



Feeding Various Zinc Forms to Sahiwal Heifers

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Effect of Three Different Forms of Zinc on Growth Performance, Digestibility, Blood Profiles and Antioxidant Status of Sahiwal Heifers

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ABSTRACT

The current study was designed to investigate the impact of dietary supplementation of organic and nano zinc on the performance of Sahiwal heifers. A total of twenty young Sahiwal heifers were randomly assigned into four groups having five animals based on body weight and age for a period of 90 days. The control group was given on a basal diet whereas, group T1 was supplemented basal diet with 40mg/kg DM Zn glycinate, group T2 was supplemented basal diet with 40mg/kg DM Zn peptide, and group T3 was supplemented with a basal diet with 20mg/kg DM nano Zn oxide. The dietary requirements of the heifers were adequately met by providing a ration consisting of concentrate, jowar (Sorghum) fodder, and wheat straw in a 50:30:20 ratio on a dry matter (DM) basis. No effect on DMI, Feed conversion ratio (FCR), Feed conversion efficiency (FCE) but ADG was significantly higher ($P < 0.05$) in the nano zinc-supplemented group. There was no significant effect ($P > 0.05$) on blood parameters except MCHC which was significantly higher in nano zinc supplemented groups. Treatment groups showed significantly higher ($P < 0.05$) plasma globulin, ALP, glucose and lower bilirubin, PUN whereas, no significant effect ($P > 0.05$) was found on albumin, total protein, triglycerides, cholesterol, ALT, AST, and creatinine. No effect on plasma minerals except serum zinc level. SOD, FRAP, and total plasma immunoglobulin concentrations were significantly higher in the treatment groups than in the control group. Finally, it may be concluded that nano Zn supplementation @20ppm and Zn pep @40ppm can be supplemented in heifer's diet for better performance without adversely affecting nutrient digestibility, blood profile, and antioxidant status.

KEYWORDS: Haematology, Heifers, Immune response, Nano zinc, Organic zinc

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INTRODUCTION

The health and wellbeing of animals are significantly impacted by mineral shortages in their feed, even when other nutrients are sufficiently present. The importance of zinc is highlighted by the observation that more than 50% of Indian soils currently exhibit zinc deficiency, with projections indicating an increase to 63% by 2025 (Gupta et al., 2016). The prevalent zinc deficit in feed and fodder crops, due to insufficient zinc levels, has negatively impacted animal health and productivity. Zinc is not retained in the body like other trace minerals, requiring consistent dietary intake to ensure optimal health and function. Zinc (Zn) is employed by the body in multiple functions, including the initiation and modulation of immune responses, antioxidant

activities, serving as a cofactor for various enzymes, spermatogenesis, steroidogenesis, vitamin A metabolism, insulin storage and secretion, energy metabolism, protein synthesis, stabilization of macromolecules, regulation of DNA transcription, and cellular division (Miller, 1970; Jackson and Lowe, 1992; Salgueiro et al., 2000; Frassinetti et al., 2006; Valle and Falchuk, 1993; Yatoo et al., 2013; Kaur et al., 2014).

Minerals, whether inorganic or organic, can be provided to address these deficiencies. Administering inorganic mineral supplements to animals may lead to deficiencies in other minerals that are only marginally available. Organic minerals also show better absorption and application than their inorganic alternatives. Nano zinc shows excellent bioavailability.

They can reduce environmental contamination, serve as alternatives to antibiotics for growth promotion, eliminate antibiotic residues in animal products, and offer pollution-free animal products at lower dosages (Schmidt, 2009). Research on zinc supplementation in native cows is limited, although numerous studies have investigated the effects of zinc from different sources on growth performance and health-related traits in various animal categories. This study investigates the impact of organic and nano Zn supplementation on the performance of Sahiwal heifers.

MATERIALS AND METHODS

Twenty Sahiwal heifers were selected from the cattle herd kept at the Livestock Farm Complex (LFC), DUVASU, Mathura and were randomly assigned into four groups (five heifers in each) on body weight and age basis. Experimental trial period was of 90 days. Control group were fed on basal diet without any zinc supplementation, whereas group T1 was supplemented with Zinc glycinate (Zn-Gly) @40 mg Zn/kg DM, group T2 was supplemented with Zinc peptide (Zn-pep) @40 mg Zn/kg DM and group T3 was supplemented with nano zinc oxide (nZnO) @20 mg Zn/kg DM. Basal diet consisted of wheat straw (particle size- 1.5 to 2.0 cm), chaffed green Jowar fodder and compounded concentrate mixture as per ICAR (2013) requirements. The composition of TMR (on %DM basis) in all treatments is Jowar fodder (30%), wheat straw (20%) and concentrate mixture (50%). Concentrate mixture was prepared by mixing 16 parts oats, 15 parts barley, 18 parts wheat bran, 17 parts gram chunni, 32 parts mustard oil cake and 2 parts mineral mixture. The analyzed chemical composition (%) was found to be in TMR were 70.25, 90.34, 3.12, 15.08, 9.70, 23.35, 48.75, 56.27, 34.24% for DM, OM, EE, CP, ASH, CF, NFE, NDF, ADF in control group, T1, T2 and T3 group, respectively. Zn concentration was 21.62 mg/kg in control, 61.62 mg/kg in T1, 61.62 mg/kg in T2 and 41.62 mg/kg in T3 TMR, respectively.

Zn-Gly procured from noreal Pvt. Ltd., purity 40%; Zn-pep procured from Alltech Pvt. Ltd., purity 20%; ZnO nano particle procured from SRL Pvt. Ltd., Maharastra, minimum assay 99.9%. All heifers were kept in a well-ventilated shed with the appropriate setup for individual feeding and watering, ensuring they did not have access to the diets of other animals. All the animals were dewormed using

the drug Fenbendazole and Ivermectin at the dose rate of 10mg/kg bodyweight, prior to the commencement of experiment.

The body weight of the experimental heifers was documented at the commencement of the trial and thereafter at fortnightly intervals. Feed, fodder, and residual ort samples were oven-dried at 60/ °C using a hot air oven and subsequently ground using a Wiley mill to pass through a 1-mm sieve. The processed samples were then analyzed for dry matter (DM), crude protein (CP), ether extract (EE), and total ash, following the standard analytical procedures outlined by the Association of Official Analytical Chemists (AOAC, 2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were quantified following the methodologies outlined by Van Soest et al. (1991). The concentrations of Calcium (Ca), Phosphorus (P), Copper (Cu), Iron (Fe), and Zinc (Zn) in feed and fodder samples were analyzed using inductively coupled plasma-optical emission spectroscopy (5800 ICP-OES Agilent, CA, USA).

Blood samples were obtained prior to the feeding and watering of heifers at 07:00 hours in heparinized vacutainer tubes (BD Franklin, USA) at 0, 30, 60, and 90 days post-supplementation. Blood samples were tested for hematological, biochemical, and hormonal characteristics. A segment of whole blood samples were used for assessing white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb) concentration, hematocrit values, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelets, and mean platelet volume, utilizing the Celltac-á (MEK-6500K) automated hematology analyzer produced by Nihon Kohden Europe. A segment of whole blood samples was employed to evaluate superoxide dismutase (SOD) activity (Madesh and Balasubramanian, 1998), FRAP activity (Benzie and Strain, 1999), and plasma total immunoglobulin using the zinc turbidity method (Mc Ewan et al., 1970). The remaining blood samples were centrifuged at 3000 rpm for 15 minutes to separate the plasma.

Plasma samples were preserved at “20°C until subsequent examination of liver and kidney function biomarkers (glucose, triglycerides, AST, ALT, ALP, bilirubin, and creatinine) and protein metabolism biomarkers (total protein, albumin, globulin, and PUN). The plasma concentrations of glucose, triglycerides, AST, ALT, ALP, bilirubin, creatinine, total protein, albumin, and PUN were measured using

an automated biochemical analyzer (BS-120 Chemistry Analyzer, Shenzhen Mindray Biochemical Electronics Co. Ltd., China) with Span Diagnostic kits (Span Diagnostic Ltd., Surat, India). The plasma globulin concentration was determined by subtracting the albumin level from the total protein content. The minerals were analyzed by inductively coupled plasma-optical emission spectroscopy (5800 ICP-OES Agilent, CA, USA).

The collected data were analysed using a mixed model for repeated measures in the Statistical Package for the Social Sciences (SPSS for Windows, V21.0; SPSS Inc., Chicago, IL, USA). The Tukey Honestly Significant Difference test was employed on treatment means, revealing a statistically significant variation across the samples. The difference was deemed significant at $P < 0.05$.

RESULTS AND DISCUSSION

There was no significant effect on dry matter intake (kg/day or % BW) in all groups. Average daily gain was significantly higher ($P < 0.05$) for nano zinc group followed by zinc peptide group. No significant effect ($P > 0.05$) of organic and nano zinc supplementation was observed on average body weight, FCR, FCE in heifers (Table 1). Seifdavati et al. (2018) documented substantial enhancements in

final weight and weight increase in calves using nano-zinc oxide supplementation. Anil et al. (2020) discovered that nano-zinc supplementation resulted in markedly greater body weight increase in calves relative to control groups. Uniyal et al. (2017) noted markedly elevated average daily gain (ADG) in guinea pigs supplemented with nanoparticles relative to those administered inorganic and organic zinc. This indicates that nano zinc oxide may demonstrate superior bioavailability and absorption relative to its organic equivalents, perhaps leading to enhanced growth performance. Zinc has an established mechanism for regulating the transcription of the growth hormone gene and affects the somatotrophic axis in multiple ways (MacDonald, 2000). Zinc strengthens the function of pituitary hormones through the action of growth hormone (GH) (Henkin et al., 1976).

Zinc deficiency has been shown to inhibit GH production from the pituitary (Root et al., 1979). Zinc significantly influences growth due to its cellular interactions with insulin-like growth factor-binding protein 3 (IGFBP-3), growth hormone (GH), and insulin-like growth factor 1 (IGF-1). IGF-1 concentrations influence the secretion of GH from the pituitary gland via a negative feedback mechanism (Guo et al., 2020).

Table 1. Effect of organic and nano zinc supplementation on dry matter intake and growth performance in heifers

Parameters	Treatment groups				SEM
	Control	T1	T2	T3	
Initial BW (kg/day)	141.6	141.2	141.6	141.3	10.6
Final BW (kg/day)	164.8	166.2	170.8	174.5	12.23
Avg BW (kg/day)	150.51	152.55	153.67	157.11	11.39
DMI (kg/day)	3.74	3.52	3.85	3.94	0.177
ADG (g/day)	276.19 ^c	297.61 ^c	347.61 ^b	395.23 ^a	23.13
FCR %	9.62	10.26	9.76	9.56	0.491
FCE %	0.115	0.260	0.133	0.127	0.029

Values bearing different superscripts (a,b,c) differ significantly ($P < 0.05$)

Table 2. Effect of organic and nano zinc supplementation on nutrient digestibility (%) of heifers

Parameters	Group				SEM
	Control	T1	T2	T3	
Initial BW(Kg)	164.8	166.2	170.8	174.5	12.234
Final BW (Kg)	167.9	169.1	174.1	180.5	12.254
BW change (kg/day)	3.1 ^{ab}	2.9 ^b	3.3 ^{ab}	6.0 ^a	0.377
Apparent Digestibility Coefficients (%)					
DM	69.80	70.03	67.61	68.56	1.006
OM	75.22	75.41	73.42	74.20	0.825
CP	71.69	71.91	69.63	70.52	0.943
EE	81.88	82.02	80.56	81.13	0.603
NDF	63.27	63.56	60.61	61.76	1.223
ADF	49.67	50.06	46.02	47.60	1.676

Values bearing different superscripts (a,b,c) differ significantly ($P < 0.01$)

The digestibility coefficients for DM, OM, CP, EE, NDF, and ADF (Table 2) were similar across all treatment groups, indicating that the incorporation of various forms of zinc in the diet did not affect nutrient digestibility. Most studies indicate that Zn supplementation, ranging from 20 to 135 mg/kg DM, did not affect the digestibility of DM and CP (Bedi, 1976; Khan, 1978; Kumar et al., 2002; James et al., 2023), as well as NDF and ADF (Salama et al., 2003) in ruminants. The lack of impact on nutrient digestibility with Zn supplementation may be attributed to the fulfilment of Zn requirements for rumen microbes by the basal diet. The outcomes of this research correlate with those of Mallaki et al. (2015) and Maan and Sihag (2014), which demonstrated that dietary zinc supplementation did not significantly affect dry matter (DM) digestibility

in Zandi lambs and goat, respectively.

Likewise, Swain et al. (2018) and Satyanarayana et al. (2017) found no significant effect of various zinc forms on ether extract (EE) digestibility in goats and buffalo heifers, respectively. Jadhav et al. (2008), Singh et al. (2024), and Mandal et al. (2007) found no significant differences in DM digestibility between zinc-supplemented groups and controls in male Murrah buffalo calves, Barbari goats, and crossbred cattle, respectively. Uniyal et al. (2017) reported a significant increase in total weight gain among guinea pigs receiving nanoparticle supplementation. Pati et al. (2024) reported a significant ($P < 0.05$) increase in average daily gain in Bengal goat kids supplemented with nano zinc.

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Table 3. Effect of organic and nano zinc supplementation on haematological parameters

Parameters	Treatment groups				SEM
	Control	T1	T2	T3	
RBC (x 10 ⁶ /μL)	7.23	7.15	6.91	7.43	0.103
Hb (g/dl)	9.48	8.76	8.70	9.21	0.134
Platelets (x10 ⁹ / L)	216.85	190.35	212.40	234.15	6.856
WBC (x10 ⁹ / L)	9.92	9.03	9.71	10.54	0.278
HCT (%)	27.71	26.34	25.85	27.04	0.374
MCV (fL)	38.59	39.52	38.03	37.22	0.488
MCH (pg)	12.64	12.94	12.62	12.39	0.158
MCHC (g/dL)	33.15 ^a	33.09 ^a	33.30 ^{ab}	33.70 ^b	0.074
MPV (fL)	5.34	5.21	5.19	5.29	0.029

Values bearing different superscripts (a,b) differ significantly (P<0.01)

The concentrations of hematological indices (Table 3) remained within the normal range for heifers following supplementation with organic and nano zinc, suggesting that the supplementation did not adversely affect the physiological status of the heifers, which indicates good health. The MCHC concentration was statistically greater (P<0.05) in the nano zinc group compared to other groups, likely due to zinc's significant role in hemoglobin formation, which is essential for improving Hb concentration up to a certain level, as suggested by Ott et al.

(1965). The mean values for RBC, Platelets, WBC, HCT, MCV, MCH, and MPV in the supplemental group did not exhibit any significant effects (P>0.05). Similar studies conducted by Anil (2020) on crossbred calves fed ZnSO₄ and nano ZnO, as well as Deori et al. (2024) and Singh et al. (2024) on goats supplemented with nano Zn, indicated no effect on hematological parameters. Ahuja et al. (2022), Pawar et al. (2023), and Gami et al. (2024) reported comparable haematological parameters in lactating Kankrej cows.

Table 4. Effect of organic and nano zinc supplementation on blood biochemical parameters

Parameters	Treatment groups				SEM
	Control	T1	T2	T3	
Glucose (mg/dl)	50.11	54.06	56.40	58.80	1.653
Triglyceride (mg/dl)	32.62	33.42	33.74	33.10	0.231
Cholesterol (mg/dl)	200.7	197.5	197.7	198.1	1.326
Total protein (g/L)	6.49	6.93	6.94	6.73	0.10
Albumin (g/L)	3.16	3.15	2.99	3.07	0.123
Globulin (g/L)	3.33 ^b	3.82 ^{ab}	3.95 ^a	3.66 ^b	0.087

Values bearing different superscripts (a,b) differ significantly (P<0.05)

Plasma glucose, cholesterol, and triglyceride levels served as biomarkers for energy and lipid metabolism, whereas plasma total protein, albumin, and globulin were utilized as biomarkers for protein metabolism (Table 4). The plasma glucose level was significantly elevated ($P < 0.05$). Increased serum glucose levels may result from changes in the molar proportion of volatile fatty acids (VFAs) in the rumen, specifically an increase in propionate production (Aliarabi and Chhabra, 2006). No differences were noted in triglyceride and cholesterol levels (Elamin et al., 2015; Kamdev et al., 2016). All biomarkers of protein metabolism are within normal physiological limits, and no significant differences ($P > 0.05$) were observed between supplemented and unsupplemented heifers, except for plasma globulin levels, which were significantly higher ($P < 0.05$) in the organic zinc supplemented groups. The findings

align with those of Kudilková et al. (2016), who similarly observed elevated levels of α -globulin in the group supplemented with organic zinc. Ramulu et al. (2015) demonstrated that zinc supplementation enhances globulin levels in buffalo calves in a dose-dependent manner, specifically at concentrations ranging from 80 to 140 ppm.

In this research, we observed significantly reduced plasma bilirubin and plasma urea nitrogen levels (Table 5) in the supplemented groups, with the highest values recorded in the control groups, consistent with the findings of Kolaskar et al. (2021) regarding BUN. No adverse effects of supplementation on liver function were observed, as indicated by comparable plasma levels of ALT and AST in both the control and zinc-supplemented groups. The plasma ALP content in the nano Zn group was markedly higher than in all other groups.

Table 5. Effect of organic and nano zinc supplementation on liver, kidney function and antioxidant status parameters

Parameters	Treatment groups				SEM
	Control	T1	T2	T3	
ALT (IU/L)	22.24	21.73	21.52	22.10	0.119
AST (IU/L)	22.10	22.38	22.39	22.73	0.133
ALP (IU/L)	148.6 ^b	142.5 ^a	143.5 ^{ab}	149.3 ^b	0.838
Bilirubin (mg/dl)	0.98 ^a	0.89 ^b	0.97 ^a	0.88 ^b	0.015
Creatinine (mg/dl)	0.91	0.90	0.90	0.92	0.003
PUN (mg/dl)	9.40 ^b	9.34 ^b	9.23 ^b	8.97 ^a	0.072
SOD (μ mol MTT formazan/mg Hb)	0.29 ^a	0.40 ^b	0.41 ^b	0.45 ^b	0.013
FRAP (μ mol/L)	1192.3 ^a	1229.3 ^a	1298.1 ^{ab}	1370.3 ^b	17.534
TIg (mg/ml)	13.56 ^a	16.24 ^b	18.19 ^c	18.35 ^c	0.334

Values bearing different superscripts (a,b) differ significantly ($P < 0.01$)

The observed difference between the treatment and control groups can be attributed to the reduced activity of ALP in the control group, rather than an increase in activity within the treatment groups. Song et al. (2021) and Dhruw (2017) observed significantly elevated serum ALP levels in nano zinc supplemented groups compared to the control group in goats. SOD, FRAP, and total plasma immunoglobulin concentrations increased across all groups over time, with treatment groups exhibiting higher levels than

the control group, particularly in the nano group. The outcomes correlate with those of Kumar et al. (2021), Nagalakshmi et al. (2016; 2017), and Alimohamady et al. (2019). Zinc serves as an essential cofactor for copper/zinc superoxide dismutase (Cu/Zn-SOD) and additionally enhances its expression (Kumar et al. 2013). Zinc is crucial for both humoral and cell-mediated immune responses (Gruber et al., 2013). Nano zinc enhances the immune function in animals.

Table 6. Effect of organic and nano zinc supplementation on plasma mineral profile

Parameters	Treatment groups				SEM	P value		
	Control	T1	T2	T3		Treatment (T)	Period (P)	T×P
Ca (mg/dl)	8.64	9.01	9.16	8.94	0.196	0.56	0.89	0.99
P (mg/dl)	4.11	4.51	4.78	4.47	0.117	0.12	0.07	0.96
Fe (mg/L)	2.60	2.64	2.57	2.63	0.04	0.79	0.89	0.61
Cu (mg/L)	1.14	1.12	1.22	1.10	0.029	0.98	0.49	0.19
Zn (mg/L)	1.40 ^a	1.94 ^b	2.14 ^b	2.27 ^b	0.078	<0.001	0.63	0.03

Values bearing different superscripts (a,b) differ significantly (P<0.01)

Plasma levels of Ca, Fe, Cu, and P demonstrated that dietary supplementation in heifers had no significant impact (Table 6). Additional researches demonstrated that zinc supplementation did not significantly influence calcium and phosphorus levels (Mahima et al., 2015), nor iron and copper levels (Smerchek et al., 2023). Serum zinc concentrations were significantly elevated (P<0.05) in this trial following supplementation compared to the control group. The application of nZn has demonstrated superior outcomes relative to traditional Zn sources and exhibits less toxicity (Wang et al., 2006; Sahoo et al., 2014b). Uniyal et al. (2017) noted that serum zinc levels were considerably elevated in groups supplemented with nano-zinc compared to other zinc sources. Kala (2013) also observed a positive association between dietary nano Zn and blood Zn levels.

CONCLUSIONS

Nano Zn supplementation @20ppm and Zn pep @40ppm can be supplemented in heifer's diet for better performance without adversely affecting nutrient digestibility, blood profile and antioxidant status.

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