B-4846
[1-9]

RESEARCH ARTICLE Indian Journal of Animal Research

Hemato-biochemical, Antioxidant Alteration in Thermal Stressed Cross-bred Cows and Mitigation using Micronutrients in Sub-tropical Zone of India

Burhan Nabi[1], S.K. Gupta[1], Makhmoor Rasool[2], Sanober Rasool[3], Afaq Amin Najar[1], Sofi Imran Ul Umar[4]

10.18805/IJAR.B-4846

ABSTRACT

Background: Stress is a serious health problem in dairy cattle resulting in decreased milk production along with disturbances in reproductive functions. India is currently losing nearly 2 per cent of the total milk production, amounting to a whooping over Rs. 2,661 crore due to rise in thermal stress among lactating cattle and buffaloes because of the global warming (Upadhyay *et al.* 2007).

Methods: The present study was conducted on 18 thermal stressed cross-bred dairy cattle for a period of about 45 days for detection and amelioration of thermal stress with the objective to evaluate clinic-haemato-biochemical, oxidative stress indices. Three different groups were taken in study with each group having 6 animals.

Result: Reduced milk yield, increased physiological parameters; respiration rate, heart rate, rectal temperature, were observed in positive control animals. Cortisol a stress hormone was estimated for evaluation and amelioration of thermal stress. Haematological examination revealed decreased Hb, PCV and lymphopenia and concomitant neutrophilia in Group 1 animals. Biochemical evaluation revealed hyperglycemia, increased total protein and plasma glycine, decreased calcium, Na, K and Cl levels while as cortisol, AST, ALT levels were found to be increased in thermally stressed dairy cattle to which no supplement was given. Oxidative indices showed decreased activity of GSH, catalase, SOD activity in thermally stressed cattle which were ameliorated with micro-nutrients and mineral supplements.

Key words: Amelioration, Cortisol, Neutrophilia, Stress, Temperature humidity index.

INTRODUCTION

Thermal stress or heat stress is a severe health problem in dairy cattle causing decrease in feed intake by 6-30% and milk production reduction by 10-35% along with derangements in reproductive functions (Das *et al.* 2016). India is currently losing nearly 2 per cent of the total milk production, amounting to a whooping over Rs 2,661 crore due to rise in thermal stress among lactating cattle and buffaloes because of the global warming (Upadhyay *et al.* 2007). There is derangement in normal physio-biochemical state of body by changing metabolic rate of normal equilibrium in response to an increased change in ambient temperature *i.e.*, animal's thermal neutral zone (Belhadj Slimen *et al.* 2016; Yazgan, 2017; Umar *et al.* 2021).

When the temperature is above the thermo-neutral zone which is <72 THI, there is production of reactive oxygen species (ROS) and corticosteroids which causes oxidative stress *in-vivo* and is among various causes for derangement in the normal physiologic state (Valko *et al.* 2007). Introduction of antioxidants or/and osmolytes for reducing the heat production load directly or indirectly is a cost effective way, chromium and betaine (an osmolyte) are among such compounds which can be used to attenuate the negative effects of environmental stress in animals (Garg and Bansal, 2000) and has an important role in maintaining water homeostasis and cell integrity (Farooqi *et al.* 2005; Hassan *et al.* 2011; Klasing *et al.* 2002; Zulkifli *et al.* 2004).

[1]Division of Veterinary Medicine, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu-181 102, Jammu and Kashmir, India.

[2]Division of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu-181 102, Jammu and Kashmir, India.

[3]Division of Veterinary and Animal Husbandry Extension, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology, Kashmir-190 006, Jammu and Kashmir, India.

[4]Division of Livestock Production and Management, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu-181 102, Jammu and Kashmir, India.

Corresponding Author: Burhan Nabi, Division of Veterinary Medicine, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu-181 102, Jammu and Kashmir, India.
Email: m.ibnnabi@gmail.com

How to cite this article: Nabi, B., Gupta, S.K., Rasool, M., Rasool, S., Najar, A.A. and Umar, S.I.U. (2022). Hemato-biochemical, Antioxidant Alteration in Thermal Stressed Cross-bred Cows and Mitigation using Micronutrients in Sub-tropical Zone of India. Indian Journal of Animal Research. DOI: 10.18805/IJAR.B-4846.

Submitted: 14-12-2021 **Accepted:** 25-05-2022 **Online:** 13-06-2022

There is less data available in India of sub-tropical zone on the supply of Chromium in animal feedstuffs as well as on the level of its supplementation to have beneficial effects on animal performance in hot and humid conditions so in view of the above stated importance study was planned to mitigate the derangements caused by thermal stress on cross-bred cows and provide successful feeding strategy to combat the growing production losses due to heat stress.

MATERIALS AND METHODS

The study was conducted on the cross-bred cattle in the dairy unit of Livestock Farm Complex, F.V.Sc and A.H., SKUAST-J, R.S.Pura, Jammu and study period was from July to August.

Study design

Plan of study was done as per given below grouping, subjected to different therapeutic protocols as mentioned below:

1. Group I, kept as positive control (normal healthy cows to which no supplement was given and provided normal feed; as per NRC 2001 *viz.*, Legume forage hay, Grass Hay and no additional supplementation was given to such animals and water ad Lib.)
2. Group II, animals were given Chromium picolinate; CrPi (0.5 ppm) and Betaine (15 g/day) orally daily for 45 days in addition to normal feed as per NRC, 2001.
3. Group III, animals were given area specific mineral mixture (60 g/day) consisting of Di Calcium Phosphate-70%, Magnesium Phosphate-27%, Potassium Iodate-0.09%, Manganese sulphate-0.50%, Sodium chloride-2%, Copper Sulphate-0.50% orally daily for 45 days in addition to normal feed as per NRC, 2001.

Observations

Observations were recorded in addition to other distant physical effects, on the basis of which the animals were considered under thermal stress as follows:

Temperature humidity index (THI) which was calculated using the formula of Thom (1959):

$$THI = 0.72 (Tdb + Twb) + 40.6$$

Tdb = Dry bulb temperature (°C)

Twb = Wet bulb temperature (°C)

Based on the below given criteria animals will be categorized into thermally stressed and in comfort zone:

THI 1 (<72): Temperature: 23±1°C and Relative Humidity: 40± 5% (Zone of Comfort).

THI 2 (78): Temperature: 35±1°C and Relative Humidity: 50± 5% (Mild Stress).

THI 3 (91): Temperature: 41±1°C and Relative Humidity: 45± 5% (High Stress).

Assessment of clinico-hematobiochemical parameters.

Following clinico-hematobiochemical parameters were analysed in the thermal stressed dairy cows:

1. The physiological parameters and production parameters like rectal temp (°F), heart rate (beats/min), respiration rate (breaths/min) and milk yield (L/day) and milk constituents (milk fat, milk Protein, total solids, solid not fat) using Neumen's lactoscan milk analyzer were recorded day 0 (pre-treatment) up-to 45 days (post treatment), after every 15 days.
2. The blood was collected aseptically from the cattle of all the three groups on day 0 (pre-treatment) up-to 45 days (post treatment), after every 15 days of feeding protocol. The blood samples were collected from jugular vein (after clipping of hair and disinfection by alcohol) in K_3- EDTA, heparinized and serum vacutainers.

a. Hematological parameters as under were estimated:

i. Hemoglobin (Hb) using Drabkin's Method.

ii. Packed Cell Volume (PCV) by microhematocrit method.

iii. Differential leucocyte count (DLC) by method described by Jain (1986).

b. Biochemical parameters as under were estimated.

i. Cortisol using DetectX Cortisol Enzyme Immunoassay Kit Method®.

ii. Betaine (Glycine derivative) by Colorimetry method as per Shah *et al.* 2007.

iii. Total Protein by Biuret method.

iv. Blood Glucose using commercially available glucometer.

v. Sodium (Na) by Colorimetric Method.

vi. Potassium (K) by Colorimetric Method.

vii. Chloride (Cl) by Ferric Thiocyanate Method.

viii. Liver enzymes; Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) by using standard kits from Transasia Bio-Medicals.

ix. Calcium (Ca) OCPC method.

c. Plasma Mineral and antioxidant parameters from heparinized blood.

i. Plasma Chromium was estimated using Atomic absorption Spectrophotometer.

ii. Catalase activity was measured as per method described by the Aebi (1983).

iii. Superoxide dismutase in erythrocyte lysate was determined by method of Marklund and Marklund (1974).

iv. Glutathione peroxidase in erythrocytes was assayed by the method of Hafeman *et al.* (1974).

Statistical analysis

Data was statistically analyzed by standard method and the significance were tested using Tukeys Multiple Range test using statistical software SPSS version 20. The significance was assayed at 5% ($P<0.05$) levels; uppercase superscripts and lowercase superscripts represents significance between and within the Groups respectively.

RESULTS AND DISCUSSION

Temperature Humidity Index (THI) at the start of trial (1st week of July) averaged 82.96±0.51 which decreased non-significantly during July, August month until 5th week of experiment thereafter THI increased up to 7th week of trial which is shown below in Table 1.

Clinico-physiological parameters

The changes clinico-physiological parameters are depicted in Table 2, the clinico-physiological parameters like respiration rate, heart rate, rectal temperature in Group 1 showed elevated pattern. Liu *et al.* (2019) also found elevated pattern in these physiological parameters in heat stressed animals but our findings are in non-significant pattern, probably cause may be shorter time period for exposure to increased ambient environment temperature and also ad-lib availability of water supply. Also, there was no significant difference of rectal temperature, heart rate, respiration rate in the post-treatment values in Group 2 and 3 as compared to Group 1 animals. The respiration rate in Group 2 animals showed decreased inclination within and also than Group 1 and Group 3 animals, probably is due to the osmo-regulatory effect of betaine which helped to offset the hyperthermia. Hassan *et al.* (2011) also found significant changes in respiration rate of betaine supplemented rabbit group as compared to the control group.

The dairy animals during the present study were not exposed to that much high ambient temperature and this might be the possible cause for the non-significant changes in all the groups with respect to heart rate, rectal temperature. AnQiang *et al.* (2009) also reported same changes in heart rate, rectal temperature in Cr supplemented group as compared to control group.

Table 1: Mean values of THI ($p<0.05$).

Week	THI
1	82.96±0.51
2	82.06±1.11
3	82.09±0.84
4	81.75±0.67
5	80.83±0.77
6	82.11±0.34
7	82.19±2.02

Haematological changes seen during study

The haemoglobin and PCV values in Group 1 animals showed decreased inclination when compared with normal values post heat stress as stated in Table 3. El-Nouty *et al.* (1990) reported same changes and the cause is haemo-dilution effect in heat stressed dairy cattle, because more water is transported inside vasculature which is due to RBC lysis either by increased attack of free radicals on its membrane or insufficient nutrient availability for Hb synthesis as the animal consumes less feed or decreases voluntary intake upon increased ambient temperature.

In Group 2 and Group 3 (Table 3) animals there is decreased Hb and PCV levels followed by spike in same values but as non-significant change. Same change has been seen by the Moonsie-Shageer and Mowat (1993) after Cr supplementation in stressed feeder calves where as possible cause for the same in Group 3 might be due to the anti-oxidants in the mineral mixture which provided substrate directly or indirectly for the various scavenging enzymes *e.g.*, Mg is used in the phosphorylation of various components of enzyme system.

Regarding the leukocyte derangement in heat stressed dairy cattle we found only significant increase of neutrophils and decrease in lymphocyte and eosinophils with the increase in THI pattern in Group 1 animals (Table 3). Our results are in accordance with Peek and Divers (2008) who

Table 2: Mean values of respiration rate, heart rate, rectal temperature ($p<0.05$).

		Group 1 (n=6)	Group 2 (n=6)	Group 3 (n=6)
Respiration rate (breaths/min)	Day 0	23.17±1.22	26.17±0.79	22.83±1.85
	Day 15	22.83±1.01	21.67±0.71	21.83±1.49
	Day 30	21.67±1.31	20.33±0.95	20.50±0.99
	Day 45	24.00±1.61	17.33±0.92	20.83±1.66
Heart rate (beats/min)	Day 0	62.17±0.54	62.17±0.54	61.83±0.95
	Day 15	62.33±0.92	62.33±0.92	62.33±0.88
	Day 30	62.83±0.70	62.83±0.70	62.50±0.85
	Day 45	62.17±0.91	62.17±0.91	62.67±0.99
Rectal temperature (°F)	Day 0	103.07±0.22	102.42±0.20	102.33±0.26
	Day 15	102.90±0.22	102.65±0.25	102.52±0.27
	Day 30	102.87±0.21	102.87±0.21	102.87±0.21
	Day 45	103.08±0.21	102.95±0.19	102.77±0.24
Parameters		**Group 1 (n=6)**	**Group 2 (n=6)**	**Group 3 (n=6)**
Hb	Day 0	11.57±0.44	11.10±0.85	11.27±0.68
	Day 15	9.78±0.43	10.35±0.69	10.23±0.71
	Day 30	9.81±0.49	10.57±0.78	10.37±0.63
	Day 45	9.43±0.21	10.77±0.55	10.50±0.46
PCV	Day 0	34.70±1.33	33.17±2.54	33.83±2.09
	Day 15	29.33±1.29	31.17±2.02	30.67±2.09
	Day 30	29.44±1.47	31.67±2.38	31.00±1.93
	Day 45	28.28±0.63	32.33±1.71	31.50±1.43

Table 3: Haematological parameters in three different groups (p<0.05).

	Group 1 (n=6)					Group 2 (n=6)					Group 3 (n=6)				
	L	N	M	E	B	L	N	M	E	B	L	N	M	E	B
Day 0	55.17±1.17	30.00±1.34	5.17±0.48	8.33±0.67	1.33±0.33	55.17±0.98	29.00±0.93	5.17±0.95	9.17±0.70	1.50±0.22	55.00±1.03	30.33±1.15	4.83±0.83	8.50±0.43	1.33±0.21
Day 15	56.17±0.60	28.67±0.88	5.50±0.50	8.50±0.76	1.17±0.17	57.33±0.71	27.17±0.87	5.00±0.37	9.33±0.71^{Aa}	1.17±0.17	56.17±1.74	28.83±1.08	4.67±0.71	8.83±0.48	1.50±0.34
Day 30	57.50±0.92	26.67±0.56	5.33±0.67	9.33±0.76	1.17±0.17	58.67±1.28	24.17±1.33	5.50±0.92	10.33±0.71	1.33±0.33	57.33±0.49	27.00±0.82	4.83±0.87	9.17±0.48	1.67±0.21
Day 45	54.83±0.83	31.00±0.86	5.33±0.49	7.83±0.48	1.00±0.26	60.50±0.85	22.83±1.22	5.33±0.95	10.67±0.67	1.00±0.26	58.00±0.58	26.00±0.77	5.00±0.37	9.67±0.67	1.33±0.21

found stress and glucocorticoids reliably alter the leukogram to create neutrophilia, lymphopenia and eosinopenia. Other likely cause for lymphopenia is the result of an immediate shift of lymphocytes from the circulating blood to other tissues, but the specific location is unknown. Other than this heat stress leads to increased secretion of glucocorticoid hormone which can induce apoptosis of lymphoid cells in mammals as stated by Schwartzman and Cidlowski (1994) while as neutrophilia might be due to a relative shift of neutrophils into circulating blood most likely contributing to this change.

The mitigation of heat stress in Group 2 and Group 3 using chromium as main micronutrient supplementation and copper and Manganese as main mineral supplements in mixture respectively has aided to reverse the changes in leucogram caused by heat stress (Table 3). Burton *et al.* (1993) also found improved lymphocyte proliferation in Cr supplemented calves and increased immune-stimulatory effects thereby ameliorating the stress leukogram which can be correlated with the decrease in the cortisol concentration. As the heat stress causes increased oxidative stress which has negative effects on leukogram, Cu and Mn are integral parts of blood antioxidant oxide dismutase, when these metals are supplied through feed in sufficient quantities, suitable antioxidant enzymes are produced in the body which ameliorate the effect of oxidative stress and thereby stress leukogram.

Changes in milk parameters after heat stress and its amelioration

In Group 1 animals there is decrease in the milk production with the increase in the THI as shown in Table 4. Previous workers like Al Reyad *et al.* 2016 reported significant decrease in milk production and significant changes in milk composition during hot ambient temperature. The cause for the decrease in milk yield might be due to a reduction in DMI which decreases the nutrients accessible for milk synthesis.

In our study the THI happened to be between moderate ranges which can be one possible cause for on-specific changes in the milk constituents despite decrease in milk production with the change in THI.

Al-Saiady *et al.* 2004 investigated effect of chelated chromium effect on the milk yield and milk composition in Holstein cows under thermal stress. The increased milk yield can possibly be described by higher dry matter intake (DMI) and proficiency of energy utilization by Cr supplementation. In our present study same effect has been seen in Group 2 animals (Table 4) with significant change in milk yield after supplementation with chromium when compared with the ongoing increase in THI.

In Group 3 animals there is positive effect on milk yield post heat stress but the changes were not significantly remarked (p<0.05) which makes our findings contrary to the West *et al.* 1991 who found feeding diets that have a high dietary cation-anion difference (DCAD) improved DMI and milk yield, our results are probably such due to the short

Table 4: Milk and milk constituents in heat stressed dairy cattle ($p<0.05$).

Parameters		Group 1 (n=6)	Group 2 (n=6)	Group 3 (n=6)
Milk yield (L/day)	Day0	7.6±0.4	7.4±0.30	7.7±0.4
	Day15	7.5±0.3	8.3±0.2	7.7±0.2
	Day30	7.7±0.2	8.5±0.3	7.9±0.2
	Day45	7.4±0.2	8.6±0.3	7.9±0.2
Total milk fat (%)	Day0	3.22±0.11	3.12±0.14	3.25±0.08
	Day15	3.13±0.04	3.17±0.10	3.35±0.08
	Day30	3.02±0.05	3.25±0.11	3.43±0.06
	Day45	3.05±0.05	3.27±0.08	3.47±0.09
SNF (%)	Day0	8.15±0.03	8.28±0.08	8.26±0.07
	Day15	8.01±0.05	8.30±0.11	8.27±0.10
	Day30	7.98±0.05	8.32±0.13	8.28±0.12
	Day45	7.92±0.06	8.41±0.17	8.30±0.10
Total solids (%)	Day0	11.36±0.13	11.40±0.14	11.51±0.10
	Day15	11.15±0.06	11.47±0.13	11.62±0.17
	Day30	11.00±0.07	11.57±0.21	11.71±0.13
	Day45	10.97±0.08	11.67±0.18	11.77±0.07
Milk total protein (%)	Day0	3.13±0.04	3.10±0.06	3.09±0.05
	Day15	3.10±0.03	3.14±0.04	3.12±0.02
	Day30	3.07±0.03	3.15±0.04	3.14±0.01
	Day45	3.08±0.03	3.15±0.01	3.14±0.02

period for supplementation of minerals under hot ambient temperature.

Changes in biochemical values with respect to increased THI

In Group 1 animals there is increased cortisol concentration due to positive effect of increased ambient temperature on hormone secretion (Table 5) and this is in agreement with Aggarwal and Upadhyay, (2013). Concurrently, the cortisol concentration in Group 2 animals decreased significantly with the mitigation by micro-nutrient chromium supplementation and betaine supplementation, Chang and Mowat (1992) and Moonsie-Shageer and Mowat (1993); Anne-Marie *et al.* (2012); Alirezaei *et al.* (2014) also found decreased serum cortisol concentration in Cr methionine and wheat bran supplemented group calves. Besides this in Group 3 animals, cortisol concentration was decreased post supplementation with the increased THI values due to the effect of the minerals which play an important role in immune status of animal and help in combating negative effects of free radical generation by providing substrate for production of suitable antioxidant enzymes which ameliorate the effect of stress. Same findings have been reported by Aggarwal and Upadhyay, (2013).

Blood glucose Group 2 animals decreased significantly as compared to the Group 1 animals (Table 5). Our findings are in consonance with the findings of Vincet (2000). The decreased blood glucose may be due to increased potency of insulin to its receptors by chromium. Similar changes in glucose level has been reported by Chang *et al.* (1996) who observed lower serum glucose concentration in chromium nicotinate supplemented group.

Same change has been seen in Group 3 animals with the increasing THI and our findings are in agreement with Aggarwal and Upadhyay, (2013) who suggested Cu and Mn play an important role in immune system and carbohydrate and lipid metabolism thereby fading the effect of stress hormone cortisol which has positive correlation with glucose.

High environmental temperature cause decrease in total plasma protein in lactating cattle which was seen in our study at peak THI values in Group 1 (Table 5), Podar and Oroian (2003) also found same results in lactating cattle. In Group 2 animals there is increased total protein concentration over increasing THI which I supported by Huang *et al.* 2006 who also found betaine supplementation (0.125% to finishing 25 pigs) increased serum basal concentrations of total protein by 9%. The decrease in protein in both the groups might be partly due to the utilization of protein in cell repair and tissue organization with the formation of lipoproteins which are important cellular constituents.

Sodium level, potassium level, chloride levels of Group 2 and Group 3 animals increased as compared to Group 1 animals (Table 5) and were in accordance with findings of Maltz *et al.* 1994. Moeckel *et al.* (2002) studied the effect of betaine and suggested betaine inhibits the activity of Ca^{2+} and Na^{+}/K^{+} ion pumps by 73 and 64%, respectively. Thus, it can be assumed that the accumulation of betaine in intestinal tissues might control water movement by decreasing the activity of water pumps in Group 2 animals. Moreover, the calcium levels also increased in Group 2 and Group 3 animals as compared to stressed ones which is supported by the findings of Moonsie-Shageer and Mowat (1993).

Plasma Cr (ppb) in Group 2 animals increased as compared to Group 1 animals and our results are in

Table 5: Biochemical profile in three different groups with change in THI ($p<0.05$).

Parameters		Group 1 (n=6)	Group 2 (n=6)	Group 3 (n=6)
Cortisol (µg/dl)	Day 0	0.81±0.03	0.83±0.04	0.89±0.07
	Day 15	0.92±0.07	0.72±0.05	0.89±0.07
	Day 30	0.97±0.04	0.65±0.03	0.83±0.08
	Day 45	0.98±0.03	0.62±0.04	0.78±0.05
Glucose (mg/dl)	Day 0	70.83±2.48	69.17±2.14	67.17±2.71
	Day 15	72.33±2.29	65.50±2.19	65.67±2.38
	Day 30	74.50±2.43	61.67±2.23	63.50±2.67
	Day 45	75.33±2.38	59.50±2.50	61.00±2.59
Total protein (g/dl)	Day 0	7.72±0.09	7.78±0.06	7.68±0.09
	Day 15	7.70±0.09	7.56±0.07	7.50±0.06
	Day 30	7.68±0.07	7.33±0.07	7.37±0.04
	Day 45	7.70±0.07	7.17±0.06	7.25±0.09
Na (mmol/L)	Day 0	138.70±2.25	139.97±2.53	138.05±2.31
	Day 15	142.23±2.11	139.97±2.53	139.13±2.48
	Day 30	140.52±2.13	146.73±2.05	141.28±2.14
	Day 45	139.85±2.23	147.22±2.01	142.72±2.17
K (mmol/L)	Day 0	4.18±0.08	4.20±0.06	4.16±0.07
	Day 15	4.07±0.07	4.82±0.15	4.42±0.11
	Day 30	4.05±0.07	4.97±0.17	4.65±0.12
	Day 45	4.12±0.03	5.10±0.18	4.77±0.05
Cl (mmol/L)	Day 0	98.56±1.67	100.92±1.32	103.00±1.67
	Day 15	96.68±0.57	101.81±1.54	103.02±1.48
	Day 30	97.11±0.33	105.30±1.22	103.13±1.11
	Day 45	97.49±0.70	105.96±0.73	103.77±0.67
ALT (U/L)	Day 0	37.12±0.62	37.29±0.54	37.18±0.82
	Day 15	38.27±0.59	33.31±0.83	36.31±0.27
	Day 30	37.71±0.63	32.53±0.55	36.16±0.95
	Day 45	37.67±0.32	29.24±0.71	35.79±1.21
AST(U/L)	Day 0	98.77±3.20	97.46±3.19	97.98±3.96
	Day 15	106.86±2.87	97.11±3.72	96.88±3.18
	Day 30	104.41±2.65	96.22±3.79	96.21±3.79
	Day 45	105.80±2.85	96.31±3.74	95.84±3.14
Cr (ppb)	Day 0	0.18±0.02	0.18±0.02	0.22±0.02
	Day 15	0.20±0.01	0.26±0.02	0.21±0.02
	Day 30	0.20±0.02	0.41±0.02	0.21±0.01
	Day 45	0.21±0.01	0.44±0.02	0.21±0.02
Ca (mg/dl)	Day 0	9.37±0.08	9.35±0.13	9.42±0.06
	Day 15	9.72±0.09	9.60±0.17	9.52±0.15
	Day 30	9.53±0.11	10.02±0.22	10.04±0.37
	Day 45	9.52±0.10	10.82±0.19	10.13±0.28
Glycine (µg/ml)	Day 0	165.9±3.1	165.3±3.2	166.7±3.3
	Day 15	166.9±4.0	165.9±3.6	167.3±3.9
	Day 30	174.3±4.9	166.4±3.9	167.3±3.9
	Day 45	180.7±6.2	167.9±3.9	169.7±3.5

agreement with Spears (1999). Supplementation of Cr resulted in dose-dependent linear increase in tissue Cr concentration which is in agreement with the study conducted by Spears (1999).

Liver enzymes ALT and AST in Group 2 animals decreased as compared to Group 1 animals which is in agreement with Nazifi *et al.* 2003 who found similar results in fat tailed sheep during high ambient temperature. The increase in the serum enzymes is probably due to the fact that enzymes are surrounded by cellular membrane, so they could not easily get across cellular membrane into blood in normal condition. But extreme condition, such as heat stress could change the cellular membrane permeability, so enzyme activity increased when animals were under heat

Table 6: Status of Metalloenzymes in three different groups with change in THI ($p<0.05$).

Parameters		Group 1 (n=6)	Group 2 (n=6)	Group 3 (n=6)
SOD	Day 0	7.41±0.49	7.61±0.78	7.57±0.61
	Day 15	5.07±0.66	9.45±1.01	8.03±0.75
	Day 30	3.76±0.79	12.53±0.99	9.57±0.61
	Day 45	3.05±0.52	14.53±0.66	10.81±0.85
GPx	Day 0	2.18±0.15	2.17±0.19	2.10±0.09
	Day 15	1.05±0.16	2.11±0.12	1.82±0.14
	Day 30	0.87±0.21	2.35±0.12	2.07±0.20
	Day 45	0.76±0.12	2.60±0.16	2.16±0.20
Catalase	Day 0	41.90±1.71	43.40±1.97	42.52±1.15
	Day 15	29.25±1.69	42.82±2.19	34.10±1.65
	Day 30	24.18±1.07	48.75±3.45	40.27±2.83
	Day 45	22.63±1.58	55.36±2.16	46.87±3.18

stress which is in agreement with Li *et al.* (2001). Sahin *et al.* (2002a, b, 2005) reported chromium picolinate supplementation (CrPic) linearly decreased serum corticosterone concentration which is possible cause for decrease in membrane degradation.

Status of metalloenzymes and their amelioration

Metalloenzymes which include superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase in Group 2 animals and Group3 animals showed significant change as compared to Group 1 animals (Table 6). Our Study is in consonance with Anne-Marie *et al.* 2012 that chromium may act as an indirect antioxidant by decreasing high levels of insulin and preventing auto-oxidation of glucose. Bhat *et al.* 2008 also reported decreased levels of metalloenzymes due high atmospheric temperature and humidity of summer which increases neuroendocrine stress and lipid peroxidation which in turn contributes to the reduced erythrocyte antioxidant response which was seen in Group 1 animals in our study. As already mentioned in the preceding sections heat stress causes increased oxidative stress and Cu and Mn being integral parts of blood antioxidants, when these metals are supplied through feed in sufficient quantities, suitable antioxidant enzymes are produced in the body which ameliorate the effect of oxidative stress which can be seen in the values of Group 3 animals.

CONCLUSION

Thermal stress is a serious health problem in dairy cattle resulting in decreased milk production along with disturbances in reproductive functions directly or indirectly associated with immunosuppression and environmental impact on physiological processes. Effective supplementation of micronutrients and minerals should be encouraged during the changing increased ambient environmental temperature which would thereby mitigate the negative effects on the health and productivity of animals and thus economic stability for dairy sector.

Conflict of interest: None.

REFERENCES

Aebi, H.E. (1983). Catalase. In: Methods in Enzymatic analysis. Academic Press. [Bergmeyer, H.U. (ed.)], New York. pp. 276-286.

Aggarwal, A. and Upadhyay, R. (2013). Heat Stress and Animal Productivity, Springer India. 113.

Al Reyad, M., Sarker, M.A.H., Uddin, M.E., Habib, R. and Rashid, M.H.U. (2016). Effect of heat stress on milk production and its composition of Holstein Friesian crossbred dairy cows. Asian Journal of Medical and Biological Research. 2(2): 190-195.

Alirezaei, M., Jelodar, G., Ghayemi, Z. and Mehr, M.K. (2014). Antioxidant and methyl donor effects of betaine versus ethanol-induced oxidative stress in the rat liver. Comparative Clinical Pathalogy. 23: 161-168.

Al-Saiady, M.Y., Al-Shaikh, M.A., Al-Mufarrej, S.I., Al-Showeimi, T.A., Mogawer, H.H. and Dirrar A. (2004). Effect of chelated chromium supplementation on lactation performance andblood parameters of Holstein cows under heat stress. Animal Feed Science and Technology. 117: 223-233.

Anne-Marie, R.A.M., Hininger, I., Benaraba, R.M.S., Ziegenfuss, T.N. and Anderson, R.A. (2012). Antioxidant Effects of a Cinnamon Extract in People with Impaired Fasting Glucose That Are Overweight or Obese. Journal of the American College of Nutrition. 28(1): 16-21.

An Qiang, L., Zhi Sheng, W. and An Guo, Z. (2009). Effect of chromium picolinate supplementation on early lactation performance, rectal temperatures, respiration rates and plasma biochemical response of Holstein cows under heat stress. Pakistan Journal of Nutrition. 8: 940-945.

Belhadj Slimen, I., Najar, T., Ghram, A. and Abdrrabba, M. (2016). Heat stress effects on livestock: molecular, cellular and metabolic aspects: A review. Journal of Animal Physiology and Animal Nutrition. 100(3): 401-412.

Bhat, S., Rao, G., Murthy Dilip, K. and Bhat Gopalakrishna, P. (2008). Seasonal variations in markers of stress and oxidative stress in rats. Indian Journal of Clinical Biochemistry. 23(2): 191-194.

Burton, J.L., Mallard, B.A., Mowat, D.N. (1993). Effects of supplemental chromium on immune responses of periparturient and early lactation dairy cows. Journal of Animal Science. 71: 1532-1539.

Chang, X., Mallard, B.A., Mowat, D.N. (1996). Effects of chromium on health status, blood neutrophil phagocytosis and *in vitro* lymphocyte blastogenesis of dairy cows. Veterinary Immunology and Immunopathology. 52: 37-52.

Chang, X. and Mowat, D.N. (1992). Supplemental chromium for stressed and growing feeder calves. Journal of Animal Science. 70: 559-565.

Das, R., Sailo, L., Verma, N., Bharti, P., Saikia, J., Imtiwati and Kumar, R. (2016). Impact of heat stress on health and performance of dairy animals: A review. Veterinary World. 9(3): 260-268.

El-Nouty, F.D., A l-Haidary, A.A., Salah, M.S. (1990). Seasonal variation in hematological values of high- and average yielding Holstein cattle in semi-arid environment. Journal of King Saud. University. 2: 173-182.

Farooqi, H.A.G., M.S. Khan, M.A. Khan, M. Rabbani, K. Pervez and J.A. Khan. (2005). Evaluation of betaine and vitamin C in alleviation of heat stress in broilers. International Journal of Agriculture and Biology. 5: 744-746.

Garg, M.C. and Bansal, D.D. (2000). Protective antioxidant effect of vitamins in streptozotocin induced stress in diabetic rats. Indian Journal of Experimental Biology. 38: 101-104.

Hafeman, D.G., Sunde, R.A. and Hoekstra, W.G. (1974). Effect of dietary selenium on erythrocyte and glutathione peroxidase in rats. Journal of Nutrition. 104: 580-587.

Hassan, R.A., Ebeid, T.A., Abd El-Lateif, A.I. and Ismail, N.B. (2011). Effect of dietary betaine supplementation on growth, carcass and immunity of New Zealand White rabbits under high ambient temperature. Livestock Science. 135: 103-109.

Huang, Q., Xu, Z., Han, X. and Li, W. (2006). Changes in hormones, growth factor and lipid metabolism in finishing pigs fed betaine. Livestock Science. 105: 78-85.

Jain, N.C. (1986). Schalm's Veterinary Hematology (No. Edition 4). Lea and Febiger.

Klasing, K.C., Adler, K.L., Remus, J.C. and Calvert, C.C. (2002). Dietary betaine increases intraepithelial lymphocytes in the duodenum of coccidia-infected chicks and increases functional properties of phagocytes. Journal of Nutrition. 132: 2274-2282.

Li, J.J., Sang, R.Z., Tian, S.J., Ma, Y.B. and Z.M. Zhou, (2001). Heat stress and variation of blood component in cattle. Ecology of Domestic Animal. 22: 56-59.

Liu, J., Li, L., Chen, X., Lu, Y. and Wang, D. (2019). Effects of heat stress on body temperature, milk production and reproduction in dairy cows: A novel idea for monitoring and evaluation of heat stress: A review. Asian-Australasian Journal of Animal Sciences. 32(9): 1332-1339.

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

Marklund, S. and Marklund, G. (1974). Involvement of superoxide anion radical in the auto oxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry. 47: 469-474.

Moeckel, G.W., Shadman, R., Fogel, J.M. and Sadrzadeh, S.M.H. (2002). Organic osmolytes betaine, sorbitol and inositol are potent inhibitors of erythrocyte membrane ATPases. Life Sciences. 71: 2413-2424.

Moonsie-Shageer, S., Mowat, D.N. (1993). Effect of level of supplemental chromium on performance, serum constituents and immune status of stressed feeder calves. Journal of Animal Science. 71: 232-238.

Nazifi, S., Saeb, M., Rowghani, E. and Kaveh, K. (2003). The influences of thermal stress on serum biochemical parameters of Iranian fat-tailed sheep and their correlation with triiodothyronine (T3), thyroxine (T4) and cortisol concentrations. Comparative Clinical Pathology. 12(3): 135-139.

Peek, S.F. and Divers, T.J. (2008). Metabolic Diseases. In: Rebhun's Diseases of Dairy Cattle. [Saunders, W.B. (ed.)]. pp. 590-605.

Podar, C., Oroian, I. (2003). The influence of high temperature on milk cows. Buletinul Universitatii-de-Stiinte-Agricole-si-Medicina-Veterinara-Cluj-Napoca,-SeriaZootehniesi Biotehnologii. 59: 130-133.

Sahin, K., Ozbey, O., Onderci, M., Cikim, G. and Aysondu, M.H. (2002b). Chromium supplementation can alleviate negative effects of heat stress on egg production, egg quality and some serum metabolites of laying Japanese quail. Journal of Nutrition. 132: 1265-1268.

Sahin, K., Sahin, N., Onderci, M., Gursu, F. and Cikim, G. (2002a). Optimal dietary concentration of chromium for alleviating the effect of heat stress on growth, carcass qualities and some serum metabolites of broiler chickens. Biological Trace Elements Research. 89: 53-64.

Sahin, N., Sahin, K., Onderci, M., Gursu, M.F., Cikim, G., Vijaya, J. and Kucuk, O. (2005). Cr picolinate, rather than biotin, alleviates performance and metabolic parameters in heat-stressed quail. British Poultry Sciences. 46: 457-463.

Schwartzman, R.A. and Cidlowski, J.A. (1994). Glucocorticoid-induced apoptosis of lymphoid cells. International Archives of Allergy and Immunology. 105(4): 347-354.

Shah, S.A., Rathod, I. S. and Kanakia, D. (2007). Colorimetry method for estimation of glycine, alanine and isoleucine. Indian Journal of Pharmaceutical Sciences. 69(3): 462-464.

Spears, J.W. (1999). Reevaluation of the metabolic essentiality of the minerals: Review. Asian-Australasian Journal Animal Sciences. 12: 1002-1008.

Umar, S., Konwar, D., Khan, A., Bhat, M.A., Javid, F., Jeelani, R., Nabi, B., Najar, A.A., Kumar, D. and Brahma, B. (2021). Delineation of temperature-humidity index (THI) as indicator of heat stress in riverine buffaloes (*Bubalus bubalis*) of a sub-tropical Indian region. Cell stress and Chaperones. 26(4): 657-669. https://doi.org/10.1007/s12192-021-01209-1.

Upadhyay, R.C., Singh, S.V., Kumar, A., Gupta, S.K. and Ashutosh. (2007). Impact of climate change on impact of Murrah buffaloes. Indian Journal of Animal Sciences. 6: 1329-1332.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M. and Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. The International Journal of Biochemistry and Cell Biology. 39(1): 44-84.

Vincent, J.B. (2000). The biochemistry of chromium. Journal of Nutrition. 130: 715-718.

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

Yazgan, K. (2017). Determining heat stress effect in Holstein dairy cattle using daily milk yield and meteorological data obtained from public weather station in Sanliurfa province of Turkey. Indian Journal of Animal Research. 51(6): 1002-1011.

Zulkifli, I., Mysahra, S.A. and Jin, L.Z. (2004). Dietary supplementation of betaine [Betafin (R)] and response to high temperature stress in male broiler chickens. Asian-Aust. Journal of Animal Sciences. 17: 244-249.