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Effect of inorganic, organic and nano-zinc supplementation on haemato-biochemical profile of lactating Kankrej cows

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Abstract
The present study was conducted to assess the effect of inorganic, organic and nano-zinc supplementation on haemato-biochemical profile of lactating Kankrej cows. Twenty-four Kankrej cows were assigned randomly into four equal groups. The four groups were as C (control): Basal diet; T_1: Basal diet + 40 mg/kg of DM of inorganic zinc supplementation; T_2: Basal diet + 40 mg/kg of DM of organic zinc supplementation and T_3: Basal diet + 20 mg/kg of DM of nano- zinc supplementation daily. The duration of experiment was of 120 days. The blood samples were collected at the end (120th day) of experimental feeding. The haemoglobin and hematocrit values were without significant (p>0.05) difference among the treatment groups. No effect (p>0.05) on red blood cells (erythrocytes, MCV, MCH and MCHC) and white blood cells (leucocytes, neutrophils, lymphocytes, monocytes and eosinophils) count were observed due to dietary addition of different sources of zinc in the diet. The serum concentrations of glucose, total protein, albumin, globulin, urea, creatinine, cholesterol, ALT and AST were not affected (p>0.05) by the dietary supplementation of inorganic, organic and nano-zinc in the diet. Based on the results, it may be concluded that supplementation of inorganic, organic and nano-zinc at the dose rate of 40, 40 and 20 mg/kg of DM, respectively, in the diet of lactating Kankrej cows did not have any adverse effect on haemato-biochemical parameters.

Keywords: Blood metabolites, haematology, Kankrej cow, zinc

Introduction
Zinc (Zn) is a crucial trace element in the animal body and cannot be stored in the body. Therefore, it requires regular dietary intake to meet physiological needs (Mandal *et al*., 2007) [14]. Zinc is an essential component in the synthesis of numerous enzymes, including alcohol dehydrogenase, alkaline phosphatase, aldolase, lactate dehydrogenase, RNA and DNA polymerases, reverse transcriptase, carboxypeptidase A, B, G, and superoxide dismutase. Zinc also plays a role in immune system function (Shinde *et al*., 2006) [21]. The normal serum Zn concentration typically ranges from 0.7 to 1.3 g/ml, and the dietary requirements of Zn for milking animals are approximately 40-80 mg/kg. Zinc is essential for various physiological functions in the body such as growth, reproduction, DNA synthesis, cell division, and gene expression (Kececi and Keskin, 2002) [11]. Zinc also plays a role in photochemical processes of vision, wound healing, ossification, and enhancing the immune system through energy production, protein synthesis, protection of membranes from bacterial endotoxins, and lymphocyte replication and antibody production (Li *et al*., 2020) [13]. Zinc deficiency has been reported in various parts of India (Gami *et al.*, 2015) [7], which is often reflected in Zn deficiency in fodder. To avoid a zinc deficiency, it can be added to ration in different forms such as inorganic salts, organic sources and nano-zinc supplements. However, effect of these different sources of zinc in the ration of lactating cows on haemato-biochemical parameters have not studied under Indian condition. Therefore, this study was conducted to assess the effect of inorganic, organic and nano-zinc supplementation on haemato-biochemical profile of lactating Kankrej cows.

Materials and Methods

Animals and Experimental design

The use of animals and the experimental procedure were approved by institutional Animal Ethics Committee (approval No. VETCOLL/IAEC/2022/20/ PROTOCOL-15). Twenty-four Kankrej cows were assigned randomly into four equal groups. The four groups were as C (control): Basal diet; T_1: Basal diet + 40 mg/kg of DM of inorganic zinc supplementation; T_2: Basal diet + 40 mg/kg of DM of organic zinc supplementation and T_3: Basal diet + 20 mg/kg of DM of nano- zinc supplementation daily. The duration of experiment was of 120 days. The blood samples were collected at the end (120^{th} day) of experimental feeding. In the treatment groups T_1, T_2, and T_3, zinc powder was thoroughly mixed into the concentrate and provided once a day during the experimental period. The experimental animals were fed as per ICAR (2013) [8] to meet the nutrient requirements.

Collection of blood samples

The blood samples were collected at the end (120^{th} day) of experimental feeding. The blood samples from external jugular vein were collected from each experimental animal in two sterilized vials one with anti-coagulant for hematological parameters and other without anti-coagulant for analysis of blood biochemical parameters.

Analysis of haematological parameters

The fresh blood samples the one with anti-coagulantwere analysed for haematological parameters *viz.*, haemoglobin, haematocrit, erythrocytes, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), leucocytes, neutrophils, lymphocytes and monocytes using automatic analyser as followed by Pawar *et al*. (2024) [19].

Analysis of blood biochemical parameters

The serum was harvested from the blood without anti-coagulant. The serum samples were analysed for glucose, total proteins, albumin, urea, creatinine, triglycerides, cholesterol, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations using commercial diagnostic kits as followed by Pawar *et al*. (2024) [19].

Statistical Analysis

All the experimental data obtained were statistically analyzed using SPSS v.20.0 (SPSS Inc., Chicago IL) as per the standard statistical method (Snedecor and Cochran, 1994) [22]. The significant differences between means of treatments were assessed by Duncun's test, and differences between treatments were declared significant at $p<0.05$.

Results and Discussion

Haematological parameters: The effect of zinc supplementation from different sources on haematological parameters of lactating Kankrej cows are given in Table 1. There was no significant difference ($p>0.05$) in the mean haemoglobin and haematocrit values among the various treatment groups. The haemoglobin value was 11.20, 10.28, 9.93 and 11.02 g/dL, while haematocrit level was 27.08, 28.99, 29.78 and 29.50%, in the control, T_1, T_2, and T_3 groups, respectively. The recorded values for haemoglobin and haematocrit fell within the normal range of 8-15 g/dL and 26-37%, respectively (Kaneko *et al.*, 2008) [10]. The supplementation of zinc from different sources did not have any effect ($p>0.05$) on red blood cells count (erythrocytes, MCV, MCH and MCHC) and white blood cell count (leucocytes, neutrophils, lymphocytes and monocytes) in Kankrej cows. Zinc is a key player influencing different blood aspects in dairy cattle, underscoring its significance in upholding overall health and metabolic function. Optimal zinc levels aid in erythropoiesis, the production of red blood cells, which is crucial for maintaining efficient oxygen transport in the blood. Maintaining the correct zinc levels contributes to normal hematocrit and hemoglobin concentrations, which are indicative of the capacity to carry oxygen and overall blood volume (Li *et al*., 2020) [13]. Zinc is vital for the growth and operation of white blood cells, including lymphocytes, neutrophils, and macrophages (Kececi and Keskin, 2002) [11], which are vital elements of the immune system.

Similar to present findings, Dass (2010) [6] in crossbred calves with a Zn supplement at a concentration of 35 ppm/kg DM in the form of either zinc sulphate or zinc propionate for duration of 180 days. The study results showed that there were no significant differences in blood hemoglobin and haematocrit among the three treatment groups. Anil *et al*., (2019) [3] in crossbred calves with the addition of 25 ppm $ZnSO_4$, 5 ppm nZnO, and 10 ppm nZnO to the basal diet, and found no significant differences in haemoglobin concentration among the groups, all of which fell within the normal range for the calves. Bakhshizadeh *et al*., (2019) [4] in dairy cattle supplemented with 60 mg of inorganic, organic, and nano Zn per kg of diet, and found no differences in hematological parameters between groups. Ulutas *et al*., (2020) [23] conducted an experimental feeding trial on 24 male goats with varying levels of Zn supplementation, and found that hematological parameters remained unaffected. Kumar *et al*., (2021) [12] investigated the effects of different Zn supplementation levels and sources on growing Haryana heifers, and concluded that blood hemoglobin concentration and packed cell volume showed non-significant effects across the various Zn supplementation groups. Similarly, Ahuja *et al.* (2022) [1] and Pawar *et al.* (2023) [20] observed similar levels of haematological parameters in Kankrej cows in lactation.

Table 1: Effect of zinc supplementation from different sources on haematological parameters of lactating Kankrej cows (n= 24)

Attributes	Dietary Treatments*				SEM	P Value
	Control	T_1	T_2	T_3		
Haemoglobin (g/dL)	11.20	10.28	9.93	11.02	0.245	0.216
Haematocrit (%)	27.08	28.99	29.78	29.50	0.549	0.312
Erythrocytes (10^6/µL)	5.89	5.18	5.47	5.50	0.137	0.347
Leukocytes (10^3/ µL)	9.75	9.92	10.40	9.63	0.455	0.946
MCV (fL)	49.23	49.62	49.00	48.30	0.833	0.961
MCH (pg)	18.73	17.72	17.47	19.07	0.601	0.768
MCHC (g/dL)	36.62	35.02	36.62	37.68	0.760	0.694
Neutrophils (10^3/ µL)	3.78	3.85	3.72	3.38	0.207	0.877
Lymphocytes (10^3/ µL)	3.43	3.82	3.70	3.47	0.227	0.930
Monocytes (10^3/ µL)	0.65	0.60	0.68	0.65	0.045	0.940

*****Control: Basal diet, T_1: Basal diet + 40 mg/kg DM/day of inorganic Zn supplementation; T_2: Basal diet + 40 mg/kg DM/day of organic Zn supplementation; and T_3: Basal diet + 20 mg/kg DM/day of nano-Zn supplementation

Blood biochemical parameters

He effect of zinc supplementation from different sources on blood biochemical parameters of lactating Kankrej cows are presented in Table 2. The serum concentrations of glucose, total protein, albumin, globulin, urea, creatinine, triglycerides, cholesterol, ALT and AST were without any significant (p>0.05) difference among the treatment groups. Their levels were within the normal physiological limits for cattle (Kaneko *et al.*, 2008) [10]. Zinc plays a significant role in influencing blood biochemical parameters in dairy cattle, highlighting its importance in various metabolic processes and overall well-being. It is engaged in insulin synthesis, secretion, and signaling, with adequate levels supporting insulin sensitivity for effective regulation of blood glucose (Zawadzka *et al.*, 2024) [24]. Maintaining a balanced zinc status is key in upholding stable blood glucose concentrations to prevent metabolic health issues like hyperglycemia or hypoglycemia, which can negatively impact milk production. Additionally, zinc influences lipid metabolism and may affect blood lipid profiles, including cholesterol and triglyceride levels.

Table 2: Effect of zinc supplementation from different sources on blood biochemical parameters of lactating Kankrej cows (n= 24)

Attributes	Dietary Treatments*				SEM	P value
	Control	T_1	T_2	T_3		
Glucose (mg/dL)	74.17	74.50	73.83	73.17	0.195	0.870
Total Proteins (g/dL)	7.52	7.23	7.97	7.37	0.170	0.476
Albumin (g/dL)	3.90	3.58	3.77	3.70	0.080	0.587
Globulin (g/dL)	3.62	3.65	4.20	3.67	0.123	0.292
BUN (mg/dL)	31.80	35.12	34.04	35.91	0.021	0.834
Creatinine (mg/dL)	1.13	1.09	1.10	1.06	0.021	0.763
Triglycerides (mg/dL)	17.68	19.90	17.77	18.28	1.021	0.875
Cholesterol (mg/dL)	287.93	306.75	328.97	305.60	7.482	0.298
ALT (U/L)	48.23	46.87	50.10	39.82	2.587	0.548
AST (U/L)	71.23	67.40	74.23	72.37	2.185	0.753

*****Control: Basal diet, T_1: Basal diet + 40 mg/kg DM/day of inorganic Zn supplementation; T_2: Basal diet + 40 mg/kg DM/day of organic Zn supplementation; and T_3: Basal diet + 20 mg/kg DM/day of nano Zn supplementation; ALT: alanine aminotransferase; AST: aspartate aminotransferase

Proper zinc levels contribute to healthy lipid metabolism, aiding in the maintenance of optimal lipid levels in the bloodstream. Ensuring adequate zinc levels is essential for proper liver function, including protein synthesis and metabolism. It supports liver health by facilitating efficient protein metabolism and preventing hepatic disorders. Maintaining a balanced zinc status is vital for normal blood protein levels, encompassing albumin and globulins necessary for various physiological functions like immune response and osmotic regulation (Mohammad *et al.*, 2012) [15]. Maintaining balanced zinc levels is crucial for promoting metabolic health, supporting immune function, and averting metabolic disorders in dairy cattle.

In a line with the present findings, Alijani *et al*., (2021) [2] administering 28 mg of Zn/kg DM in the form of ZnO, Zn-methionine, or nano-ZnO in male sheep. The results indicated that the levels of blood glucose, total protein, triglycerides, SGPT, and SGOT were not significantly (P>0.05) altered by zinc supplementation from various sources. Correspondingly, Kumar *et al*., (2021) [12] provided 50 mg Zn/kg DM as inorganic Zn, 25 mg Zn/kg DM as nano-Zn, and 50 mg Zn/kg DM as nano-Zn in growing heifers, which resulted in non-significant differences in plasma glucose, total cholesterol, triglyceride, ALP, ALT, AST, bilirubin, creatinine, and urea nitrogen levels. Furthermore, Budak, (2023) [5] supplemented 20 mg/kg of N-ZnO in ewes, where the results showed that albumin and total protein levels were within the normal range across all groups. Pati *et al*., (2024) [17] supplemented 25 and 50 ppm of nano-Zn in male kids, respectively, over a 90-day experimental period. The blood metabolites levels reported in the present study was also in agreement with the findings of previous studies conducted in Kankrej cows during lactation (Joshi *et al*., 2021; Pawar *et al.*, 2021) [9, 20]. The findings suggested that zinc supplementation did not affect significantly blood protein concentration, urea, and creatinine levels, which remained within the normal range.

Conclusion

Based on the findings, it may be concluded that supplementation of inorganic, organic and nano-zinc at the dose rate of 40, 40 and 20 mg/kg of DM, respectively, in the diet of lactating Kankrej cows did not have any adverse effect on haemato-biochemical parameters.

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