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Effect of feeding various sources of Zn on antioxidant enzyme activities and blood biochemical parameters

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Abstract

This experiment was conducted to evaluate the effect of replacing inorganic zinc with lower level of organic and nano form of Zn on antioxidant activities and blood biochemical parameters of growing crossbred calves. For this experiment, fifteen crossbred calves were randomly divided into three treatment groups (n=5). These calves were provided with TMR containing 50:50 roughage to concentrate ration as a basal diet along with fixed 2 kg of green fodder. Along with basal diet toppings of different Zn sources were provided to different treatment group animals *i.e.*, ZnSO₄ (@ 40 ppm) in T1, Zn-glycinate (@ 20 ppm) in T2 and nano-ZnO (@ 10 ppm) in T3 per kg DM feed offered, for the period of 98 days. Among the groups, no significant ($p>0.05$) difference was observed in antioxidant activities and blood biochemical parameters except serum albumin level. Serum albumin level was observed to be comparatively higher ($p<0.05$) in group T3 compared to other two groups. It indicated that supplementation of ZnSO₄ (@ 40ppm), Zn-glycinate (@ 20ppm) and nano-ZnO (@ 10 ppm) gave comparable ($p>0.05$) results among the groups without any adverse effects on growing crossbred calves.

Keywords: Antioxidant activities, blood biochemical parameters, Zn-glycinate, Nano-ZnO

Introduction

Zinc (Zn) is the second most important trace element in the animal body with diverse functions like nutrient metabolism, appetite control, regulation of the immune system, oxygen free radical scavenging and in transcription factors. Also, Zn cannot be stored in the body (Mandal *et al.*, 2007) [9] therefore; it has to be regularly supplied in the ration to meet the physiological needs. Animal body contains about 10 to 50 µg of Zn/g, while plasma contains 12 to 16 µM/100 ml (Zalewski *et al.*, 2005) [22].

Zn is the only metal encountered in all 6 enzyme classes established by the International Union of Biochemistry (Vallee and Falchuk, 1993) [20]. Zn is also required for structural and functional integrity of more than 2000 transcription factors and 300 enzymes. Further-more, Zn enzymes are involved in the synthesis and/or breaking down of carbohydrates, lipids, proteins, and nucleic acids, and encompass all known classes of enzymes (Mandal *et al.*, 2007; Liu *et al.*, 2011) [9,8]; hence, almost all metabolic pathways are in some way dependent on at least one Zn requiring protein (Ranasinghe *et al.*, 2015) [15]. One of the most important functions of Zn is its participation in the anti-oxidant defence system. Zn is an essential component of the antioxidant enzyme superoxide dismutase and is needed for the synthesis of metallothionein, which may scavenge free radicals (Spears & Weiss, 2008) [18]. Which also indicates that deficiency of zinc can also increase oxidative damage to cell membranes caused by free radicals (Prasad & Kucuk, 2002) [14].

Absorption of inorganic Zn in the body is very less and differs with both site of absorption in gastro intestinal tract and age of the animal. Whereas, Chelated minerals are minerals bound to a chelating agent, which are typically organic compounds or amino acids which improves the absorption and prevents minerals from interacting with other compounds (Cao *et al.*, 2000) [2]. It is also noteworthy that trace minerals in nano form come with the characteristic property of large surface area, particle size in the range of 1-100 nm which accounts for its own kind of better bioavailability in biological systems (Bunglavan *et al.*, 2014) [1]. Considering the background and above facts and to reduce the inclusion levels and environmental pollution present study was conducted to assess the effect of various sources of Zn supplementation on blood biochemical parameters and antioxidant status of growing crossbred calves.

Materials and Methods

With the approval of Committee for the Purpose of Control and Supervision of Experiments

on Animals (CPCSEA), New Delhi, this experiment was conducted on Fifteen male crossbred calves (average body weight of 93.39±3.19 kg, 8-10 months old) at Animal Nutrition Research Station (ANRS), College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat, India. These fifteen calves were divided randomly into three treatment groups of five animals in each. Animals were offered total mixed ration (Table 1) having 50:50 roughage to concentrate ratio along with fixed quantity of 2 kg green fodder as a basal diet to all the calves for 98 days of experimental period (excluding the adaptation period and pre-experimental feeding of 21 days) as per ICAR (2013) feeding standards. In addition to this, toppings of various sources of Zn like ZnSO₄ (@ 40 ppm) as an inorganic source, Zn-glycinate (@ 20 ppm) as an organic source and nano ZnO (@ 10 ppm) as nano particle form of Zn was supplemented to T1, T2 and T3, respectively. Thus, the level of Zn in T2 and T3 was adjusted to 50 and 25% level of T1, respectively. Animals were kept in well ventilated barn having individual feeding facilities. Fresh water was offered *ad libitum* twice daily to all the animals. Quantity of ration provided was adjusted individually on the basis of body weight changes recorded biweekly. Deworming of all the animals was carried out using broad spectrum anthelmintic, before initiation of the experiment.

Blood was collected from all crossbred calves at starting of an experiment (0th day) and at the end of an experiment (98th day). Blood was collected in the morning before feeding and watering with all aseptic measures from jugular veins using in clot activator vials. Serum was separated by centrifugation of blood at 2000 rpm for 8 minutes in REMI research centrifuge machine. Separated serum was collected in sterilised Eppendorf tubes for further analysis. Blood biochemical parameters were analysed from serum in Mindry BS-120 automatic chemistry analyser with coral clinical system kit. Whereas, antioxidants parameters like superoxide dismutase (SOD), glutathione peroxidase (GPx) and lipid hydroperoxide (LPO) were estimated by using Cayman ELISA kit and TECANINFINITE M NANO ELISA plate reader.

Statistical Analysis

The experimental data were subjected to one-way analysis of variance (ANOVA) as per the methods of Snedecor and Cochran (1994) [17]. The Completely randomized design was followed. Treatment means were compared using the standard error of the difference between means for significance ($p < 0.05$).

Results and Discussion

Antioxidant Parameters

Serum was collected from all the experimental calves at the end of an experiment to evaluate the effect of different sources of Zn on antioxidant status (Table 2) of experimental animals. Mean glutathione peroxidase (GPx) activity without any significant ($p > 0.05$) difference obtained was 331.37±41.10, 372.42±40.39 and 414.90±50.58 nmol/min/ml in the groups T1, T2 and T3, respectively. Similarly, no significant ($p > 0.05$) difference was observed among the groups in serum activities of superoxide dismutase (SOD) and lipid hydroperoxide (LPO) with the values 1.13±0.11, 1.06±0.09 and 1.03±0.10 U/ml for SOD and 0.84±0.11, 1.15±0.28 and 0.72±0.15 µM for LPO in the groups T1, T2 and T3, respectively. These results indicated that reducing the

level of Zn-glycinate and nano-ZnO by 50 and 75% does not have any adverse effect on blood biochemical parameters of growing crossbred calves.

In accordance to us, Cope *et al.* (2009) [3] found no any difference between higher and lower supplementation level of inorganic and organic Zn on SOD concentration. However, in contrary to us Parashuramulu *et al.* (2015) [13] found improved antioxidant activities in higher Zn (@ 140 ppm) supplemented group compared to @ 80 ppm Zn supplemented group. Nagalakshmi *et al.* (2017) [12] found that SOD, catalase and Glutathione reductase activities were comparable among the groups whereas, Glutathione Peroxidase concentration was higher in organic Zn supplemented group. Mishra *et al.* (2017) [11] also stated that antioxidant enzyme activities were comparable among the inorganic ZnSO₄ (@ 40 ppm) and nano ZnO (@ 20 ppm and @10ppm) supplemented groups however, it was significantly higher compared to the control.

Blood Biochemical Parameters

Blood biochemical parameters at the starting of an experiment (Table 3) were comparable ($p > 0.05$) among the groups. Similarly, on 98th day of an experiment except serum albumin level all other blood biochemical parameters were comparable ($p > 0.05$) among the groups with the values 5.91±0.18, 6.26±0.18 and 6.45±0.06 g/dl for total protein; 2.41±0.11, 2.57±0.14 and 2.64±0.05 g/dl for serum globulin; 0.72±0.05, 0.66±0.08 and 0.66±0.05 mg/dl for creatinine; 12.79±0.73, 12.26±0.78 and 12.28±1.00 mg/dl for urea; 142.14±5.21, 141.42±3.97 and 137.71±4.70 U/L for alkaline phosphatase activity; 50.81±2.05, 53.92±2.06 and 56.05±1.48 U/L for SGOT; 26.38±3.08, 29.30±2.12 and 28.58±0.89 U/L for SGPT and 8.35±0.31, 8.71±0.54 and 8.52±0.29 for serum calcium level in the groups T1, T2 and T3, respectively. However, Serum albumin level was significantly higher ($p < 0.05$) in T3 (3.81±0.02) compared to T2 (3.69±0.05) and T1 (3.50±0.12). These results indicated that reducing the level of Zn-glycinate and nano-ZnO to 50 and 25% does not have any adverse effect on blood biochemical parameters of growing crossbred calves. Also, numerically higher results were obtained in the group supplemented with nano-ZnO. Similar results were also observed by Spears (1989) [18], Wright & Spears (2004) [21], Dass *et al.* (2009) [4] and Hassan *et al.* (2016) [6] where no significant difference was observed among the groups. However, Dass *et al.* (2009) [4] observed higher alkaline phosphatase activity in ZnSO₄ supplemented group compared to control. Whereas, Shakweer *et al.* (2010) [16] and Gaafar *et al.* (2011) [5] stated higher ($p < 0.05$) plasma albumin and globulin level in Zn methionine supplemented groups. Mishra *et al.* (2017) [11] and Kumar *et al.* (2018) [7] also observed comparable ($p > 0.05$) results in Zn supplemented groups, however, higher plasma albumin and higher total protein, albumin and globulin level was observed in Zn supplemented groups compared to control in both the experiments respectively.

Table 1: Ingredient's composition of TMR

Ingredients	Quantity (kg/100kg)
Jowar straw	50
Soybean meal	20
Maize	10
DORB	10
Molasses	9
Mineral mixture	1

Table 2: Antioxidant Status of Crossbred calves on 98th day of experiment

Parameters	Treatments			SEM	CD	CV%
	T1	T2	T3			
GPx (nmol/min/ml)	331.37±41.10	372.42±40.39	414.90±50.58	44.27	NS	26.54
SOD (U/ml)	1.13±0.11	1.06±0.09	1.03±0.10	0.10	NS	20.94
LPO (µM)	0.84±0.11	1.15±0.28	0.72±0.15	0.19	NS	48.49

Table 3: Blood biochemical parameters at 0th day of experiment

Parameters	Treatments			SEM	CD	CV%
	T1	T2	T3			
Total protein (g/dl)	5.64±0.10	5.88±0.13	5.74±0.20	0.149	NS	5.80
Albumin (g/dl)	3.37±0.10	3.47±0.08	3.37±0.10	0.095	NS	6.25
Globulin (g/dl)	2.28±0.03	2.41±0.13	2.36±0.12	0.102	NS	9.68
A/G Ratio	1.48±0.04	1.46±0.09	1.44±0.06	0.065	NS	9.91
Creatinine (mg/dl)	0.75±0.08	0.70±0.11	0.77±0.01	0.082	NS	24.73
Urea (mg/dl)	13.04±0.37	13.09±0.56	13.00±0.60	0.522	NS	8.94
Alkaline phosphatase (U/L)	115.32±6.75	114.52±6.24	117.66±5.80	6.276	NS	12.12
SGOT (U/L)	56.73±1.49	55.93±3.40	58.02±1.65	2.347	NS	9.22
SGPT (U/L)	27.33±1.42	28.38±1.80	29.13±1.95	1.737	NS	13.73
Calcium (mg/dl)	8.41±0.50	8.57±0.69	6.78±1.74	0.543	NS	14.32

Table 4: Blood biochemical parameters at 98th day of experiment

Parameters	Treatments			SEM	CD	CV%
	T1	T2	T3			
Total protein (g/dl)	5.91±0.18	6.26±0.18	6.45±0.06	0.151	NS	5.45
Albumin (g/dl)	3.50 ^b ±0.12	3.69 ^{ab} ±0.05	3.81 ^a ±0.02	0.78	0.241	4.76
Globulin (g/dl)	2.41±0.11	2.57±0.14	2.64±0.05	0.110	NS	9.71
A/G Ratio	1.48±0.08	1.45±0.07	1.44±0.03	0.061	NS	9.43
Creatinine (mg/dl)	0.72±0.05	0.66±0.08	0.66±0.05	0.062	NS	20.34
Urea (mg/dl)	12.79±0.73	12.26±0.78	12.28±1.00	0.844	NS	15.17
Alkaline phosphatase (U/L)	142.14±5.21	141.42±3.97	137.71±4.70	4.659	NS	7.42
SGOT (U/L)	50.81±2.05	53.92±2.06	56.05±1.48	1.881	NS	7.85
SGPT (U/L)	26.38±3.08	29.30±2.12	28.58±0.89	2.216	NS	17.65
Calcium (mg/dl)	8.35±0.31	8.71±0.54	8.52±0.29	0.397	NS	10.40

Mean with different superscript (a to b) in row differ significantly ($P < 0.05$) showing treatment effect

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