SPSS Inc., Chicago, IL, USA). The model used for each analysis 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} = observation, μ = overall mean, A_l = animal effect, H_i = house effect as main plot (i = NS, MF), $A(H)_{il}$ = main plot error (animal l in house i), B_j = treatment effect (rbST) as a split plot (j = with and without rbST administration), $(HB)_{ij}$ = interaction effect between treatment and house , $A(HB)_{ijl}$ = split plot error (animal l in house i and treatment j), Cov_k = covariate effect and e_{ijk} = residual error.

RESULTS

Ambient temperature, relative humidity, temperature humidity index, rectal temperature and respiration rate

The mean values of ambient temperature in NS and MF barns, respiratory rate and rectal temperature of animals are shown in Table 2. At the period of hottest daily temperature (13.00 to 14.00 h), ambient temperature and temperature humidity index (THI) of the MF barn were significantly lower (p<0.05), while relative humidity in the MF barn was significantly higher than in the NS barn throughout the periods of study. The mean values of both respiratory rate and the rectal temperature of cows under MF were significantly lower than under NS with or without treatment

Table 2. Ambient temperature, relative humidity, temperature humidity index, rectal temperature and respiration rate in animals treated with rbST under normal shade (NS) and cooling with misters and fans (MF) at different stages of lactation

	Lactating period	Treatments				SEM	Effects[1]		
		NS	NS+rbST	MF	MF+rbST		MF	rbST	MF×rbST
Ambient temperature (°C)	Early	34.6	35.3	31.6	29.8	0.95	0.001	0.578	0.224
	Mid	33.4	33.1	30.7	30.0	0.61	0.004	0.439	0.753
	Late	32.5	32.3	27.8	30.1	0.81	0.001	0.229	0.16
Relative humidity (%)	Early	56.0	51.0	60.2	75.5	3.31	0.006	0.159	0.015
	Mid	57.6	60.0	71.5	75.4	4.48	0.017	0.503	0.871
	Late	60.0	61.8	79.2	64.4	4.35	0.054	0.173	0.093
Temperature humidity index (THI)	Early	85.5	85.3	82.2	81.8	1.13	0.002	0.816	0.924
	Mid	83.9	84.1	82.7	82.1	0.75	0.043	0.778	0.671
	Late	83.3	83.2	79.2	80.6	0.72	0.001	0.369	0.354
Rectal temperature (°C)	Early	38.8	39.0	38.0	38.2	0.07	0.001	0.023	0.886
	Mid	39.4	39.9	38.6	38.9	0.13	0.005	0.011	0.245
	Late	39.1	39.3	38.5	38.8	0.10	0.057	0.023	0.565
Respiration rate (breath/min)	Early	72.0	78.0	54.0	63.2	2.52	0.001	0.017	0.544
	Mid	70.4	73.4	52.8	55.0	1.22	0.001	0.065	0.751
	Late	71.6	78.4	52.2	57.6	1.16	0.001	0.004	0.657

SEM = Standard error of the mean.

[1] p-values for the effects; MF = Mister-fan cooling effect, rbST = rbST effect, MF×rbST = Interaction effect of MF and rbST.

Table 3. Dietary dry matter intake (DMI), water intake, milk yield and mammary blood flow (MBF) in animals treated with rbST under normal shade (NS) and cooling with misters and fans (MF) at different stages of lactation

	Lactating period	Treatments				SEM	Effects[1]		
		NS	NS+rbST	MF	MF+rbST		MF	rbST	MF×rbST
DMI (kg/d)	Early	6.12	7.04	7.20	8.22	0.32	0.043	0.016	0.879
	Mid	6.16	7.62	8.92	9.98	0.52	0.001	0.042	0.709
	Late	7.36	7.76	8.48	9.16	0.31	0.010	0.122	0.666
Water intake (kg/d)	Early	10.70	11.28	12.26	12.76	0.96	0.163	0.590	0.967
	Mid	9.28	10.10	11.18	12.32	1.18	0.134	0.430	0.895
	Late	8.10	9.68	8.88	11.04	1.39	0.295	0.216	0.840
Milk yield (kg/d)	Early	10.81	12.30	12.19	12.82	0.25	0.580	0.002	0.146
	Mid	9.19	10.44	11.58	12.70	0.36	0.222	0.002	0.413
	Late	8.24	9.73	9.38	12.30	0.54	0.362	0.003	0.217
MBF (ml/min)	Early	2,551	2,904	3,158	4,129	206	0.180	0.018	0.184
	Mid	2,204	2,636	2,333	2,914	236	0.617	0.076	0.766
	Late	1,984	3,361	2,569	2,843	98	0.929	0.001	0.001

SEM = Standard error of the mean.

[1] p-values for the effects; MF = Mister-fan cooling effect, rbST = rbST effect, MF×rbST = interaction effect of MF and rbST.

of rbST. Both cooled and non-cooled cows showed significant increases in RR and RT after rbST treatment in all stages of lactation.

Dietary dry matter intake, water intake, milk yield and mammary blood flow

Effects of supplemental rbST and MF cooling on dietary DMI, water intake, milk yield and MBF are shown in Table 3. The DMI of cows housed in the MF barn with or without treatment of rbST was significantly higher than of cows housed in the NS barn in all stages of lactation. Cows treated with rbST under either MF or NS showed significantly increased DMI compared with the pre-treatment period. The highest values of DMI were apparent in cooled cows treated with rbST. Water intake was not significantly different among cows; however, the mean values of water intake of cooled cows tended to be higher than those of non-cooled cows. Cows treated with rbST under either MF or NS showed significantly increased water intake compared with the pre-treatment period. There was no evidence of interaction of cooling system and rbST treatment on water intake. Milk yield was significantly increased ($p<0.01$) during rbST administration. The milk yields of cooled cows were slightly higher than those of non-cooled cows. The milk yield of cows treated with rbST was significantly higher in all stages of lactation. Cows treated with rbST under either MF or NS showed significantly increased MBF compared with the pre-treatment period. There was no evidence of interaction of cooling system and rbST treatment on MBF.

Total body water, extracellular fluid, plasma volume, blood volume and hematocrit

Effects of supplemental rbST and MF cooling on total body water (TBW), extracellular fluid (ECF), plasma volume (PV), blood volume (BV) and hematocrit are shown in Table 4. The absolute value of TBW and as a percentage of body weight were significantly increased ($p<0.01$) by rbST treatment in all stages of lactation. The application of MF cooling did not affect the level of TBW. Mean values of ECF, PV and BV, either as absolute values or as a percentage of body weight, in cows without rbST showed no significant differences between NS and MF barns in all stages of lactation, except that cows in MF barn showed higher absolute PV values during late lactation. The absolute values of ECF, PV and BV markedly increased during rbST treatment in all stages of lactation. The values of ECF, PV and BV as a percentage of body weight of cows with rbST treatment tended to increase, but there were no significant differences as compared with the pre-treatment period. Neither rbST treatment nor MF cooling affected the hematocrit values.

Renal hemodynamics of crossbred Holstein cows supplemented with rbST

Effects of supplemental rbST and MF cooling on renal hemodynamics are shown in Table 5. No significant changes of GFR, ERPF, ERBF and FF were apparent in cows during rbST treatment or under the application of MF fans cooling in all stages of lactation. The rate of urine flow tended to decrease in cows treated with rbST by an average 14.9% compared with the pre-treatment period under either MF or NS barn conditions.

Urinary and fractional excretion of electrolytes, osmolar clearance and free water clearance of crossbred Holstein cows

Effects of supplemental rbST and MF cooling on urinary and fractional (FE) excretion of electrolytes,

Table 4. Total body water (TBW), extracellular fluid (ECF), plasma volume (PV), blood volume (BV) and packed cell volume (Hct) in animals treated with rbST under normal shade (NS) and cooling with misters and fans (MF) at different stages of lactation

	Lactating period	Treatments				SEM	Effects[1]		
		NS	NS+rbST	MF	MF+rbST		MF	rbST	MF×rbST
TBW (L)	Early	262.7	312.2	280.6	323.9	7.92	0.426	0.001	0.709
	Mid	263.9	312.6	272.9	336.5	7.74	0.512	0.001	0.375
	Late	267.3	321.7	275.9	330.3	13.15	0.712	0.006	0.999
TBW (L/100 kg)	Early	74.5	83.5	72.1	76.6	2.81	0.520	0.026	0.800
	Mid	71.7	84.6	66.2	76.5	1.78	0.374	0.001	0.503
	Late	68.6	85.1	65.0	74.8	3.80	0.349	0.090	0.410
ECF (L)	Early	97.9	104.3	111.1	123.1	3.79	0.171	0.010	0.086
	Mid	97.3	116.7	108.6	123.6	6.97	0.114	0.049	0.759
	Late	106.3	115.3	103.3	130.4	6.84	0.467	0.040	0.238
ECF (L/100 kg)	Early	27.67	27.83	26.02	30.26	0.64	0.852	0.013	0.018
	Mid	26.17	31.94	26.36	28.12	2.20	0.330	0.138	0.396
	Late	24.97	30.42	24.37	29.48	1.16	0.409	0.004	0.885
PV (L)	Early	18.85	20.88	17.00	19.55	1.11	0.185	0.047	0.627
	Mid	19.08	19.72	20.45	23.79	0.66	0.100	0.018	0.079
	Late	19.16	21.94	22.07	26.14	1.07	0.023	0.035	0.918
PV (L/100 kg)	Early	5.36	5.48	4.44	5.07	0.28	0.211	0.226	0.401
	Mid	4.98	5.14	5.17	6.19	0.28	0.253	0.068	0.170
	Late	4.83	5.62	5.30	5.97	0.35	0.077	0.069	0.862
BV (L)	Early	25.36	26.8	23.20	25.82	1.33	0.437	0.166	0.668
	Mid	24.96	25.54	25.45	31.83	1.30	0.096	0.028	0.056
	Late	25.34	29.09	28.62	31.96	1.79	0.076	0.044	0.892
BV (L/100 kg)	Early	7.18	7.03	6.27	6.82	0.36	0.385	0.594	0.356
	Mid	6.50	6.65	6.82	7.91	0.29	0.342	0.064	0.142
	Late	6.38	7.45	6.68	7.93	0.25	0.093	0.002	0.727
Hct (%)	Early	25.69	22.06	22.17	22.42	1.17	0.502	0.187	0.137
	Mid	23.48	22.70	22.59	22.41	0.66	0.696	0.489	0.662
	Late	24.34	24.40	23.08	22.80	0.95	0.424	0.607	0.564

SEM = Standard error of the mean.

[1] p-values for the effects; MF = Mister-fan cooling effect, rbST = rbST effect, MF×rbST = Interaction effect of MF and rbST.

Table 5. Glomerular filtration rate (GFR), effective renal plasma flow (ERPF), effective renal blood flow (ERBF), filtration fraction (FF) and urine flow rate in animals treated with rbST under normal shade (NS) and cooling with misters and fans (MF) at different stages of lactation

	Lactating period	Treatments				SEM	Effects[1]		
		NS	NS+rbST	MF	MF+rbST		MF	rbST	MF×rbST
GFR (ml/min/kg)	Early	2.03	1.95	1.63	1.45	0.09	0.076	0.188	0.599
	Mid	2.12	2.07	1.51	1.44	0.07	0.065	0.204	0.773
	Late	1.96	2.01	1.51	1.50	0.06	0.172	0.847	0.671
ERPF (ml/min/kg)	Early	6.01	5.83	5.43	5.44	0.23	0.592	0.715	0.682
	Mid	6.57	6.56	5.82	5.56	0.33	0.074	0.692	0.708
	Late	6.28	6.43	4.87	4.75	0.40	0.092	0.977	0.746
ERBF (ml/min/kg)	Early	8.14	7.45	6.94	6.98	0.37	0.481	0.400	0.349
	Mid	8.62	8.49	7.51	7.17	0.42	0.077	0.597	0.806
	Late	8.38	8.57	6.29	6.06	0.48	0.081	0.972	0.672
FF (%)	Early	34.7	33.6	30.7	27.3	1.3	0.092	0.112	0.387
	Mid	32.2	30.5	26.5	25.8	1.6	0.192	0.456	0.748
	Late	31.0	31.5	30.7	31.3	1.0	0.917	0.598	0.944
Urine flow rate (ml/min)	Early	17.65	13.34	16.08	13.96	2.52	0.916	0.238	0.675
	Mid	14.94	13.88	16.29	13.78	2.12	0.927	0.425	0.742
	Late	17.61	15.88	10.52	8.48	4.55	0.146	0.748	0.979

SEM = Standard error of the mean.

[1] p-values for the effects; MF = Mister-fan cooling effect, rbST = rbST effect, MF×rbST = interaction effect of MF and rbST.

osmolar clearance and free water clearance are shown in Table 6. The urinary and fractional excretion of sodium tended to decrease during rbST administration in cows housed in either NS or MF barns. The significant effect of rbST on decreases in both urinary and fractional excretion of sodium was apparent ($p<0.01$) in mid-lactation. Potassium excretion was decreased ($p<0.05$) during rbST treatment in early and mid-lactation, and tended to decrease in cows treated with rbST administration under MF cooling in both early and mid lactation. The effect of cooling system and rbST treatment on changes in fractional excretion of potassium was significantly apparent ($p<0.05$) in early lactation. Chloride excretion and fractional excretion of chloride tended to decrease during rbST administration, but was not statistically different ($p>0.05$) in all stages of lactation. Osmolar clearance decreased significantly ($p<0.05$) during rbST treatment in both early and mid-lactation, while free water clearance was not significantly affected ($p>0.05$) by rbST treatment or the application of MF throughout lactation.

Plasma electrolyte concentrations and plasma osmolarity

Effects of supplemental rbST and MF cooling on the concentrations of plasma electrolytes and on plasma osmolarity are shown in Table 7. There were no changes in the concentrations of plasma Na^+, K^+, Cl^- and osmolarity in cows treated with rbST or when housed in the MF barn.

DISCUSSION

In the present study, the temperature-humidity index (THI) derived from ambient temperature and humidity taken in both barns ranged from 79-85 throughout all stages of lactation. Cows in both groups would be subjected to moderate heat stress (Fuquay, 1981), since the onset of heat stress is about 72 THI (Armstrong, 1994), indicating that application of misters and fans was not sufficient to completely eliminate heat stress in cows in the present study. However, THI might not accurately reflect heat stress in MF evaporative cooling systems that deliver a pressurized spray

Table 6. Urinary electrolyte excretion, fractional excretion of electrolytes, osmolar clearance and free water clearance in animals treated with rbST under normal shade (NS) and cooling with misters and fans (MF) at different stages of lactation

	Lactating period	Treatments				SEM	Effects[1]		
		NS	NS+rbST	MF	MF+rbST		MF	rbST	MF×rbST
Na^+ excretion (μmol/min)	Early	485	384	614	465	90	0.344	0.215	0.801
	Mid	613	514	613	411	43	0.713	0.008	0.272
	Late	653	515	442	393	202	0.226	0.288	0.176
Fractional Na^+ excretion (%)	Early	0.52	0.37	0.73	0.57	0.06	0.154	0.044	0.911
	Mid	0.49	0.41	0.66	0.48	0.04	0.318	0.007	0.177
	Late	0.95	0.52	0.56	0.46	0.19	0.396	0.249	0.425
K^+ excretion (μmol/min)	Early	1,611	1,471	1,500	1,010	80	0.087	0.004	0.060
	Mid	1,612	1,457	2,066	1,281	143	0.624	0.011	0.052
	Late	1,620	1,309	1,547	1,476	199	0.799	0.349	0.587
Fractional K^+ excretion (%)	Early	45.6	53.7	55.6	45.2	3.58	0.941	0.750	0.032
	Mid	43.2	36.3	60.4	47.3	5.45	0.129	0.103	0.587
	Late	54.2	40.9	42.7	50.6	5.50	0.440	0.658	0.087
Cl^- excretion (μmol/min)	Early	487	300	360	258	114	0.484	0.241	0.717
	Mid	366	375	639	439	108	0.372	0.399	0.362
	Late	722	324	437	409	121	0.666	0.115	0.165
Fractional Cl^- excretion (%)	Early	0. 63	0. 47	5.80	0.46	0.13	0.874	0.284	0.867
	Mid	0. 42	0. 42	0.86	0.64	0.14	0.171	0.456	0.464
	Late	0. 91	0. 40	0.86	0.86	0.13	0.663	0.097	0.097
Osmolar clearance (ml/min)	Early	23.50	15.89	17.39	14.95	1.69	0.283	0.018	0.166
	Mid	22.20	19.59	22.99	16.16	1.48	0.624	0.013	0.191
	Late	22.68	19.59	20.96	18.88	1.83	0.637	0.194	0.788
Free water clearance (ml/min)	Early	-5.84	-2.55	-1.31	-0.99	3.10	0.463	0.576	0.645
	Mid	-7.26	-5.72	-6.70	-2.38	3.26	0.766	0.395	0.682
	Late	-5.09	-3.71	-9.60	-7.43	3.95	0.419	0.665	0.923

SEM = Standard error of the mean.

[1] p-values for the effects; MF = Mister-fan cooling effect, rbST = rbST effect, MF×rbST = Interaction effect of MF and rbST.

Table 7. Plasma sodium, chloride, potassium, osmolarity in animals treated with rbST under normal shade (NS) and cooling with misters and fans (MF) at different stages of lactation

	Lactating period	Treatments				SEM	Effects[1]		
		NS	NS+rbST	MF	MF+rbST		MF	rbST	MF×rbST
Plasma Na^+ (mEq/L)	Early	139.4	139.6	138.0	139.2	0.79	0.570	0.400	0.543
	Mid	140.0	140.6	139.2	139.0	0.59	0.426	0.744	0.518
	Late	139.4	139.2	140.4	139.8	0.50	0.582	0.447	0.700
Plasma K^+ (mEq/L)	Early	4.76	4.56	4.44	4.54	0.09	0.345	0.602	0.142
	Mid	4.64	4.86	4.58	4.64	0.07	0.580	0.077	0.279
	Late	4.52	4.40	4.64	4.64	0.08	0.444	0.473	0.473
Plasma Cl^- (mEq/L)	Early	101.4	100.2	97.6	100.0	1.23	0.380	0.637	0.180
	Mid	100.0	99.0	101.0	101.0	0.35	0.431	0.195	0.195
	Late	100.6	101.0	101.6	100.4	1.12	0.889	0.730	0.495
Plasma osmolarity (mOsm/kg)	Early	275.0	272.4	275.8	277.0	2.53	0.333	0.789	0.473
	Mid	275.8	276.4	281.0	279.2	1.57	0.176	0.713	0.467
	Late	276.2	279.4	281.2	280.0	2.11	0.374	0.648	0.327

SEM = Standard error of the mean.

[1] p-values for the effects; MF = Mister-fan cooling effect, rbST = rbST effect, MF×rbST = Interaction effect of MF and rbST.

with considerable air movement above the cow's back, resulting in higher humidity but also causing the cooling effect. The significant lower rectal temperatures and respiratory rates of cooled cows at the period of hottest daily temperatures (1300 to 1400 h) showed a partial alleviation of heat stress from MF cooling, which was confirmed by an increase in milk production compared with non-cooled animals throughout all stages of lactation. However, administration of exogenous bovine somatotropin in crossbred HF animals under high environment temperature could increase milk yield (Chaiyabutr et al., 2007), indicating that thermal stress alone was not an extra-mammary factor causing a reduction in milk production in crossbred HF animals.

An increase in milk yield accompanying elevations of TBW, ECF, blood volume and PV in rbST treated cows with or without MF cooling throughout lactation confirmed the previous reports of Maksiri et al. (2005) and Chaiyabutr et al. (2007) that exogenous rbST in the crossbred cow exerts a galactopoietic action, in part, through increases in body fluids and MBF in distribution of nutrients to the mammary gland for milk synthesis. Similar results for increases in both milk secretion and MBF during administration of exogenous growth hormone were also reported in goats (Hart et al., 1980) and *Bos taurus* cows (Davis et al., 1988). The marked increase in MBF has been shown to be associated with an increase in the level of plasma insulin-like growth factor-I (IGF-I) during prolonged treatment with rbST in crossbred cows (Chaiyabutr et al., 2005). The effect of rbST on MBF is thought to be indirectly mediated via IGF-I (Bauman, 1992), since infusion of IGF-I into the pudic artery of lactating goats has been shown to increase MBF and milk yield on the infused side (Prosser et al., 1990; 1994). However, in the present study, the pattern of progressive decline in milk yield as lactation advanced was apparent even with a higher level of MBF during rbST administration under conditions with or without MF. The decline in milk yield as lactation advances without facilitating MBF during rbST treatment could be attributed to a local change within the mammary gland.

The action of rbST can affect higher blood flow to the mammary gland, but it seems unlikely to affect blood flow to the kidneys, despite a high level of TBW and ECF during rbST administration. With respect to renal hemodynamics, no alterations of glomerular filtration rate (GFR), effective renal plasma flow (ERPF), effective renal blood flow (ERBF) and filtration fraction (FF) were apparent in rbST-treated cows housed in NS or MF barns at all stages of lactation. The different action of rbST on renal blood flow compared to the mammary gland indicates that the kidneys were able to regulate RBF and GFR constantly during experimental periods. It is probable that the kidneys of the ruminant respond differently from the mammary gland to the high level of endogenous IGF-I, secretion of which could be inferred during rbST administration in crossbred cows (Chaiyabutr et al., 2005). The action of IGF-I may appear directly in the blood vessels, but be more pronounced in the mammary gland of ruminants. The present findings seemingly contradict studies of differences among species in the effect of IGF-I on hyperfiltration rate in the kidney. An infusion of IGF-I, or recombinant human IGF-I, has been shown to decrease renal vascular resistance and increased GFR and RBF both in man (Guler et al., 1989; Hirschberg et al., 1993; Jaffa et al., 1994; Giordano and Defronzo, 1995) and rat models (Inishi et al., 1997). The different response in ruminants is an interesting finding that deserves further investigation.

In the present results, the effect of rbST on kidney function would more directly influence the renal tubular part of the nephron rather than change renal hemodynamics. The GFR and filtered load for Na^+, K^+ and Cl^- ions (GFR× plasma ion concentration) of rbST-treated cows remained constant, while the absolute values of urinary excretion of these ions, including FE_{Na}, FE_K and $FE_{Cl,}$ decreased as compared with pre-treatment values under either NS or MF barn conditions. On the other hand, these results obviously indicate an elevation of renal tubular reabsorption of ions in rbST-treated cows. These changes are similar to studies in a rat model by Dimke et al. (2007) which showed that the urinary excretion of sodium, potassium, and chloride ions and urine flow rate decreased in rats treated with growth hormone. An increase in renal tubular reabsorption of sodium ion was also reported in Lewis dwarf rats treated with somatotropin (Wyse et al., 1993). However, the mechanism of action of growth hormone on kidney function is still unsettled. Physiologically, the renal tubular reabsorption of ions is generally known to be under hormonal control. Several studies demonstrated that growth hormone increased sodium and water reabsorption via stimulation of the renin-angiotensin-aldosterone system (Ho and Weissberger, 1990; Cuneo et al., 1991; Herlitz et al., 1994; Moller et al., 1995; Moller et al., 1997). Growth hormone also activates an increase in the plasma aldosterone concentration coinciding with increases in IGF-I and renin-angiotensin (Hanukoglu et al., 2001). The interaction of these hormones on kidney function in ruminants is still speculative.

In the present findings, the increase, and thereby restoration, of body fluids in rbST-treated animals might not be the result of water consumption, although water intakes were higher in rbST-treated cows as compared with non-treated cows. Higher water intake would be attributed to the higher DM intake during the treatment of rbST (MacFarlane et al., 1959). According to the classical view, an increased plasma sodium concentration and plasma osmolality will stimulate vasopressin secretion and thirst which leads to enlarged plasma volume. However, in the present results, both the plasma sodium concentration and plasma osmolality of cows were maintained constant during rbST supplementation in NS or MF barns. This is likely explained by the fact that sodium ion is the osmotic factor of ECF and water is required in proportion to the amount of body fluids produced. Thus, the secretion of vasopressin acting on water reabsorption from the distal tubules and the collecting ducts of the kidneys might not be expected to occur to save water and thereby increase the ECF in rbST-treated cows. No significant change of the CH2O values was also independent of any direct effect of the rbST on free-water formation. In the present results, the observed decrease in excretion of electrolytes during supplemental rbST would create lower osmotic diuretic effect resulting in the decline in rate of urine flow. These findings would be supported by estimation of C_{osm} which was decreased during supplemental rbST. It is known that the sodium ion is the main cation in the ECF and it plays the dominant role in regulation of body fluid homeostasis by its osmotic action. It can be postulated from the present findings that, an increase in the renal tubular reabsorption of electrolytes (Na^+, K^+, Cl^-) during rbST administration would increase the number of electrolytes in the body composition. This is a part of the effects of enlarged body fluid volume arising from its colligative properties with exerting osmotic forces for retaining body water. It would be an explanation for an increase in body fluid volume during rbST administration.

In conclusion, cows supplemented with rbST and housed under misters and fan cooling could increase milk yield in all stages of lactation. Application of misters and fans alone did not affect kidney function in terms of both renal hemodynamics and electrolyte excretion. An increase in body fluid volume during rbST supplementation appears partly due to changes in renal tubular function by stimulating reabsorption of electrolytes without changes in renal hemodynamics. Further studies are required for a better understanding of the mechanisms of exogenous bovine somatotropin on segmental tubular sodium handling in the kidney in relation to aldosterone and vasopressin activation in the regulation of body fluid volume in crossbred dairy cattle.

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