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Different Responses of Oxidative Stress Index in the Plasma of Crossbred Holstein Cattle During Cooling and Supplemental Recombinant Bovine Somatotropin

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Abstract: The objective of this study was to investigate the effect of supplemental recombinant bovine somatotropin and cooling management on changes in oxidative stress index in the plasma of crossbred Holstein cattle at different stages of lactation. Ten primiparous crossbred 87.5% Holstein cattle were randomly divided into 2 groups of five animals each which were housed in Normal Shaded (NS) as non-cooled cows and in shaded with Misters and Fans (MF) as cooled cows. The cows in each group were supplemented of recombinant bovine Somatotropin (rbST) in each stage of lactation with three consecutive subcutaneous injections with 500 mg of rbST in every 14 days. The ambient temperature at 13:00 h in the MF barn was significantly lower while relative humidity was higher than that of the NS barn. Cows under NS barn showed high rectal temperatures and respiration rate as compared with cows under the MF barn. Milk yield significantly increased in both cooled and non-cooled cows treated with rbST in each stage of lactation. The plasma concentrations of Sulfhydryl (SH) residue and Thiobarbituric Acid Reactive Substance (TBARS) did not alter in both groups of cows with or without rbST supplementation but the ascorbic acid concentrations in the plasma and in milk of cows under NS barn were significantly lower than those of cows under MF barn during early and mid lactation. The present study, demonstrates that changes in antioxidant reserve of ascorbic acid in both plasma and milk of dairy cows would be an appropriate and sensitive indicator of oxidative stress in crossbred dairy cows during exposure to high temperatures.

Key words: Bovine somatotropin, crossbred Holstein cattle, misty-fan cooling, oxidative stress index, sulfhydryl, NS barn

INTRODUCTION

Low milk yield is one of the most problems for dairy animal production in the tropic. Thermal effect with heat stress is probably, the most important factor and is widely recognized as one of the leading causes on the performance of dairy cows. It affects their dry matter intake, feed efficiency and milk production (Fuquay, 1981; Shibata and Mukai, 1979) including disruption their homeostasis and metabolism. The effect of heat stress is known to induce oxidative stress which induces production of Reactive Oxygen Species (ROS) (Mitchell and Russo, 1983; Loven, 1988). The high production of reactive oxygen species and a decrease in antioxidant defense, leads to cause of many diseases (Halliwell and Gutteridge, 1990) and leading to the onset of health disorders in cattle (Miller *et al*., 1993).

Oxidative stress markers can be divided into nonenzymatic antioxidants and antioxidant enzymes such as Superoxide Dismutase (SOD), catalase and glutathione peroxidase (Zalba *et al*., 2001). The plasma concentration of vitamin C in ruminants is an oxidative stress indicator which is affected by heat stress. A negative correlation between rectal temperatures and ascorbic acid concentrations of *Bos taurus* cattle in the hot season was reported (Tanaka *et al*., 2007). The different responses of radical scavengers for antioxidant mechanisms may be due to interaction with factors that involve in the

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activation of oxidative stress. Different breeds of dairy cows may show different responses of the level of oxidative stress marker during heat stress. Temperate breeds (*Bos taurus*) have higher milk production but they also have inherent disadvantageous traits particular low heat tolerance as compared with crossbred cows containing *Bos indicus* gene which have high heat tolerance in the tropical environment. Few data are available for studies of the oxidative status in crossbred cows under high ambient temperatures although, a number of studies have been reported in heat stressed exotic Holstein dairy cows (Bernabucci *et al*., 2002; Lakritz *et al*., 2002; Tanaka *et al*., 2007).

The previous studies in crossbred Holstein cattle (Chaiyabutr *et al*., 2007) have shown that growth hormone act as a regulator of fluid homeostasis with an increase in total body water after rbST administration. Increment of body temperature during bST treatment has been noted (Johnson *et al*., 1991). The restoring body fluids involving heat dissipation mechanism during rbST administration in crossbred Holstein cattle under high ambient temperatures led to raise the question of whether rbST treated cows show a novel mechanism to defend heat stress which need for further investigation.

As described previous, there are many reports examining the effect of heat stress alone on oxidative stress index in many species animals including poultry (Altan *et al*., 2003; Mujahid *et al*., 2005). Oxidative stress can be monitored with several biomarkers (antioxidants and pro-oxidants) in the plasma. However, little information of oxidative status regarding crossbred Holstein cattle given exogenous rbST under high temperatures is available. Therefore, the aim of the present study was to examine, the effect of cooling and supplemental rbST on the responses of oxidant defenses in crossbred Holstein cows in the tropic.

Changes in antioxidative stress markers in plasma for concentrations of Sulfhydryl (SH) residue and Thiobarbituric Acid Reactive Substance (TBARS) including concentrations of ascorbic acid in both plasma and milk were studied.

MATERIALS AND METHODS

Animals and management: Animal managements in the present study were similar to those in the previous study (Chaiyabutr *et al*., 2011). Ten primiparous, non pregnant lactating crossbred cattle, containing 87.5% Holstein (HF) genes were used and divided into 2 groups of five animals each. Cows in the 1st group were housed in open-sided barn with a tiled roof in Normal Shaded house (NS) as the non-cooled cows. Cows in the 2nd group were housed in open-sided barn of normal shaded house with two sets of Misty Fan cooling (MF) as cooled cows. Each system of misty fan cooling consisted of a 65 cm. Diameter blade fan circulating 81 m^3 min^{-1} of air with oscillation coverage of 180°. The amount of water discharged from 4 splay heads was 7.5 L h^{-1} and size of mist droplet 0.01 mm. Animals were exposed to MF for 45 min at 15th min intervals from 06:00-18:00 h. At night, animals were exposed to MF for 15 min at 45th min intervals from 18:00- 06:00 h.

Cows in both groups were offered, the same ration of Total Mixed Ration (TMR) *ad libitum* twice a day around 06:00 and 17:00 h when the animals were milking. Ingredient and chemical compositions of feed are shown in Table 1. Meteorological data of both barns were performed using dry bulb thermometer for measurement of the ambient temperature. The relative humidity at NS and MF barns were read by psychrometric chart depending on wet and dry bulb temperature.

Ambient temperatures, humidity, rectal temperature and respiratory rate were measured weekly at 09:00 and 13:00 h on specified day. A Temperature-humidity Index (THI) was calculated from the average ambient temperature of dry and wet bulb temperatures according to McDowell (1972) as follow: THI = 0.72 (wb+db)+40.6. Where; wb = wet bulb temperature and db = dry bulb temperature expressed in °C. Rectal temperature and respiratory rates were measured at different parts of the day with electronic thermometers and observing flank movements, respectively.

The procedures used in the present study were performed in accordance with national animal care guidelines of National Research Council of Thailand and were pre-approved by the faculty ethics committee.

Experimental design: The design of experimental protocol in cooled and non-cooled cows was also similar as that of the previously described (Chaiyabutr *et al*., 2011). Briefly,

Table 1: Feed ingredients and chemical compositions of the diet

Ingredients	Kg (as fed basis)
Pine apple waste	50.0
Soybean meal	23.0
Rice bran	3.0
Cotton seed	20.0
Lime stone	1.4
Di-calcium phosphate	1.4
Sodium bicarbonate	0.3
Potassium chloride	0.1
Mineral and vitamin premix	0.8
Total	100.0
Chemical composition	
Dry matter (%)	39.1
Ash (DM%)	7.3
Organic matter (DM%)	92.7
Crude protein (DM%)	18.0
Acid detergent fiber (DM%)	20.1
Neutral detergent fiber (DM%)	33.9

the experiments were carried out throughout lactating period in each group. The experiment in each group was divided into 3 phases, namely early (day 60 postpartum), mid (day 120 postpartum) and late lactating periods (Day 180 postpartum).

The pretreatment study was conducted on the starting day of each phase. At the end of the pretreatment within the same day, the animal was injected with the 1st dose subcutaneous injection of 500 mg of recombinant bovine Somatotropin (rbST) (POSILAC, Monsanto, USA). Subsequently, the animal was injected with two consecutive doses injections of rbST every 2 weeks. Thereafter within 2 days after the 3rd injection, the treatment study was conducted. The pretreatment, 3 doses of injections and the treatment periods were performed during the 1st 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.

Determinations of plasma concentrations of SH residue, TBARS, albumin and concentration of ascorbic acid in both plasma and milk: On each specified day after the morning milking at around 10 h, blood sample from each cow was collected from the coccygeal vessel by venopuncture into the tube containing heparin 25 i.u. mL^{-1} blood. Plasma was separated by centrifugation (3500 × g, 15 min) immediately after sampling and kept at -40°C until analysed.

The concentration of Sulfhydryl residue (SH), the Thiobarbituric Acid-Reactive Substances (TBARS) concentration and the total ascorbate concentration in plasma were measured by spectrophotometry as described in details by Tanaka *et al.* (2007). In brief, the concentration of plasma Sulfhydryl (SH) residue was measured via the method of Motchnik *et al.* (1994) using Dithionitrobenzene (DTNB) in the reaction. The TBARS concentration in the plasma, a breakdown product of lipid peroxidation was carried out with an extraction solution contained pyridine and 1-buthanol using the concentration of malondialdehyde as the standard (Ohkawa *et al.*, 1979; Chitra *et al.*, 2003). The milk sample was collected from each animal at the morning milking on the day of study of each period.

Milk aliquots were kept at -40°C until the day of analysis of ascorbic acid in milk. Briefly, the milk samples were centrifuged at 12,000 rpm for 15 min at 4°C. The aqueous phase below the solidified fat layer was removed for the measurement of ascorbic acid concentration. The total ascorbate concentration in both plasma and milk was measured by the oxidation of ascorbic acid with copper ions to form dehydroascorbic acid and diketogulonic acid which react with 2, 4-dinitrophenylhydrazine to form the derivative bis-2, 4dinitrophenylhydrazone (Omaye *et al.*, 1979). The total protein and albumin concentrations in the plasma were determined by an automatic analyzer (Operator Manual BT 2000 Plus, Biotecnica Instruments S.P.A Via Licenza, Rome, Italy).

Statistical analyses: The statistic analyses were performed using General Linear Model (GLM) procedures of statistical software package SPSS (SPSS for windows, V14.0; 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, 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
e_{ijk} = Residual error

Means values were used to evaluate the effect for all variables. Statistical significance was declared at $p<0.05$ and trends were declared at $0.05<p\leq0.10$. Duncan's new multiple range tests were used to detect the statistical significance between different treatment groups. The unpaired t-test was also done for comparison of average meteorological data between cooling and non-cooling barns.

RESULTS

Ambient temperature, relative humidity, temperature humidity index, rectal temperature and respiration rate in animals treated with rbST under Normal Shade (NS) and Misters and Fans cooling (MF) at different stages of lactation: Mean values of environmental parameters in NS and MF barns at morning (09:00 h) and afternoon (13:00 h) are shown in Fig. 1. Ambient temperatures at 09:00 h were not different between NS and MF barns while ambient temperatures in the afternoon of NS barn were significantly higher than those of MF barn

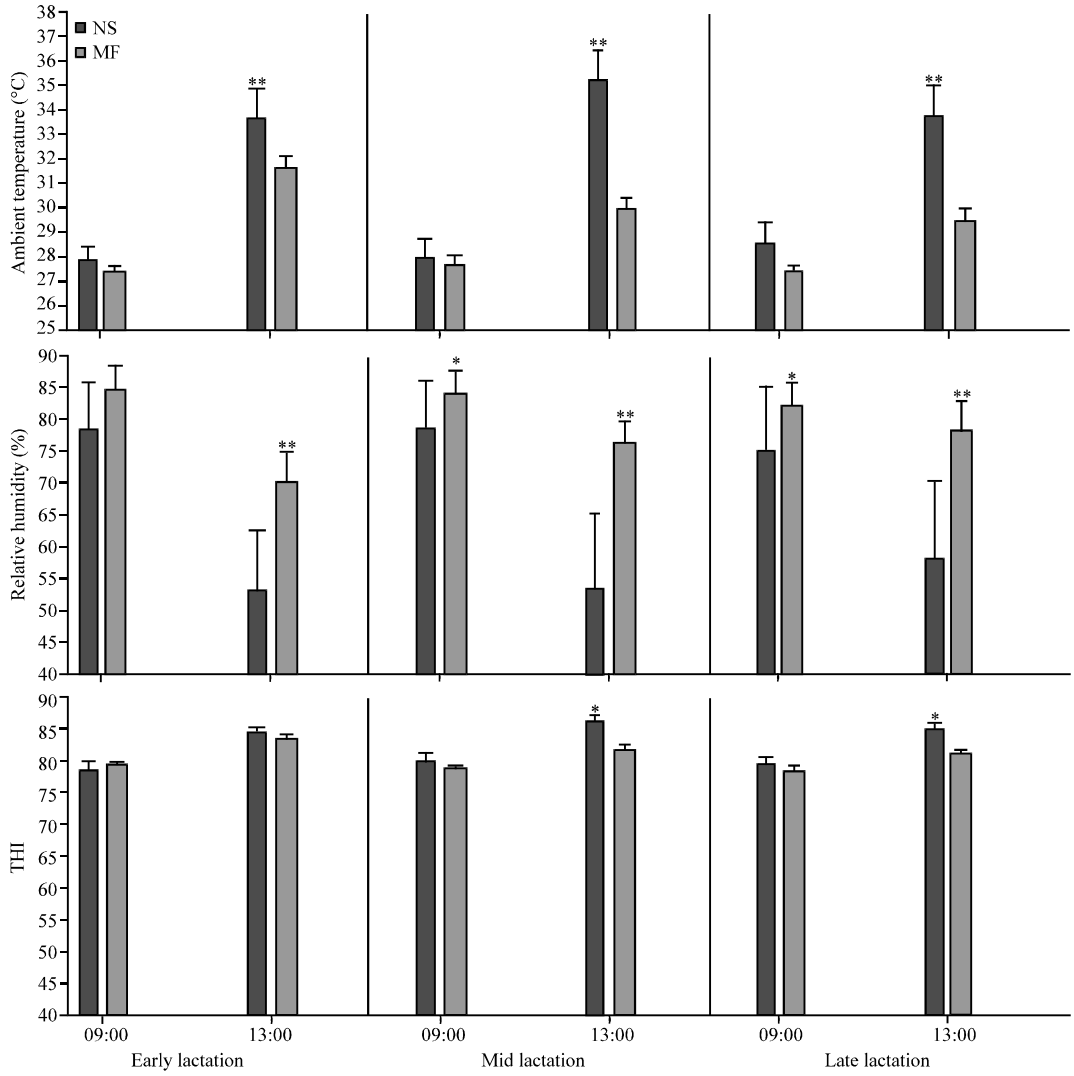

Fig. 1: Ambient temperature, relative humidity and temperature humidity index at morning (09:00 h) and afternoon (13:00 h) in Normal Shade (NS) and Misters and Fans cooling (MF) barns at different stages of location. Comparisons were made between mean values of measurement at the same time of both barns: values marked with significant differences using unpaired t-test, *p<0.05, **p<0.01

(average 37.7 vs. 31.6°C) throughout experimental periods. The values of relative humidity of NS barn were significantly lower than those of MF barn throughout experimental periods. Values of THI in both barns ranged from 77.9-85.5 which the THI of MF barn in the afternoon were significantly lower than those of NS barn during mid and late stage of lactation.

In afternoon, cows without rbST housed under MF barn showed significantly lower rectal temperature and the respiration rate than those of cows housed under NS barn (Fig. 2). Rectal temperature and the respiration rate were increased after rbST supplementation in both cooled and non-cooled cows when compared with pre-treatment in each stage of lactation.

Effects of misty fan cooling and supplementation with rbST on milk yield, plasma concentrations of sulfhydryl residue, lipid peroxidation-end product (TBARS), plasma albumin and plasma and milk concentrations of ascorbate of crossbred Holstein cows: Milk yield significantly increased after supplemental rbST as compared with the pretreatment period in each stage of lactation in both cooled and non-cooled cows. The decline in milk yield were apparent as lactation advanced in both cooled and non-cooled cows whether supplemental rbST or not.

The concentrations of ascorbic acid in both plasma and milk of non-cooled cows were lower than those of cooled cows. These changes were significantly apparent

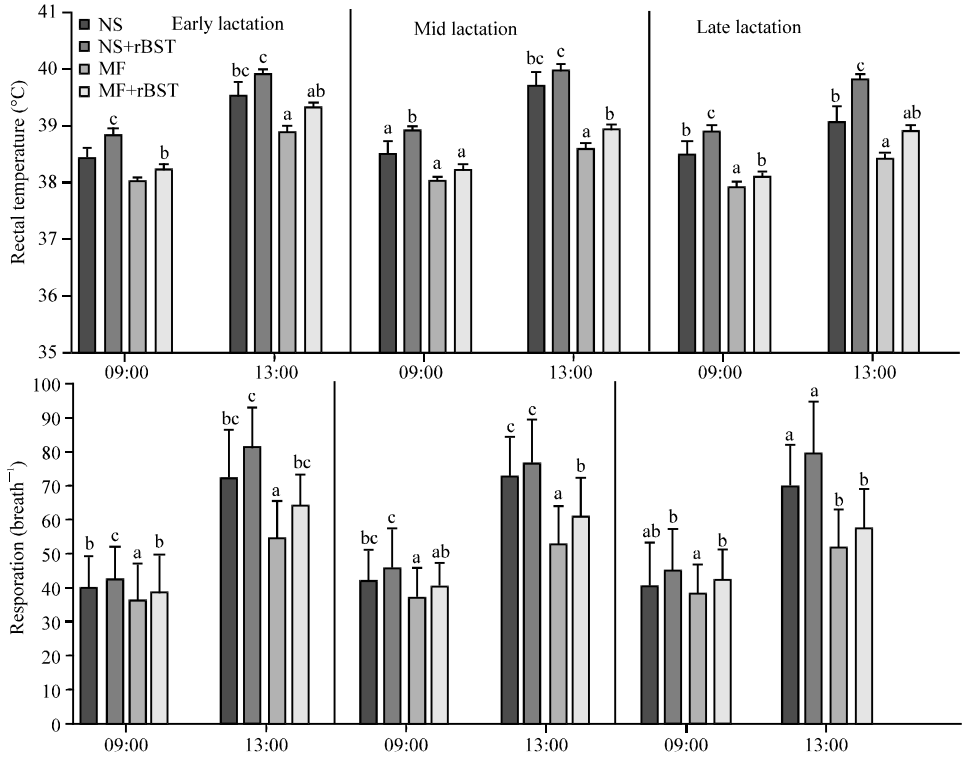

Fig. 2: Mean values of rectal temperature (a) and respiration rate (b) in cows treated with rbST under Normal Shade (NS) and Misters and Fans cooling (MF) at morning (09:00 h) and (13:00 h) in different stages of location. Duncan's new multiple range tests were used to detect the statistical significance between different treatments. Means with the same letter in each time of measurement are not significantly different ($p<0.05$)

Table 2: Effects of misty fan cooling and supplementation with rbST on milk yield, plasma and milk concentrations of ascorbic acid of crossbred Holstein cows

		NS		MF		[1]Effect			
Parameters	Stages of lactation	Pre	rbST	Pre	rbST	SEM	MF	rbST	MF x rbST
Milk yield (kg day^{-1})	Early	10.65	11.95	11.88	12.38	0.317	0.633	0.040	0.313
	Mid	9.25	10.14	11.46	12.94	0.276	0.237	0.003	0.319
	Late	8.04	9.81	9.14	11.78	0.593	0.432	0.006	0.487
Plasma ascorbic acid (μmol L^{-1})	Early	22.26	22.20	32.10	30.62	1.980	0.023	0.708	0.730
	Mid	20.91	19.29	25.48	28.79	1.610	0.065	0.612	0.164
	Late	22.21	25.01	25.25	26.62	1.730	0.440	0.262	0.691
Milk ascorbic acid (μmol L^{-1})	Early	105.90	158.80	166.10	179.60	22.000	0.157	0.170	0.397
	Mid	137.40	125.70	228.40	208.20	24.600	0.015	0.534	0.868
	Late	143.90	132.90	168.30	202.00	29.900	0.437	0.821	0.211

SEM = Standard Error of the Mean; [1]p values for the effects; MF = Misty Fan cooling effect, rbST = rbST effect, MF x rbST = Interaction effect of MF and rbST

Table 3: Effects of misty fan cooling and supplementation with rbST on plasma concentrations of lipid peroxidation end product (TBARS), sulfhydryl residue, and plasma albumin concentration of crossbred Holstein cows

		NS		MF		[1]Effect			
Parameters	Stages of lactation	Pre	rbST	Pre	rbST	SEM	MF	rbST	MF x rbST
TBARS (nmol mL^{-1})	Early	1.69	2.11	2.61	2.06	0.110	0.243	0.590	0.003
	Mid	1.72	2.29	1.79	1.92	0.240	0.539	0.181	0.400
	Late	1.89	2.16	1.89	2.28	0.160	0.814	0.075	0.729
Sulfhydryl residue (μmol L^{-1})	Early	393.21	363.23	365.67	361.59	17.010	0.763	0.346	0.468
	Mid	379.95	363.12	378.93	389.64	14.890	0.742	0.842	0.382
	Late	388.62	419.26	375.87	418.22	19.940	0.872	0.105	0.777
Albumin	Early	636.60	635.10	630.00	634.60	10.330	0.901	0.890	0.779

Table 3: Continued

Parameters	Stages of lactation	NS		MF		[1]Effect			
		Pre	rbST	Pre	rbST	SEM	MF	rbST	MF x rbST
(μmol L^{-1})	Mid	630.30	614.90	616.90	627.10	10.720	0.755	0.765	0.294
	Late	642.90	648.70	625.90	621.40	13.540	0.604	0.715	0.831
SH residue/	Early	0.62	0.58	0.59	0.57	0.027	0.818	0.308	0.496
Albumin	Mid	0.60	0.59	0.61	0.61	0.031	0.765	0.465	0.344
	Late	0.61	0.65	0.60	0.66	0.026	0.804	0.543	0.431

SEM = Standard error of the mean; [1]p values for the effects ; MF = Misty Fan cooling effect, rbST = rbST effect, MF x rbST = interaction effect of MF and rbST

in early and mid-lactation while it was not affected by rbST supplementation (Table 2). Another antioxidative component, plasma concentration of the SH residue and the concentration of TBARS, oxidation products of polyunsaturated lipid did not significantly differ between cooled and non-cooled cows with or without rbST supplementation.

The plasma albumin concentration did not differ between cooled and non-cooled cows with or without rbST supplementation (Table 3).

DISCUSSION

The values of ambient temperature in NS barn recording at 13:00 h were significantly higher than those of MF barn throughout the experimental periods. Values of the daytime THI in both barns ranged from 77.9-85.5 which were higher than the threshold level (THI 72) of comfortable zone (Armstrong, 1994). It indicates that cows in both groups were subjected to moderate heat stress (Fuquay, 1981). However both respiratory rate and rectal temperature of cows under MF were affected by the cooling effect in comparison with those of non-cooled cows which indicate a partial alleviation of heat stress by MF system especially in the afternoon.Milk yield of cows under NS barn in each stage of lactation were smaller than those of cows in MF barn. The reduction in milk production in a hot environment is usually attributed to a fall in feed intake. However, cows in both groups in the present study were fed similar diet throughout studies. Therefore, the low milk yields of cows under NS barn as compared with those of cows under MF barn would attribute to direct effect of high temperatures even cows were fed with fixed level of feed intake (Wayman *et al*., 1962).

The previous studies in 87.5% crossbred Holstein cattle in the tropic showed that the decline in milk yield as lactation progressed to mid and late lactation would concomitant to the decrease in the concentration of plasma bST (Chaiyabutr *et al*., 2000). Cows under NS and MF barns receiving rbST, significantly increased milk yields after treatment in each stage of lactation and decreased as lactation advanced to mid and late lactation. These results confirm that an increase in milk yield in response to rbST administration will not be sustained indefinitely (Bauman, 1992) and it is influenced by the stage of lactation (Phipps *et al*., 1991). However, rbST is one of the factors capable of stimulating milk production efficiency in crossbred lactating animals in the tropical environment.

It is known that heat stress, induces oxidative stress resulting increase in formation of ROS and/or decreased antioxidant reserve. Oxidative stress affecting to cells by the oxidizing of macromolecules resulting from increased formation of ROS have been studied in heat-stressed cells (Flanagan *et al*., 1998) and in cultured tissue model (Skibba *et al*., 1990). In the present study in different stages of lactation, clarifications of the oxidative stress of crossbred cows under moderate heat stress and supplemental rbST with determinations of the alteration of plasma biomarkers were performed. Under high ambient temperatures, a marked increase in the respiratory rates of cows under NS barn might increase oxygen pressure of blood which might be the cause of alteration of oxidative status. Crossbred Holstein cows under moderate heat stress (high THI) in NS barn seemed to induce oxidative stress leading to reduction of the plasma ascorbic acid concentration in early and mid lactation. Reduction of the plasma ascorbic acid concentration in late lactation was less pronounced.

Ascorbic acid is known as an antioxidative vitamin serving as a water-soluble reductant to donate a free molecule of hydrogen that detoxifies the harmful ROS generated by the body to a less toxic form during heat stress (Frei *et al*., 1989).

Changes in the antioxidative activity of ascorbic acid during heat stressed is also observed in Bos Taurus dairy cattle (Tanaka *et al*., 2007) when the body ascorbic acid is exhausted (Altan *et al*., 2003; Tauler *et al*., 2003). Cows have not been considered to need dietary ascorbic acid because they are able to synthesize a high enough level of ascorbic acid in themselves. The significant reduction of ascorbic acid concentrations in plasma of non-cooled cows during early lactation and mid lactation were apparent independent on the effect of rbST. A reduction of antioxidant activity in plasma during mid lactation has

also been observed in heat-stressed Holstein cows (Harmon *et al*., 1997). The reductions of the concentration of ascorbic acid in plasma have also been observed in calves under heat stressful conditions of cowshed environments (Cummins and Brunner, 1991). High ambient temperatures in NS barn increased the rectal temperature of cows up to 39.9°C in mid lactation. Negative relationship of ascorbic acid concentration in plasma and rectal temperature of dairy cows has also been reported (Tanaka *et al*., 2007). It is possible in the present study that high milk yield in early lactation have more metabolic activity and produce more body heat production (West, 1994).

The combination of body heat increment either consequence of rbST treatment (Cole and Hansen, 1993) and high environmental temperatures would lead to sensitive to oxidative stress. In the present results, there was dissociation among plasma biomarkers of oxidative status.

The components of SH residue, Thiobarbituric Acid Reactive Substance (TBARS) as an index of lipid peroxidation and the plasma albumin concentration were not affected in cows under NS barn as compared with cows under MF barn whether supplemental rbST or not. The study of Calamari *et al*. (1999) in *Bos tuarus* cows showed that a reduction of plasma lipid soluble antioxidants (vitamin E and β-carotene) and an increase of plasma TBARS under moderately heat-stressed during summer were apparent particularly in mid-lactation.

In the present results, the concentration of SH residues in plasma of both cooled and non-cooled cows supplemental rbST were closed to the plasma albumin concentrations indicating that most protein SH groups are found on plasma albumin (Radi *et al*., 1991).

Albumin is thought to be one of resources of SH residue in the plasma. The reversible oxidations of sulfur residues are common and fundamentally important in the control of cell functions (Moran *et al*., 2001). SH residues are abundant both inside and outside the cell as non-protein and protein SH groups which predominate over the oxidized form. Albumin possess only one SH residue in a molecule (Carter and Ho, 1994). It was observed that no alterations in the ratio of SH residues to albumin concentrations were apparent in plasma of both cooled and non-cooled cows supplemental rbST. It is possible that crossbred cows using in the present study containing indigenous genes have heat tolerance.

Cows did not expose to severe heat stress which a marked increase in plasma total solids including plasma protein concentrations causing of alteration of plasma albumin concentration have been noted in both cattle and buffalo under severe heat exposure (Bianca, 1957; Chaiyabutr *et al*., 1987). In the present results, there was dissociation among plasma biomarkers of oxidative status. The plasma SH concentration did not show pattern as the plasma ascorbic acid concentration although, oxidized ascorbic acid by oxidative stress has been reported to be reduced using the reducing equivalent of SH residues in rat plasma (Vethanayagam *et al*., 1999). It is possible that the oxidation of the SH residues by oxidative stress did not utilize the same mechanism as that of ascorbic acid oxidation. An ascorbic acid concentration in the plasma of dairy cows seems to be important in reducing its equivalent in body fluid and is sensitive to oxidative stress. These results suggest that ascorbic acid concentrations in cow plasma are sensitive to high environmental temperature.

The TBARS concentrations in plasma of crossbred cows in both groups were not derived from oxidative stress under moderately heat stress although, the concentration of a breakdown product of lipid peroxidation, TBARS is known to be one of the oxidative stress markers in the plasma (Flemming *et al*., 1997). However, Armstrong and Browne (1994) reported that the thiobarbituric acid test would be considered to be a good general indicator of oxidative stress rather than a marker of lipid peroxidation. Weak negative effects of moderate heat stress on the plasma SH and TBARS levels for oxidative status were apparent in crossbred cows.

It is possible that the primiparous cows in both groups using in the present study may be less affected than multiparous cows during exposure to high environmental temperatures (Folman *et al*., 1979). Other findings (Miller *et al*., 1993) have also shown that an imbalance of the oxidative status during different stages of lactation was observed in Holstein cows.

CONCLUSION

From the present results, different plasma biomarkers using for determination of the effect of heat stress on oxidative status suggest that measurement of plasma level of TBARS is not sensitive to the oxidant-antioxidant balance in crossbred dairy cows exposure to high environmental temperatures. The study of antioxidant reserve of ascorbic acid concentration in plasma is an appropriate and sensitive model to study the oxidative status of crossbred dairy cows during exposure to high temperatures.

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REFERENCES

Altan, O., A. Pabuccuoglu, S. Konyalioglu and H. Bayracktar, 2003. Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. Br. Poult. Sci., 44: 545-550.

Armstrong, D. and R. Browne, 1994. The analysis of free radicals, lipid peroxides, antioxidant enzymes and compounds related to oxidative stress as applied to the clinical chemistry laboratory. Adv. Exper. Med. Biol., 366: 43-58.

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

Bauman, D.E., 1992. Bovine somatotropin: Review of an emerging animal technology. J. Dairy Sci., 75: 3432-3451.

Bernabucci, U., B. Ronchi, N. Lacetera and A. Nardone, 2002. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. J. Dairy Sci., 85: 2173-2179.

Bianca, W., 1957. The effect of repeated short exposure to heat on the volume and hydration of the blood of the calf. Brit. Vet. J., 113: 227-241.

Calamari, L., M.G. Maianti, F. Amendola and G. Lombardi, 1999. On some aspects of the oxidative status and on antioxidants in blood of dairy cows during summer. Proceedings of the 13th Associazione Scientifica Produzioni Animali Congress, June 21-24, Piacenza, Italy, pp: 449-451.

Carter, D.C. and J.X. Ho, 1994. Structure of serum albumin. Adv. Protein Chem., 45: 153-203.

Chaiyabutr, N., C. Buranakarl, V. Muangcharoen, P. Loypetjra and A. Pichaicharnarong, 1987. Effects of acute heat stress on changes in the rate of liquid flow from the rumen and turnover of body water of swamp buffalo. J. Agric. Sci. (Camb.), 108: 549-553.

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., 145: 173-184.

Chaiyabutr, N., S. Komolvanich, S. Preuksagorn and S. Chanpongsang, 2000. Plasma levels of hormones and metabolites as affected by the forages type in two different types of crossbred Holstein cattle. Asian-Aust. J. Anim. Sci., 13: 1359-1366.

Chaiyabutr, N., D. Boonsanit and S. Chanpongsang, 2011. 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. Asian-Aust. J. Anim. Sci., 24: 230-238.

Chitra, K.C., C. Latchoumycandane and P.P. Mathur, 2003. Induction of oxidative stress by bisphenol A in the epididymal sperm of rats. Toxicol., 185: 119-127.

Cole, J.A. and P.J. Hansen 1993. Effect of administration of recombinant bovine somatotropin on the responses of lactating and nonlactating cows to heat stress. J. Am. Vet. Med. Assoc., 203: 113-117.

Cummins, K.A. and C.J. Brunner, 1991. Effect of calf housing on plasma ascorbate and endocrine and immune function. J. Dairy Sci., 74: 1582-1588.

Flanagan, S.W., P.L. Moseley and G.R. Buettner, 1998. Increased flux of free radicals in cells subjected to hyperthermia: Detection by electron paramagnetic resonance spin trapping. FEBS Lett., 431: 285-286.

Flemming, N., B.B. Mikkelsen, B.N. Jesper, R.A. Helle and G. Philippe, 1997. Plasma malondialdehyde as biomarker for oxidative stress: Reference interval and effects of life-style factors. Clin. Chem., 43: 1209-1214.

Folman, Y., A. Berman, Z. Herz, M. Kaim, M. Rosenberg, M. Mamen and S. Gordin, 1979. Milk yield and fertility of high-yielding cows in a sub-tropical climate during summer and winter. J. Dairy Sci., 46: 411-425.

Frei, B., L. England and B.N. Ames, 1989. Ascorbate is an outstanding antioxidant in human blood plasma. Proc. Natl. Acad. Sci. USA., 86: 6377-6381.

Fuquay, J.W., 1981. Heat stress as it affects animal production. J. Anim. Sci., 52: 164-174.

Halliwell, B. and J.M.C. Gutteridge, 1990. Role of free radicals and catalytic metal ions in human disease: An overview. Method Enzymol., 186: 1-85.

Harmon, R.J., M. Lu, D.S. Trammel and B.A. Smith, 1997. Influence of heat stress and calving on antioxidant activity in bovine blood. J. Dairy Sci., 80: 264-264.

Johnson, H.D., R. Li, W. Manula, K.J. Spencer-Johnson, B.A. Becker, R.J. Collier and C.A. Baile, 1991. Effects of somatotropin on milk yield and physiological responses during summer farm and hot laboratory conditions. J. Dairy Sci., 74: 1250-1262.

Lakritz, J., M.J. Leonard, P.A. Eichen, G.E. Rottinghaus, G.C. Johnson and D.E. Spiers, 2002. Whole-blood concentrations of glutathione in cattle exposed to heat stress or a combination of heat stress and endophyte-infected tall fescue toxins in controlled environmental conditions. Am. J. Vet. Res., 63: 799-803.

Loven, D.P., 1988. A role for reduced oxygen species in heat induced cell killing and the induction of thermotolerance. Med. Hypotheses, 26: 39-50.

McDowell, R.E., 1972. Improvement of Livestock in Warm Climates. W.H. Freeman and Co., San Francisco, CA.

Miller, J.K., E. Brzezinska-Slebodzinska and F.C. Madsen, 1993. Oxidative stress, antioxidants and animal function. J. Dairy Sci., 76: 2812-2823.

Mitchell, J.B. and A. Russo, 1983. Thiols, thiol depletion and thermosensitivity. Rad. Res., 95: 471-485.

Moran, L.K., J.M. Gutteridge and G.J. Quinlan, 2001. Thiols in cellular redox signaling and control. Cur. Med. Chem., 8: 763-772.

Motchnik, P.A., B. Frei and N. Ames, 1994. Measurement of antioxidants in human blood plasma. Method Enzymol., 234: 269-279.

Mujahid, A., Y. Yoshiki, Y. Akiba and M. Toyomizu, 2005. Superoxide radical production in chicken skeletal muscle induced by acute heat stress. Poult. Sci., 84: 307-314.

Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.

Omaye, S.T., T.D. Turnbull and H.E. Sauberlich, 1979. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods Enzymol., 62: 3-11.

Phipps, R.C., T. Madakadze, D. Mutsvangwa, L. Hard and G.D.E. Kerchove, 1991. Use of bovine somatotropin in the tropics: The effect of sometribove on milk production of *Bos indicus*, dairy crossbred and *Bos taurus* cows in Zimbabwe. J. Agric. Sci., 117: 257-263.

Radi, R., K.M. Bush, T.P. Cosgrove and B.A. Freeman, 1991. Reaction of xanthine oxidase-derived oxidants with lipid and protein of human plasma. Arch. Biochem. Biophys., 286: 117-125.

Shibata, M. and A. Mukai, 1979. Effect of heat stress and hay-concentrate ratios on milk production, heat production and some physiological responses of lactating cows. Jap. J. Zootech. Sci., 50: 630-637.

Skibba, J.L., R.H. Powers, A. Stadnicka and J.H. Kalbfleisch, 1990. Lipid peroxidation caused by hyperthermic perfusion of rat liver. Biochem. Pharmacol., 40: 1411-1414.

Tanaka, M., Y. Kamiya, M. Kamiya and Y. Nakai, 2007. Effect of high environmental temperatures on ascorbic acid, sulfhydryl residue and oxidized lipid concentrations in plasma of dairy cows. Anim. Sci. J., 78: 301-306.

Tauler, P., A. Aquilo, I. Gimeno, E. Fuentespina, J.A. Tur and A. Pons, 2003. Influence of vitamin C diet supplementation on endogenous antioxidant defense during exhaustive exercise. Eur. J. Physiol., 446: 658-664.

Vethanayagam, J.G., E.H. Green, R.C. Rose and A.M. Bode, 1999. Glutathione-dependent ascorbate recycling activity of rat serum albumin. Free Rad. Biol. Med., 26: 1591-1598.

Wayman, O., H.D. Johnson, C.P. Merilan and I.L. Berry, 1962. Effect of ad libitum or force-feedind of two rations on lactating dairy cows subject to temperature stress. J. Dairy Sci., 45: 1472-1478.

West, J.W., 1994. Interactions of energy and bovine somatotropin with heat stress. J. Dairy Sci., 77: 2091-2102.

Zalba, G., G. San Jose and M. Moreno, 2001. Oxidative stress in arterial hypertension: Role of NAD (P)H oxidase. Hypertension, 38: 1395-1399.