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Effect of zinc supplementation on physiological and oxidative stress status of peri-parturient Karan Fries cows during heat stress condition

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Abstract
Present study was conducted to investigate effect of Zinc supplementation on physiological and oxidative stress status of Karan Fries (KF) cows during heat stress condition. Cows were categorized as Control, T1 and T2 group having 6 animals each. Cows of control group were fed basal diet whereas T1 and T2 group were supplemented with 80 ppm and 120 ppm Zn, respectively along with basal diet from 45 days pre-partum to 45 days post-partum. Physiological parameters viz. rectal temperature, respiration rate, pulse rate and skin temperature were recorded fortnightly. Blood samples were collected fortnightly for estimation of levels of plasma Zn and oxidative stress parameters viz. superoxide dismutase and total anti-oxidant activity. THI indicated that all three groups of cows experienced heat stress during study period. Plasma Zn level was significantly (p<0.05) higher in supplemented group and no significant effect of Zn supplementation on physiological parameters was observed. SOD activity and total anti-oxidant capacity was significantly (p<0.05) higher in supplemented groups. It can be concluded that KF cows could cope efficiently with heat stress by releasing more antioxidant when supplemented with dietary Zn.

Keywords: cow, heat stress, oxidative, physiological, zinc

Introduction
In India, crossbred cattle are the highest milk producer and play an important role in dairy industry [1]. However, their heat dissipation ability is poor which limits their productivity under tropical conditions. This can be attributed to their higher metabolic rate, larger body size and low density of sweat glands as compared to indigenous breeds [2]. High ambient temperature compromise performance and productivity as it results in reduced feed intake and nutrient utilization, poor growth rate, feed efficiency and milk production which leads to economic losses in dairy animals [3]. Heat stress also leads to oxidative stress associated with a reduced antioxidant status in the animals as reflected by increased oxidative damage and lowered plasma concentrations of antioxidants. There are many antioxidants which plays a critical role in protecting various cells of the body against the damaging effects of free radicals [4]. However, activity of antioxidants decline due to the excess production of endogenous free radicals during heat stress [5].

Zinc (Zn) is an essential nutrient and a very important trace element involved in a wide range of activities. It acts as cofactor for more than 200 enzymes and promotes biochemical reactions in body [6]. One of the most important functions of Zn is its participation in the anti-oxidant defense system. It is a required component of the antioxidant enzyme superoxide dismutase and is needed for the synthesis of metallothionein, which may scavenge free radicals [7]. Zn deficiency increases oxidative damage of cell membranes caused by free radicals [8]. The major sources of Zn in the mineral supplements formulated for animal feeding are inorganic salts like Zn sulphate ($ZnSo_4$), Zn oxide (ZnO) and Zn chloride ($ZnCl_2$) [9]. The modifications in the physiological and metabolic functions of the KF cows can help in improving their welfare and future productivity during thermal stress [10]. The metabolic status of cows can be modified by supplementing with dietary Zn to ameliorate heat stress. It has been reported that supplementation of Zn in diet of crossbred animals results in improved reproductive performance and hormonal status of peri-parturient KF cows during heat stress condition [11, 12]. However, reports on effect of supplemental Zn on physiological parameters and oxidative

stress status of crossbred cows during heat stress are scanty. Therefore, the present investigation was undertaken to assess the effect of Zn supplementation on physiological parameters and antioxidant status of peri-parturient KF cows during heat stress condition.

Materials and Methods

Animal's selection, management and experimental protocol

Experiment was approved by Institutional Animal Ethics Committee constituted as per the article number 13 of the CPCSEA-rules, laid down by the Government of India. Present experiment was carried out at Livestock Research Centre, ICAR-National Dairy Research Institute, Karnal during hot humid season. 18 pregnant KF cows at peri-parturient period were selected from and divided into three groups of 6 animals each on basis of expected producing ability (EPA), parity and body weight. Experimental animals were kept in separate pens and were maintained as per the standard conditions of feeding and management followed at NDRI, Karnal. All the animals were fed a ration consisting of concentrate mixture and roughages as per ICAR (2013). Cows were divided into 3 group viz. C (Control), T1 (Treatment1) and T2 (Treatment 2). Control group was fed basal di*et al*one, however T1 and T2 groups were supplemented with 80 ppm and 120 ppm Zn, respectively as $ZnSo_4$ (food grade) along with basal diet from 45 days pre-partum to 45 days postpartum. The peri-parturient KF cows were fed according to recommendations of ICAR (2013).

Temperature humidity index (THI) estimation

Meteorological variables in terms of dry and wet bulb temperatures were recorded throughout the experimental period in morning and evening. Temperature humidity index (THI) was calculated using the formula of Johnson *et al.*, (1963) [13].

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

Where: Tdb = dry bulb temperature (°C) and Twb = wet bulb temperature

Physiological responses

Rectal temperature was recorded using digital thermometer by keeping the thermometer in contact with rectal mucosa for about 2 minutes. Respiration rates of each animal were recorded from visual observation of inward and outward movement of the flank. Pulse rate (PR) of the animals were counted by feeling the pulsation of middle coccygeal artery at the base of the tail. The peripheral skin temperature at different anatomical sites of the experimental animals, viz, forehead, ear, dorsal, ventral and flank regions were recorded using non-contact telethermometer (Raytek, Model Raynger ST2L, M/s. Surrey Scientific, and Surrey, U.K.) by keeping it 2-3 inches away from the surface of the desired site.

Blood collection

Blood samples were collected from jugular vein in 10 ml heparinized tubes at 7:30 AM before offering feed on the days -45, -30, -15, 0, + 15, + 30 and + 45 with respect to expected date of calving. Day '0' represent the day of calving of experimental animals. The plasma was harvested within one hour after sampling following centrifugation at 3000 rpm for 30 minutes at 4 °C and plasma collected and stored at -20 °C for further analysis.

Estimation of Zn in plasma

Plasma Zn was estimated with the help of Atomic absorption spectrophotometer using acetylene as fuel and air as an oxidant.

Estimation of antioxidants

Plasma total antioxidant activity and plasma superoxide dismutase (SOD) was determined in the plasma of experimental animals by using standard kits.

Statistical analysis

Statistical analysis was done by one way ANOVA using the SPSS statistical software program (version 22.0). The data were reported as mean (±SE), and differences were regarded as significant at $p<0.05$. Post hoc difference between treatments groups were examined by the Duncan test**.**

Results and Discussion

Temperature humidity index (THI)

Data pertaining to THI of morning, evening and overall THI during study period has been presented in Table 1.The average THI values recorded during month of July was 81.13, during August was 83.4 and during September was 78.9. The observed values of THI indicated that all three groups of experimental cows experienced heat stress during the entire study period because it exceeds the upper critical limit (72 THI units) for crossbred cattle.

Table 1: Average THI Value of Experimental Sheds

Month	Db(M) (^{0}C)	Wb(M) (^{0}C)	THI(M)	Db(E) (^{0}C)	Wb(E) (^{0}C)	THI(E)	Over all THI
July	27.6	25.5	78.83	31.1	28.4	83.44	81.13
August	28.8	26.7	80.56	34.3	29.1	86.24	83.4
September	25.9	23.8	76.38	30.2	26.5	81.42	78.9

Plasma Zn status

Results pertaining to plasma Zn status of KF cows from different groups have been presented in Table 2 .The mean (± SE) values of plasma Zn level at 45 days pre-partum was 0.94 ± 0.02, 0.96 ± 0.03 and 0.93±0.01ppm in control, T1 and T2 groups, respectively. At calving, the plasma Zn level decreased by 34.2% in control group whereas the reduction was only 17.0% and 3.3% in T1 and T2 groups, showing effect of Zn supplementation. After calving, plasma Zn level was significantly ($p<0.01$) higher in supplemented groups throughout study period. Our results are in agreement with findings of Maurya *et al.*, (2013) [14] who reported that supplementation of 60 ppm Zn to dairy cows results in higher Zn concentration in treatment groups compared to control (1.68 ± 0.03 vs 1.52 ± 0.03 ppm).

Table 2: Fortnightly Mean (± SE) plasma Zn concentration (ppm) of KF cows

Days	Control	T1	T2	P-value
-45	0.94 ± 0.02	0.96 ± 0.03	0.93 ± 0.01	NS
-30	0.82^{a} ± 0.01	1.02^{ab} ± 0.12	1.19^{b} ± 0.07	*P*<0.05
-15	0.64^{a} ± 0.01	0.99^{b} ± 0.06	1.12^{b} ± 0.04	*P*<0.01
0	0.60^{a} ± 0.01	0.82^{b} ± 0.05	1.05^{b} ± 0.06	*P*<0.01
15	0.74^{a} ± 0.05	0.90^{b} ± 0.02	1.31^{b} ± 0.05	*P*<0.01
30	0.86^{a} ± 0.02	1.11^{b} ± 0.06	1.49^{c} ± 0.08	*P*<0.01
45	0.97^{a} ± 0.07	1.21^{b} ± 0.04	1.59^{c} ± 0.04	*P*<0.01
^{a,b,c}Means having different superscripts within a row differ significantly (*P*<0.05)				

Physiological Parameters

In present study, no significant variation was observed in the physiological parameters viz. rectal temperature, respiration rate, pulse rate and skin temperature in any of the group of KF cows during heat stress condition. The mean (± SE) values of rectal temperature of morning, afternoon, and evening at 45 days pre-partum (101.12 ± 0.14, 101.08 ± 0.4 and 100.75 ± 0.28), (101.25 ± 0.16, 101.23 ± 0.17 and 101.41 ± 0.30), (101.55 ± 0.26,101.43 ± 0.21 and 102.05 ± 0.28) in control, T1 and T2 groups, respectively indicate that there was no significant difference in rectal temperature among any of the experimental groups. At the time of calving, rectal temperature slightly increases in all three groups of cows which is perhaps due to calving stress. It is probably the first study in which supplemental Zn has been tried to alleviate heat stress.

The mean (± SE) values of pulse rate of morning, afternoon, and evening at 45 days pre-partum (52.33 ± 0.33, 54 ± 1.58 and 53.33 ± 0.55), (58.66 ± 2.12, 57.16 ± 1.30 and 57.83 ± 1.53) and (73 ± 1.36, 73 ± 0.93 and 71.16 ± 2.98) in control, T1 and T2 groups, respectively indicate no significant variation in pulse rate among any of groups. Similar trend was followed during the entire experimental period. Pulse rate, a criteria for assessing heat stress, do not show any significant difference in Zn supplemented group as compared to control.

The mean ± SE of respiration rate of morning, afternoon, and evening at 45 days pre-partum (54.83 ± 1.6, 54.66 ± 1.49 and 54.5 ± 1.58) 44.5 ± 1.58, 41 ± 1.93 and 42.33 ± 0.66) and 75.5 ± 1.60, 75 ± 1.31 and 69.83 ± 2 in control, T1 and T2. groups respectively indicate that there was no significant difference in respiration rate in any of the experimental groups. Similar trend was followed during the entire experimental period.

The overall mean (± SE) values of skin temperature of morning, noon and evening in control, T1, and T2 (36.08 ± 0.48, 36.01 ± 0.46 and 36.27 ± 0.49 °C), (36.27 ± 0.49, 36.14 ± 0.48 and 36.10 ± 0.52 °C) and (36.87 ± 0.42, 36.67 ± 0.44 and 36.69 ± 0.43 °C) in control, T1 and T2 groups respectively, indicate no significant variation in skin temperature in any of the experimental groups. Similar trend was followed during the entire experimental period. No traceable literature could be found for comparison of our result.

Superoxide dismutase (SOD)

Results pertaining to plasma superoxide dismutase level (IU/ml) in control and Zn supplemented groups of KF crossbred cows has been presented in the Table 3. The mean (± SE) of plasma SOD activity at 45 days pre-partum was 142.08±2.08, 140.41±3.4 and 144.7± 10.52 in control, T1 and T2 group, respectively (*p*>0.05). Afterwards, a reducing trend in plasma SOD activity was observed in all three groups up to day of calving. However, proportion of reduction of SOD activity was less in Zn supplemented groups compared to control. On the day of calving, lowest values of plasma SOD activity was observed in all three groups. After calving, increasing trend in SOD activity was observed in all group which was significantly (*p*<0.01) different. This increment in SOD activity was much more in supplemented groups especially 120 ppm Zn supplemented group. Our results indicate that plasma SOD activity is increased by Zn supplementation which might be due to the fact that Zn is an intrinsic constituent of superoxide dismutase, a major scavenger of free radicals, present in the cytoplasm of many types of cells and in the extracellular space [7]. Our results are in agreement with Phondba *et al.*, (2012) [15] who found improved values of plasma SOD by supplementing Zn to cattle.

Table 3: Fortnightly average SOD activity (IU/ml) of KF cows

Days	Control	T1	T2	p-value
-45	142.08 ± 2.08	140.41 ± 3.4	144.7 ± 10.52	NS
-30	134.23^{a} ± 2.58	136.23^{a} ± 5.64	142.88^{b}± 7.83	*P*<0.01
-15	119.83^{a} ± 5.33	127^{a} ± 6.13	137.33^{b} ± 6.88	*P*<0.01
0	80.53^{a} ± 7.79	110.03^{b} ± 9.77	119.55^{c} ± 9.14	*P*<0.01
15	101.83^{a} ± 3.27	120.2^{b} ± 6.2	140.41^{c} ± 3.40	*P*<0.01
30	106.36^{a} ± 2.82	131.21^{b} ± 7.77	161.55^{c} ± 8.10	*P*<0.01
45	111.86^{a} ± 3.65	146.36^{b} ± 9.16	195.22^{c} ± 2.34	*P*<0.01
^{a,b,c}Means having different superscripts within a row differ significantly (*P*<0.01)				

Total anti-oxidant capacity (TAC)

The mean of plasma total anti-oxidant capacity (ng/ml) at 45 days pre-partum in three respective groups were 10.37, 9.80 and 10.89 (Table 4). On the day of calving, significant difference (*p*<0.05) in the mean of plasma total anti-oxidant capacity (ng/ml) was observed between control, T1 and T2 and was similar (*p* ranging from <0.05 to <0.01) up to 45 days postpartum. In our study, supplementation of Zn improved plasma total antioxidant capacity in supplemented groups as compared to control which might be due to fact that Zn are free radical scavengers which protect the body defense system against excessively produced free radicals and stabilize health status of the animal. The reduced plasma total antioxidant capacity in the control group suggested that the temperature to which animals were exposed during hot humid months was stressful. Supplementation of 80 ppm and 120 ppm Zn

increased plasma total antioxidant capacity, indicating supplementation of Zn relieved the severity of heat stress in KF cows. Out of two doses used, 120 ppm Zn has shown better response in terms of amelioration of heat stress. This might be due more absorption and bioavailability of Zn in blood as evidenced by the higher plasma Zn concentration in 120 ppm Zn supplemented group. Our results are in agreement with Gaál *et al.*, (2006) [16] who reported 20% lower total antioxidant activity in dairy cows at calving compared to pre and post calving periods. Contrary to our study, Phondba *et al.*, (2012) [15] found that supplementation of 80 ppm Zn did not affect the plasma total antioxidant capacity in crossbred cows.

Table 4: Fortnightly average plasma total antioxidants activity (ng/ml) of KF cows.

Days	Control	T1	T2	P-value
-45	10.37 ± 0.12	9.80 ± 0.33	10.89 ± 2.26	NS
-30	7.24 ± 0.43	8.21 ± 1.34	9.97 ± 1.80	NS
-15	6.66 ± 0.35	7.24 ± 0.34	8.21 ± 1.34	NS
0	2.53^{a} ± 0.23	3.73^{b} ± 0.45	5.67^{c} ± 0.22	$P<0.05$
15	4.98^{a} ± 0.24	7.85^{b} ± 0.34	9.82^{c} ± 0.32	$P<0.01$
30	5.04^{a} ± 0.34	9.57^{b} ± 1.84	13.13^{c} ± 1.18	$P<0.01$
45	6.14^{a} ± 0.24	10.89^{b} ± 2.26	15.20^{c} ± 3.16	$P<0.05$
^{a,b,c}Means having different superscripts within a row differ significantly ($P<0.05$)				

Conclusion

It can be concluded that KF cows could cope efficiently with heat stress by releasing more antioxidant (SOD and TAC) when supplemented with dietary Zn. However no significant variation was observed in physiological parameters by Zn supplementation.

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Conflict of interest

No potential conflict of interest was reported by the author

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