Asian-Aust. J. Anim. Sci.
Vol. 24, No. 2 : 230 - 238
February 2011

www.ajas.info

# Effects of Cooling and Exogenous Bovine Somatotropin on Hematological and Biochemical Parameters at Different Stages of Lactation of Crossbred Holstein Friesian Cow in the Tropics

**N. Chaiyabutr*, D. Boonsanit and S. Chanpongsang[1]**
Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand

**ABSTRACT :** Effects of cooling and supplemental recombinant bovine somatotropin (rbST) on hemato-biochemical characteristics were studied at different stages of lactation of crossbred Holstein Friesian cows in a tropical environment. Ten primiparous cows were divided into two groups of five animals each. The first group was housed as the non-cooled animals in an open-sided barn with a tiled roof in a normal shaded house (NS), while the second group was housed as cooled cows in an open-sided barn with a tiled roof under misty fan cooling (MFC). Three injections with rbST (500 mg per dose) at each stage of lactation (early, mid and late lactation) significantly increased total milk yield as compared with pretreatment in both cooled and non-cooled cows. Milk fat was significantly increased, while total solids, solid not fat, milk protein and lactose were not affected by the rbST treatment. Hematological parameters, plasma proteins, albumin, glucose, triglyceride, cholesterol, creatinine, alkaline phosphatase (ALP), plasma inorganic phosphate and the activities of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were not affected by supplemental rbST in cooled and non-cooled cows. Supplementation of rbST caused a significant decrease in plasma urea concentration, while plasma FFA concentrations significantly increased in both cooled and non-cooled cows. The results of the present study suggest that exogenous rbST is efficacious in increasing milk yield without adverse effects on lactating crossbred Holstein cows in a tropical environment. (**Key Words :** Cooling, Recombinant Bovine Somatotropin, Hematology, Blood Chemistry, Crossbred Holstein Freisian)

## INTRODUCTION

Low milk yield and short persistency of lactation of dairy cattle is still the major problem for dairy practices in the tropics. Low productivity of crossbred dairy cattle is influenced by a large number of factors. A high environmental temperature is known to be one of the problems for animal production in tropical areas. Heat stress affects the performance of dairy cows by reducing their dry matter intake, feed efficiency and milk production (Shibata and Mukai, 1979; Fuquay, 1981; Collier et al., 1982; Johnson, 1987; Huber, 1994). Environmental modifications have been performed in attempts to alleviate severe heat stress in dairy cattle for example, water spray and fans (Armstrong et al., 1985; 1993), evaporative cooling, (Armstrong et al., 1985; 1988; 1993; Ryan et al., 1992; Chaiyabutr et al., 2008a). In dairy cows, milk synthesis is dependant on hormonal stimuli and the provision of nutrients from the blood to the mammary gland to sustain milk production. A previous study in crossbred cattle containing 87.5% Holstein Friesian (HF) genes found that a short persistency of lactation accompanied decreases in plasma bovine somatotropin level (bST) and blood flow to the mammary gland during the transition period from early to mid-lactation (Chaiyabutr et al., 2000). However, cows treated with rbST exhibited increased milk yield which coincided with increases in both total body water and mammary blood flow (Chaiyabutr et al., 2007). Administration of bST has been shown to be one of the management strategies that are needed to minimize the effects of heat stress and that will maintain sufficient DMI to sustain the potentially increased milk yields (West, 1994). An increase in total body water in bST-treated cows may play a central role for both lactation and heat dissipation. However, controversy exists on the use of bST due to effects on animal health. A number of studies have noted the effect of bST on bovine health in terms of risk of

* Corresponding Author : N. Chaiyabutr. Fax: +662-2520737, E-mail: Narongsak.C@Chula.ac.th
[1] Department of Animal Husbandry, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand.
Received July 2, 2010; Accepted September 3, 2010

clinical mastitis, reduction in fertility and risk of developing clinical signs of lameness (Dohoo et al., 2003). The adverse effects of exogenous bST have also been reported in other animal species (Skarda et al., 1992). The changes in relative mass of many organs and tissue growth following bST administration have also been reported (Moallem et al., 2004), which may reflect changes in bodily functions. According to this information, blood analysis will be a useful tool and a priority for diagnosis and health monitoring of animals in veterinary medicine (Payne and Payne, 1987) in prevention of metabolic disease or other disorders during intensive milk production. Few data are available for the additive effects of supplemental rbST and cooling on both health and productivity of crossbred dairy cattle in tropical environments. Therefore, the aim of the present study was to determine effects of supplemental rbST on hematological values and variability of metabolic parameters of crossbred dairy cows housed under shade with or without misters and fans at different stages of lactation. The results of this study will provide values of hematology and blood metabolites for systematic health monitoring and nutritional status and suggest possible preventive measures to reduce the risk of adverse effects during rbST supplementation in crossbred dairy cattle under high environmental temperatures.

## MATERIALS AND METHODS

### Animals and management

Ten primiparous, non-pregnant crossbred cattle, containing 87.5% Holstein Friesian (HF) genes were assigned randomly into two groups of five animals each. Animal management was previously described in detail by Boonsanit et al. (2010). Briefly, cows in the first group were housed in a normal shaded house (NS) as the non-cooled animals and cows in the second group were housed in a normal shaded house 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 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.

The diet was fed *ad libitum* twice daily as the same total mixed ration (TMR) to both groups in equal portions at about 06:00 h and 17:00 h when the animals were milking throughout the experiments. Ingredient and chemical compositions of the diet are shown in Table 1. The ambient temperature and humidity were recorded inside the barn, using a dry and wet bulb thermometer placed above the feeding lane. The relative humidity in NS and MFC barns was read by psychrometric chart depending on wet and dry bulb temperature. A temperature-humidity index (THI) was calculated from the average ambient temperature of dry and wet bulb temperatures according to McDowell (1972), as follows: THI = 0.72 (wb+db)+40.6 where wb = wet bulb temperature and db = dry bulb temperature expressed in °C.

**Table 1.** Ingredients and nutrient composition of the total mixed ration (TMR) administered during the experimental periods

| Ingredients | kg |
|---|---|
| Pine apple waste | 50 |
| Soybean meal | 23 |
| Rice bran | 3.0 |
| Cotton seed | 20 |
| Limestone | 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 |
| | ------------ % DM ---------- |
| Organic matter | 92.7 |
| Crude protein | 18.0 |
| Acid detergent fiber | 20.1 |
| Neutral detergent fiber | 33.9 |

### Experimental design

The experimental design in cooled and non-cooled cows was the same as that of Boonsanit et al. (2010). The studies in pre-treatment and treatment periods in each cow were carried out in early- (day 75 post-partum), mid- (day 135 post-partum) and late lactation (day 195 post-partum). After the pre-treatment study, each cow was injected subcutaneously with three doses of 500 mg of recombinant bovine somatotropin (rbST) (Posilac, Monsanto, USA) in each 2-week period. The pre-treatment, 3 doses of injections, and the treatment periods were performed during the first 30 days and the same procedures were followed for each stage of lactation. No experiments were conducted after the last 30 days of the experiment in order to allow milk yield from the effect of rbST treatment to return to the control level (Kirchgessner et al., 1991). Cows were milked twice daily using a milking machine and daily milk yield was recorded. On the same day as blood sampling, respiration rate (RR) and rectal temperature (RT) were recorded (between 13:00 and 14:00 h) by counting flank movements during two 30-s cycles and using a digital thermometer, respectively.

The procedures used in the present study were carried out 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.

**Samples collection and chemical analysis**

On specified days of the study, blood samples were taken at around 11:00 h in order to avoid daily variations in activities of both the pre-treatment and the treatment periods at each stage of lactation. Two blood samples (one for whole blood, one for plasma) were collected from the coccygeal vessel by venipuncture with a 21-gauge needle into heparinized tubes, and the samples were immediately placed in an ice bath until plasma separation. Just before centrifugation, blood samples were analysed for hemoglobin, erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), leukocyte count (WBC), leukocyte differential count for lymphocytes, monocytes and granulocytes and platelets by automated *in vitro* diagnostic medical equipment (Model Mythic18 (IVD) C2 Diagnostics; Montpellier, France). Blood samples were centrifuged in microhematocrit tubes at 12,000 rpm for 5 min, and the spun packed cell volume (PCV) was measured. The remaining blood was centrifuged at 3,500 g for 10 min to separate plasma, which was stored at -20°C for subsequent analysis.

The frozen plasma samples were used to determine the following parameters; urea nitrogen (BUN), creatinine, inorganic phosphorus, total protein, albumin, glucose, total cholesterol and triglycerides. Enzyme activities were determined for alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase. All determinations of blood chemistry were conducted on plasma at 37°C, with an automatic clinical chemistry analyzer (Operator Manual BT 2000 Plus, Biotecnica Instruments S.P.A Via Licenza, Rome, Italy). Plasma free fatty acids were determined by colorimetry after plasma extraction with chloroform, heptane and methanol and TAN solution containing 1-(2-Thiazolylazo)-2-naphthol (Sigma-Aldrich) as described by Wang et al. (2004). Milk samples from morning milking were used to determine milk composition using Milkoscan (Milko-Scan 133B, A/S N. Foss Electric, Hillerød, Denmark).

**Statistical analysis**

Individual cow data 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 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, $\mu$ = overall mean, $A_l$ = Animal effect, $H_i$ = house effect as main plot ($i$ = NS, MFC), $A(H)_{il}$ = main plot error (animal $l$ in house $i$), $B_j$ = treatment effect (rbST) as a split plot ($j$ = with and without rbST supplementation), $(HB)_{ij}$ = 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.

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 AND DISCUSSION

**Climatic parameters**

The average results for the climatic parameters in NS and MFC barns are reported in Table 2. The results obtained in the present study were similar to the values in our

**Table 2.** Comparative measurements of ambient temperature, relative humidity and temperature humidity index in normal shade (NS) and misty-fan cooling (MFC) barns at different stages of lactation

| | Stages of lactation | Treatments | | p-value |
|---|---|---|---|---|
| | | NS (n = 5) | MFC (n = 5) | (NS vs. MFC) |
| Ambient temperature (°C) | Early | 34.9±0.58 | 30.7±0.62 | p<0.001 |
| | Mid | 33.3±0.51 | 30.4±0.40 | p<0.002 |
| | Late | 32.4±0.58 | 29.0±0.49 | p<0.002 |
| Relative humidity (%) | Early | 53.5±2.52 | 67.9±3.55 | p<0.011 |
| | Mid | 58.8±3.62 | 73.5±2.64 | p<0.011 |
| | Late | 60.9±3.01 | 71.8±3.98 | p<0.069 |
| Temperature humidity index (THI) | Early | 85.4±0.50 | 81.9±0.76 | p<0.006 |
| | Mid | 84.0±0.44 | 82.4±0.53 | p<0.044 |
| | Late | 83.2±0.44 | 79.9±0.46 | p<0.001 |

Mean±SE.

previous report by Boonsanit et al. (2010). The period of hottest daily temperature (13:00 to 14:00 h), ambient temperature and temperature humidity index (THI) of the MFC barn were significantly lower, while relative humidity in the MFC barn was significantly higher than that of the NS barn throughout the periods of study. Cows in both groups were housed in both NS and MFC barns which had a THI ranging from 79-85 throughout the study period, which is considered the upper critical THI for lactating exotic dairy cows (Fuquay, 1981; Armstrong, 1994). The THI was >72 which is considered moderate heat stress and indicates that the effect of misters and fans was insufficient to completely eliminate heat stress in the cows. However, THI might not accurately reflect heat stress in misty fan evaporative cooling systems that deliver a pressurized spray above the cow's back, resulting in higher humidity but also causing a cooling effect. Boonsanit et al. (2010) reported that a partial alleviation of heat stress from misty fan cooling was evident from the significant lower rectal temperatures in cooled cows (average 38.5°C vs. 39.3°C) and respiratory rates (average 56 vs. 74 breaths/min) at the period of hottest daily temperatures. Milk yield was significantly increased during rbST supplementation in both cooled and non-cooled cows. The milk yields of cooled cows were slightly higher than those of non-cooled cows. This is in accordance with the results reported during cooling of *Bos taurus* cows (Tarazon et al., 1999). It is possible that the difference between day and night temperatures in the present study would affect milk yield by heat stress, because animals were exposed to a high THI during the hottest part of the day. The concentrations of milk protein, lactose, total solid (TS) and solid-not-fat (SNF) were not affected by supplemental rbST in either cooled or non-cooled cows at each stage of lactation. These findings indicate that an increase in milk production of cross-bred cows would be governed by both MFC and rbST supplementation at all stages of lactation. Similar effects were observed by Keister et al. (2002). The concentration of milk fat of cooled and non-cooled cows was significantly increased by supplementation of rbST in early and mid-lactation, while it had a tendency to increase in late lactation. A similar increase in milk fat concentration due to rbST administration has been previously reported (West et al., 1991). The synthesis of milk fat arises from both blood lipids and from *de novo* synthesis of fatty acids within the mammary epithelial cells. An increase in milk fat after rbST injection was associated with the increased yield of long-chain fatty acids characteristic of plasma free fatty acids and body fat (Chaiyabutr et al., 2008b).

**Haematological and plasma biochemical values**

The objective of the present study was to evaluate the effect of rbST administration and cooling with misters and fans on haematological (Table 4) and plasma biochemical

**Table 3.** Milk yield and milk compositions of cows treated with rbST and housed under normal shade (NS) and misty-fan cooling (MFC) barns at different stages of lactation

| Parameter | Stages of lactation | NS | | MFC | | SEM | Effect[1] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Pre | rbST | Pre | rbST | | MFC | rbST | MFC×rbST |
| Milk yield (kg/d/cow) | 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 |
| Protein (gm/dl) | Early | 3.37 | 3.61 | 3.48 | 3.63 | 0.15 | 0.788 | 0.227 | 0.790 |
| | Mid | 3.79 | 3.84 | 4.09 | 4.26 | 0.15 | 0.104 | 0.466 | 0.695 |
| | Late | 4.25 | 4.03 | 4.30 | 4.32 | 0.17 | 0.518 | 0.586 | 0.499 |
| Fat (gm/dl) | Early | 3.27 | 4.29 | 3.89 | 4.76 | 0.325 | 0.757 | 0.004 | 0.325 |
| | Mid | 3.53 | 4.25 | 3.87 | 4.44 | 0.593 | 0.732 | 0.013 | 0.593 |
| | Late | 4.27 | 4.58 | 4.11 | 5.15 | 0.732 | 0.301 | 0.075 | 0.732 |
| Lactose (gm/dl) | Early | 4.74 | 5.09 | 5.00 | 4.89 | 0.846 | 0.411 | 0.140 | 0.846 |
| | Mid | 4.85 | 4.82 | 4.82 | 4.91 | 0.820 | 0.698 | 0.452 | 0.820 |
| | Late | 4.77 | 4.78 | 4.41 | 4.72 | 0.358 | 0.186 | 0.217 | 0.358 |
| SNF (gm/dl) | Early | 8.61 | 9.39 | 9.18 | 9.22 | 0.614 | 0.079 | 0.110 | 0.614 |
| | Mid | 9.34 | 9.37 | 9.61 | 9.87 | 0.071 | 0.385 | 0.474 | 0.071 |
| | Late | 9.72 | 9.37 | 9.41 | 9.74 | 0.906 | 0.953 | 0.179 | 0.906 |
| TS (gm/dl) | Early | 13.24 | 12.79 | 13.42 | 14.54 | 0.401 | 0.703 | 0.381 | 0.401 |
| | Mid | 14.87 | 13.42 | 14.92 | 15.05 | 0.590 | 0.215 | 0.147 | 0.590 |
| | Late | 14.68 | 14.40 | 15.22 | 16.09 | 0.447 | 0.722 | 0.494 | 0.447 |

SEM = Standard error of the mean.

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

**Table 4.** Effects of cooling and rbST supplementation on hematological values of crossbred Holstein cows housed in normal shade (NS) and shade plus misty-fan cooling (MFC) barns at different stages of lactation

| Parameter | Stages of lactation | NS | | MFC | | SEM | Effect[1] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Pre | rbST | Pre | rbST | | MFC | rbST | MFC×rbST |
| RBC | Early | 5.07 | 4.39 | 4.76 | 4.31 | 0.37 | 0.648 | 0.170 | 0.757 |
| (×10$^6$/μl) | Mid | 4.71 | 4.44 | 4.87 | 4.41 | 0.37 | 0.801 | 0.351 | 0.791 |
| | Late | 4.65 | 4.39 | 4.34 | 4.90 | 0.23 | 0.858 | 0.533 | 0.109 |
| Hb | Early | 7.72 | 7.51 | 7.58 | 7.88 | 0.32 | 0.751 | 0.885 | 0.445 |
| (g/dl) | Mid | 7.66 | 8.24 | 8.16 | 7.38 | 0.55 | 0.741 | 0.861 | 0.253 |
| | Late | 8.04 | 7.71 | 7.48 | 8.00 | 0.13 | 0.862 | 0.508 | 0.013 |
| Hct | Early | 23.4 | 22.5 | 23.2 | 24.7 | 0.95 | 0.427 | 0.753 | 0.225 |
| (%) | Mid | 22.9 | 25.3 | 24.9 | 22.2 | 1.20 | 0.748 | 0.865 | 0.062 |
| | Late | 23.6 | 22.7 | 22.5 | 23.6 | 0.35 | 0.801 | 0.119 | 0.002 |
| MCV | Early | 46.72 | 53.04 | 43.40 | 52.10 | 2.38 | 0.528 | 0.013 | 0.630 |
| (fl) | Mid | 49.02 | 54.80 | 45.70 | 49.44 | 3.06 | 0.298 | 0.158 | 0.747 |
| | Late | 49.22 | 49.32 | 45.73 | 50.48 | 1.70 | 0.811 | 0.191 | 0.208 |
| MCH | Early | 15.84 | 17.38 | 14.46 | 17.10 | 1.16 | 0.452 | 0.110 | 0.649 |
| (pg) | Mid | 16.32 | 17.96 | 16.20 | 16.50 | 0.88 | 0.526 | 0.301 | 0.467 |
| | Late | 17.44 | 18.88 | 16.66 | 17.62 | 1.50 | 0.457 | 0.447 | 0.877 |
| MCHC | Early | 33.72 | 33.03 | 33.40 | 32.86 | 1.03 | 0.845 | 0.567 | 0.942 |
| (g/dl) | Mid | 33.48 | 33.00 | 35.82 | 35.72 | 1.31 | 0.097 | 0.831 | 0.888 |
| | Late | 35.42 | 38.70 | 33.26 | 34.14 | 2.17 | 0.098 | 0.366 | 0.595 |
| Platelets | Early | 413.6 | 383.2 | 512.2 | 401.8 | 70.67 | 0.587 | 0.348 | 0.587 |
| (10$^3$/μl) | Mid | 699.4 | 287.6 | 697.8 | 279.8 | 174.68 | 0.983 | 0.045 | 0.986 |
| | Late | 321.0 | 478.0 | 588.4 | 279.4 | 125.23 | 0.853 | 0.561 | 0.100 |
| WBC | Early | 15.67 | 9.34 | 9.34 | 11.98 | 2.68 | 0.612 | 0.511 | 0.132 |
| (×10$^3$/μl) | Mid | 8.16 | 13.80 | 9.58 | 7.55 | 2.12 | 0.309 | 0.419 | 0.108 |
| | Late | 9.43 | 12.42 | 13.56 | 17.84 | 1.09 | 0.253 | 0.010 | 0.569 |
| Lymphocyte | Early | 27.76 | 16.78 | 18.30 | 19.32 | 3.79 | 0.565 | 0.226 | 0.152 |
| (%) | Mid | 15.03 | 18.23 | 15.70 | 18.45 | 4.53 | 0.944 | 0.536 | 0.962 |
| | Late | 19.13 | 20.23 | 25.85 | 25.18 | 3.05 | 0.256 | 0.947 | 0.781 |
| Monocyte | Early | 6.90 | 3.88 | 5.38 | 6.33 | 1.57 | 0.846 | 0.534 | 0.254 |
| (%) | Mid | 5.63 | 5.58 | 5.80 | 5.33 | 1.84 | 0.986 | 0.889 | 0.913 |
| | Late | 2.08 | 3.23 | 4.03 | 4.33 | 0.63 | 0.125 | 0.292 | 0.524 |
| Granulocyte | Early | 68.18 | 79.48 | 75.10 | 71.48 | 3.07 | 0.927 | 0.258 | 0.051 |
| (%) | Mid | 75.08 | 79.58 | 78.50 | 76.15 | 6.49 | 1.000 | 0.874 | 0.616 |
| | Late | 90.10 | 72.05 | 69.83 | 68.88 | 5.32 | 0.150 | 0.124 | 0.159 |

SEM = Standard error of the mean.

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

values (Table 5) of crossbred Holstein cattle in a tropical environment. The haematological values did not show any specific disorder during rbST supplementation in both cooled and non-cooled cows. However, significant increases in MCV of red blood cells were apparent in early lactation in both cooled and non-cooled cows given supplemental rbST ($p<0.05$), whereas PCV values were not altered. This indicates that rbST supplementation would increase body fluids in both ECF and ICF. An increase in extracellular fluid and plasma volume during rbST administration in crossbred Holstein cattle has been noted (Chaiyabutr et al., 2007). These changes might be the cause of variations in haematological and plasma biochemical values during hemodilution at each stage of lactation. The concentrations of platelets were not affected by cooling alone but were decreased by rbST supplementation, which may have been due to expansion of extracellular fluid and plasma volumes during rbST administration.

The rbST supplementation in both cooled and non-cooled cows had no effects on plasma levels of triglyceride, glucose and protein at each period of lactation. However, concentrations of FFA in plasma were not affected by cooling but were significantly increased by rbST supplementation especially in mid- and late lactation in

**Table 5.** Effects of cooling and rbST supplementation on blood chemistry values of crossbred Holstein cows housed in normal shade (NS) and shade plus misty-fan cooling (MFC) barns at different stages of lactation

| Parameter | Stages of lactation | NS | | MFC | | SEM | Effect[1] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Pre | rbST | Pre | rbST | | MFC | rbST | MFC×rbST |
| ALT (IU/L) | Early | 15.02 | 14.58 | 15.06 | 12.50 | 1.73 | 0.717 | 0.410 | 0.556 |
| | Mid | 16.50 | 14.77 | 15.80 | 17.42 | 2.64 | 0.685 | 0.984 | 0.544 |
| | Late | 15.60 | 17.20 | 16.80 | 17.08 | 3.41 | 0.880 | 0.790 | 0.851 |
| AST (IU/L) | Early | 58.74 | 60.92 | 71.28 | 58.06 | 3.53 | 0.538 | 0.157 | 0.061 |
| | Mid | 63.54 | 49.60 | 68.06 | 70.98 | 5.22 | 0.035 | 0.322 | 0.145 |
| | Late | 62.28 | 57.70 | 60.00 | 78.18 | 4.25 | 0.358 | 0.148 | 0.028 |
| Alkaline phosphatase (IU/L) | Early | 103.98 | 122.60 | 88.90 | 92.38 | 8.55 | 0.375 | 0.233 | 0.402 |
| | Mid | 132.04 | 92.38 | 102.72 | 122.60 | 19.49 | 0.985 | 0.625 | 0.165 |
| | Late | 77.00 | 118.60 | 115.58 | 126.20 | 9.69 | 0.110 | 0.086 | 0.587 |
| BUN (mg/dl) | Early | 11.96 | 7.64 | 10.56 | 7.96 | 0.938 | 0.773 | 0.006 | 0.386 |
| | Mid | 13.44 | 9.14 | 10.72 | 7.01 | 1.248 | 0.188 | 0.012 | 0.819 |
| | Late | 12.00 | 9.20 | 10.00 | 7.47 | 0.789 | 0.385 | 0.010 | 0.866 |
| Creatinine (mg/dl) | Early | 1.46 | 1.54 | 1.41 | 1.49 | 0.05 | 0.759 | 0.141 | 1.000 |
| | Mid | 1.41 | 1.68 | 1.52 | 1.61 | 0.07 | 0.912 | 0.077 | 0.255 |
| | Late | 1.59 | 1.71 | 1.48 | 1.47 | 0.09 | 0.327 | 0.548 | 0.482 |
| Total protein (g/dl) | Early | 8.52 | 8.79 | 9.23 | 8.82 | 0.18 | 0.428 | 0.718 | 0.092 |
| | Mid | 9.11 | 8.49 | 8.57 | 8.97 | 0.29 | 0.956 | 0.706 | 0.114 |
| | Late | 8.70 | 8.13 | 8.98 | 8.88 | 0.22 | 0.460 | 0.179 | 0.327 |
| Albumin (g/dl) | Early | 4.27 | 4.22 | 4.18 | 4.21 | 0.06 | 0.820 | 0.868 | 0.513 |
| | Mid | 4.18 | 4.08 | 4.14 | 4.20 | 0.07 | 0.755 | 0.765 | 0.294 |
| | Late | 4.27 | 4.31 | 4.18 | 4.19 | 0.06 | 0.604 | 0.715 | 0.831 |
| Cholesterol (mg/dl) | Early | 183.00 | 169.60 | 128.10 | 136.14 | 5.40 | 0.092 | 0.633 | 0.083 |
| | Mid | 153.80 | 143.50 | 150.60 | 154.96 | 11.33 | 0.811 | 0.800 | 0.536 |
| | Late | 162.60 | 169.60 | 141.32 | 170.80 | 9.45 | 0.999 | 0.117 | 0.055 |
| Triglyceride (mg/dl) | Early | 14.24 | 13.73 | 13.36 | 12.72 | 0.84 | 0.755 | 0.516 | 0.939 |
| | Mid | 14.72 | 13.80 | 12.82 | 13.50 | 1.60 | 0.671 | 0.942 | 0.631 |
| | Late | 11.84 | 12.54 | 14.84 | 18.68 | 1.68 | 0.140 | 0.245 | 0.408 |
| Free fatty acid (mg/dl) | Early | 3.70 | 3.88 | 4.72 | 6.78 | 0.90 | 0.239 | 0.247 | 0.323 |
| | Mid | 3.14 | 4.63 | 4.45 | 4.82 | 0.35 | 0.576 | 0.029 | 0.150 |
| | Late | 2.42 | 3.62 | 4.21 | 6.20 | 0.40 | 0.147 | 0.004 | 0.362 |
| Glucose (mg/dl) | Early | 90.18 | 84.62 | 88.36 | 85.80 | 3.35 | 0.904 | 0.260 | 0.666 |
| | Mid | 91.44 | 78.56 | 84.58 | 94.86 | 6.24 | 0.308 | 0.840 | 0.100 |
| | Late | 79.90 | 84.66 | 96.58 | 98.18 | 2.18 | 0.123 | 0.183 | 0.489 |
| Inorganic phosphate (mg/dl) | Early | 6.38 | 6.34 | 5.75 | 7.09 | 0.40 | 0.916 | 0.141 | 0.123 |
| | Mid | 7.10 | 6.77 | 6.48 | 6.70 | 0.47 | 0.644 | 0.906 | 0.572 |
| | Late | 6.44 | 7.40 | 6.53 | 6.84 | 0.45 | 0.640 | 0.197 | 0.493 |

SEM = Standard error of the mean.

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

both cooled and non-cooled cows. These results were in accordance with marked increases in plasma FFA levels observed in high-producing lactating cows following administration of large doses of growth hormone (Kronfeld, 1965). These results indicate the greater mobilization of adipose tissue to supply extra nutrients for the increased milk production. An elevation of plasma FFA concentration did not depend on the stage of lactation, although cows in early lactation generally suffer a negative energy balance from stimulating mobilization of adipose tissue for an increase in the concentrations of plasma FFA. Cows in both groups in the present study were fed balanced diets, which would imply no mobilization of fat tissue. Thus, the lipolytic activity would be a function of rbST treatment *per se* instead of the associated changes in energy balance. As lactation advanced, the milk yield declined and thus cows would gain body fat in preparation for the coming lactation. The effect of supplemental rbST on an increase in the plasma concentrations of FFA of both cooled and non-cooled cows might not directly exert acute lipolytic effects on adipose tissue. An alteration of lipogenesis or lipolysis by bST may occur indirectly via its anti-insulin effects (McDowell, 1991).

Supplementation of rbST in both cooled and non-cooled cows showed no effect on plasma protein levels at each stage of lactation. The concentrations of total plasma proteins of both cooled and non-cooled cows, either with or without rbST, averaged 8.13-9.23 g/dl. The present results did not agree with other reports that protein concentration in blood during bST administration will be influenced by changes in relative body mass of many organs and tissue growth for the role in protein metabolism (Moallem et al., 2004). Among proteins, plasma albumin concentration had an average value of 4.14-4.31 g/dl of total protein, which is similar to values found in the literature (Kaneko et al., 1997). In the present study, no effects of rbST and misty fan cooling were apparent on plasma albumin concentration. Plasma albumin concentration in cows is influenced by their physiological stage and it is closely connected with nutrition and the amount of nitrogen intake (Roil et al., 1974). Cows in both groups were equally well-fed throughout the periods of study, which indicates that dietary protein was not limiting and that liver function was likely to be normal. In the present study, no differences were observed in plasma BUN concentrations between non-cooled cows and cooled cows without rbST, although several reports have shown that animals exposed to high temperatures had increased plasma levels of urea (Ronchi et al., 1999) and creatinine (Broucek et al., 1986; Chaiyabutr, and Johnson, 1991). However, the concentrations of BUN were significantly decreased by rbST in both cooled and non-cooled cows, while no significant changes in the creatinine concentrations were apparent. These results are in agreement with studies in lactating *Bos taurus* dairy cows treated with GH, which showed reduced plasma urea concentrations as compared with control cows (Cheli et al., 1998; Santos et al., 2000). The reduction of plasma urea concentration might not reflect the crude protein intake which was higher in cows given supplemental rbST. The decreased plasma concentrations of urea during supplementation of rbST might be due to the effect of bovine somatotropin via N-actyl glutamate, on carbamyl phophate synthetase, a key enzyme in the urea cycle (Oddy and Lindsay, 1986). In the present study, exposure of cows to moderate heat stress appears not to have caused a higher utilization of amino acids as energy source with increased urea level or mobilization of protein from muscle mass with subsequent creatinine delivery into the plasma (Fekry et al., 1989). This indicates that the kidneys were able to perform their normal function.

It is known that the activity of aminotransferases in the blood is important in acting as a catalyst in connecting the metabolism of amino acids and carbohydrates. Changes in aminotransferase activities in the blood can be a consequence of their activities in liver cells and also a reflection of cell structure damage. High activities of AST and ALT are most often found in acute and chronic liver disorder, e.g. fatty liver syndrome in dairy cows (Fekry et al., 1997), starvation and the appearance of ketosis during early lactation (Steen, 2001), even in a subclinical disorder (Meyer and Harvey, 1998). However, the liver is known to be the source of synthesis and secretion of IGF-I in ruminants, which are dependent on the availability of both plasma growth hormone level and some nutritional factors (Clemmons and Underwood, 1991). Animals with a lower nutritional state (Hodgkinson et al., 1991) or a negative energy balance, have reduced hepatic production IGF-I (Ketelsleger et al., 1995; Steen, 2001), which would not be expected to occur in the present study. The present results did not show any changes of liver function for the activity of the plasma alanine aminotransferase (ALT) (12.5-17.4 IU/L) and plasma aspartate aminotransferase (AST) (49.6-78.2 IU/L) during rbST supplementation in both cooled and non-cooled cows, although an increase in plasma IGF-I concentration, which originates primarily from liver, has been shown to be affected by rbST administration in cows (Chaiyabutr et al., 2005). In late lactation, both plasma cholesterol levels and plasma alkaline phosphatase activity had a tendency to increase by supplemental rbST of both cooled and non-cooled cows. These variations of plasma cholesterol levels and plasma alkaline phosphatase (ALP) activity in both cooled and non-cooled cows were within normal values (Kaneko et al., 1997). It did not confirm the hypothesis that a reduction of plasma cholesterol concentration and plasma ALP activity would coincide with a reduction in liver activity in cows exposed to high temperatures (Abeni et al., 2007). Enzyme activity of ALP has been indicated to be a quick and reliable blood marker for heat stress (Abeni, et al., 2007). Our previous study, (Boonsanit et al., 2010) showed no alterations of plasma concentrations of $K^+$, $Na^+$, and $Cl^-$ in both cooled and non-cooled cows with or without rbST, although it has been reported that, during heat stress, $K^+$ losses from sweating cause negative electrolyte balance with impaired absorption of $Na^+$ and $Cl^-$ from the rumen coinciding with the depression of rumen fermentation (Maltz et al., 1994). It is probable that the crossbred cows in both groups experienced moderate heat stress and were on the borderline between moderate and high heat stress in the hotter periods of the tropical environment. In conclusion, the present data of both haematological and plasma biochemical values of the crossbred Holstein cows, with or without supplemental rbST under NS and MFC barns, were within normal physiological limits in comparison with those of clinically healthy cows (Schalm et al., 1975; Kaneko et al., 1997). Overall, the results of the present study suggest that supplemental rbST is efficacious in increasing milk yield without adverse effects on lactating crossbred Holstein cattle in a tropical environment.

## ACKNOWLEDGMENTS

This study was supported in part by The Thailand Research Fund (BRG498004). D. Boonsanit is the recipient a grant from Walailak University, Nakornsrithammarach province, Thailand.

## REFERENCES

Abeni, F., L. Calamari and L. Stefanini. 2007. Metabolic condition of lactating dairy cows during the hot season in the Po Valley. 1. Blood indicators of heat stress. Int. J. Biometeorol. 52:87-96.

Armstrong, D. V. 1994. Heat stress interaction with shade and cooling. J. Dairy Sci. 77:2044-2050.

Armstrong, D. V., F. Weirsma, T. J. Fuhrman, J. M. Tappan and S. M. Cramer. 1985. Effect of evaporative cooling under a corral shade on reproduction and milk production in a hot climate. J. Dairy Sci. 68:167(Abstr.).

Armstrong, D. V., S. K. DeNise, F. J. Delfino, E. J. Hayes, P. J. Grundy, S. Montgomery and A. Correa. 1993. Comparing three lactational performances of Holstein cows in hot weather. J. Dairy Sci. 64:844-849.

Armstrong, D. V., M. E. Wse, M. T. Torabi, F. Wiersma, R. Hunter and E. Kopel. 1988. Effect of different cooling systems on milk production of late lactation Holstein cows during high ambient temperature. J. Dairy Sci. 71(Suppl. 1):212.

Boonsanit, D., Chanpongsang and N. Chaiyabutr. 2010. 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. Asian-Aust. J. Anim. Sci. 23:355-365.

Broucek, J., M. Kovalcikova and K. Kovalcik. 1986. The effect of high temperatures on feed intake, milk production and biochemical traits. In: Proceedings of the 37th Annual Meeting of EAAP, Vol. I, pp. 596-597.

Cebra, C. K., F. B. Garry, D. M. Getzy and M. J. Fettman. 1997. Hepatic lipidosis in anorectic, lactating Holstein cattle: a retrospective study of serum biochemical abnormalities. J. Vet. Intern. Med. 11:231-237.

Chaiyabutr, N. and H. D. Johnson. 1991. The regulation of body fluids and electrolytes of buffalo in a hot environment. In: Physiological aspects of digestion and metabolism in ruminants (Ed. T. Tsuda, Y. Sasaki and R. Kawashima) Academic Press Inc. pp. 297-319.

Chaiyabutr, N., S. Preuksagorn, S. Komolvanich and S. Chanpongsang. 2000. Comparative study on the regulation of body fluids and mammary circulation at different states of lactation in crossbred Holstein cattle feeding on different types of roughage. J. Anim. Physiol. Anim. Nutr. 83:74-84.

Chaiyabutr, N., S. Thammacharoen, S. Komolvanich and S. Chanpongsang. 2005 Effects of long-term administration of recombinant bovine somatotropin on milk production and insulin like growth factor-I and insulin in crossbred Holstein cows. J. Agric. Sci. (Cambridge), 143:311-318.

Chaiyabutr, N., S. Thammacharoen, S. Komolvanich and S. Chanpongsang. 2007. Effects of long-term exogenous bovine somatotropin on water metabolism and milk yield in crossbred Holstein cattle. J. Agric. Sci. (Cambridge) 145:173-184.

Chaiyabutr, N., S. Chanpongsang and S. Suadsong. 2008a. Effects of evaporative cooling on the regulation of body water and milk production in crossbred Holstein cattle in a tropical environment. Int. J. Biometeorol. 52: 575-585.

Chaiyabutr, N., S. Thammacharoen, S. Komolvanich and S. Chanpongsang. 2008b. Effects of long-term exogenous bovine somatotropin on glucose metabolism and the utilization of glucose by the mammary gland in different stages of lactation of crossbred Holstein cattle. Anim. Sci. J. 79(5):561-574.

Cheli, F., A. Baldi, L. Gregoretti, F. Rosi, D. Cattaneo and V. Dell'Orto. 1998. Changes of plasma insulin, urea, amino acids and rumen metabolites in somatotropin treated dairy cows. Amino Acids 15:187-194.

Clemmons, D. R. and L. E. Underwood. 1991. Nutritional regulation of IGF-I and IGF binding proteins. Annu. Rev. Nutr. 11: 393-412.

Collier, R. J., D. K. Beede, W. W. Thatcher, L. A. Israel and C. J. Wilcox. 1982. Influences of environment and its modifications on dairy animal health and production. J. Dairy Sci. 65:2213-2227.

Dohoo, I. R., K. Leslie, L. Descôteaux and W. Shewfelt. 2003. A meta-analysis review of the effects of recombinant bovine somatotropin. Can. J. Vet. Res. 67(4):241-251.

Fekry, A. E., A. F. Abdelaa, M. K. Shebaita and M. A. I. Salem. 1989. Is creatinine a good indicator for meat production in fat-tailed sheep? In: Ruminant production in dry subtropics: constraints and potential. EAAP Publication n. 38, pp. 150-252.

Fuquay, J. W. 1981. Heat stress as it affects animal production. J. Anim. Sci. 52:164-169.

Hodgkinson, S. C., J. J. Bass and P. D. Gluckman. 1991. Plasma IGF-I binding proteins in sheep: effect of recombinant growth hormone treatment and nutritional status. Domestic Anim. Endocrinol. 8:343-351.

Huber, J., T. G. Higginbotham, R. A. Gomez-Alarcon, R. B. Taylor, K. H. Chen, S. C. Chan and Z. Wu. 1994. Heat stress interactions with protein, supplemental fat and fungal cultures. J. Dairy Sci. 77:2080-2090.

Johnson, H. D. 1987. Bioclimate effects on growth, reproduction and milk production. In: Bioclimatology and Adaptation of Livestock (Ed. H. D. Johnson). Elsevier Sci. Publ., Amsterdam, pp. 35-57.

Kaneko, J. J., J. W. Harvey and M. L. Bruss. 1997. Clinical biochemistry of domestic animals. 5th ed. Academic press. London, New York, Tokyo.

Ketelsleger, J. M., D. Maiter, M. Maes, L. E. Underwood and J. P. Thicssen. 1995. Nutritional regulation of insulin-like growth factor-I. Metabolism 44:50-57.

Kirchgessner, M., W. Windisch, W. Schwab and H. L. Muller. 1991. Energy metabolism of lactating dairy cows treated with prolonged-release bovine somatotropin or energy deficiency. J. Dairy Sci. 74:35-43.

Keister, Z. O., K. D. Moss, H. M. Zhang, R. A. Teegerstrom, R. J. Eding and L. A. Ax. 2002. Physiological responses in thermal stressed Jersey cows subjected to different management strategies. J. Dairy Sci. 85:3217-3224.

Kronfeld, D. S. 1965. Growth hormone-induced ketosis in the cow. J. Dairy Sci. 48:342.

Maltz, E., N. Silanikove, U. Shalit and A. Berman. 1994. Diurnal fluctuations in plasma ions and water intake of dairy cows as affected by lactation in warm weather. J. Dairy Sci. 77:2630-2639.

McDowell, G. H. 1991. Somatotropin and endocrine regulation of metabolism during lactation. J. Dairy Sci. 74:44-62.

McDowell, R. E. 1972. Improvement of livestock in warm climates. W.H. Freeman and Co., San Francisco, CA.

Meyer, D. J. and J. W. Harvey. 1998. Evaluation of hepatobiliary system and skeletal muscle and lipid disorders. In: Veterinary Laboratory Medicine. Interpretation and Diagnosis. 2nd edn. (Ed. D. J. Meyer and J. W. Harvey). W. B. Sounders. Philadelphia, London, Toronto, Montreal, Sydney, Tokyo. pp. 157-187.

Moallem, U., G. E. Dahl, E. K. Duffey, A. V. Capuco, D. L.Wood, K. McLeod, R. L. Baldwin, VI and R. A. Erdman. 2004. Bovine somatotropin and rumen-undegradable protein effects in prepubertal dairy heifers: Effects on body composition and organ and tissue weights. J. Dairy Sci. 87:3869-3880.

Oddy, V. H. and D. B. Lindsay. 1986. Metabolic and hormonal interactions and their potential effectson growth. In: Control and Manipulation of Animal Growth (Ed. P. J. Buttery, N. B. Haynes and D. B. Lindsay). Butterworths, London, England. pp. 231-248.

Payne, J. M. and S. Payne. 1987. The metabolic profile test. Oxford University Press. Oxford, New York, Tokyo. pp. 36-49.

Roil, M. R., G. W. Suckling and J. Mattingley. 1974. Serum total protein and albumin levels in grazing sheep. N.Z. Vet. J. 22: 232-236.

Ronchi, B., U. Bernabucci, N. Lacetera, A. Verini Supplizi and A. Nardone. 1999. Distinct and common effects of heat stress and restricted feeding on metabolic status of Holstein heifers. Zoot. Nutr. Anim. 25:11-20.

Ryan, P. D., M. P. Boland, E. Kopel, D. Armstrong, L. Minyakasi, R. A. Godke and R. H. Ingraham. 1992. Evaluating two different evaporative cooling management systems for dairy cows in hot, dry climates. J. Dairy Sci. 75:1052-1059.

Skarda, J., E. Markalous, J. Slaba, P. Krejci, O. Skardova and J. Zednik. 1992. Effect of methionyl bovine somatotropin in a prolonged-release vehicle on milk production, hormone profiles and health in dairy cattle. J. Dairy Res. 59:499.

Schalm, O. W., N. C. Jain and E. J. Carroll. 1975. Veterinary hematology, 3 rd edn. Lea and Febiger, Philadelphia, USA.

Shibata, M. and A. Mukai. 1979. The effect of environmental temperature on the relationship between ruminal volatile fatty acids and heat production of dry cows. Jpn. J. Zootech. Sci. 50:265-270.

Steen, A. 2001. Field study of dairy cows with reduced appetite in early lactation: clinical examinations, blood and rumen fluid analyses. Acta Vet. Scand. 42:219-228.

Santos, J. E. P., J. T. Huber, C. B. Theurer, C. B. Nussio, L. G. Nussio, M. Tarazon and D. Fish. 2000. Effects of grain processing and bovine somatotropin on metabolism and ovarian activity of dairy cows during early lactation. J. Dairy Sci. 83:1004-1015.

Tarazon, H. M., J. T. Hubaer, J. Santos, H. Mena, L. Nusso and C. Nussio. 1999. Effect of bovine somatotropin and evaporative cooling plus shade on lactation performance of cows during summer heat stress. J. Dairy Sci. 82:2352-2357.

Weller, P. A., M. J. Dauncey, P. C. Bates, J. M. Brameld, P. J. Buttery and R. S. Gilmour. 1994. Regulation of porcine insulin-like growth factor-I and growth hormone receptor m-RNA expression by energy status. Am. J. Physiol. 266:E776-785.

Wang, A. S., D. F. Jan, K. J. Chen, D. W. Yang and Y. K. Fan. 2004. Dietary supplementation of increased milk fat percentage without affecting ruminal characteristics in Holstein cows in a warm tropical environment. Asian-Aust. J. Anim. Sci. 17:213-220.

West, J. W. 1994. Interactions of energy and bovine somatotropin with heat stress. J. Dairy Sci. 71:2091-2102.

West, J. W., B. G. Mullinix and T. G. Sandifer. 1991. Effects of bovine somatotropin on physiologic responses of lactating Holstein and Jersey cows during hot, humid weather. J. Dairy Sci. 74:840-851.