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Impact of Nano Zinc Supplementation During Transition Period on Reproductive Performance, Serum Biochemical Indices and Metabolic Profile in Purebred Sahiwal Cows

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Abstract

Micronutrients are essential for physiological functions and are especially crucial during the transition period. Zinc is one of such nutrients that plays a vital role in metabolism, immunity, endocrine regulation, reproductive physiology, and uterine health. Nano-zinc, due to its superior bioavailability, offers improved physiological benefits at lower supplementation levels. Twenty-four pregnant purebred Sahiwal cows were randomly assigned to four groups receiving a balanced diet without zinc (T0), zinc oxide at 40 ppm (T1), and nano zinc oxide at 20 ppm (T2) and 40 ppm (T3). Nano-zinc supplementation did not significantly affect reproductive performance but showed a non-significant reduction in the postpartum interval to estrus by 11–20%, and service period by 9.66–16.78%, and an increase in the conception rate compared to other treatments. The concentration of serum zinc varied significantly ($P \leq 0.05$) among treatments, with the highest levels observed in the 40 ppm nano-zinc group during both the prepartum and postpartum periods, despite its superior bioavailability. Administration of nano zinc-supplemented groups caused a non-significant increase in cholesterol concentration as compared to other groups and contributed to better reproductive performance. Compared to the different treatments group feeding with nano zinc at 40 ppm showed significantly ($P \leq 0.01$) lower concentrations of AST and ALT during prepartum and postpartum periods.

Introduction

Adequate intake of zinc is crucial for meeting physiological needs, maintaining animal health, and supporting reproduction. Supplementing livestock diets with zinc is a common practice to prevent or address deficiencies and to mitigate biochemical and clinical disorders. Zinc acts as a signaling factor and plays a role in DNA synthesis (Hara et al. 2017). Zinc plays a pivotal role in various enzymes, which are essential for maintaining liver function. Zinc plays a crucial role in nutrient metabolism; therefore, it is necessary for growth and immune function. Lipid metabolism is compromised, and plasma cholesterol is increased in Zn deficiency (Shen et al. 2007). Zinc deficiency can lead to reduced feed intake, poor feed conversion, and impaired growth performance. In mature animals, inadequate zinc levels can lead to reproductive problems. Zinc plays a crucial role in oocyte maturation, fertilization, and the development of both the embryo and placenta (Garner et al. 2021; Wilson et al. 2017). Supplementation of Zn at an effective concentration modulates endocrine hormone levels during transition periods and improves reproductive efficiency in cows (Akil et al. 2025).

The deficiency of zinc is common in ruminants, as the soil, feed, and fodder in many parts of India are reported to be zinc-deficient (Kumar et al. 2024). The absorption coefficient of Zn in ruminants varies in a wide range (NRC 2001), and performance is affected by the sources of Zn. Inorganic zinc has been shown to improve body weight, whereas a better effect on reproduction was observed from alternative sources of zinc (Fulton et al. 2023). Organic sources, such as Zn lysine, are recommended to have greater bioavailability than inorganic salts, like sulfate forms (Kinal and Slupczynska 2011). Nano Zn is reported to have a higher absorption rate due to its higher dissolution rate in the acidic pH of the stomach and surface activity (Cho et al. 2013). Nano Zn has been demonstrated to have added

advantages over Zn salt, particularly in terms of feed intake, digestibility, blood Zn concentration, growth-stimulating and immunomodulatory effects, lactation performance, Zn concentrations in milk and plasma, and hematological profiles (Abdollahi et al. 2020; Cai et al. 2021; Swain et al. 2016). Zinc deficiency has been frequently observed during the transition phase (Maurya et al. 2014). Supplementation with nano-zinc at a reduced dose improved the quality and quantity of milk, while also helping to maintain the udder health of dairy cattle (Nandanwar et al. 2022). The effect of nano-Zn supplementation on the reproductive performance of indigenous Sahiwal cattle is not well studied. The present research work was conducted to investigate the effect of supplementing nano-Zn during the transition phase of purebred Sahiwal cows on reproductive performance, hormonal profile, and blood biochemical parameters.

Materials and Methods

Animals, Treatments and Management

Twenty-four pregnant pure-bred Sahiwal cows in first to fifth parity (300–350 Kg body weight) from Bull Mother Experimental Farm (BMEF) of the College of Veterinary Science and Animal Husbandry, Durg, Chhattisgarh, were grouped randomly into four treatment groups according to parity. Cows were kept in naturally ventilated conventional barn housing with a concrete floor with paddy straw as bedding material for the duration of the trial. The pens were cleaned daily, and manure was removed periodically to keep them clean and dry. All cows in each pen had access *to ad libitum* fresh drinking water three times daily. The experimental cows were dewormed with fenbendazole @ 7.5 mg/kg. All experimental cows were given chaffed green fodder (Hybrid Napier, Sudan, Berseem, and local grasses), dry fodder (paddy straw), and a concentrate mixture (20% Crude protein, 0.93% Ether extract) in a shed and were left for 4 hours of grazing in the morning. The cows in each group were fed individually in the pen. During the experimental period, the control group (T0) was fed a basal diet without Zn supplementation, whereas the treatment groups were given a basal diet supplemented with Zn at 40 ppm from ZnO (T1), 20 ppm from nano Zn (T2), and 40 ppm from nano Zn (T3). Nano ZnO was characterised using a particle size analyzer and a scanning electron microscope (Fig. 1). The treatment diets were administered during the transition period, from 30 days prior to the expected date of calving and through 30 days after the actual date of calving.

Studied Parameters

Reproductive Traits

Postpartum reproductive traits studied in this work included the postpartum interval to estrus (PPIE), service period, and conception rate during the first 200 days postpartum. After parturition, all experimental animals were observed regularly for signs of heat for the first 24 hours, both in the morning and evening. The interval from parturition to the onset of heat symptoms was recorded to determine the

days from calving to the first heat. The dates of calving and conception were recorded for all animals to calculate the service interval. The conception rate was calculated using the formula: no. of pregnant animals X 100/no. of artificial inseminations.

Biochemical Analysis

For analysis of biochemical parameters, blood samples were collected from the jugular vein in clot-activator vials in the early morning before feeding and watering. The sampling was done on -21, -14, -7, -5, -3, -1 days prepartum and on the day of calving (day 0) as well as on + 1, +3, + 5, +7, + 14, +21 postpartum from individual animals. The clot-activator vials containing blood samples were kept in the rack at a 45° angle at room temperature for 60–120 minutes, followed by centrifugation at 1000 rpm for 10 minutes, and clear supernatant serum was decanted into pre-labelled Eppendorf tubes and deep frozen (-20°C) till further analysis. Serum Zn concentration was determined by an Atomic Absorption Spectrophotometer after digestion in tri acid solution (Nitric acid: Sulphuric acid: Perchloric acid- 9:1:1). Serum samples were analyzed for cholesterol, AST, ALT in a semi-auto analyser using commercial diagnostic kits (Biolab Diagnostics Pvt. Ltd., Biosar, Maharashtra, India).

Statistical Analysis

For interpretation of the results, the data were subjected to a completely randomized design (CRD) for one-way analysis of variance. The significance of differences among the groups was analyzed by Duncan's multiple range test (Snedecor and Cochran 1994).

Results

Reproductive Traits

The postpartum interval to estrus (PPIE), average service period, and conception rate in Sahiwal cows under different dietary zinc supplementation levels are presented in Table 1.

Table 1
Effect of zinc supplementation on reproductive performance of Sahiwal cows

	Group				
Parameter	T_0	T_1	T_2	T_3	Sig. level
PPIE (Days)	128.83 ± 12.65	113.50 ± 7.31	102.66 ± 8.01	108.16 ± 9.32	NS
Service period (Days)	182.66 ± 10.77	165.00 ± 8.50	152.00 ± 6.08	158.00 ± 6.33	NS
Conception rate (%)	36 ± 11.48	44 ± 13.93	62.5 ± 12.59	55 ± 11.19	NS

Postpartum Interval to Estrus

The mean values of postpartum interval to estrus (PPIE) in T0, T1, T2, and T3 groups were 128.83, 113.50, 102.66, and 108.16 days, respectively. There was no significant difference between the treatment and control groups. However, compared to the control group, zinc supplementation reduced PPIE by 11.09%, 20.31%, and 16.04% in T1, T2, and T3, respectively.

Service Period Interval

The average interval of service period of Sahiwal cows was 182.66, 165.00, 152.00, and 158.00 days in T0, T1, T2, and T3 groups, respectively. Zinc supplementation in T1, T2, and T3 groups reduced the service period by 9.66%, 16.78% and 13.50%, respectively, compared to the control group. However, there was no significant difference between the treatment and control groups.

Conception Rate

The conception rate averaged 36%, 44%, 62.5% and 55% in T0, T1, T2, and T3 groups, respectively. There was a non-significant difference in the mean values of conception rate among the groups of Sahiwal cows. However, the nano zinc-supplemented groups (T2 and T3) showed a better conception rate compared to the control and inorganic zinc-supplemented groups.

Serum Zn

The effect of different zinc sources on the concentration of serum zinc in Sahiwal cows is shown in Table 2. Serum zinc concentration was significantly affected by the treatments and the overall experimental period ($P \leq 0.05$). The highest concentration was recorded in the 40 ppm nano zinc (T3) group of Sahiwal cows. The concentration of serum zinc gradually decreased towards calving and reached its lowest level on the third day postpartum across all treatment and control groups. Significantly higher ($P \leq 0.05$ and $P \leq 0.01$) serum zinc concentrations were observed at 1, 3, and 7 days prepartum, as well as at calving and on 5, 7, 14, and 21 days postpartum in the 40 ppm nano zinc group

compared to other groups. Overall, the mean serum zinc concentration during the prepartum period was higher than during the postpartum period across all treatments.

Table 2
Mean values (± SE) of serum zinc (ppm) in Sahiwal cows

Periods of observations	Serum zinc (ppm) in Sahiwal cows				Sig. level
Days relative to calving	T_0	T_1	T_2	T_3	
-21	1.55 ± 0.07	1.62 ± 0.05	1.66 ± 0.07	1.71 ± 0.09	NS
-14	1.50 ± 0.04	1.55 ± 0.07	1.62 ± 0.05	1.73 ± 0.05	NS
-7	1.34^{c}±0.02	1.39^{bc}±0.08	1.54^{ab}±0.03	1.59^{a}±0.07	*
-5	1.25 ± 0.08	1.31 ± 0.02	1.38 ± 0.04	1.44 ± 0.08	NS
-3	1.05^{b}±0.05	1.18^{ab}±0.04	1.26^{a}±0.08	1.31^{a}±0.02	*
-1	1.02^{b}±0.06	1.06^{b}±0.04	1.17^{a}±0.04	1.20^{a}±0.03	*
At Calving	0.96^{b}±0.07	0.98^{b}±0.03	1.13^{a}±0.02	1.23^{a}±0.02	**
+ 1	0.90^{b}±0.06	0.93^{b}±0.05	1.04^{ab}±0.05	1.15^{a}±0.03	*
+ 3	0.80 ± 0.08	0.85 ± 0.09	0.93 ± 0.05	1.05 ± 0.04	NS
+ 5	0.89^{b}±0.07	0.93^{b}±0.05	1.04^{ab}±0.02	1.14^{a}±0.01	**
+ 7	0.93^{b}±0.06	0.96^{b}±0.07	1.07^{ab}±0.02	1.21^{a}±0.03	**
+ 14	1.08^{b}±0.05	1.15^{b}±0.05	1.32^{a}±0.02	1.45^{a}±0.04	**
+ 21	1.14^{b}±0.04	1.19^{b}±0.03	1.54^{a}±0.03	1.57^{a}±0.07	**
Mean	1.10^{b}±0.06	1.16^{b}±0.06	1.28^{ab}±0.06	1.36^{a}±0.06	*
Prepartum Mean	1.28 ± 0.09	1.35 ± 0.08	1.43 ± 0.08	1.49 ± 0.08	NS
Postpartum Mean	0.95^{b}±0.05	1.00^{b}±0.05	1.15^{ab}±0.09	1.26^{a}±0.08	*

$^{a,b,\,c}$- Mean with different superscript in a row differ significantly *($P \leq 0.05$), **($P \leq 0.01$)

Cholesterol

Table 3
Mean values (± SE) of cholesterol (mg/dl) in Sahiwal cows

Periods of observations	Cholesterol (mg/dl) in Sahiwal cows				Sig. level
Days relative to calving	T_0	T_1	T_2	T_3	
-21	116.33 ± 2.47	118.62 ± 2.99	119.02 ± 2.76	121.18 ± 3.31	NS
-14	109.00 ± 3.41	113.77 ± 2.35	116.09 ± 2.45	118.01 ± 2.83	NS
-7	111.69 ± 2.05	109.16 ± 2.73	111.09 ± 2.44	110.00 ± 3.10	NS
-5	103.61^{b}±2.23	105.13^{b}±2.17	107.38^{ab}±2.13	113.06^{a}±2.45	*
-3	98.10^{b}±2.43	100.73^{ab}±2.35	104.08^{ab}±1.75	107.00^{a}±2.01	*
-1	97.04^{b}±1.85	99.03^{ab}±2.08	103.81^{a}±2.43	105.16^{a}±2.11	*
At Calving	96.90^{b}±2.19	99.27^{b}±1.74	102.69^{ab}±2.09	108.00^{a}±2.96	*
+ 1	99.24 ± 1.88	101.95 ± 2.17	106.03 ± 2.20	108.00 ± 2.96	NS
+ 3	105.05^{c}±2.23	103.55^{bc}±2.24	120.02^{a}±3.24	112.66^{ab}±2.58	**
+ 5	105.10^{b}±1.64	112.01^{ab}±2.49	118.01^{a}±3.05	110.17^{ab}±3.25	*
+ 7	116.00^{b}±2.53	109.30^{b}±3.13	129.07^{a}±3.18	128.06^{a}±3.17	**
+ 14	121.01^{c}±3.29	124.08^{bc}±3.19	133.03^{ab}±2.87	136.03^{a}±2.94	**
+ 21	129.02 ± 3.02	135.70 ± 3.53	138.70 ± 2.98	141.86 ± 3.14	NS
Mean	108.31 ± 2.78	110.17 ± 2.99	116.07 ± 3.27	116.86 ± 3.25	NS
Prepartum Mean	105.96 ± 3.14	107.74 ± 3.10	110.24 ± 2.57	112.40 ± 2.55	NS
Postpartum Mean	112.57 ± 4.63	114.43 ± 5.32	124.14 ± 4.82	122.79 ± 5.90	NS

^{a,b,c}- Mean with different superscript in a row differ significantly*($P \leq 0.05$) **($P \leq 0.01$)

The mean serum cholesterol concentration during the transition period in Sahiwal cows under different dietary zinc supplementation is presented in Table 3. The mean cholesterol concentration in Sahiwal cows for the T0, T1, T2, and T3 groups was 108.31, 110.17, 116.07, and 116.86 mg/dL, respectively. The prepartum means of cholesterol in the T0, T1, T2, and T3 groups were 105.96 ± 3.14, 107.74 ± 3.10, 110.24 ± 2.57, and 112.40 ± 2.55 mg/dL, respectively. The postpartum values were 112.57, 114.43, 124.14, and 122.79 mg/dL, respectively. The overall cholesterol concentration during the prepartum period was lower than that during the postpartum period in all groups. The treatment-related differences were more pronounced during early and mid-lactation (3, 5, 7 and 14 postpartum). During these periods,

cows in T2 and T3 exhibited significantly higher cholesterol concentrations compared to T0 and T1. Decline in serum cholesterol concentration approaching calving, followed by a gradual and significant increase during the postpartum period, with treatment-related differences being more pronounced during early and mid-lactation.

Aspartate Aminotransferase (AST)

The AST enzyme in the liver plays a role in the metabolism of amino acids. The AST test may help diagnose and monitor liver problems. These enzymes are released when the liver is damaged and the concentration of serum AST is increased. The mean serum concentration of AST during the transition period in Sahiwal cows under different dietary zinc supplementation has been shown in Table 4. The mean concentration of AST of Sahiwal cows in T0, T1, T2, and T3 groups was 90.99 ± 1.89, 84.61 ± 1.34, 81.46 ± 1.14, and 79.19 ± 1.38 U/L, respectively. The prepartum mean of AST of Sahiwal cows in T0, T1, T2 and T3 groups were 85.15 ± 1.91, 80.88 ± 1.85, 78.53 ± 1.85 and 75.39 ± 1.87 U/L, respectively and the postpartum values were 96.64 ± 1.29, 88.16 ± 0.81, 84.28 ± 0.28 and 83.00 ± 0.77 U/L, respectively. Significant differences were observed in overall mean as well as postpartum and prepartum mean values. Significant differences were observed in the overall mean, as well as in prepartum and postpartum mean values among the groups. The prepartum and postpartum mean concentrations of AST were found to be significantly ($P \leq 0.05$ and $P \leq 0.01$, respectively) lower in the T3 and T2 groups. The mean concentration of serum AST was significantly ($P \leq 0.01$) lower in the T3 group, followed by the T2 and T1 groups, compared to the control group. Increased AST activity from prepartum to postpartum period, with consistently higher enzyme activity in T0, suggests greater metabolic and hepatic load around calving and early lactation without Zn supplementation.

Table 4
Mean values (± SE) of AST conc. (U/L) in Sahiwal cows

Periods of observations	AST (U/L) in Sahiwal cows				Sig. level
Days relative to calving	T_0	T_1	T_2	T_3	
-21	76.24^{a}±1.93	72.80^{ab}±1.35	70.05^{b}±2.12	67.05^{b}±2.40	*
-14	83.74^{a}±2.17	78.38^{ab}±1.82	76.72^{b}±2.00	73.31^{b}±1.18	**
-7	87.57^{a}±2.06	83.00^{ab}±2.85	80.28^{ab}±2.81	77.03^{b}±1.97	*
-5	87.17 ± 2.47	82.82 ± 3.07	81.05 ± 1.43	76.82 ± 2.81	NS
-3	87.42 ± 1.87	83.25 ± 2.09	81.38 ± 2.06	79.09 ± 2.27	NS
-1	88.78^{a}±2.14	85.06^{ab}±2.44	81.71^{b}±1.99	79.04^{b}±1.90	*
At Calving	92.17^{a}±3.05	85.71^{ab}±3.01	82.10^{b}±3.06	79.24^{b}±2.21	*
+ 1	93.14^{a}±2.41	85.13^{b}±2.70	83.04^{b}±2.45	80.40^{b}±2.80	*
+ 3	95.18^{a}±2.38	87.01^{b}±2.50	84.25^{b}±2.11	82.06^{b}±1.81	**
+ 5	95.18^{a}±1.93	87.86^{b}±1.99	84.74^{b}±1.97	83.05^{b}±2.20	**
+ 7	95.18^{a}±1.93	88.91^{ab}±2.14	84.81^{b}±2.44	85.37^{b}±2.46	*
+ 14	101.12^{a}±2.90	91.03^{b}±2.96	84.81^{b}±2.44	85.01^{b}±3.05	**
+ 21	100.08^{a}±2.60	89.02^{b}±2.20	84.06^{b}±2.87	82.12^{b}±3.17	**
Mean	90.99^{a}±1.89	84.61^{b}±1.34	81.46^{bc}±1.14	79.19^{c}±1.38	**
Prepartum Mean	85.15^{a}±1.91	80.88^{ab}±1.85	78.53^{b}±1.85	75.39^{b}±1.87	*
Postpartum Mean	96.64^{a}±1.29	88.16^{b}±0.81	84.28^{c}±0.28	83.00^{c}±0.77	**

^{a,b,c}- Mean with different superscript in a row differ significantly*($P \leq 0.05$) **($P \leq 0.01$)

Alanine Aminotransferase (ALT)

The function of ALT is to convert alanine into pyruvate for cellular energy production. High levels of these enzymes can be a sign that the liver is injured. The mean serum concentration of ALT during the transition period in Sahiwal cows under different dietary zinc supplementation has been shown in Table 5. The mean concentration of ALT of Sahiwal cows in T0, T1, T2, and T3 groups was 37.03 ± 1.65, 34.06 ± 1.54, 28.72 ± 0.86, and 28.61 ± 0.79 U/L, respectively. The serum enzyme ALT level increased gradually up to 3 days postpartum, then remained stable from 21 days into the prepartum period,

followed by a decline thereafter in all treatment groups. The overall ALT concentration during the prepartum period was lower than that during the postpartum period in all groups. The prepartum and postpartum mean concentration of ALT was found significantly ($P \leq 0.01$) lower in the T2 and T3 groups. There was a significant variation in the mean serum ALT concentration of Sahiwal cows across the treatments ($P < 0.01$), with the T3 and T2 groups having significantly lower concentrations ($P \leq 0.01$), followed by the T1 group, and then the control group.

Table 5
Mean values (± SE) of ALT conc. (U/L) in Sahiwal cows

Periods of observations	**ALT (U/L) in Sahiwal cows**				**Sig. level**
Days relative to calving	**T_0**	**T_1**	**T_2**	**T_3**	
-21	28.10 ± 2.04	25.61 ± 1.76	23.82 ± 1.23	23.61 ± 0.81	NS
-14	29.40^{a}±1.16	26.82^{ab}±2.06	22.92^{b}±1.39	23.90^{b}±0.76	*
-7	30.20 ± 2.18	27.78 ± 1.86	25.88 ± 1.94	26.96 ± 1.70	NS
-5	31.80 ± 2.34	28.91 ± 1.29	27.05 ± 2.14	26.53 ± 1.89	NS
-3	34.07 ± 2.82	30.05 ± 2.17	28.42 ± 1.92	27.82 ± 1.92	NS
-1	36.09 ± 2.77	33.74 ± 2.73	29.05 ± 1.85	28.61 ± 1.98	NS
At Calving	37.85 ± 2.33	35.90 ± 3.29	30.12 ± 2.41	30.01 ± 1.74	NS
+ 1	39.35 ± 2.96	37.18 ± 1.64	31.39 ± 2.53	31.00 ± 2.17	NS
+ 3	46.08^{a}±3.41	41.68^{ab}±3.32	33.18^{b}±2.73	32.94^{b}±2.05	**
+ 5	44.38^{a}±3.11	40.36^{ab}±2.97	32.02^{b}±2.37	32.06^{b}±2.25	**
+ 7	43.28^{a}±2.87	39.54^{a}±2.56	31.07^{b}±2.16	30.52^{b}±2.40	**
+ 14	41.27^{a}±3.28	38.47^{a}±3.16	30.26^{b}±1.88	29.95^{b}±2.06	*
+ 21	39.60^{a}±3.02	36.81^{a}±2.18	28.46^{b}±1.95	28.10^{b}±2.04	**
Mean	37.03^{a}±1.65	34.06^{a}±1.54	28.72^{b}±0.86	28.61^{b}±0.79	**
Prepartum Mean	31.61^{a}±1.22	28.81^{ab}±1.17	26.19^{b}±1.00	26.23^{b}±0.83	**
Postpartum Mean	42.32^{a}±1.10	39.00^{b}±0.76	31.06^{c}±0.65	30.76^{c}±0.69	**

^{a,b,c}- Mean with different superscript in a row differ significantly*($P \leq 0.05$) **($P \leq 0.01$

Discussion

Zinc plays a crucial role in reproductive physiology, and its involvement in ovulation, follicular development, and hormone regulation has been well-documented in livestock species (Abdel Monem and El-shahat 2011; Garner et al. 2021; Wada and King 1986). Zinc deficiency has been associated with impaired oocyte development and suboptimal reproductive performance (Liu et al. 2024). Several studies have reported significant improvements in reproductive traits following zinc supplementation, including reduced days to first estrus and service interval in Holstein Friesian cows (Riad et al. 2018), shortened postpartum estrus interval with nano ZnO supplementation at higher doses (Kundu et al. 2014), and improved estrus onset in goats and ewes receiving elevated dietary zinc levels ((Abdel Monem and El-shahat 2011; Habeeb et al. 2013). These findings suggest that zinc, particularly when supplied in more bioavailable forms or at higher concentrations, can positively influence reproductive efficiency.

In the present study, dietary zinc supplementation especially in nano form resulted in numerically shorter postpartum interval to estrus and service period, as well as higher conception rates compared to the control group; however, these differences were not statistically significant. The observed trends are consistent with earlier reports indicating improved reproductive outcomes following zinc supplementation (Anchordoquy et al. 2019; El-Nour et al. 2010; Riad et al. 2018), although the magnitude of response may depend on factors such as dose, source of zinc, physiological status, baseline mineral status, and sample size. Anchordoquy et al. (2019) reported enhanced pregnancy rates in beef cows following zinc supplementation, which was attributed to increased plasma progesterone concentration and improved preovulatory follicle development, whereas other studies reported limited or no effects on conception rate following single-dose trace mineral supplementation (Vanegas et al. 2004). These variable responses highlight the complexity of zinc-mediated reproductive regulation.

Zinc may influence reproductive performance through multiple biological pathways, including its role in vitamin A metabolism, antioxidant defense, immune modulation, and endocrine regulation. Adequate zinc status supports antioxidative immunity through superoxide dismutase activity and enhances innate immune responses, which are critical for uterine health and embryo survival during the postpartum period (Bonaventura et al. 2015; Fair 2015; Wang et al. 2013). Improved immune competence and metabolic adaptation during the transition period may indirectly support reproductive recovery and fertility. Reports of increased conception rates in zinc-supplemented Holstein Friesian cows and Baladi goats (El-Nour et al. 2010; Riad et al. 2018), as well as improved reproductive performance with combined trace mineral supplementation (Ahola et al. 2004), further support zinc's role in reproductive efficiency.

Serum Zn: The present results are consistent with previous studies showing that nano zinc supplementation leads to higher blood or serum zinc concentrations than inorganic or organic zinc sources due to its greater bioavailability (Pomport et al. 2021; Swain et al. 2016). Similar increases in plasma zinc levels following zinc supplementation during the pre- and post-calving periods have been reported in Sahiwal cows by Maurya et al. (2014). In addition, Wahyuningsih et al. (2025) observed that improved zinc status in Holstein cows was associated with enhanced reproductive performance,

indicating a close relationship between zinc levels and fertility. Zinc plays a key role in the regulation of the hypothalamic–pituitary axis (Baltaci et al. 2019), and adequate zinc levels may support the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), thereby influencing ovarian function (Garner et al. 2021). Therefore, maintaining optimal serum zinc concentrations through nano zinc supplementation may contribute to endocrine balance and improved reproductive performance in dairy cows.

Plasma cholesterol: Higher serum cholesterol levels observed in nano zinc–supplemented groups highlight the important role of zinc in lipid metabolism and steroid hormone synthesis, which are critical during the periparturient period. A decline in serum cholesterol concentration towards calving, as observed in this study, has also been reported by Yadav et al. (2019) and Piccione et al. (2012). Similar findings were reported by Abayawansa et al. (2013), who observed a continuous decrease in serum cholesterol concentration until calving in buffaloes. The gradual increase in cholesterol levels after parturition may be associated with increased steroid hormone synthesis and mammary gland preparation for lactation (Patel Pankaj et al. 2020). Variations in cholesterol patterns, as reported by Vasantha et al. (2020), may be due to differences in breed, nutritional status, and physiological demands during early lactation. Overall, the findings suggest that nano zinc supplementation, particularly at 40 and 60 ppm levels, supports better lipid metabolism and may enhance reproductive efficiency by improving cholesterol availability for steroidogenesis for improved ovarian cyclicity and fertility.

Serum AST activity increased during the prepartum period and reached peak values around the second week after calving. This is in accordance with the findings of Walter et al. (2022), who reported peak AST activity around 10 days postpartum in multiparous cows. Yadav et al. (2019) also observed variations in AST levels during early lactation, which may be attributed to breed or physiological differences. Higher AST activity during the postpartum period reflects increased hepatic load and tissue metabolism associated with milk production. The comparatively lower prepartum AST levels may be due to reduced metabolic stress, while elevated postpartum values could be related to increased tissue damage and oxidative stress following parturition, as reported by Maurya et al. (2014) and Walter et al. (2022). Both prepartum and postpartum mean AST concentrations were significantly lower in the nano zinc supplemented groups (T2 and T3), indicating a hepatoprotective effect of zinc due to its antioxidant properties and role in maintaining cell membrane integrity. Similar reductions in serum AST levels following zinc supplementation were reported by Gaafar et al. (2011) and Riad et al. (2018) in Friesian cows. However, Hegazy et al. (2018) reported increased AST levels in rats with ZnO nanoparticle supplementation, suggesting species-specific responses to nano Zn.

The present study showed that serum ALT activity increased around calving, indicating higher hepatic stress during this period. Similar findings were reported by Maurya et al. (2014), who observed significantly higher ALT levels at 3 days postpartum in Sahiwal cows. The increase in ALT activity around calving may be attributed to increased lipid mobilization, higher metabolic load on the liver, and oxidative stress associated with parturition and the onset of lactation, as also reported by Angeli et al. (2019). Lower ALT concentrations during the prepartum period compared to the postpartum period were also

observed, which agrees with the findings of Abdelrazek et al. (2018) and Yadav et al. (2019). Patel Pankaj et al. (2020) further reported a gradual increase in ALT levels during early lactation in crossbred cows. The decline in ALT levels after the early postpartum peak may indicate recovery of liver function following initial lactation stress. Significantly lower ALT concentrations in the nano zinc–supplemented groups (T2 and T3) suggest a hepatoprotective role of zinc. These findings are in agreement with Riad et al. (2018) and Gaafar et al. (2011), who reported reduced serum ALT levels in zinc-supplemented Friesian cows. Supplementing the diet with nano zinc (20 and 40 ppm) positively affected the concentration of zinc in serum, indicating improved bioavailability of zinc and other biochemical parameters. Nano Zn supplementation at 40 ppm in the diet of Sahiwal cows improved the other biochemical parameters, such as cholesterol, AST, and ALT. However, supplementation with 20 ppm of nano-Zn in the diet only resulted in a non-significant improvement in the postpartum interval to estrus, service period, and conception rate. Supplementation of Zn in either form is beneficial during the transition period in Sahiwal cows; however, nano-Zn supplementation has an edge over ZnO. However, to decide the feasible dose of nano Zn supplementation, a larger number of such investigations are required.

Declarations

Ethical approval

Appropriate permission and procedure were followed for the collection of blood samples from the concerned authorities. All the procedures were performed under the relevant guidelines and regulations. The research work has been approved by the Institutional Animal Ethics Committee, College of Veterinary Science and Animal Husbandry, Anjora, Durg, Dau Shri Vasudev Chandrakar Kamdhenu Vishwavidyalay, Durg, Chhattisgarh, India.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Author contributions

All authors of this paper contributed to conducting research. Methodology, data collection and analysis were performed by Arvind K. Nandanwar, Dhirendra Bhonsle, Sonali Prusty and Vandana Bhagat. The first

draft of the manuscript was written by Arvind K. Nandanwar. All authors read and approved the final manuscript.

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Data availability

All data generated in this study are included in this published article.

References

1. Anchordoquy JM, Anchordoquy JP, Galarza EM, Farnetano NA, Giuliodori MJ, Nikoloff N, Fazzio LE, Furnus CC (2019) Parenteral zinc supplementation increases pregnancy rates in beef cows. Biol Trace Elem Res 192(2):175–182. https://doi.org/10.1007/s12011-019-1651-8
2. Abayawansa WD, Prabhaka S, Singh RAK, Brar PS (2013) Seasonal variations in blood metabolic profiles during peri and early postpartum period in winter and summer calved buffaloes. Ind J Anim Reprod 34(1):34–37. https://www.journals.acspublisher.com/index.php/ijar/article/view/3995
3. Abdel Monem UM, El-Shahat KH (2011) Effect of different dietary levels of inorganic zinc oxide on ovarian activities, reproductive performance of egyptian baladi ewes and growth of their lambs. Bulg J Vet Med 14(2): 116–123. https://www.cabidigitallibrary.org/doi/pdf/10.5555/20113201993
4. Abdelrazek H, Ismail TA, El-Azzazi FE, Elsayed DH (2018) Hematological and metabolic alerations in egyptian buffaloes during transition period. Egypt Acad J Biolog Sci C Physiol Mol Biol 10(1): 69–78. https://doi.org/10.21608/EAJBSC.2018.13657
5. Abdollahi M, Rezaei J, Fazaeli H (2020) performance, rumen fermentation, blood minerals, leukocyte and antioxidant capacity of young Holstein calves receiving high surface Zno instead of common ZnO. Arch Anim Nutr 74(3):189–205. https://doi.org/10.1080/1745039X.2019.1690389
6. Ahola JK, Baker DS, Burns PD, Mortimer RG, Enns RM, Whittier JC, Geary TW Engle TE (2004) Effect of copper, zinc and manganese supplementation and source on reproduction, mineral status and performance in grazing beef cattle over a two year period. J Anim Sci 82: 2375–2383. https://doi.org/10.2527/2004.8282375x
7. Akil S, Damayanti E, Samsudewa D, Hapila A, Iskandar H (2025) Effects of zinc supplementation on reproductive performance in cattle: a review. J Bioteknol Biosains Indones 12(1):163–170. https://doi.org/10.55981/jbbi.2025.11335

8. Ali HA, Ezzo OI, El-Ekllnawy KE (1998) Effect of zinc supplementation on reproductive performance of barki ewes under practical field condition. Vet Med J Giza 46(1): 77–87. https://doi.org/10.21608/VMJG.1998.377239
9. Angeli E, Rodriguez FM, Rey F, Stantiago G, Matiller V, Ortega HH, Hein GJ (2019) Liver fatty acid metabolism associated with reproductive performance of dairy cattle. Anim Reprod Sci 208:106104. https://doi.org/10.1016/j.anireprosci.2019.06.016
10. Arelovich HM, Owens FN, Horn GW, Vizcarra JA (2000) Effects of supplemental zinc and manganese on ruminal fermentation, forage intake, and digestion by cattle fed prairie hay and urea. J Anim Sci 78: 2972–2979. https://doi.org/10.2527/2000.78112972x
11. Baltaci AK, Mogulkoc R, Baltaci SB (2019) The role of zinc in the endocrine system. Pak J Pharm Sci 32(1): 231–239.
12. Bonaventura P, Benedetti G, Albarede F, Miossec P (2015) Zinc and its role in immunity and inflammation. Autoimmun Rev 14(4):277–285. https://doi.org/10.1016/j.autrev.2014.11.008
13. Brand IA, Kleineke J (1996) Intracellular zinc movement and its effect on the carbohydrate metabolism of isolated rat hepatocytes. J Biol Chem 271(4)1941–1949. https://doi.org/10.1074/jbc.271.4.1941
14. Cai J, Miao C, Chen Y, Xie Y, Liu J, Wang D (2021) Nano-sized zinc addition enhanced mammary zinc translocation without altering health status of dairy cows. Anim Nutr J 7(4):1024–1030. https://doi.org/10.1016/j.aninu.2021.06.003
15. Cho WS, Kang BC, Lee JK, jeong J, Che JH (2013) comparative absorption, distribution, and excretion of titanium dioxide and zinc oxide nanoparticles after repeated oral administration. Part Fibre Toxicol 10:9. https://doi.org/10.1186/1743-8977-10-9
16. De K, Pal S, Prasad S, Dang AK (2014) Effect of micronutrient supplementation on the immune function of crossbred dairy cows under semi-arid tropical environment. Trop Anim Health Prod 46(1):203–211. https://doi.org/10.1007/s11250-013-0477-1
17. El-Nour HHM, Abdel Rahman HMA El-Wakeel SA (2010) Effect of zinc methionine supplementation on reproductive performance, kids performance, minerals profile and milk quality in early lactating baladi goats. World Appl Sci J 9(3): 275–282. https://www.idosi.org/wasj/wasj9(3)/8.pdf
18. Fair T (2015) The contribution of the maternal immune system to the establishment of pregnancy in cattle. Front Immunol 6(7):1–8. https://doi.org/10.3389/fimmu.2015.00007
19. Fulton JO, Blair AD, Underwood KR, Daly RF, Gonda MG, Perry GA, Wright CL. The Effect of Copper and Zinc Sources on Liver Copper and Zinc Concentrations and Performance of Beef Cows and Suckling Calves. Vet Sci 10(8):511. https://doi.org/10.3390/vetsci10080511
20. Gaafar HMA, Bassiouni MI, Ali MFE, Shitta AA, Shamas ASE (2011) Effect of zinc methionine supplementation on productive performance of lactating friesian cows. Span J Rural Dev 53–62. https://doi.org/10.5261/2011.GEN1.05
21. Garner TB, Hester JM, Carothers A, Diaz FJ (2021) Role of zinc in female reproduction. Biol Reprod 104(5):976–994. https://doi.org/10.1093/biolre/ioab023

22. Habeeb AAM, El-Tarabany AA, Gad AE (2013) Effect of zinc levels in diet of goats on reproductive efficiency, hormonal levels, milk yield and growth aspects of their kids. Global Veterinaria, 10(5): 556–564. https://doi.org/10.5829/idosi.gv.2013.10.5.7367
23. Hara T, Takeda TA, Takagishi T, Fukue K, Kambe T, Fukada TJ (2017) Physiological roles of zinc transporters: molecular and genetic importance in zinc homeostasis. J Physiol Sci 67(2):283–301. https://doi.org/10.1007/s12576-017-0521-4
24. Hedley CF, Kristen EG, Krishna G, Chunli H, Steven AZ, Actions and Interactions of Thyroid Hormone and Zinc Status in Growing Rats. J Nutr 131(4):1135–1141. https://doi.org/10.1093/jn/131.4.1135
25. Hegazy AA, Ahmed MM, Shehata MA, Abdelfattah MM (2018) Changes in rats liver structure induced by zinc oxide nanoparticles and the possible protective role of vitamin E. International Journal of Human Anatomy, 1(3): 1–16. https://doi.org/10.14302/issn.2577-2279.ijha-18-2384
26. Kinal S, Slupczynska M (2011) The bioavailability of different chemical forms of zinc in fattening lambs. Archiv Tierzucht 54(4): 391–398. https://doi.org/10.5194/aab-54-391-2011
27. Kumar B, Ram H, Schoenau J (2024) Agronomic biofortification of fodder maize (zea mays l.) with zinc for improving herbage productivity and its quality. *Agronomy 14*(5):912. https://doi.org/10.3390/agronomy14050912
28. Kundu MS, De AK, Jeyakumar S, Sunder J, Kundu A, Sujatha T (2014) Effect of zinc supplementation on reproductive performance of teressa goat. Vet World 7(6):380–383. http://dx.doi.org/10.14202/vetworld.2014.380-383
29. Lemaire K, Chimienti F, Schuit F (2012) Zinc transporters and their role in the pancreatic β-cell. J Diabetes Invest 3(3):202–211. http://doi.org/10.1111/j.2040-1124.2012.00199.x
30. Li X, Guan Y, Shi X, Ding H, Song Y, Li C, Liu R, Liu G (2013) Effects of high zinc levels on the lipid synthesis in rat hepatocytes. Biol Trace Elem Res 154: 97–102. https://doi.org/10.1007/s12011-013-9702-z
31. Liu WJ, Li LS, Lan MF, Shang JZ, Zhang JX, Xiong WJ, Lai XL, Duan X (2024) Zinc deficiency deteriorates ovarian follicle development and function by inhibiting mitochondrial function. J Ovarian Res 17(1):115. https://doi.org/10.1186/s13048-024-01442-z
32. Lukaski HC, Hall CB, Marchello M (1992) Impaired thyroid hormone status and thermoregulation during cold exposure of zinc deficient rats. Horm Metab Res 24(8):363–366. https://doi.org/10.1055/s-2007-1003336
33. Maurya PK, Aggarwal A, Singh SV, Chandra G, Singh AK, Chaudhari BK (2014) Effect of vitamin e and zinc on cellular antioxidant enzymes in karan fries cows during transition period. Indian J Anim Res 48(2): 109–119. https://doi.org/10.5958/j.0976-0555.48.2.025
34. Nandanwar AK, Bhonsle D, Prusthy S, Rajendran D, Thakre A (2022) Effect of nano zinc supplementation on hematological parameters and body condition score during transition period in sahiwal cows. Indian J Anim Nutr 39(2):163–173. https://doi.org/10.5958/2231-6744.2022.00021.4
35. NRC (2001) *Nutrient requirements of dairy cattle*. 7th rev. ed. Vol.: National Press Academy, Washington, DC

36. Patel Pankaj A, Khatti A, Paul A, Mahato D, Maurya VP Singh G (2020) Effect of vitamin E and selenium supplementation on serum metabolites concentration during peri-parturient stress period in crossbred cows. Int. J Curr Microbiol Appl Sci 9(6):3758–3763. https://doi.org/10.20546/ijcmas.2020.906.444
37. Piccione G, Messina V, Marafioti S, Casella S, Giannetto C, Fazio F (2012) Changes of some haematochemical parameters in dairy cows during late gestation, postpartum, lactation and dry periods. Vet Med Zootech 58(80): 59–64.
38. Pomport PH, Warren HE Taylor-Pickard J (2021) Effect of total replacement of inorganic with organic sources of key trace minerals on performance and health of high producing dairy cows. J Appl Anim Nutr 9(1): 23–30. https://doi.org/10.3920/JAAN2020.0018
39. Ranasinghe P, Pigera S, Galappatthy P, Katulanda P, Constantine GR (2015) Zinc and diabetes mellitus: understanding molecular mechanisms and clinical implications. DARU J Pharm Sci 23:44. https://doi.org/10.1186/s40199-015-0127-4
40. Riad WA, El-Esawy GS, Mohy El-Dein AMA, Ali MFE. Gaafar HMA (2018) Effect of supplementary chelated zinc and manganese methionine on productive and reproductive performance of friesian cows. Egypt. J Agric Res 96(1): 289–302. https://doi.org/10.21608/ejar.2018.132846
41. Severo JS, Morais JBS, de Freitas TEC, Andrade ALP, Feitosa MM, Fontenelle LC, de Oliveira ARS, Cruz KJC, Marreiro, D. do N. (2019). The role of zinc in thyroid hormones metabolism. Int J Vitam Nutr Res 89(1–2):80–88. https://doi.org/10.1024/0300-9831/a000262
42. Shen H, MacDonald R, Bruemmer D, Stromberg A, Daugherty A, Li XA, Toborek M, Hennig B (2007) Zinc deficiency alters lipid metabolism in LDL receptor deficient mice treated with rosiglitazone. J Nutr 137(11):2339–45. https://doi.org/10.1093/jn/137.11.2339
43. Snedecor GW, Cochran W G (1994) Statistical methods. 8th edition. Iowa State University Press, Iowa, USA. https://doi.org/10.3102/10769986019003304
44. Swain PS, Rao SBN, Rajendran D, Dominic G, Selvaraju S (2016) Nano zinc, an alternative to conventional zinc as animal feed supplement: A review. Anim Nutr 2(3):134–141. https://doi.org/10.1016/j.aninu.2016.06.003
45. Vanegas JA, Reynolds J, Atwill ER (2004) Effects of an injectable trace mineral supplement on first-service conception rate of dairy cows. J Dairy Sci 87(11):3665–3671. https://doi.org/10.3168/jds.S0022-0302(04)73505-5
46. Vasantha SKI, Tej NK, Saikiran BVS, Lavanya S, Sivaiah K, Mutha RM Srinivasa PCH (2020) Hematology and biochemical changes in ongole cows one week before and one week after parturition in relation to THI. Pharma Innov J 9(1):318–324.
47. Wada L, King JC (1986) Effect of low zinc intakes on basal metabolic rate, thyroid hormones and protein utilization in adult men J Nutr 116(6):1045–1053. https://doi.org/10.1093/jn/116.6.1045
48. Wahyuningsih, Qomariyah N, Hidayah CN, Nurasih AD, Ifani M, Prihambodo TR (2025) Zinc supplementation on boosting reproductive efficiency, milk production and blood profile in holstein: a

meta-analysis perspective. Adv Anim Vet Sci 13(6):1200–1209. https://dx.doi.org/10.17582/journal.aavs/2025/13.6.1200.1209

49. Walter LL, Gartner T, Gernand E, Wehrend A, Donat K (2022) Effects of parity and stage of lactation on trend and variability of metabolic markers in dairy cows. Animals 12(8):1008. http://doi.org/10.3390/ani12081008
50. Wang C, Liu X, Liu Y, Zhang Q, Zhenyu Y, Juang B, Zhang P, Li N, Cao X (2013) Zinc finger protein 64 promotes toll-like receptor-triggered proinflammatory and type I interferon production in macrophages by enhancing p65 subunit activation. J Biol Chem 288(34):24600–24608. https://doi.org/10.1074/jbc.M113.473397
51. Wilson RL, Leemaqz, SY, Goh Z, McAninch D, Jankovic-Karasoulos T, Leghi GE, Phillips JA, Mirabito Colafella K, Tran C, O'Leary S, Buckberry S, Pederson S, Robertson SA, Bianco-Miotto T, Roberts CT (2017) Zinc is a critical regulator of placental morphogenesis and maternal hemodynamics during pregnancy in mice. Sci Rep 7(1):15137. https://doi.org/10.1038/s41598-017-15085-2
52. Yadav BK, Singh VK, Singh SK (2019) Lipid mobilization and serum metabolites dynamics of sahiwal cows during the transition period. Biol Rhythm Res 52(9):1354–1371. https://doi.org/10.1080/09291016.2019.1629094

Figures

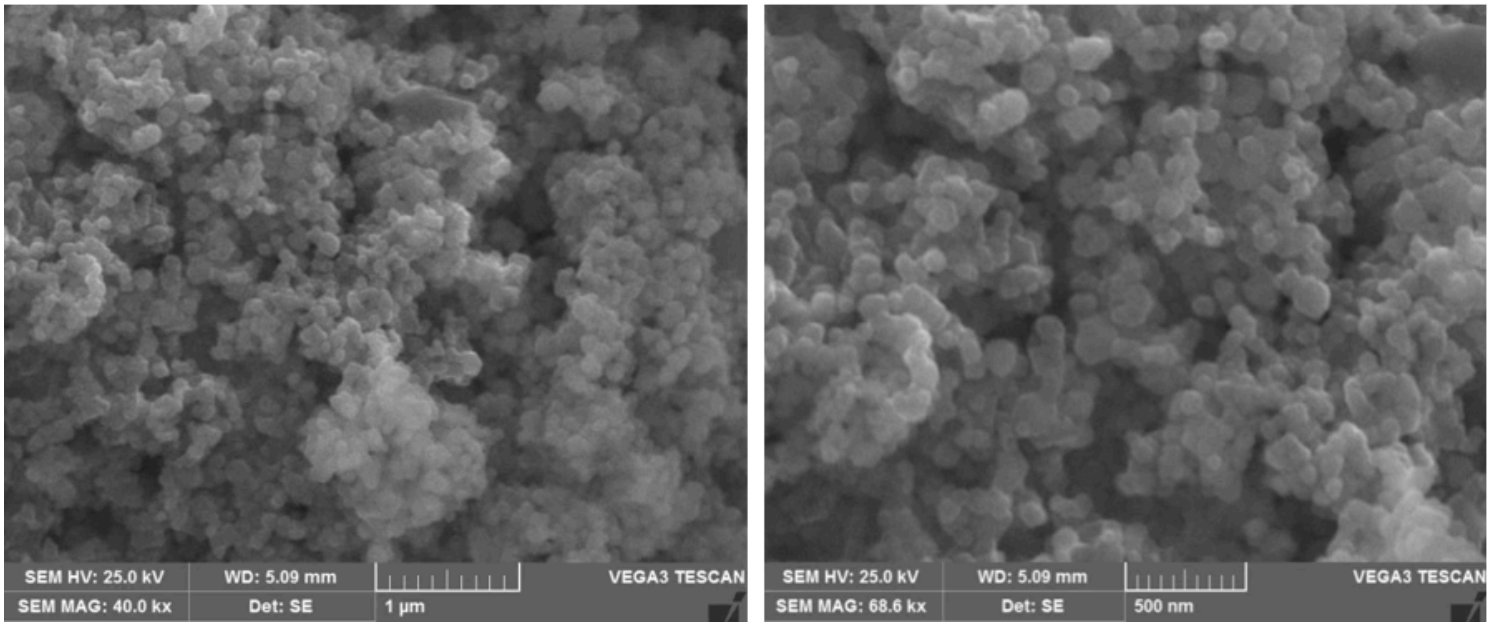


Figure 1

SEM image of nano zinc oxide (NZnO)