Indian J. Anim. Nutr. 2025. 42 (1): 47-53
doi: 10.56093/IJAN.v42i1.7
Indian Journal of Animal Nutrition

Effect of Organic Zinc Supplementation on Productive and Reproductive Performance of Transition crossbred Cows

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ABSTRACT

Experiment was conducted for 150 d on forty Holstein crossbred cows (530±30 kg b. wt.) and the experimental period had included one-month prior parturition and four months after parturition and were distributed randomly into four treatments and fed super Napier green fodder, paddy straw and concentrate mixture to meet ICAR (2013) nutrient requirements except for mineral mixture. Four experimental rations were formulated viz. BD, Zn_i, Zn_g, and Zn_m in which BD ration had no supplemented zinc, Zn_i (zinc sulphate), Zn_g (zinc glycinate) and Zn_m (zinc methionate). All the forms of zinc were supplemented as per the NRC (2001) nutrient requirements for dairy cattle. Significantly ($P<0.05$) higher concentration of SOD (units/mg protein) was recorded in Zn_m ration fed cows followed by Zn_g, Zn_i and BD rations. The mean GPx (units/ml) concentration was significantly ($P<0.05$) lower in the Zn_m ration fed cows followed by Zn_g, Zn_i and BD rations. Milk yield (kg/day) was increased significantly ($P<0.05$) in the Zn_m ration fed cows. Estrogen (pg/ml) and progesterone (ng/ml) concentrations were significantly ($P<0.05$) higher in Zn_m ration fed cows when compared to other groups. Reproductive performance was improved by supplementing zinc methionate (Zn_m) and it had shown the best performance in comparison to other treatment groups. With the results, it is inferred that zinc methionate supplementation has improved the productive and reproductive performance by ameliorating the transition stress in crossbred cows.

KEYWORDS: Antioxidants, Crossbred cows, Organic zinc, Reproductive performance, Stress, Transition period.

Article received: 11 November 2024; Article accepted: 02 March 2025

INTRODUCTION

The "transition period," the period from three weeks before to three weeks after calving, is extremely stressful for dairy cows or any other livestock species. Oxidative stress in a living organism is a result of an imbalance between reactive oxygen metabolites (ROM) production and neutralizing capacity of antioxidant mechanisms. Oxidative stress leads to peroxidative damage of lipids and other macromolecules with consequent alteration of cell membranes and other cellular components, and can also lead to the modification of important physiological and metabolic functions.

Bakhshizadeh et al. (2019) experimented with different sources of zinc as zinc oxide, zinc glycine and zinc nano @ 60 mg zinc per kg diet in Holstein dairy cows and reported that there was significant effect of time on DMI and milk yield, with an increase in milk yield being observed. Nazari et al. (2019) revealed that, supplementation of trace minerals (as per NRC 2001) had greater antioxidant trace mineral concentration of Cr, Cu, Fe and Zn which were associated with early postpartum period with normal luteal activity, earlier resumption of cyclicity, decreased pregnancy loss and increased conception rate in Holstein dairy cows.

There were lack of reports on the effect of organic compounds in roughage-based diets and only a limited number of studies have investigated the importance of organic zinc supplementation in transition cows. Hence, the present work was carried out to evaluate the amelioration of transition stress in crossbred cows with organic zinc supplementation.

MATERIALS AND METHODS

The protocols adopted in this experimental study were approved by the Institutional Animal Ethics Committee (IAEC), College of Veterinary Science, Hyderabad and is in accordance with the guidelines for the care and use of Laboratory animals with IAEC vide approval No.22/25/ C.V.Sc, Hyd. IAEC. Forty advanced pregnant multiparous crossbred cows (about one-month pre-partum) with an average body weight of 530±20 kg was selected from farmers of nearby villages of Korutla and allotted randomly into 4 groups of 10 animals each. All the cows were housed in well-ventilated hygienic stalls and stall fed throughout the experimental period. The animals were treated for both ecto and endo parasites well before the initiation of the study.

Conventional practice of feeding concentrates and roughages separately was followed throughout the experiment. Chopped green super Napier (*Pennisetum purpureum*) and paddy straw were used as sources of roughage for feeding of experimental animals in all the groups. Concentrate feed ingredients viz., maize, wheat bran, cotton seed cake, moong dal chunni, mineral mixture and salt were used. Concentrate feed ingredients were maize (average 3.5 kg), wheat bran (average 1.75 kg), cotton seed cake (average 1kg), moong dal chunni (1.75kg), mineral mixture and salt. The cows had free access to fresh and wholesome clean drinking water all the time.

Four different rations were fed to four treatment groups as mentioned below. The particulars are BD, basal diet without Zn supplementation; $Zn_{i,}$ Basal diet + inorganic Zn (Zinc sulphate); $Zn_{g,}$ Basal diet + Zinc glycinate; $Zn_{m,}$ Basal diet + Zinc methionate; Zinc supplementation was done through oral route by feeding the animals individually by preparing the mineral mixture. Mineral mixture was prepared with premixer in feed plant. The mineral mixture was prepared separately for four treatments by adding different sources of zinc and as per zinc bio-availability. All the cows were fed basal diet (roughages and concentrates) in accordance with the ICAR (2013) nutrient requirements for the experimental period of 150 days i.e., 1-month before calving to 4-months after calving. Zinc was supplemented to the mineral mixture prepared, as per NRC (2001) nutrient requirements for dairy cattle in different diets of experiment viz. without zinc (BD) Zn_i with zinc sulphate (27 % of zinc - Ven Vet chemicals), Zn_g with zinc glycinate (26 % of zinc- Avitech) and Zn_m with zinc methionate (16% of zinc-Novus company) .

The blood samples were collected at 15 days before parturition and on 0^{th}, 15^{th}, 30^{th} and 60^{th} day of post calving and the separated serum was used for further analysis. The proximate analysis of feeds was performed as per the procedures described by AOAC (2019). Mineral estimation was done by atomic absorption spectrophotometer using wet digestion procedures. Di-acid procedure was used for feed minerals like Cu, Zn, Fe, Mn, Co, Mg estimations by atomic absorption spectrophotometer using Air/Acetylene gas. Tri acid digestion procedures were used for serum minerals like Cu, Zn, Fe, Mn, Co, Mg estimations by atomic absorption spectrophotometer using Air/Acetylene gas.

Serum antioxidant parameters SOD concentration was estimated as per the procedure detailed by Madesh and Balasubramanian (1998). The GPx activity was determined by the method proposed by Paglia and Valentine (1967). The Milk yield was recorded from individual animals daily up to four months postpartum. The reproductive performance of cows was recorded as part of routine farm management, the postpartum cows were observed twice a day by trained farm personnel. The animals were examined for various gynecological parameters like involution of uterus, % of animals affected with mastitis and endometritis. These parameters were done using intrauterine probe (scanner).

The data were analyzed using General Linear Model procedure of Statistical Package for Social Sciences (SPSS) 20^{th} version and comparison of means was done using Duncan's multiple range test (Duncan, 1955) and significance was considered at $P<0.05$. All the statistical procedures were carried out as per the procedures of Snedecor and Cochran (1994) by programming and processing in computer. Two-way ANOVA was used to analyze the various parameters and if there was an interaction between the diet and time period was observed, one way ANOVA was done and mentioned in the results.

RESULTS AND DISCUSSION

Zinc concentration (mg/kg) in different feed ingredients offered to the animals during experimental period was super Napier green fodder (10.52), paddy straw (34.36), maize grain (21.58),wheat bran (69.41), cotton seed cake(undecorticated) (55.30), moong dal chunni (32.1).

Serum Zinc Concentrations

The decreased plasma zinc concentration (Table 1) on the day of calving might be due to redistribution of zinc in various tissues and this is in agreement with other experiments conducted by Maurya et al. (2014) and Chandra et al. (2014) in Karan Fries cows and buffaloes, respectively. Mean serum zinc concentration was 6.59 %, 18.68 %, 30.76 % higher in CB cows fed Zn_i, Zn_g and Zn_m, respectively when compared to BD fed cows. This might be due to increased bioavailability of zinc in organic sources than inorganic and without zinc supplementation.

Table 1. Serum zinc concentration (μmoles/l) of crossbred transition cows fed conventional rations with supplementation of different sources of zinc and at different time intervals

Day	Zn concentration (μmoles/l)				Mean	P-Value		
	BD	Zn_i	Zn_g	Zn_m	±SEM	Treatment	Period	T*P
-15	1.71	1.72	1.73	1.76	1.73^{c} ±0.13	0.0001	0.001	0.924
0	0.87	0.94	1.16	1.39	1.09^{b} ±0.09			
15	0.73	0.81	0.98	1.09	0.90^{ab} ±0.11			
30	0.69	0.75	0.83	0.92	0.79^{a} ±0.12			
60	0.54	0.65	0.72	0.82	0.68^{a} ±0.15			
Mean ±SEM	0.91^{A} ±0.13	0.97^{AB} ±0.12	1.08^{B} ±0.14	1.19^{BC} ±0.16				

a,b,c Values bearing different superscripts in columns differ significantly
A,B,C Values bearing different superscripts in rows differ significantly

Serum antioxidant enzymes

Super Oxide Dismutase (SOD)

The results of the present study are in agreement with the results reported by Bernabucci et al. (2005) who showed an increased activity of SOD during the last 3 weeks of pregnancy and rapidly declined SOD activity after calving (Table 2). Chandra et al. (2013) reported an increase in the SOD activity due to increased level of free radicles during the last three weeks and declined after calving till day 60 postpartum in Karan Fries crossbred cows supplemented with vitamin E and Zn.

Table 2. Super Oxide Dismutase (SOD) (units/mg of protein) in crossbred cows at different time intervals with supplementation of different sources of zinc in conventional rations

Day	SOD (units/mg of protein)				Mean±	P-Value		
	BD	Zn_i	Treatment	Treatment	SEM	Treatment	Period	D*P
-15	6.48	7.10	7.34	7.27	7.05^{a}±0.13	0.044	0.0001	0.0001
0	8.03	7.46	7.52	7.85	7.72^{c}±0.10			
15	7.67	7.36	7.09	7.42	7.38^{b}±0.13			
30	7.43	7.26	7.56	7.33	7.40^{b}±0.13			
60	6.60	7.20	7.41	7.31	7.13^{a}±0.09			
Mean±SEM	7.24^{A}± 0.14	7.28^{AB}± 0.08	7.38^{AB}± 0.12	7.44^{B}± 0.11				

a,b,c Values bearing different superscripts in columns differ significantly
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Glutathione peroxidase (GPx)

The GPx concentration was increased at the time of calving in all the groups when compared to prepartum values (Table 3). The increase in the concentration of GPx was least in the Zn_m followed by Zn_g, Zn_i and BD. After parturition there was decrease in the concentration of GPx in all the groups but it was less observed in the cows fed Zn_m followed by Zn_g, Zn_i and BD . Irrespective of supplementation of zinc, the GPx concentration was increased at the time of parturition and decreased regressively after calving. The increase was less in zinc methionate supplemented group when compared to basal diet and other supplemented groups. The present results are in agreement with Maurya et al. (2014) who supplemented zinc and vitamin E in Karan Fries cows.

Table 3. Glutathione peroxidase (GPx) enzyme (units/ml) in crossbred cows at different time intervals with supplementation of different sources of zinc in conventional rations

Day	Glutathione Peroxidase (units/ml)				Mean± SEM	P-Value		
	BD	Zn_i	Treatment	Treatment		D	P	D*P
-15	602.69	576.22	560.72	550.89	572.63^{a}±9.84	0.0001	0.0001	0.005
0	691.16	614.41	589.08	581.89	619.13^{d}±9.12			
15	648.06	605.71	581.89	573.95	602.40^{c}±9.01			
30	607.22	594.75	577.73	567.14	586.71^{b}±8.37			
60	602.65	584.92	571.31	558.07	579.25^{ab}±8.85			
Mean±SEM	630.36^{C}± 9.32	595.20^{B}± 8.66	576.14^{A}± 9.12	566.39^{A}± 9.05				

a,b,c Values bearing different superscripts in columns differ significantly
A,B,C Values bearing different superscripts in rows differ significantly

Milk Yield

Increased (P<0.05) milk yield in cows fed Zn_m (Table 4) is in agreement with the report of Kellogg et al. (2004) in which Zn methionine increased the lactation performance by increasing milk yield (P<0.01). Zinc plays an important role in maintaining skin health and integrity and has been implicated in reducing somatic cell count in some herds (Kellogg et al., 2004) and zinc is involved with keratin lining of the teat canal that physically traps the bacteria and prevents migration into mammary gland (Boland 2003). The improved performance of the cows supplemented with zinc methionine may be attributed to the superior bioavailability of zinc in this form.

Table 4. Milk yield (kg/d) in crossbred cows with supplementation of different sources of zinc in conventional rations

Month	Average milk yield (kg/d)				P Value
	BD	Zn_i	Zn_g	Zn_m	
1st month	19.43	20.04	20.24	20.62	0.619
2nd month	20.64	21.30	21.44	21.70	0.769
3rd month	20.60	20.70	23.11	24.01	0.009
4th month	19.66	20.00	22.63	23.77	0.002
Mean±SEM	20.08^{A}±1.13	20.51^{A}±1.23	21.85^{AB}±1.15	22.52^{B}±0.52	0.033

A,B Values bearing different superscripts in rows differ significantly

Reproductive parameters

The supplementation of zinc had shown reduced number of days for coming to heat after calving in crossbred cows (Table 5). In agreement with the present study, Patel et al.(2017) stated that, supplementation of zinc at various levels decreased no. of days to show first heat after calving in Karan Fries cows, In corroboration with the present study, Patel et al. (2017) reported decreased number of animals faced difficulty in shedding the fetal membranes, number of inseminations per conception, service period, number of mastitis cases and number of metritis cases with the supplementation of zinc at various levels in Karan Friess cows. Kassab et al. (2020) reported the importance of selenium, Zn and Mn as their deficiency have been linked to abnormal oestrus cycle, impaired ovulation and decreased conception rates. Concomitantly, Ahola et al. (2004) also found that, cows received trace minerals (Zn, Cu and Mn) had higher pregnancy rates than non-supplemented cows. Zinc is involved in the steroidogenesis and thus helps in improving the reproductive efficiency of the animals.

Table 5. Reproductive parameters in crossbred cows with supplementation of different sources of zinc in conventional rations

Parameter	BD	Zn_i	Zn_g	Zn_m	P Value
First heat after calving(days)	61.80^{C}	55.50^{B}	52.30^{A}	51.80^{A}	0.0001
Percentage of animals faced difficulty in expulsion of placenta after calving	0.4^{B}	0.2^{AB}	0.1^{AB}	0.0^{A}	0.012
Average number of inseminations per conception	1.4	1.3	1.2	1.1	0.131
Service period (days)	84.30^{C}	78.30^{B}	74.80^{A}	72.90^{A}	0.0001
Involution of uterus (days)	55.70^{D}	47.70^{C}	44.70^{B}	42.30^{A}	0.0001
% of mastitis cases	0.4^{B}	0.2^{AB}	0.1^{AB}	0.0^{A}	0.012
% of endometritis cases	0.5	0.4	0.1	0.0	0.153

A,B,C Values bearing different superscripts in rows differ significantly

Zinc oxide supplementation showed higher pregnancy rate in Does (Kundu et al., 2014) and concluded that inclusion of Zn @ 50, 100 and 150 ppm increased pregnancy rates compared to controls. In corroboration with present study Maurya et al. (2011) and Chandra et al. (2013) reported reduced mastitis cases with vitamin E and Zn supplementation. In general, free radicals cause cell damage and might influence the functions of immune cells resulting in increased mastitis risk (Politis et al., 2004). Miller et al. (1993) suggested that, cows with retained placenta had lower total antioxidant status. Reduced incidence of mastitis in the present study might be due to zinc trapping the bacteria on the teat surface and prevented their entry into mammary gland.

CONCLUSION

The crossbred cows exhibited better performance with supplementation of organic zinc sources, with a significant improvement observed in the group supplemented with zinc methionine compared to those receiving zinc glycinate and inorganic zinc sources. This enhanced performance could be attributed to the higher bioavailability of zinc in zinc methionine. Supplementing with zinc methionine reduced oxidative stress in the cows during the transition period, which, in turn, improved both their productive and reproductive performance.

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