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Effect of Supplemental Chromium, Vitamin E and Selenium on Biochemical and Physiological Parameters of Holstein Friesian Calves under Heat Stress

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ABSTRACT

Background: Heat stress has been a major concern in tropical, sub-tropical and arid areas affecting the performance of farm animals. Along with vitamin E and Selenium, chromium is gaining importance in combating heat stress.

Methods: Twenty-four calves of 7- 8 months old with an average body weight of 172.79±4.39 kg were distributed randomly into four dietary treatment groups (T_0, T_1, T_2 and T_3). The calves of control group (T_0) were fed on basal diet *i.e.* total mixed ration (TMR). The basal diet of T_1, T_2 and T_3 on per kg DM basis were supplemented vitamin E (500 IU) + selenium (0.3 mg), chromium propionate (0.5 mg) and chromium propionate (0.5 mg) + vitamin E (500 IU) + selenium (0.3 mg), respectively. Ambient air temperature and humidity were measured for calculation of THI. Blood samples were collected fortnightly and the rectal temperature (RT), respiration rate (RR) and pulse rate (PR) of all the experimental animals were recorded twice daily to study the biochemical and physiological parameters.

Result: Supplementation of chromium along with vitamin E and selenium decreased significantly ($P<0.05$) the cortisol levels and increased the albumin concentration. Chromium supplemented groups (T_2 and T_3) showed greater ($P<0.05$) increase in mean total protein concentration. A significant reduction in RT was observed in all the supplemented groups compared to control. Whereas, the PR and RR differed significantly in T_3 group compared to control.

Key words: Calves, Chromium propionate, Heat stress, Selenium, Vitamin E.

INTRODUCTION

Livestock rearing is a major continuous income generating activity for the rural households (Mahla *et al*., 2015). Climatic variables like humidity and temperature have an intense effect on the performance of the animals. The Thermoneutral zone (TNZ) is comfortable for the animal, the core temperature is generally within physiological limits and body temperature regulation is achieved only by sensible heat loss (Charles, 1994; IUPS, 2001). Dairy animal's TNZ lies in the range of 16°C-25°C, within which a physiological body temperature of 38.4-39.1°C is maintained by them (Yousef, 1985). Animals are able to adjust to adverse climate by means of acclimatization and adaptation (Roy and Collier, 2012). Extreme climatic conditions that cannot be compensated by thermoregulatory mechanisms result in thermal stress (Roland *et al*., 2016). Calves and heifers generate less metabolic heat and have greater body surface area relative to body mass, hence efficiently dissipating body heat and are thus considered to be more tolerant of heat stress than mature cattle (West, 2003), still they suffer from heat stress to some degree (Wang *et al*., 2020). Temperature-Humidity Index (THI) is a parameter widely used to describe heat load on animals and is a good indicator of stressful thermal climatic conditions (Das, 2018; Habeeb *et al*., 2018).

Heat stress brings about changes in immunity and hormonal concentrations. Primary indicators of immune response comprise of protein, glucose, packed cell volume, hemoglobin, red blood cells and white blood cell concentrations in blood which get changed due to thermal stress (Das *et al.,* 2016). The cortisol level is an important indication of heat stress during summer season (Sinha *et al*., 2019). The heat stress induced changes potentially enhance

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the formation of reactive oxygen species (El-Kholy, *et al*., 2018). Reactive oxygen species induce oxidative stress and damage the cellular macromolecules causing lipid peroxidation, nucleic acid and protein alterations (Adwas *et al*., 2019). The animal shows several physiological adaptation mechanisms to cope up the adverse climate condition. Some of the physiological determinants of adaptations to heat stress are respiration rate, rectal temperature, pulse rate, skin temperature and sweating rate (Indu and Pareek, 2015). Respiration rate is one of the ideal biomarkers of heat stress. In homeostasis, PR reflects the circulation and metabolic status (Rashamol *et al*., 2020).

Dietary modifications could help animals to retain their normal physiological and blood biochemical profile thereby maintaining the general wellbeing of the animals. Antioxidants, both non-enzymatic and enzymatic, offer required defense against oxidative stress resulted due to thermal stress (Kumar *et al.,* 2011). Vitamin E and selenium are documented antioxidants (Domoslawska *et al*., 2018). Chromium is gaining worldwide importance for combating heat stress adverse effects and it is believed as a promising agent. Hence, this study was performed for investigating the chromium propionate effects on physiological as well as the biochemical profile of calves under thermal stress.

MATERIALS AND METHODS

Site of study

The current study 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 547meters altitude above the sea level. Analysis of blood and feed sample was carried out at ICAR-CRIDA (ICAR-Central Research Institute for Dryland Agriculture), Santoshnagar, Hyderabad.

Animals and diet

The trial was performed for a 90d period during peak summer season (April 15 to July 15) in the year 2017 on 24 H.F calves with an average body weight of 172.96±4.39 kg and aged 7-8 months . They were randomly distributed into four groups of six animals in each group considering their body weights. Calves either received a basal diet (T_0) or were supplemented with vitamin E + selenium (T_1; 500IU vitamin E + 0.3 mg selenium per kg DM); chromium propionate (T_2; 0.5 mg chromium per kg DM) and chromium propionate + vitamin E + selenium (T_3; 0.5 mg chromium per kg DM+ 500 IU vitamin E+ 0.3 mg selenium per kg DM) and fed with a total mixed ration (TMR) as basal diet. All the calves were stall fed, reared under standard management conditions and housed in tie stalls with fans. All the groups were fed total mixed ration (TMR) diet twice daily. Fifty per cent of the daily feed allocation was provided in the morning feeding at 8.00 am and fifty per cent in the evening at 4.00 pm during the trial period. The TMR contained chopped paddy straw, maize fodder (sweet corn with cobs and grains in milk stage) and concentrate mixture (Table 1) with roughage: concentrate ratio of 70:30. The basal diet was formulated to contain all the necessary nutrients to meet the nutrient requirements as recommended by ICAR (2013). Chromium propionate, vitamin E and selenium were supplemented to the individual animal daily in the morning in the form of a 20 g jaggery bolus. The calves had free access to fresh wholesome clean drinking water all the time.

Temperature humidity index (THI)

Ambient air temperature and humidity were measured for calculation of THI. Dry bulb and wet bulb temperatures were recorded twice a day at 10.00AM and 3.00 PM using dry and wet bulb thermometers placed about 1.5 m above the ground level. The degree of heat stress was determined according to mean THI values measured. THI was estimated for the entire study period as per Bianca (1962).

$$THI= (0.35xT_{db} + 0.65xT_{wb})\ x1.8+32$$

Where

T_{db} represents Dry bulb temperature and T_{wb} represents Wet bulb temperature (°C).

Blood collection

Blood samples were collected fortnightly from the jugular vein of all the experimental animals, prior to the morning feeding with the help of sterilized needles. Each time two blood samples were collected from each 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. The samples were refrigerated at 8°C and transported immediately to the laboratory for analysis. Within an hour, the blood samples were centrifuged for 10 minutes at 3500 rpm and blood serum was collected and stored -20°C for further use.

Whole blood samples were analyzed using automatic haematology analyzer (ABX Micros 60, USA). Thyroid profile and cortisol were estimated using chemi luminescent immuno assay method with FUJI DRI-CHEM NX 500V (Japan). Alkaline phosphatase, aspartate amino aransferase, alanine amino transferase, total protein, albumin, cholesterol, triglycerides, glucose, urea, creatinine, were analyzed by kits (Bio systems, Spain) using blood biochemical semi auto analyzer (ichem~168, Spain).

Table 1: Ingredient Composition of concentrate mixture fed to Holstein Friesian calves.

Ingredients	Qty (kg)/T
Maize	32.0
Wheat bran	32.0
Soyabean meal	30.0
Limestone powder	2.0
Salt	1.0
Mineral mixture	2.0
Sodium bicarbonate	1.0

Physiological responses measurement

Physiological responses including rectal temperature (RT), respiration rate (RR) and pulse rate (PR) of all the experimental animals were recorded twice daily at 10.00am and 3.00pm during the entire length of the experimental period of three months. The rectal temperature was measured by inserting clinical thermometer per rectum and observation was recorded after one min. The respiration rate was recorded by observing the flank movements for one minute in which each inward and outward movement of the flank was counted as one complete respiration. The pulse rate of the animals was recorded by observing the pulsation of middle coccygeal artery at the base of the tail.

Chemical and statistical analysis

Samples of TMR were collected, dried overnight in a hot air oven at 100±5°C and then ground in laboratory Willey mill and preserved in airtight containers for further chemical analysis. The proximate analysis and fiber fractions of feeds was performed as per the procedures described by AOAC (2005) and Van Soest *et al.* (1991) methods respectively.

Statistical analysis of the data was carried out according to the procedures suggested by Snedecor and Cochran (1994). Least-square Analysis of variance was used to test the significance of various treatments and the difference between treatments means was tested for significance by Duncan's new multiple range and F Test (Duncan, 1955).

RESULTS AND DISCUSSION

Chemical composition of the feed

The chemical composition of experimental rations fed to the H.F calves is presented in Table 2. The per cent of crude protein in the TMR, maize fodder and paddy straw fed to the growing calves were 13.90, 8.68 and 3.40 percent on a dry matter basis, respectively.

Environmental conditions and microenvironment

Temperature humidity index (THI) at the start of experiment (1^{st} week of April) averaged 75.83±0.22 in the morning (10.00 AM) and 80.44±0.85 in the afternoon (3.00PM) and the THI decreased with the decrease in environmental temperature in July to 72.25±0.31 and 76.69±0.48 in the morning and afternoon, respectively. The mean THI during 90 days of experimental period 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 May month with 83.48±1.41 THI value in the afternoon (Fig 1).

Table 2: Chemical composition of experimental feeds (% DM) fed to Holstein Friesian calves.

Nutrient	TMR	Concentrate mixture	Paddy straw	Maize fodder
		Proximate composition		
Dry matter	54.50	93.61	95.35	23.96
Organic matter	89.04	89.86	85.54	92.67
Crude protein	13.90	20.84	3.40	8.68
Ether extract	2.02	3.22	1.24	1.91
Crude fibre	14.02	6.16	31.68	25.12
Total ash	10.96	10.14	14.46	7.33
Nitrogen free extract	59.10	59.64	49.22	56.96
		Van Soest fibre fractions		
Neutral detergent fiber	59.85	56.37	73.56	75.99
Acid detergent fiber	22.05	14.94	51.09	37.70

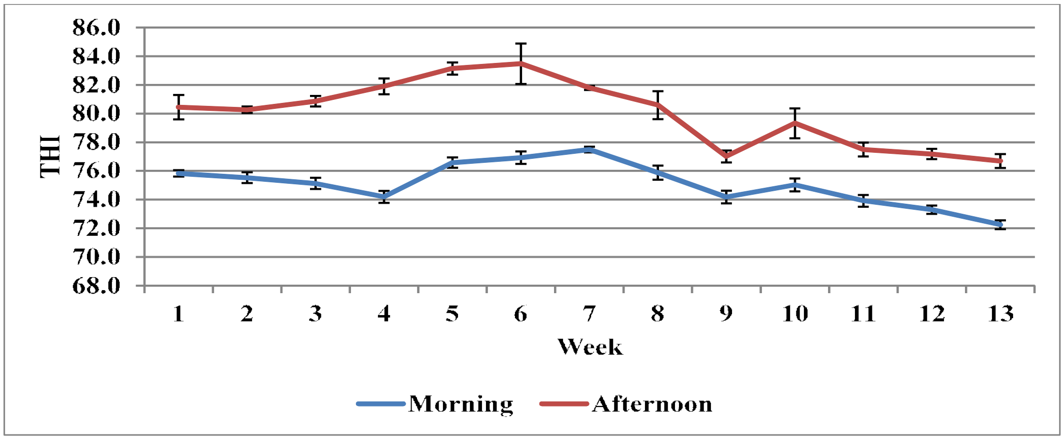


Fig 1: Mean THI values recorded during experimental period.

Biochemical profile

Hematological profile has been depicted in Table 3. Overall mean values for the hematological profile were comparable among calves fed experimental rations. No significant difference was found in groups T_0, T_1, T_2 and T_3. Similar findings were reported by Kumar *et al*. (2016) and Shinde *et al*. (2009) who did not find any effect of supplementation of chromium and vitamin E with selenium respectively, on calves.

In the present study, hormonal profile revealed (Table 4) that in T_1 group supplemented with vitamin E and selenium the cortisol values were numerically lower than control. This lower cortisol levels could be due to reduced production of reactive oxygen metabolites by supplemental vitamin E and selenium (Gupta *et al*., 2005). Chromium supplementation reduced the cortisol levels in groups T_2 (P>0.05) and T_3 groups (P<0.05). Obtained results are consistent with the research studies of Chang and Mowat (1992) who suggested

Table 3: Haematological profile of Holstein Friesian calves fed on experimental rations.

Parameter	Dietary groups†			
	T_0	T_1	T_2	T_3
Hb (g%)	11.30±0.08	11.34±0.10	11.43±0.06	11.51±0.11
PCV (g%)	32.49±0.22	32.92±0.29	32.57±0.18	33.11±0.35
RBC (10v /µl)	7.54±0.10	7.58±0.09	7.63±0.07	7.72±0.04
Platelets (10^3/µl)	397.00±6.27	399.17±3.62	402.25±6.88	408.46±3.78
MCV (%)	43.17±0.82	42.86±0.50	43.51±0.41	43.01±0.51
MCH (%)	15.01±0.23	15.03±0.14	14.97±0.16	14.94±0.18
MCHC (%)	34.79±0.37	35.00±0.41	34.45±0.29	35.44±0.37
Leucocytes (10^3 µl)	10.56±0.09	10.42±0.08	10.46±0.16	10.38±0.16
Granulocytes (%)	41.10±0.82	42.30±0.82	41.92±0.98	43.42±0.94
Lymphocytes (%)	52.59±0.71	50.19±1.19	51.62±0.77	50.23±1.27
Monocytes (%)	4.37±0.13	4.05±0.14	4.22±0.05	4.32±0.14

† Dietary groups included feeding of the basal TMR (T_0); TMR along with supplemental vitamin E and selenium (T_1; vitamin E at 500 IU/d and selenium at 0.3 mg/kg DM); TMR along with supplemental chromium propionate (T_2; chromium at 0.5 mg/kg DM); and TMR along with supplemental chromium propionate, vitamin E and selenium (T_3; chromium at 0.5 mg/kg DM**,** vitamin E at 500 IU/d and selenium at 0.3 mg/kg DM)

Table 4: Blood biochemical profile of Holstein Friesian calves fed on experimental rations.

Parameter	Dietary groups†			
	T_0	T_1	T_2	T_3
Cortisol (µg/dl)	3.85±0.06^a	3.71±0.07 ^{ab}	3.68±0.06 ^{ab}	3.62±0.07 ^{b}
Triiodothronine (ng/ml)	1.59±0.04	1.62±0.04	1.64±0.04	1.69±0.04
Thyroxine (ng/ml)	79.35±2.00	80.73±1.96	82.76±1.86	84.17±2.38
T3:T4	50.18±0.35	50.46±0.84	51.29±0.88	50.35±0.93
ALP (U/L)	132.67±5.06	125.99±6.61	126.24±6.90	120.78±6.98
ALT(U/L)	39.22±4.34	37.35±3.92	37.35±3.74	36.60±3.89
AST(U/L)	103.14±4.67	90.57±5.64	87.22±5.35	89.49±5.69
Total protein(g/dl)	6.76±0.03^a	6.83±0.03 ^{ab}	6.91±0.04 ^{b}	6.95±0.06 ^{b}
Serum albumin(g/dl)	3.96±0.06^a	4.09±0.07 ^{ab}	4.14±0.06 ^{ab}	4.18±0.07 ^{b}
Globulin(g/dl)	2.79±0.03	2.74±0.05	2.77±0.01	2.77±0.03
Albumin:globulin	1.45±0.04	1.53±0.05	1.52±0.03	1.53±0.04
Cholesterol(mg/dl)	74.67±2.79	77.71±2.77	80.49±2.74	82.45±2.67
Triglycerides(mg/dl)	50.10±8.63	54.32±10.27	55.27±10.50	56.33±11.03
Glucose (mg/dl)	52.75±0.38	52.34±0.31	51.89±0.33	51.80±0.36
Urea (mg/dl)	8.63±0.12	8.60±0.12	8.53±0.12	8.55±0.09
Creatinine (mg/dl)	1.18±0.02	1.13±0.02	1.15±0.01	1.15±0.02

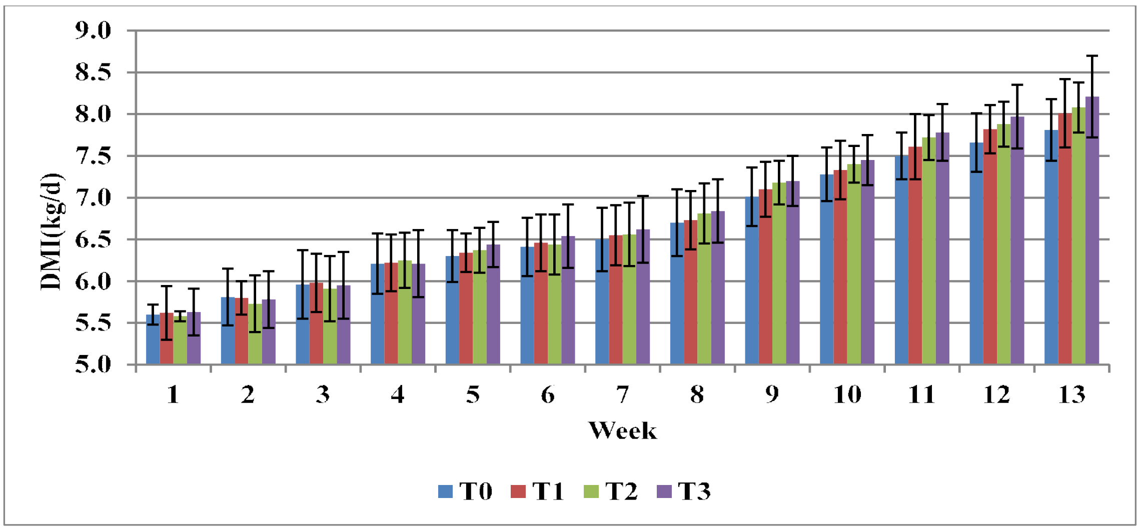
* $^{a, b,c}$ means with different superscripts row-wise vary significantly (P<0.05).

† Dietary groups included feeding of the basal TMR (T_0); TMR along with supplemental vitamin E and selenium (T_1; vitamin E at 500 IU/d and selenium at 0.3 mg/kg DM); TMR along with supplemental chromium propionate (T_2; chromium at 0.5 mg/kg DM); and TMR along with supplemental chromium propionate, vitamin E and selenium (T_3; chromium at 0.5 mg/kg DM**,** vitamin E at 500 IU/d and selenium at 0.3 mg/kg DM).

that chromium potentiates the insulin action that may reciprocally prevent cortisol. The present results agreed with the findings of Soltan *et al.* (2012) and Kumar *et al.* (2016) in buffalo calves with chromium supplementation. There was no significant difference among the groups in Triiodothyronine (T_3), Thyroxin (T_4) activity and T_4: T_3 in calves fed ration T_0, T_1, T_2 and T_3.

The mean values for the liver enzymes activity ALP, AST and ALT didn't vary significantly (p> 0.05) between the experimental group which was in accordance with the results of Nejad *et al.* (2016), Patil *et al*. (2017) with chromium supplementation and Shinde *et al.* (2009), Hala *et al.* (2014) with vitamin E and selenium in calves and kids, respectively. The T0 group fed only on basal diet showed higher values for the liver enzymes activity indicating more leakage of these enzymes into the extracellular fluids which might be due to heat stress-induced oxidative damage to the liver cells.

Overall mean total serum protein was significantly (P<0.05) high in the T_3 group followed by T_2, T_1 and T_0 indicating the critical role of chromium. This could be due to the decrease of serum cortisol concentration or an increase of sensitivity tissue to insulin (Roginski and Mertz, 1969). The role of insulin is proved at increase of synthesis of proteins (Roginski and Mertz, 1969). The findings of the current research corroborated with the results of Hala *et al.* (2014) and Pechova *et al.* (2002). Perusal of the table indicates that the albumin values were greater in chromium supplemented groups compared to T_0 and T_1. Khalili *et al*. (2011) suggested an increase in the synthesis of amino acid in the liver probably through insulin due to chromium supplementation. The results are in agreement with Nejad



Dietary groups included feeding of the basal TMR (T_0); TMR along with supplemental vitamin E and selenium (T_1; vitamin E at 500 IU/d and selenium at 0.3 mg/kg DM); TMR along with supplemental chromium propionate (T_2; chromium at 0.5 mg/kg DM); and TMR along with supplemental chromium propionate, vitamin E and selenium (T_3; chromium at 0.5 mg/kg DM**,** vitamin E at 500 IU/d and selenium at 0.3 mg/kg DM)

Fig 2: Dry matter intake (kg/d) of Holstein Friesian calves fed on experimental rations during the experimental period.

Table 5. Physiological parameters of Holstein Friesian calves fed on experimental rations.

Parameter		Dietary groups†			
		T_0	T_1	T_2	T_3
DMI (kg/d)		6.67±0.20	6.74±0.22	6.76±0.23	6.82±0.23
Rectal temperature	Morning	101.69±0.03	101.66±0.03	101.63±0.03	101.60±0.03
	Afternoon	102.67±0.05^{c}	102.42±0.04^{b}	102.39±0.03^{b}	102.23±0.07^{a}
Respiration rate	Morning	36.22±1.23	35.38±1.19	35.36±1.22	34.20±1.16
	Afternoon	43.24±0.56^{b}	41.72±0.56 ^{ab}	41.65±0.46 ^{ab}	40.56±0.59 ^{a}
Pulse rate	Morning	70.76±0.59	70.10±0.52	70.26±0.58	69.46±0.62
	Afternoon	74.26±0.78^{b}	73.17±0.70^{ab}	72.71±0.70 ^{ab}	71.89±0.77^{a}

* $^{a,\ b,c}$ means with different superscripts row-wise vary significantly (P<0.05).

† Dietary groups included feeding of the basal TMR (T_0); TMR along with supplemental vitamin E and selenium (T_1; vitamin E at 500 IU/d and selenium at 0.3 mg/kg DM); TMR along with supplemental chromium propionate (T_2; chromium at 0.5 mg/kg DM); and TMR along with supplemental chromium propionate, vitamin E and selenium (T_3; chromium at 0.5 mg/kg DM**,** vitamin E at 500 IU/d and selenium at 0.3 mg/kg DM).

et al. (2016) who found relatively higher albumin levels in Holstein steers supplemented with chromium. The mean value for serum globulin and albumin: globulin ratio didn't vary significantly among the groups.

No difference (p>0.05) was observed among the groups in triglyceride and cholesterol concentration in the current study. Nejad *et al.* (2016) also found no deviation in cholesterol and triglyceride values in Holstein steers supplemented chromium from that of the control group. Similarly, Shinde *et al.* (2009) also revealed that total cholesterol and triglycerides concentration was statistically similar in vitamin E and selenium supplemented group and the group devoid of supplementation. Overall mean values of the serum glucose, urea as well as creatinine concentration were similar across the treatments (P>0.05). No effect of chromium (Pechova *et al.*, 2002; Nejad *et al.*, 2016) and vitamin E and selenium (Tahmasbi *et al*., 2012; Alhidary *et al*., 2015) was observed on glucose in previous studies during summer. Likewise, no effect on blood urea nitrogen and creatinine was observed in trials conducted by Zhang *et al.* (2014) with chromium supplementation and Hala *et al.* (2014) with vitamin E and selenium supplementation.

Feed intake and physiological measures

The results indicated that the DMI did not differ significantly (p> 0.05) among four groups of calves fed experimental rations but relatively higher DMI was found in the supplemented groups compared to control group (Fig 2). Increased DMI may be attributed to the lower stress levels as reflected by lower serum cortisol level in the supplemented calves. The RT, PR as well as RR did not vary significantly (P>0.05) in the morning among the four experimental groups (Table 5). However, in the afternoon calves in the control group had higher RT, PR and RR compared to supplemented groups with a significant (P<0.05) variation in T3 group. Lower serum cortisol levels in T_1, T_2 and T_3 group calves indicate heat stress mitigation potential of dietary supplemented chromium and vitamin E with selenium by lowering the oxidative stress at the cellular level and thus resulting in better thermoregulation and lower RT, PR and RR in calves in T_1, T_2 and T_3 groups. Chauhan *et al.* (2016) also reported a reduction in RT (P = 0.08) and RR in heat-stressed lambs fed supra nutritional selenium and vitamin E levels. Yari *et al*. (2010) also concluded that chromium supplementation lowered the respiration rate in calves.

CONCLUSION

Dietary supplementation of chromium reduced the thermal stress impacts in Holstein Friesian calves more effectively than vitamin E and selenium. Further, supplementation of vitamin E and selenium along with chromium had significant additional benefit which is reflected in the physiological variables and the cortisol levels in the present study. Hence, supplementation of chromium along with vitamin E and selenium could be more beneficial to mitigate negative impacts of heat stress and improving the performance of H.F. calves.

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