Indian Journal of Animal Sciences **92** (7): 858–864, July 2022/Article
https://doi.org/10.56093/ijans.v92i7.109736

Effect of dietary chromium, vitamin E and selenium supplementation on biochemical and physiological parameters of Holstein Friesian cows under heat stress

J RAZIA SULTANA[1✉], A SARAT CHANDRA[1], T RAGHUNANDAN[1], M GNANA PRAKASH[1], M VENKATESWARLU[1] and D B V RAMANA[2]

PV Narsimha Rao Telangana Veterinary University, Hyderabad, Telangana 500 030 India

Received: 21 January 2021; Accepted: 2 May 2022

ABSTRACT

A study was carried out for a period of 90 days during the summer season with the inclusion of chromium propionate and vitamin E with selenium, in the feed to investigate their effect on 24 uniparous lactating Holstein Friesian cows in an early phase of lactation. Cows were randomly assigned to four supplemental doses of 0 (T0); 500 IU/animal/day of vitamin E and 0.3 mg/kg DM of selenium (T1); 0.5 mg/kg DM of chromium propionate (T2) and 0.5 mg/kg DM of chromium propionate, 500 IU/animal/day of vitamin E and 0.3 mg/kg DM of selenium (T3). The mean THI value in the morning was 75.10±0.42 and 80.01±0.64 in the afternoon indicating that the animals were under mild to moderate heat stress. Increase in the DMI was found in the supplemented groups with a significant difference between control and T3 group. Milk yield differed significantly between the control and supplemented groups. The hematological profile did not vary significantly among the groups. Supplementation of chromium along with vitamin E and selenium decreased the cortisol level and glutathione peroxidase activity signigicantly in the T3 group. Chromium supplemented groups (T2 and T3) showed greater increase in mean total protein, albumin concentration and A/G ratio. However, the mean values for thyroid profile, liver enzymes, superoxide dismutase, catalase, globulin, total cholesterol, triglycerides, glucose, urea, creatinine were comparable among the four groups. Statistical analysis revealed that the rectal temperature, respiratory rate, and pulse rate in supplemented groups were significantly reduced. The findings showed that chromium supplementation plays a significant role in alleviating thermal stress and is further more beneficial when supplemented with proven antioxidants vitamin E and selenium.

Keywords: Chromium propionate, Dairy cows, Heat stress, Holstein Friesian, Selenium, Vitamin E

Climate change influences the sustainability of the livestock production system. A major part of our country is characterized as humid tropic and is exposed to extended intervals of humidity and high ambient temperature. High air temperature and humid climate lead to an imbalance between the metabolic heat output inside the animal body and its dissipation to the surroundings resulting in heat stress (Das *et al*. 2016). Cows decrease their feed intake to mitigate heat stress (Ammer *et al*. 2018) thereby leading to a gradual decline in milk production (Liu *et al*. 2019). Heat stress has several direct and indirect effects on dairy animal health. It can disrupt normal metabolism as well as affect the physiological, hormonal, and immune status of animals (Abd El-Hack *et al*. 2019, Saeed *et al*. 2019). In addition, heat stress increases antioxidant enzyme activities, namely superoxide dismutase, catalase and glutathione peroxidase in response to increased reactive oxygen species levels (Belhadj *et al*. 2016). Increased environmental temperature also effects other serum parameters like serum protein concentration, urea, creatinine, glucose, cholesterol, etc. affecting the general well-being and performance of the animals.

Nutritional changes may support animals to retain homeostasis or avoid nutrient deficiencies that result from heat stress. It is well known that vitamin E and selenium can scavenge the free radicals resulting in reduced oxidative stress. The micronutrients vitamin E and selenium have a joint biological function and it is established that they act synergistically and affect biological processes, mainly antioxidant and immunity (Dalia *et al*. 2018). Chromium is one of the important micronutrients which plays an important role in metabolism of ruminants (Lashkari *et al*. 2018). Chromium restores productivity, performance, antioxidant profile and immune status while decreasing lipid peroxidation in animals after exposure to heat stress (Bin-Jumah *et al*. 2020). Chromium can be added at levels up to 0.50 mg Cr/kg DM (Spears 2010, Lloyd *et al*. 2010). Requirement of supplemental vitamin E for lactating cows is 500 IU (Spears 2011) and selenium at the rate of 300 µg/kg DM (Mehdi and Dufrasne 2016). Now-a-days, chromium is gaining worldwide importance

Present address: [1]PV Narsimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, Telangana. [2]ICAR-Central Research Institute for Dryland Agriculture, Hyderabad, Telangana. ✉Corresponding author email: israrazia@gmail.com

and is being incorporated in the animals diet to combat the adverse effects of heat stress. Hence, the current research was conducted to examine the effect of chromium and vitamin E with selenium on biochemical and physiological parameters of Holstein Friesian cows under heat stress.

MATERIALS AND METHODS

Site of study: The current research was performed at Kapila Agro Farm, a commercial dairy farm situated at Timmareddypally village of Kondapak Mandal in Siddipet district, Telangana State, located at 17° 58' 25.7" North and 78° 51' 56.5" East, at 547 m altitude above sea level. Analysis of blood and feed sample was carried out at ICAR-CRIDA (Central Research Institute for Dryland Agriculture), Santoshnagar, Hyderabad.

The study was carried out in accordance with the regulations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and approved by the Institutional Animal Ethical Committee of College of Veterinary Science, Hyderabad with No.I/2018/-7/IAEC/ C,V,Sc, Hyd., dated 16/07/2018.

Animals and diet: The trial was conducted in the year 2017 for a period of 90 days during the peak period of summer season (April 15 to July 15) on 24 uniparous lactating Holstein Friesian cows in their early stages of lactation (56.54±4.15 days) with an average body weight of 450.92±2.87 kg, and producing an average of 16.63±0.22 kg of milk per day with a butterfat content of 3.70±0.04. Cows were randomly divided into four dietary categories of six animals each considering the stage of lactation. T0 group received a basal diet, T1 was supplemented with 500 IU/animal/day of vitamin E and 0.3 mg/kg of DM Selenium; T2 received 0.5 mg/kg DM of chromium propionate, and T3 was supplemented with 0.5 mg/kg DM of chromium propionate, 500 IU/animal/day of vitamin E and 0.3 mg/kg DM of selenium. All the cows were housed in well ventilated hygienic stalls and stall-fed throughout the experimental period. TMR was configured to provide all the nutrients required to satisfy the maintenance and production requirements as recommended by ICAR (2013). The TMR contained chopped paddy straw, maize green (sweet corn with cobs and grains in milk stage) and concentrate mixture with roughage: concentrate ratio of 70:30. The ingredient composition of the concentrate mixture fed to the animals is given in Table 1. All the groups were fed with TMR diet thrice, i.e at 5.30 AM, 10.30 AM and 4.00 PM during the experimental period. Chromium propionate, vitamin E and selenium were supplemented to the individual animal daily in the morning in the form of a jaggery bolus. All the time, the cows had free acess to fresh and wholesome clean drinking water.

Table 1. Composition (%) of concentrate mixture for Holstein Friesian cows

Ingredient	g/kg
Maize	30.0
Soybean meal	9.0
Cotton seed meal	10.0
Rapeseed meal	15.0
Deoiled rice bran	20.0
Distiller's dried grains with soluble (?)	6.0
Urea	0.7
Fat boost	2.5
Limestone powder	1.8
Salt	1
Mineral mixture	2
Sodium bicarbonate	2
	100

Temperature humidity index (THI): For THI calculation, ambient air temperature and humidity were measured using dry bulb and wet-bulb thermometer twice a day at 10.00 AM and 3.00 PM placed about 1.5 m above the ground level. The level of heat stress was determined based on the mean THI values calculated. THI was estimated for the entire study period as per Bianca (1962).

$$THI = (0.35\times T_{db} + 0.65\times T_{wb}) \times 1.8 + 32$$

where, T_{wb}, temperature of Wet bulb (^{0}C); T_{ub}, temperature of Dry bulb.

Feed intake and milk yield: Feed offered and feed refusal were monitored thrice weekly for each group of cows and feed intake was calculated according to the DM content of the diet. Cows were completely milked with milking machine twice daily at 5.00 AM and 5.00 PM throughout the experimental period and the daily milk yield was recorded from each cow.

Blood collection: Blood samples from the jugular vein of all the experimental animals were obtained fortnightly, prior to the morning feeding with the help of sterilized needles. Each time two blood samples were obtained from every animal, one sample of 5 ml in heparinized vacutainer, for hematology and the other (10 ml) in the vacutainer coated with clot activator for serum separation and was sent to the laboratory for further use.

Measurement of physiological responses: Physiological responses including rectal temperature (RT), respiration rate (RR) and pulse rate (PR) of all the experimental animals were recorded at 10.00 AM and 3.00 PM during the entire length of the experimental period of three months. The rectal temperature was measured by inserting a clinical thermometer per rectum and observation was recorded after 1 min. A flank movement was observed for 1 min to record the respiration rate, in that each movement in and out of the flank being recorded as one full breath. The pulse rate of the animals was reported by observing the pulsation of a center coccygeal artery at the bottom of the tail.

Chemical and statistical analysis: Samples of TMR were collected, dried over-night at 100±5°C in a hot air oven, and then ground in laboratory Willey mill and stored for further use in airtight containers. The proximate analysis of feeds was carried out as per the procedures described by AOAC (2005). Statistical data analysis was performed according to Snedecor and Cochran's (1994) recommended procedures. Least-square analysis of variance was used to test the significance of various treatments and the difference between treatment means was tested for significance by Duncan's new multiple range and F Test (Duncan 1955).

Table 2. Chemical composition of experimental feed (% DM) fed to the Holstein Friesian cows

Chemical composition	TMR	Concentrate mixture	Paddy straw	Maize
Proximate composition				
DM	53.82	93.61	95.35	23.96
OM	86.59	86.56	85.54	92.67
CP	17.19	21.25	3.40	8.68
EE	1.48	2.27	1.24	1.91
CF	13.31	8.32	31.68	25.12
TA	13.41	13.44	14.46	7.33
NFE	54.61	54.72	49.22	56.96
Van Soest fibre fractions				
NDF	59.91	60.12	83.56	75.99
ADF	21.88	21.03	51.06	37.70

RESULTS AND DISCUSSION

Chemical composition of feedstuffs: The chemical composition of experimental rations fed to the lactating H F cows is given in Table 2. The crude protein per cent in the total mixed ration fed to the lactating cows was 17.19 on the basis of dry matter. In the present study, the CP values for the maize fodder green and paddy straws were 8.68 and 3.4 %, respectively.

Environmental conditions and microenvironment: THI is a calculated index that incorporates the effects of environmental temperature with relative humidity to assess the heat stress. According to Armstrong (1994), THI<71 is a thermal comfort zone, 72 to 79 is mild heat stress, 80 to 90 is moderate heat stress and >90 is severe heat stress. The mean THI value for 90 days was 75.10±0.42 in the morning and 80.01±0.64 in the afternoon indicating the animals were under mild to moderate stress. High THI was found in the month of May with a THI value of 83.48±1.41 in the afternoon. Kumar *et al*. (2016), who recorded THI 88.80 in the month of May, noted similar findings.

Feed intake: The average dry matter intake (DMI) was 13.22±0.13, 13.48±0.11, 13.56±0.12 and 13.63±0.11 kg/d, respectively in lactating cows fed experimental diets T0, T1, T2 and T3. An increase of 0.26 kg (P>0.05), 0.34 kg (P>0.05) and 0.41 kg (P<0.05) DMI was observed in the supplemented groups T1,T2 and T3, respectively, compared to control group. Chauhan *et al*. (2014) also reported an increase in feed intake (P<0.01) with the supplementation of vitamin E and selenium in animals during heat stress compared to those unsupplemented, indicating a positive effect of supplementation. Kargar *et al*. (2018) and Pantelic *et al*. (2018) suggested that chromium requirement rise during thermal heat load due to increased chromium urinary excretion, which in turn result in diminished feed intake. Results recorded by Jin *et al.* (2012) and Deka *et al.* (2015) showed improvement in DMI with chromium supplementation revealing the beneficial impact of chromium on DMI in early lactating cows under heat stress.

Milk yield: The average milk yield in T0, T1, T2 and T3 groups was 15.73±0.12, 16.04±0.07, 16.14±0.06 and 16.28±0.06, respectively showing a significant (P<0.05) difference between the control and supplemented groups. An increase in milk yields compared with control was recorded when cows were given vitamin E and selenium (Hisham *et al*. 2016). Sadri *et al*. (2009) and Jin *et al*. (2012) also observed an increase in milk yield with chromium supplementation in lactating cows. Winkelman and Overton (2012) reported an increase in the ingestion of feed, DM effectiveness and a decline in insulin levels, leading to a significant augmentation in milk secretion as a result of increased mammary nutrient flow with chromium.

Hematological profile: The hematological profile is given in Table 3. The values did not differ significantly in

Table 3. Hematological profile of Holstein Friesian cows fed with different experimental rations (n=42)#

Parameter	Dietary group†			
	T0	T1	T2	T3
Hb (g%)	10.63±0.09	10.69±0.09	10.76±0.03	10.81±0.04
PCV (g%)	32.13±0.25	32.17±0.42	32.28±0.21	32.40±0.17
RBC (10^6/µl)	6.68±0.02	6.72±0.09	6.80±0.06	6.84±0.10
Platelets (10^3/µl)	333.58±5.79	340.42±6.57	351.46±9.78	356.42±8.70
MCV (%)	48.29±0.46	48.08±0.46	47.66±0.33	47.60±0.96
MCH (%)	15.79±0.23	16.00±0.30	15.98±0.22	15.53±0.17
MCHC (%)	33.11±0.34	33.36±0.35	33.39±0.22	33.44±0.20
Leucocytes (10^3µl)	10.04±0.07	9.88±0.06	10.00±0.88	9.86±0.09
Granulocytes (%)	39.44±1.15	40.62±1.31	40.29±1.17	41.87±0.72
Lymphocytes (%)	55.40±1.40	54.63±1.13	53.32±1.34	52.66±1.01
Monocytes (%)	4.37±0.13	4.05±0.14	4.32±0.14	4.22±0.05

#Values are Mean±SE of fortnightly values for a period of three months with six animals in each group. †Dietary groups included feeding of the basal TMR (T0); TMR along with supplemental vitamin E @ 500 IU/d and selenium @ 0.3 mg/kg DM (T1); TMR along with supplemental chromium @ 0.5 mg/kg DM (T2) and TMR along with supplemental chromium @ 0.5 mg/kg DM, vitamin E @ 500 IU/d and selenium @ 0.3 mg/kg DM.

the supplemented groups but were relatively higher than the control group which might be because of less oxidative stress and better adaptation to the environment as a result of supplemented antioxidants. The findings of the current research are consistent with the results of Sivakumar *et al*. (2010) who observed an increase in Hb per cent and PCV per cent in the selenium supplemented group and Calamari *et al*. (2014) who found an improvement in lymphocytes and eosinophil values on Se yeast supplementation. Khalili *et al.* (2011), Agustin *et al.* (2012), Zhang *et al.* (2014) and Patil *et al.* (2017) also reported that chromium supplementation did not affect blood hematology significantly.

Hormonal profile: The hormonal profile is given in Table 4. In the present study, the cortisol level in the T3 group supplemented with chromium as well as vitamin E with selenium reduced significantly ($P<0.05$), whereas, T1 and T2 group supplemented selenium with vitamin E and chromium, respectively also had lower values when compared to T0. Chang and Mowat (1992) suggested that chromium potentiates the action of insulin which may inversely inhibit cortisol. Vitamin E and selenium reduce the reactive oxygen metabolites, contributing to the reduction in concentration of cortisol as well as oxidative stress (Gupta *et al*. 2005). Similar to our findings, earlier scientists reported a decrease in cortisol level with supplementation of chromium (Soltan 2010, Baiomy 2012) and supplementation of vitamin E with Selenium (Gupta *et al*. 2005, Sathya *et al*. 2007, Dimri *et al.* 2010). There was no major difference between the groups in Triiodothyronine (T_3), Thyroxin (T_4) activity, and T_4: T_3 in cows fed ration T0, T1, T2, and T3.

The mean values for the activity of the liver enzymes ALP, ALT, and AST did not vary among the experimental groups. Our results are in accordance with the findings of Lai *et al*. (2009) and Patil *et al*. (2017) (Chromium) and Ganie *et al*. (2012), Khalifa *et al.* (2016) (Selenium and vitamin E) who did not report any significant effect of supplementation. The values were higher in the control group showing oxidative damage to the liver cells caused by heat stress-that resulted in more leakage of these enzymes into the extracellular fluids.

The activity of all the estimated antioxidant enzymes glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase was comparatively lesser in the supplemented groups. GSH-Px activity was least in T3 ($P<0.05$) followed by T1, T2, and T0 designating the important role of vitamin E and selenium in the activity of GSH-Px. It is well recognized that vitamin E and selenium can scavenge the free radicals resulting in reduced oxidative stress. GSH-Px is a selenium-dependent antioxidant enzyme that converts H_2O_2 to water (Ganaie *et al*. 2013) regulating the reactive oxygen species and redox balancing in almost all the tissues (Bizerea *et al*. 2018). Our results corroborated with the outcomes of Parmar *et al.* (2015) who found a non-significantly higher level of SOD activity in cows of the control group during stress induced by pregnancy and parturition compared to the treatment group supplemented with selenium and vitamin E. Khatti *et al.* (2017) found a decrease in catalase activity in vitamin E-Selenium supplemented group of cows. Reduction in the antioxidant enzyme activity in the supplemented group indicates the beneficial effects of chromium along with non-enzymatic antioxidants during the thermal stress.

Cows of the treatment group supplemented with chromium (T2 and T3) showed significantly ($P<0.05$) greater values for serum total proteins and serum albumin than T0 group. Our findings agreed with the studies on chromium supplementation by Khalili *et al.* (2011) and Baiomy (2012) in Holstein cows and buffaloes, respectively. According to Khalili *et al.* (2011), the increase in serum albumin might be due to increase in amino acid synthesis in the liver, suggesting that chromium may improve amino acid synthesis possibly via insulin. Patil *et al.* (2017) also observed significantly higher ($P<0.05$) albumin levels in buffaloes supplemented with chromium. T1 group supplemented with vitamin E and selenium, also showed comparatively higher levels of total proteins and albumin than control group. Reduced levels in the control group might be due to the increased cortisol which increases the serum-free amino acids through inhibition of collagen formation, reducing amino acid intake via muscle, and inhibit protein synthesis. Our results disagreed with outcomes of Khalifa *et al*. (2016) who did not find any difference in the total protein and albumin concentration in Holstein cattle supplemented with chromium, selenium and vitamin E, respectively.

The mean values for serum globulin for the treatment groups were comparable to that of the control group. The findings of the current research corroborated with the outcomes of Patil *et al.* (2017) and Khalifa *et al*. (2016) who also did not find any significant difference in globulin concentration in buffaloes and Holstein dairy cows supplemented chromium and administered vitamin E and selenium, respectively. Albumin: globulin ratio in the T2 as well as T3 groups was significantly ($p<0.05$) higher and is in continuation with the trend of increased albumin concentration in the cows fed diets supplemented with chromium.

The dietary supplementation of chromium and vitamin E with selenium did not have a major impact on the total serum cholesterol and triglyceride level which is in compliance with the results reported by Baiomy (2012) (with chromium supplementation) and Khalifa *et al.* (2016) (with selenium-vitamin E injection in cows). Overall mean values of the serum glucose, urea as well as creatinine concentration among the lactating cows fed experimental rations were comparable. The results are consistent with the findings of Zhang *et al*. (2014) who also did not find any significant variations in blood urea and creatinine concentration in Holstein Friesian cows on account of chromium supplementation.

Physiological responses: In the present study, the mean RT, RR, and PR did not differ significantly (Table 5)

Table 4. Hormonal profile of Holstein Friesian cows fed with different experimental rations

Parameter	Dietary group†			
	T0	T1	T2	T3
Cortisol (μg/dl)	4.48±0.07[b]	4.27±0.13[ab]	4.23±0.10[ab]	4.10±0.04[a]
Triiodothronine (ng/ml)	1.31±0.03	1.35±0.05	1.37±0.05	1.40±0.06
Thyroxine (ng/ml)	65.90±2.75	68.07±2.79	69.87±2.74	71.31±2.95
T3:T4	51.87±1.07	51.41±1.03	51.25±0.68	51.11±0.62
ALP (U/L)	134.72±6.49	127.42±6.43	123.69±5.97	121.52±6.33
ALT (U/L)	45.53±3.63	41.04±3.68	38.35±3.57	36.63±3.18
AST (U/L)	92.10±4.47	84.35±4.30	83.79±4.56	81.59±4.85
GSH-Px (U/ml)	629.96±12.36[b]	601.07±9.16[ab]	611.99±7.64[ab]	596.34±10.23[a]
SOD (U/ml)	6.94±0.08	6.68±0.08	6.74±0.08	6.66±0.11
Catalase activity (μm of H_2O_2 consumed/min/mg of sample)	16.31±0.11	16.11±0.15	15.98±0.15	15.97±0.18
Total protein (g/dl)	7.25±0.02[a]	7.31±0.04[ab]	7.35±0.03[b]	7.39±0.03[b]
Serum albumin (g/dl)	3.92±0.05[a]	4.06±0.05[ab]	4.12±0.07[b]	4.16±0.08[b]
Globulin (g/dl)	3.33±0.03	3.25±0.02	3.23±0.05	3.23±0.05
Albumin:globulin	1.19±0.02[a]	1.26±0.02[ab]	1.30±0.04[b]	1.31±0.02[b]
Cholesterol (mg/dl)	81.88±2.48	83.36±2.44	84.76±2.33	85.69±2.74
Triglycerides (mg/dl)	36.81±1.68	41.05±1.91	38.38±1.96	42.29±2.25
Glucose (mg/dl)	57.14±0.51	56.68±0.47	56.02±0.49	55.88±0.50
Urea (mg/dl)	10.05±0.14	9.91±0.15	9.98±0.13	9.92±0.13
Creatinine (mg/dl)	1.81±0.01	1.79±0.02	1.76±0.02	1.74±0.02

[* a, b,c]Means with different superscripts row wise differ significantly (P<0.05). The values are Mean±SE of fortnightly values for a period of three months with six animals in each group. †Dietary groups included feeding of the basal TMR (T0); TMR along with supplemental vitamin E @ 500 IU/d and selenium @ 0.3 mg/kg DM (T1); TMR along with supplemental chromium @ 0.5 mg/kg DM (T2) and TMR along with supplemental chromium @ 0.5 mg/kg DM, vitamin E @ 500 IU/d and selenium @ 0.3 mg/kg DM.

among the groups in the morning. However, in the afternoon, the values were greater in the control group compared to the supplemented groups indicating that the dietary supplementation of chromium and vitamin E with selenium had significant (P<0.05) effect in aiding thermoregulation. The outcomes of this current research corroborated with the results of Sivakumar *et al*. (2010) who registered a significant (P<0.05) decline in rectal temperature and respiration rate in antioxidant supplemented group of goats compared to the heat-stressed group without any supplementation. Similarly, Lai *et al*. (2009) studied the effect of adding chromium as chromium picolinate and found non-significantly higher values in the control group compared to the supplemented group, indicating a positive role of chromium.

It can be concluded from the present study that chromium supplementation (0.5 mg/kg DM) alone or in combination with vitamin E (500 IU/animal/day) and selenium (0.3 mg/kg DM) alleviates the negative effects of thermal stress which is reflected in the physiological and hormonal profile, in turn improving the DMI and milk yield leading to the better health and productivity of livestock.

ACKNOWLEDGEMENTS

The authors are grateful to the University Grants Commission for providing financial support (MANF 2017-18 F1-17.1/2017-18/MANF-2017-18-TEL-84155) for this experiment and also to ICAR-CRIDA and NICRA project for providing facilities for nutritional analysis of feeds and feed ingredients.

Table 5. Physiological parameters of Holstein Friesian cows fed with different experimental rations (n=552)[#]

Parameter		Dietary group†			
		T0	T1	T2	T3
Rectal temperature (°F)	Morning	101.03±0.07	100.95±0.06	100.92±0.06	100.88±0.06
	Afternoon	101.92±0.17[b]	101.56±0.09[a]	101.54±0.07[a]	101.46±0.04[a]
Respiration rate (bpm)	Morning	30.07±0.36	29.63±0.37	29.40±0.39	29.30±0.43
	Afternoon	40.10±0.87[b]	37.56±0.97[a]	37.46±0.90[a]	37.07±0.70[a]
Pulse rate (beats/min)	Morning	55.43±0.75	54.33±0.69	54.31±0.72	53.26±0.75
	Afternoon	61.31±0.75[b]	59.58±0.81[ab]	59.35±0.80[ab]	58.89±0.75[a]

[* a, b,c]Means with different superscripts row wise differ significantly (P<0.05). [#]The values are Mean±SE of daily values for a period of three months with six animals in each group. †Dietary groups included feeding of the basal TMR (T0); TMR along with supplemental vitamin E @ 500 IU/d and selenium @ 0.3 mg/kg DM (T1); TMR along with supplemental chromium @ 0.5 mg/kg DM (T2) and TMR along with supplemental chromium @ 0.5 mg/kg DM, vitamin E @ 500 IU/d and selenium @ 0.3 mg/kg DM.

REFERENCES

Abd El-Hack M E, Alagawany M and Noreldin A E. 2019. Managerial and nutritional trends to mitigate heat stress risks in poultry farms, pp. 325&338. Negm A M and Abu-hashim M (Eds). Sustainability of Agricultural Environment in Egypt: Part II: Soil–Water-Plant Nexus. Springer International Publishing.

Agustin F, Toharmat T, Evvyernie D, Taniwiryon D and Tarigan S. 2012. Blood profile of lactating cows supplemented with organic chromium and *Ganoderma lucidum*. *Pakistan Journal of Nutrition* **11**: 893–99.

Ammer S, Lambertz C, Von Soosten D, Zimmer K, Meyer U, Dänicke S and Gauly M. 2018. Impact of diet composition and temperature–humidity index on water and dry matter intake of high-yielding dairy cows. *Journal of Animal Physiology and Animal Nutrition* **102**(1): 103–13.

AOAC. 2005. *Official Methods of Analysis*. Association of Official Analytical Chemists, 18th (Ed.). Benjamin Franklin Station, Washington, DC.

Armstrong D. 1994. Heat stress interaction with shade and cooling. *Journal of Dairy Science* **77**(7)*:* 2044–50.

Baiomy A A. 2012. Effect of chromium methionine supplementation on milk production, composition and some blood metabolites of lactating buffaloes. *Journal of Animal and Poultry Production*, *Mansoura University* **3**(8): 415–21.

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): 401–12.

Bianca W. 1962. Relative importance of dry bulb and wet bulb temperatures in causing heat stress in cattle. *Nature* **195**: 251–52.

Bin-Jumah M, Abd El-Hack M E, Abdelnour S A, Hendy Y A, Ghanem H A, Alsafy S A, Khafaga A F, Noreldin A E, Shaheen H, Samak D, Momenah M A, Allam A A, AlKahtane A A, Alkahtani S, Abdel-Daim M M and Aleya L. 2020. Potential use of chromium to combat thermal stress in animals. *Science of the Total Environment* **707**: 135996.

Bizerea T O, Dezsi S G, Marginean O, Stroescu R, Rogobete A, Bizerea-Spiridon O and Ilie C. 2018. The link between selenium, oxidative stress and pregnancy induced hypertensive disorders. *Clinical Laboratory* **64**(10): 1593.

Calamari L U I G I, Abeni F and Bertin G. 2014. Metabolic and hematological profiles in mature horses supplemented with different selenium sources and doses. *Journal of Animal Science* **88**(2): 650–59.

Chang X and Mowat D. N. 1992. Supplemental chromium for stressed and growing feeder calves. *Journal of Animal Science* **70**(2): 559–65.

Chauhan S S, Celi P, Leury B J, Clarke I J and Dunshea F R. 2014. Dietary antioxidants at supranutritional doses improve oxidative status and reduce the negative effects of heat stress in sheep. *Journal of Animal Science* **92**: 3364–74.

Dalia A M, Loh T C, Sazili A Q, Jahromi M F and Samsudin A A. 2018. Effects of vitamin E, inorganic selenium, bacterial organic selenium, and their combinations on immunity response in broiler chickens. *BMC Veterinary Research* **14**(1): 1–10.

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

Deka R S, Mani V, Kumar M, Shiwajirao Z S and Kaur H. 2015. Chromium supplements in the feed for lactating Murrah buffaloes *(Bubalus bubalis)*: Influence on nutrient utilization, lactation performance and metabolic responses. *Biological Trace Element Research* **168**(2): 362–71.

Dimri U, Ranjan R, Sharma M C and Varshney V P. 2010. Effect of vitamin E and selenium supplementation on oxidative stress indices and cortisol level in blood in water buffaloes during pregnancy and early postpartum period. *Tropical Animal Health and Production* **42**(3): 405–10.

Duncan D B. 1955. Multiple range and multiple F tests. *Biometrics* **11**: 1–42.

Ganaie A H, Ghasura R S, Mir N A, Bumla N A, Sankar G and Wani S A. 2013. Biochemical and physiological changes during thermal stress in bovines: A review. *Iranian Journal of Applied Animal Science* **3**(3): 423–30.

Gupta S, Gupta H K and Soni J. 2005. Effect of vitamin E and selenium supplementation on concentrations of plasma cortisol and erythrocyte lipid peroxides and the incidence of retained fetal membranes in crossbred dairy cattle. *Theriogenology* **64**(6): 1273–86.

Hisham Khalifa H, Safwat M A, Sysy E, Abd ElFatah M and Al-Metwaly M A E. 2016. Effect of selenium and vitamin E supplementation as a nutritional treatment for some physiological and productive traits of Holstein dairy cows under Egyptian summer conditions. *Journal of Egyptian Academic Society for Environmental Development. D, Environmental Studies* **17**(1): 97–113.

Indian Council of Agricultural Research. 2013. *Nutrient Requirements of Cattle and Buffalo.*

Jin X, Li S and Zhang W. 2012. Effect of chromium propionate supplementation on lactation performance and blood parameters of dairy cows. *Journal of Animal Veterinary Advances* **11**(16): 3031–35.

Kargar S, Mousavi F, Karimi-Dehkordi S and Ghaffari M H. 2018. Growth performance, feeding behaviour, health status, and blood metabolites of environmentally heat-loaded Holstein dairy calves fed diets supplemented with chromium. *Journal of Dairy Science* **101**(11): 9876–87.

Khalifa H H, Safwat M A, El Sysy M A I and Al-Metwaly M A. 2016. Effect of selenium and vitamin E supplementation as a nutritional treatment for some physiological and productive traits of Holstein dairy cows under Egyptian summer conditions. *Journal of the Egyptian Academy of Environmental Development* **17**(1): 97–113.

Khalili M, Foroozandeh A D and Toghyani M. 2011. Lactation performance and serum biochemistry of dairy cows fed supplemental chromium in the transition period. *African Journal of Biotechnology* **10**(50):10304-10.

Khatti A, Mehrotra S, Patel P K, Singh G, Maurya V P, Mahla A S and Kumar H. 2017. Supplementation of vitamin E, selenium and increased energy allowance mitigates the transition stress and improves postpartum reproductive performance in the crossbred cow. *Theriogenology* **104**:142–48.

Kumar S, Singh S V, Singh A K, Maibam U and Upadhyay R C. 2016. Influence of chromium propionate supplementation on feed intake, growth rate, haematological and antioxidant enzymes profile in Sahiwal calves during summer season. *Indian Journal of Animal Nutrition* **33**(1): 59–63.

Lai A, Wang Z and Zhou A. 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**(7): 940–45.

Lashkari S, Habibian M and Jensen S K. 2018. A review on the role of chromium supplementation in ruminant nutrition—

effects on productive performance, blood metabolites, antioxidant status, and immunocompetence. *Biological Trace Element Research* **186**(2): 305–21.

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.

Lloyd K E, Fellner V, McLeod S J, Fry R S, Krafka K, Lamptey A and Spears J W. 2010. Effects of supplementing dairy cows with chromium propionate n milk and tissue chromium concentrations. *Journal of Dairy Science* **93**: 4774–80.

Mehdi Y and Dufrasne I. 2016. Selenium in cattle: A review. *Molecules* **21**: 545.

Pantelić M, Jovanović L J, Prodanović R, Vujanac I, Đurić M, Ćulafić T, Vranjes-Duric S, Koricanac G and Kirovski D. 2018. The impact of the chromium supplementation on insulin signalling pathway in different tissues and milk yield in dairy cows. *Journal of Animal Physiology and Animal Nutrition* **102**(1): 41–55.

Parmar V L, Panchasara H H, Sutaria P, Sutaria T V, Sadhu D B and Prajapati H B. 2015. Synergism of vitamin E and selenium on oxidative stress during transition period in Kankrej cattle. *Indian Journal of Veterinary Sciences and Biotechnology* **10**(3): 1–3.

Patil A K, Verma A K, Singh P, Das A and Gaur G K. 2017. Effect of molasses based multinutrients and chromium supplementation on the haematological and blood biochemical Profile in lactating Murrah buffaloes. *Journal of Animal Research* **7**(2): 1–9.

Sadri H, Ghorbani G R, Rahmani H R, Samie A H, Khorvash M and Bruckmaier R M. 2009. Chromium supplementation and substitution of barley grain with corn: Effects on performance and lactation in periparturient dairy cows. *Journal of Dairy Science* **92**(11): 5411–18.

Saeed M, Abbas G, Alagawany M, Kamboh A A, Abd El-Hack M E, Khafaga A F and Chao S. 2019. Heat stress management in poultry farms: A comprehensive overview. *Journal of Thermal Biology* **84**: 414–25.

Sathya A, Prabhakar S, Sangha S P and Ghuman S P. 2007. Vitamin E and selenium supplementation reduces plasma cortisol and oxidative stress in dystocia-affected buffaloes. *Veterinary Research Communication* **31**(7): 809–18.

Sivakumar A V N, Singh G and Varshney V P. 2010. Antioxidants supplementation on acid-base balance during heat stress in goats. *Asian Australasian Journal of Animal Sciences* **23**(11): 1462–68.

Snedecor G W and Cochran W G. 1994. *Statistical Methods*. (8th) Edn. Iowa State University Press, Ames, Iowa, USA-50010.

Soltan M A. 2010. Effect of dietary chromium supplementation on productive and reproductive performance of early lactating dairy cows under heat stress. *Journal of Animal Physiology and Animal Nutrition* **94**(2): 264–72.

Spears J W. 2010. Chromium supplementation in cattle diets. *Florida Ruminant Nutrition Symposium;* Gainesville, FL, USA. 143–55.

Spears J W. 2011. Role of mineral and vitamin status on health of cows and calves. *WCDS Advances in Dairy Technology* **23**: 287–97.

Winkelman L A and Overton T R. 2012. The effects of increasing doses of 2 preparations of long-acting insulin on short-term plasma profiles of glucose and insulin in lactating dairy cows. *Journal of Dairy Science* **95**(12): 6974–82.

Zhang F J, Weng X G, Wang J F, Zhou D, Zhang W, Zhai C C, Hou Y X and Zhu Y H. 2014. Effects of temperature-humidity index and chromium supplementation on antioxidant capacity, heat shock protein 72, and cytokine responses of lactating cows. *Journal of Animal Science* **92**(7): 3026–34.