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Effect of Micro-nutrients Supplementation and Seasonal Variability on Production Performance, Antioxidants and Immune Status in Crossbred Heifers

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ABSTRACT

Twenty four Karan Fries crossbred heifers were allocated into 4 groups of 6 animals each to evaluate the effects of micronutrient supplementation on animal performance during summer and winter seasons.The animals were fed concentrate mixture, maize fodder and wheat straw to meet nutrient requirements (ICAR, 2013). In group G_1, the mineral mixture added in concentrate mixture was devoid of iodine while in groups G_2 and G_3, iodine was supplemented at 0.25 and 0.5 ppm of diet. In group G_4, a micro-nutrient mixture was supplemented that contained chromium, niacin, vitamin E and Zn at 1.5, 600, 40 and 40 ppm of dietary DM, respectively. Daily feed intake, body weight gain, feed conversion ratio were similar in different groups irrespective of seasons.Activities of glutathione peroxidase, super oxide dismutase and catalase did not vary due to treatments and seasons. However, overall ferric reducing ability of plasma values was reduced ($P<0.05$) in group G_4 in both seasons and the values were lower ($P<0.05$) in winter than summer. The thiobarbuturic reactive substances values were also comparatively higher ($P<0.01$) in summer. Plasma total immunoglobulins concentration was higher ($P<0.05$) in winter irrespective of groups. Hence, micronutrient supplementation could help to mitigate oxidative stress in crossbred heifers during summer.

Key words: Antioxidant status, Crossbred heifers,Immunity, Performance, Seasonal variation

INTRODUCTION

In tropical countries, environmental temperature varies greatly during winter and summer seasons. Increased ambient temperature, humidity and radiation may lead to increased heat gain in comparison to heat loss from the body and cause thermal stress in animals (Cooke *et al.,* 2020). Heat stress results in reduced gut motility, ruminal contractions, saliva secretion and depressed appetite (Zha *et al.,* 2009) and also induces oxidative stress which results in altered physiological, antioxidant enzyme activity and immunity in cattle (Pradhan *et al.,* 2015; Das *et al.,* 2016). Therefore, maintaining better performance during period of seasonal stress induced immunosuppression is critically important. Nutritional management is one of the key factors in reducing the effects of thermal stress wherein certain micro-nutrients play crucial roles. Supplementation of micro-nutrients improved growth, physiological responses and immune functions in animals under heat stress (Keshri *et al.,* 2019). Oxidative stress can be ameliorated by antioxidants (Higdon and Frei, 2003). Anti- oxidative functions could be either enzymatic like catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) or non- enzymatic (vitamins C, A, E and minerals like Cu, Zn, chromium *etc.*). Changes in metabolic activity of animals during seasonal variation in ambient temperature and air humidity are related to thyroid activity. Supplementation of iodine during high environmental temperature reduced feed intake and decreased the synthesis of thyroid hormones in cattle, sheep and goats (ARC, 1980). Pattanaik *et al.* (2001) reported that iodine supplementation at 0.05 and 0.075 mg/animal/d had no significant impact on utilization of

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nutrients, and seasonal variations affected thyroid gland activity which is associated with air humidity and ambient temperature. Provision of high levels of iodine (0, 2.5, 5, 7.5 mg/kg) in the diet of Holstein cows decreased DM intake but no significant differences were observed between the groups (Norouzian *et al.,* 2009). The information on the effects of supplementation of combination of micronutrients on antioxidant status in different seasons is scanty. Therefore, the present investigation was undertaken to study the effect of micronutrient mixture (Zn, Cr, vitamin E and niacin) and iodine supplementation on growth performance, antioxidant enzyme activity and immunity status in crossbred heifers during summer and winter seasons.

MATERIALS AND METHODS

The study was conducted at Livestock Research Centre (LRC) at ICAR-NDRI, Karnal, Haryana (India) which is located at an altitude of 250 m above msl. The maximum and minimum ambient temperature in this area goes up to 48°C and 4°C, respectively with a diurnal variation to the order of 15-20°C. The average annual rainfall is 700 mm, most of which is received from early July to mid September. This study was conducted under the recognized standards of the Institutional Animal Ethics Committee (IAEC) constituted as per the article number 13 of the Committee for Purpose of Control and Supervision of Experimentation on Animals (CPCSEA) rules (Government of India).

Twenty four female KF calves were selected from LRC of ICAR-NDRI, Karnal. Based on their body weight and age they were distributed into 4 groups of 6 animals in each group as per randomised block design (RBD). All calves were fed experiment diet in form of total mixed ration (TMR) comprising of concentrate mixture, maize (green fodder) and wheat straw mixed in the ratio of 40:40:20 (on DM basis) to meet the requirements (ICAR, 2013). The concentrate mixture (maize 35, ground nut cake 15, soybean meal 15, wheat bran 32, mineral mixture 2 and common salt 1%) of group G_1 consisted of mineral mixture which was devoid of iodine. The animals in groups G_2 and G_3 were supplemented with iodine at a level of 0.25 and 0.5 ppm of dietary DM while in group G_4, a micronutrient mixture was provided supplying Zn, Cr, vitamin E and niacin at 40, 1.5, 40 and 600 ppm of dietary DM, respectively.

The dry bulb and wet bulb temperatures were recorded daily at 07:30 h and 14:30 h using Zeal dry bulb wet bulb thermometer and THI was calculated using formula (NRC, 1971) as given below:

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

Tdb= Dry bulb temperature

Twb= Wet bulb temperature

During 150 days of feeding trial, DM intake (DMI) and body weight of the animals were recorded at fortnightly intervals. Feed offered, residue leftover and DMI of the animal were recorded daily. Recording of body weights was done in morning hours before feed and water intake. Blood samples were collected at monthly intervals from the animals by jugular vein puncture in heparinised vacutainer and tubes were rotated between the palms to ensure proper mixing of blood and anticoagulants and were brought to the laboratory after placing over ice. Thereafter in order to separate plasma, samples were centrifuged for 20 min. at 3000 rpm. The plasma samples were stored at -20°C for estimation of catalase, glutathione peroxidase (GPx) and superoxide dismutase (SOD), ferric reducing ability of plasma (FRAP), thiobarbituric acid reducing substances (TBARS) and total immunoglobulins.

Dry matter, organic matter (OM), crude protein (CP), ether extract (EE) and total ash were determined as per AOAC, 2005 and neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined as per Van Soest *et al.* (1991). Urine samples were analyzed for nitrogen content (AOAC, 2005). Concentration of zinc (Zn), copper (Cu), iron (Fe), manganese (Mn) and chromium (Cr) in feed samples were determined in an air-acetylene flame on an atomic absorption spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan) after digestion in tri-acid mixture (HNO_3:$HClO_4$:H_2SO_4"3:2:1 ratio). Sulphur was estimated in feed sample by turbidimetric method

(Massoumi and Cornfield, 1963).

For haemolysate preparation, 2 ml of whole blood was collected in vials containing acid citrate dextrose (300 µl/2 ml blood) as anticoagulant and centrifuged for 15 min at 4°C at 2000 rpm to separate buffy coat and plasma. The erythrocyte pellet (packed RBC) was washed with phosphate buffer saline (PBS) solution (NaCl, 137 mM; KCl, 2.7 mM; Na_2HPO_4, 10 mM and KH_2PO_4, 1.8 mM), pH was adjusted to 7.4. The packed RBC was mixed with an equal volume of PBS to form RBC suspension. Haemolysate (1:20 dilution) was prepared by mixing 0.5 ml RBC suspension with 4.5 ml of stabilizing solution (EDTA, 2.7 mM and 0.7 mM, 2-mercaptoethanol).The levels of SOD, catalase, GPx, FRAP and TBARS in blood plasma was estimatedusing the procedures of Madesh and Balasubramanian (1998), Aebi (1984), Pagliaand andValentine (1967), Benzie and Strain (1999) and Mc Ewan and Fisher (1970), respectively.

The data were analyzed by two-way ANOVA and difference between the means were compared by Tuckey's-B multiple range test as per Snedecor and Cochran (1994) using software package SPSS version 20.0 (2012).

RESULTS AND DISCUSSION

The chemical composition of feed ingredients (proximate and cell wall constituents) *viz.* concentrate mixture, maize fodder and wheat straw are presented in Table 1. There were significant (P<0.05) seasonal variations in terms of THI. The THI ranged from 61.42±0.6 to 85.60±0.48 and (Fig. 1). The THI was lower in winter as compared to summer (March to May). The THI values were higher during 6th to 10th fortnight. When THI exceeded 76, the animals showed heat stress (Srikandakumar *et al.,* 2003).

Supplementing iodine (G_2 0.25, G_3 0.5 mg kg/DM) and micronutrient mixture (G_4, Zn, Cr, vitamin E and niacin at 40, 1.5, 40 and 600 ppm, respectively) did not affect feed intake, daily gain and feed conversion ratio irrespective of seasonal variation in THI (Table 2). However, an improvement was observed in live weight gain of goats on iodine supplementation at 0.05 and 0.075 mg/animal/d (Pattanaik *et al.,* 2001). Supplementation of inorganic source of Zn at 20 (Wright and Spears, 2004), 100 and 200 ppm (Malcolm-Callis *et al.,* 2000) or Zn as propionate at 35 ppm (Mandal *et al.,* 2008) did not affect body weight gain in calves. Jadhav *et al.* (2008) supplemented Zn at 35 and 70 ppm to the basal diet containing about 35 ppm Zn in male Murrah buffalo calves and found that ADG was higher (P<0.05) in 70 ppm Zn fed group (601.4 g) as compared control (547.4 g) and 35 ppm Zn (566.7 g) supplemented group. Zinc supplementation at 60 ppm in either organic or inorganic form to the basal diet containing 40 ppm Zn to Holstein Friesian calves for 90 d after birth did not cause any significant difference in their growth performance (Array *et al.,* 2002). Supplementary Zn at 35 and 70 ppm through $ZnSO_4$ to the basal diet containing 32.5 ppm Zn did not affect nutrient intake and body weight gain in crossbred calves (Mandal *et al.,* 2008). Wright and Spears (2004) showed that weight gain, feed intake and feed efficiency of calves were not markedly affected by addition of $ZnSO\text{-}_4$ in Holstein male calves.

Feed intake improved in lactating buffaloes supplemented with inorganic Cr at 1.5 ppm in the ration (Deka *et al.,* 2015). Halder *et al.* (2009) reported that intake of DM, OM and CP was not affected by the level of supplemental Cr but digestibility of these nutrients tended to increase linearly with the level of Cr in the diet. N retention increased proportionately with the level of supplemental Cr. When rumen protected niacin was supplemented at 12 g/d to lactating dairy

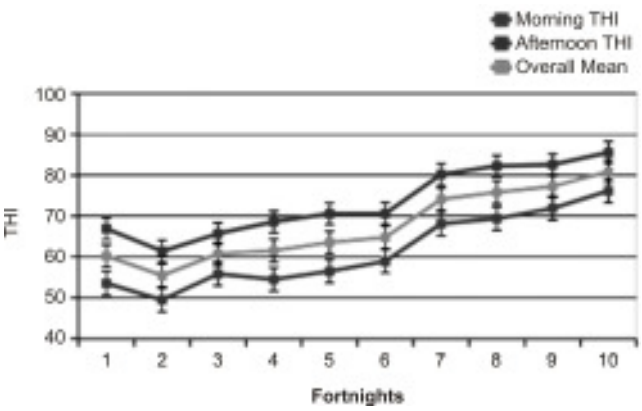

Fig. 1. THI values during different fortnights

Table 1. Chemical composition (% DM basis) of feed ingredients

Parameter	Concentrate mixture	Maize fodder	Wheat straw
Dry matter	90.99	13.98	92.26
Organic matter	92.74	89.38	88.44
Crude protein	20.34	10.75	2.27
Total ash	8.26	10.62	11.56
Ether extract	3.99	2.66	0.71
Neutral detergent fibre	33.96	47.55	72.97
Acid detergent fibre	13.81	36.21	54.62
Total digestible nutrients*	68.38	62.66	45.34
Mineral composition			
Zn (ppm)	49.56	30.20	6.08
Cu (ppm)	26.12	16.66	2.16
Mn (ppm)	56.42	32.85	2.38
Cr (ppm)	0.84	1.76	0.37
Fe (ppm)	402.5	380.83	208.41
Iodine (ppm)	0.32	0.25	0.19
S (%)	0.29	0.21	0.15

(* = calculated as per NRC, 2001)

cows during heat stress there was no significant effect on DM intake (Zimbelman *et al.,* 2013). There was no any significant effect of supplementing 185 IU of vitamin E/kg of DM in comparison to diet supplemented with 15 IU/kg DM on calves' DMI, average daily weight gain and feed conversion efficiency (Cusack *et al.,* 2005).Samanta *et al.* (2006) also did not observe any significant effect on weight. gain in calves supplemented with 125 IU of vitamin E/day when compared with control group. Likewise, supplementation of vitamin E at 40 and 20 mg/kg DM had no positive effect on growth (Dass *et al.,* 2009).

The activity of SOD, GPx and catalase was found to be similar among groups and also there were no significant differences between seasons (Table 3). It was reported that SOD activity increased ($P<0.05$) under heat stress conditions (Manish *et al.,* 2011). Under the heat stress conditions, cellular oxidative damage and lipid peroxidation increase due to reactive oxygen species generation. Supplementation of Zinc may have many nutritional benefits for livestock but has an inhibitory effect on the spontaneous lipid peroxidation. Zinc is present as cofactor for SOD and reduces superoxide radicals to H_2O_2 through cascading enzyme

Table 2. Effect of micronutrients supplementation on dry matter intake and feed conversion ratio in Karan Fries heifers

Variable	Group			
	G_1	G_2	G_3	G_4
Initial body weight (kg)	116.18±16.35	117.65±11.81	115.15±13.60	116.61±14.04
Final body weight (kg)	197.87±19.09	201.06±14.68	197.86±16.39	199.66±13.12
Feed intake (kg/d)	4.49±0.23	4.56±0.23	4.70±0.27	4.59±0.24
Average daily gain (g)	543.55±9.98	542.99±10.81	551.37±10.47	549.72±24.79
Feed conversion ratio (kg feed consumed/kg gain)	8.23±0.35	8.21±0.37	8.50±0.44	8.28±0.38

systems (Chan *et al.,* 1998). Supplementation of vitamin E (Lallawmkimi *et al.,* 2013) and niacin (Khan *et al.,* 2013) in heat stressed cows reduced plasma SOD activity compared to the un-supplemented group. Aliarabi and Chabbra (2006) reported that 40 ppm of chelated Zn supplementation in crossbred calves increased ($P<0.05$) SOD activity. The SOD and catalase activities were lower in vitamin E supplemented (100 IU and 150 IU/kg) groups of Murrah buffalo calves (Dass *et al.,* 2009). Serum SOD activity was not affected but serum GPx activity was significantly decreased by iodine supplementation (Qin *et al.,* 2011).

The GPx and catalase activity was not affected due to micronutrient supplementation or seasons. Similarly, Khan *et al.* (2013) reported that the plasma catalase activity showed no significant difference between the periods on niacin supplementation to lactating cows. However, supplementation of Zn at higher level increased GPx and catalase activity in calves fed basal diet with 140 ppm supplemental Zn compared to those fed control diet (Parashuramulu *et al.,* 2015). In present study, supplementation of iodine and micronutrient mixture had no marked effect on antioxidant status *viz.*, SOD, catalase and GPx in heat stressed crossbred heifers.

The TBARS levels during winter and summer in groups G_1, G_2, G_3 and G_4 and monthly variation in TBARS activity in different treatment groups under winter and seasons are presented in (Table 3). Plasma TBARS values were found to be lower ($P<0.05$) during winter than summer season. The blood lipid peroxidation activity was lower ($P<0.05$) in group G_4 supplemented

Table 3. Effect of micronutrients supplementation on antioxidant status in different groups of Karan Fries heifers

Season	Group				Period mean	P value		
	G_1	G_2	G_3	G_4		T	S	T×S
			Superoxide dismutase activity (Unit/g Hb/min)					
W	2012±142	2005±157	2151±141	2054±148	2055±72	0.85	0.64	0.74
S	2155±170	2229±134	2297±176	2178±231	2227±89			
Overall mean	2083±110	2117±103	2224±112	2116±136	2135±58			
			Blood glutathione peroxidase activity (µmole NADPH oxidized/g Hb/min)					
Winter	21.80±1.25	21.31±1.25	23.57±1.71	21.00±1.36	22.23±0.70	0.10	0.10	0.94
Summer	23.39±1.34	24.29±1.40	24.85±1.29	22.32±2.11	24.17±0.78			
Overall Mean	22.59±0.91	22.80±0.96	24.21±1.06	21.66±1.24	22.82±0.53			
			Blood catalase activity (µmol of H_2O_2 consumed/min/mg Hb)					
Winter	109.54±5.29	111.41±5.52	112.02±6.29	111.80±2.61	111.19±2.61	0.91	0.70	0.97
Summer	110.49±8.91	113.44±7.79	117.12±5.26	110.79±3.53	112.96±3.53			
Overall Mean	110.02±5.11	112.43±4.71	114.57±4.07	111.08±2.19	112.08±2.19			
			Plasma TBARS values (nmol/mg protein)					
Winter	5.35±0.29	4.94±0.20	5.03±0.27	4.50±0.33	4.95^{A}±0.12	<0.01	<0.01	0.15
Summer	6.63±0.18	6.02±0.25	6.23±0.30	4.75±0.17	6.29^{B}±0.16			
Overall Mean	5.99^{b}±1.00	5.48^{b}±0.91	5.63^{b}±0.94	4.62^{a}±0.77	5.43±0.45			
			Ferric reducing ability of plasma (µmol/l)					
Winter	1327±60	1309±34	1428±53	1221±41	1321^{A}±25	0.003	0.01	0.87
Summer	1375±50	1397±70	1505±52	1348±41	1406^{B}±27			
Overall Mean	1351^{ab}±38	1353^{ab}±39	1467^{b}±37	1284^{a}±30	1364±19			

Values bearing different superscripts in a row (a, b) and column (A, B) differ significantly ($P<0.05$)

Table 4. Plasma total imunoglobulins levels (mg/mL) in different groups as affected by micronutrients supplementation in Karan Fries heifers

Season	Group					Significance		
	G_1	G_2	G_3	G_4	Overall mean	T	S	T×S
				Superoxide dismutase activity (Unit/g Hb/min)				
Winter	27.03±1.04	28.73±1.51	27.11±0.32	28.47±1.28	27.83^{B}±0.44	0.40	0.03	0.34
Summer	22.77±0.73	22.87±0.91	22.59±0.68	22.09±0.62	22.58^{A}±0.17			
Overall Mean	24.77±0.73	25.80±1.53	24.85±1.06	25.28±1.56	25.20±0.14			

^{A,B} Values bearing different superscripts in a column differ significantly (P<0.05)

with micronutrient mixture, however, no significant interaction was observed between the treatment effect and seasonal variation. Overall mean of blood plasma FRAP activity (µmol/l) during winter and summer seasons differed significantly (P<0.05) (Table 3) and group G_4 showed the lowest activity and significantly higher value was found in group G_1. Zade *et al.* (2014) observed that Cr supplementation increased peripheral blood glucose concentration along with decreased level of NEFA and TBARS. It was suggested that addition of $CrCl_3$ might have positive effects on antioxidant status and reduced the susceptibility of blood to free radical oxidative damage. The FRAP activity was higher during summer. In the present study, FRAP activity was reduced in response to micronutrient supplementation which is an indicative of oxidative stress mitigation. Similarly, the FRAP activity was found to be lower on vitamin E supplementation in Murrah buffalo calves (Dass *et al.,* 2009).

The supplementation of micronutrients did not have any significant effect on plasma total Ig, however, total Ig levels were found to be higher (P<0.05) in winter than in summer (Table 4). Higher levels of total Ig levels during winter than in summer season indicated that a prolonged exposure to severe heat stress was responsible for a decline of immune cell's reactivity (Cook *et al.,* 2002). They suggested that vitamin E enhanced the ability of immune cells of the mammary gland to produce more immunoglobulin. Dietary supplementation of Zn at 500 ppm level in grower pigs improved gamma globulin concentration (Borah *et al.,* 2014). Serum concentration of IgM and IgG increased by 13.2 and 20.6% due to Cr supplementation 200 µg/kgDM as nano-Cr (Wang *et al.,* 2007). Deka *et al.* (2014) also reported improved plasma total Ig and IgG in peri-parturient Murrah buffaloes due to supplementation of inorganic Cr at 1.5 ppm of dietary DM.

CONCLUSIONS

The feed intake and growth performance was not affected by seasons in Karan-Fries heifers in winter and summer irrespective of micronutrients inclusion in the diet. Micronutrient supplementation could mitigate oxidative stress and reduced FRAP and TBARS activity in cross bred heifers under summer stress conditions.

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REFERENCES

Aebi, H.1984. Catalase *in vitro*. In: *Methods in Enzymology* (L. Packer; ed.). Academic Press, New York.105: 121-126.

Aliarabi, H. And Chhabra, A. 2006. Effect of inorganic and chelated zinc supplementation on the performance of crossbred calves. *Indian J. Anim. Nutr*. 21: 141-145.

AOAC. 2005.*Official Methods of Analysis*. 18th edn. Association of Official Analytical Chemists, Washington, DC, USA.

ARC. 1980. *Nutrient Requirements of Ruminant Livestock*. Commonwealth Agricultural Bureaux, Slough, UK.

Array, J.L., Oberbauer, A.M., Famula, T.R., Garnett, I., Oltjen, J.W., Imhoof, J., Kehrli., M.E.and Jr., Graham, T.W. 2002.

Growth of Holstein calves from birth to 90 days: The influence of dietary zinc and BALD status. *J. Anim.Sci*. 80: 545-552.

Benzie, I.F. and Strain, J.J. 1999. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol*. 299:15-27.

Borah, S., Sarmah, B.C., Chakravarty, P., Naskar, S., Dutta, D.J. and Kalita, D. 2014. Effect of zinc supplementation on serum biochemicals in grower pig. *J. Appl. Anim. Res*. 42:244-248.

Chan, P.H., Kawase, M., Murakami, K., Chen, S.F., Li, Y., Calagui, B., Reola, L., Carlson, E. and Epstein, C.J. 1998. Overexpression of SOD1 in transgenic rats protects vulnerable neurons against ischemic damage after global cerebral ischemia and reperfusion. *J. Neurosci*. 18:8292-8299.

Cooke, R.F., Daigle, C.L., Moriel, P., Smith, S.B., Tedeschi, L.O. andVendramini, J.M. 2020. Cattle adapted to tropical and subtropical environments: social, nutritional and carcass quality considerations. *J. Anim. Sci*. 98:14.

Cook-Wiens, E. And Grotewiel, M.S. 2002. Dissociation between functional senescence and oxidative stress resistance in Drosophila. *Exp. Gerontol*. 37:1347-1357.

Das, S., Gautam, N., Dey, S.K., Maiti, T. and Roy, S. 2009. Oxidative stress in the brain of nicotine-induced toxicity: Protective role of *Andrographis paniculata*and vitamin E. *Appl. Physiol. Nutr. Metabol*. 34: 124-135.

Das, R., Sailo, L., Verma, N., Bharti, V., Saikia, J. and Kumar, R. 2016. Impact of heat stress on health and performance of dairy animals: A review. *Vet.World*. 9:260-268.

Deka, R.S., Mani, V., Kumar, M., Zade, S.S., Tyagi, A.K. and Kaur, H. 2014. Body condition, energy balance and immune status of periparturient Murrah buffaloes (*Bubalus bubalis*) supplemented with inorganic chromium. *Biol. Trace Elem. Res*. DOI 10.1007/s12011-014-0069-6.

Deka, R.S., Mani, V., Kumar, M., Zade, 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.*Biol. Trace Elem. Res*. 168: 362-371.

Higdon, J.V. and Frei, B. 2003. Obesity and oxidative stress. *Arterioscler. Thromb.Vasc. Biol*. 23: 365-367.

Jadhav, S.E., Garg, A.K. and Dass, R.S. 2008. Effect of graded levels of zinc supplementation on growth and nutrient utilization in male buffalo (*Bubalus bubalis*) calves. *Anim.Nutr. Feed Technol*.8: 65-72.

Keshri, A., Bashir, Z., Kumari, V., Prasad, K., Joysowal, M., Singh, M., Singh, D., Tarun, A. and Shukla, S. 2019. Role of micronutrients during peri-parturient period of dairy animals: A review. *Biol. Rhythm Res*. 9:1-3.

Khan, M., Ringseis, R., Mooren, F.C., Krüger, K., Most, E. and Eder, K. 2013. Niacin supplementation increases the number of oxidative type I fibers in skeletal muscle of growing pigs. *BMC Vet. Res*. 9:177.

Lallawmkimi, M.C., Singh, S.V., Upadhyay, R.C. and De, S. 2013. Impact of vitamin E supplementation on heat shock protein 72 and antioxidant enzymes in different stages of Murrah buffaloes during seasonal stress. *Indian J. Anim. Sci*. 83: 909-915.

Madesh, M. and Balasubramanian, K.A. 1998. Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. *Indian J. Biochem. Biophys*. 35:184-188.

Malcolm-Callis, K.J., Duff, G.C., Gunter, S.A., Kegley, E.B. and Vermeire, D.A. 2000. Effects of supplemental zinc concentration and source on performance, carcass characteristics, and serum values in finishing beef steers. *J. Anim. Sci*. 78:2801-2808.

Mandal, G.P., Dass, R.S., Garg, A.K., Varshney, V.P. and Mondal, A.B. 2008. Effect of zinc supplementation from inorganic and organic sources on growth and blood biochemical profile in crossbred calves. *J. Anim. Feed Sci*.17:147-156.

Manish, K., Jindal, R. and Nayyar, S. 2011. Influence of heat stress on antioxidant status in Beetle goats. *Indian J. Small Rumin*. 17:178-181.

Massoumi, A. and Cornfield, A.H. 1963. A rapid method for determining sulphate in water extracts of soils. *Analyst*. 88: 321-322.

McEvan, A.D. and Fisher, E.W. 1970. A turbidity test for the estimation of immunoglobulins levels in neonatal calf serum. *Clin. Chem. Acta*. 17:155-163.

Norouzian, M.A., Valizadeh, R., Azizi, F., Hedayati, M., Naserian, A.A. and ShahroodI, F.E.2009. The effect of feeding different levels of potassium iodide on performance, G3 and G4 concentrations and iodine excretion in Holstein dairy cows. *J. Anim. Vet. Adv*. 8:111-114.

NRC. 1971. A Guide to Environmental Research on Animals. Natl. Acad. Sci., Washington, DC, USA.

Paglia, D.E. and Valentine, W.N. 1967. Studies on quantitative and qualitative characterization of erythrocyte gluthathione peroxidase. *J. Lab. Clin. Med*. 70: 158-168.

Parashuramulu, S., Nagalakshmi, D., Rao, D.S., Kumar, M.K. and Swain, P.S. 2015. Effect of zinc supplementation on antioxidant status and immune response in buffalo calves. *Anim. Nutr. Feed Technol*. 15: 179-188.

Pattanaik, A.K., Khan, S.A., Varshney, V.P. andBedi, S.P.S. 2001. Effect of iodine level in mustard (*Brassica juncea*) cake-based concentrate supplement on nutrient utilisation and serum thyroid hormones of goats. *Small Rumin. Res*. 41: 51-59.

Pradhan, N., Mukherjee, J., Biswas, U., Sarathi, J.P., Pakira, M. and Guha, C. 2016.Infradian rhythm in hematology, cell mediated immunity and serum micronutrient concentration around peripartum periods in high yielding crossbred cows before and after micro-nutrient supplementation. *Biol. Rhythm Res*. 47: 315-327.

Qin, F., Zhu, X., Zhang, W., Zhou, J., Zhang, S. and Jia, Z. 2011. Effects of dietary iodine and selenium on nutrient digestibility, serum thyroid hormones and antioxidant status of Liaoning cashmere goats. *Biol.Trace Elem. Res*. 143: 1480-1488.

Samanta, A.K., Dass, R.S., Rawat, M., Mishra, S.C. and Mehra, U.R. 2006. Effect of dietary vitamin E supplementation on serum alpha- tocopherol and immune status of crossbred calves. *Asian-Australas. J. Anim. Sci*.19: 500-506.

Snedecor, G.W. and Cochran, W.G. 1994.*Statistical Methods*. 8th ed. Affiliated East. West Press, New Delhi, India.

Srikandakumar, A., Johnson, E.H. and Mahgoub, O. 2003. Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in Omani and Australian Merino sheep. *Small Rumin. Res*. 49: 193-198.

Van Soest, P.J., Robertson, J.B. and Lewis, B. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74: 3583-3597.

Wang, M.Q., Xu, Z.R., Zha, L.Y. and Lindemann, M.D. 2007. Effects of chromium nanocomposite supplementation on blood metabolites, endocrine parameters and immune traits in finishing pigs. *Anim. Feed Sci. Technol*. 139: 69-80.

Wright, C.L. and Spears, J.W. 2004. Effect of zinc source and dietary level on zinc metabolism in Holstein calves. *J. Dairy Sci.* 87:1085-1091.

Zade, S., Mani, V., Deka, R.S., Kumar, M., Kaur, H., Kewalramani, N. and Tyagi, A.K. 2014. Energy metabolites, lipid variables and lactation performance of periparturient Murrah buffaloes (*Bubalus bubalis*) fed on diet supplemented with inorganic chromium. *Biol. Trace Elem. Res*. 159:115-127.

Zha, L.Y., Zeng, J.W. and Chu, X.W. 2009. Efficacy of trivalent chromium on growth performance, carcass characteristics and tissue chromium in heat-stressed broiler chicks. *J. Sci. Food Agric*.89: 1782-1786.

Zimbelman, R.B., Collier, R.J. and Bilby, T.R. 2013. Effects of utilizing rumen protected niacin on core body temperature as well as milk production and composition in lactating dairy cows during heat stress. *Anim. Feed Sci. Technol*.180: 26-33.

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