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## Effect of organic zinc supplementation to ameliorate the transition stress and reduce the days to first heat in crossbred cows

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### Abstract

A study was carried out to evaluate the effect of supplementing different sources of zinc in crossbred cows during transition and early phase of lactation. 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<sub>i</sub>, Zn<sub>g</sub>, and Zn<sub>m</sub> in which BD ration had no supplemented zinc in it, Zn<sub>i</sub> (zinc sulphate), Zn<sub>g</sub> (zinc glycinate) and Zn<sub>m</sub> (zinc methionate). All the forms of zinc were supplemented as per the NRC (-2001) nutrient requirements for dairy cattle. Blood samples were collected at -15, 0, +15, +30 and +60 d post calving (0 indicates day of calving) and analyzed for serum zinc concentration during the experimental study. The zinc content in the various feed ingredients were analyzed. As part of routine management, the postpartum cows were observed twice a day by trained farm personnel for the occurrence of estrous based on vaginal discharges and behavioural signs. The mean values of serum zinc concentration (µmoles/ liter) in crossbred transition cows for BD, Zn<sub>i</sub>, Zn<sub>g</sub> and Zn<sub>m</sub> fed rations was 0.91, 0.97, 1.08 and 1.19, respectively and the values were significantly ( $p < 0.05$ ) different among the groups. The first heat after calving were 61.80, 55.50, 52.30 and 51.80 for BD, Zn<sub>i</sub>, Zn<sub>g</sub> and Zn<sub>m</sub>, respectively. With the supplementation of organic zinc sources the anoestrus period has been decreased in Zn<sub>g</sub> and Zn<sub>m</sub> ration fed cows compared to without zinc and inorganic zinc treated animals.

**Keywords:** Transition period, stress, crossbred cows, organic zinc, first heat after calving

### Introduction

The “transition period” is the period from three weeks before to three weeks after calving, is extremely stressful for dairy cows or any other livestock species. Since decades, different attempts have been made by animal nutritionists to understand the transition physiology of the cows as to formulate various feeding management tactics to alleviate the transition stress. The postpartum normalization of reproductive activity is certainly of vital importance to achieve early gestation, but it is necessary to understand the main events that trigger impairment in metabolism of cow in order to implement feeding management strategies that can contribute to better productive and reproductive performance of the cows.

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.

The transition period is particularly important for health and subsequent performance of dairy cows, which are exposed to drastic physiological changes and metabolic stress. High yielding animals require feeding strategies which guarantee the right contribution of all microelements such as zinc, manganese, copper, cobalt, iodine and selenium along with macronutrients viz. energy and protein.

Deficiency of zinc in soils of tropical areas, including India is common, and this phenomenon decreases the zinc content in feed & fodder crops and thereby dietary Zn level for farm animals. Zinc (Zn) influences various biological functions by being a co-factor for more than 300 metallo enzymes (Chasapis *et al.*, 2012) [4]. Besides this, Zn is essential for humoral and cellular immune responses and also plays an important role in antioxidant defense system

(Oteiza, 2012) [11]. Similarly, Zn is required for female reproductive system and necessary for normal ovulation and fertilization (Tian and Diaz, 2011) [15]. Zinc protects the female reproductive system from reactive oxygen species (ROS) by lowering the oxidative stress. In addition to this, several researchers have noticed beneficial effect of higher levels of Zn supplementation than recommendations on antioxidant status, immunity (Shinde *et al.*, 2007 and Nagalakshmi *et al.*, 2016) [13, 9] and reproduction. Zn is deficient in most parts of soils in India (Gowda *et al.*, 2009 and Nagalakshmi *et al.*, 2016) [6, 9] and due to its importance in various functions, it is a common practice by farmers and feed manufacturers to supplement with a large safety margin to avoid deficiency. But excess supplementation of Zn could lower its absorption and increases the excretion of other minerals such as Cu (Spears, 1996) [14], hence it causes mineral imbalance which could adversely affect the animal performance. To avoid this problem, the concept of organic minerals was developed in which mineral is in a chemically inert form, more stable and less prone to interactions, so absorbed and circulated to target tissues very efficiently (Spears, 1996) [14] thereby, decreasing the mineral excreted and hence lowering the requirement.

The most widely used products for zinc supplementation are inorganic (zinc sulfate and zinc oxide) and recently, organically bound zinc supplements are being used in animal diets. Knowledge on organic zinc compounds is still incomplete. Supplementing organic forms of zinc improve production responses compared with those observed in ruminants that are supplemented with inorganic zinc.

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 *viz.* zinc glycinate and zinc methionate in transition cows.

## Materials and Methods

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. The cows had free access to fresh and wholesome clean drinking water all the time.

Four different rations were fed to four treatment groups mentioned below

Treatment group	rations offered
BD	Basal diet without Zn supplementation
Zn <sub>i</sub>	Basal diet + inorganic Zn (Zinc sulphate)
Zn <sub>g</sub>	Basal diet + Zinc glycinate
Zn <sub>m</sub>	Basal diet + Zinc methionate

\*All the forms of Zn were supplemented as per NRC (2001) nutrient requirements for dairy cattle.

Zinc supplementation was done through oral route by feeding the animals individually by preparing the mineral mixture.

This mineral mixture was prepared with pre mixer in feed plant, Department of Animal Nutrition, College of Veterinary Science, Rajendranagar, Hyderabad. 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) [7] 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) [10] nutrient requirements for dairy cattle in different diets of experiment *viz.* in the basal diet (BD) no zinc was added, in the Zn<sub>i</sub> diet-zinc sulphate (27% available) from Ven vet chemicals, Zn<sub>g</sub> diet-zinc glycinate (26%) from Avitech and Zn<sub>m</sub> diet-zinc methionate (16%) from Novus company.

The blood samples were collected at 15 days before parturition and on 0<sup>th</sup>, 15<sup>th</sup>, 30<sup>th</sup> and 60<sup>th</sup> day of post calving, blood was drawn from the cow's jugular vein. The animals were restrained and the area was sterilized with surgical spirit. About 10 ml of blood was collected from all of the experimental cows in the morning in a clean, dry, sterilized test tube without adding the anticoagulant, centrifuged at 3000 rpm for 15 minutes to separate serum and then transferred into sterilized small plastic Eppendorf tubes (2 ml) and stored at -20 °C in deep freeze for further analysis. As part of routine management, the postpartum cows were observed twice a day by trained farm personnel for the occurrence of estrous based on vaginal discharges and behavioral signs. A gynaeco-clinical examination of the genitalia confirmed the occurrence of estrous, and the time between calving and the first occurrence of estrous and was recorded accordingly.

## Mineral Estimation

### Di-acid digestion (AOAC-2019)

The di-acid digestion method is the most frequently used procedure for feed minerals like Cu, Zn, Fe, Mn, Co, Mg estimations by atomic absorption spectrophotometer by using Air/Acetylene gas. 0.5g of sample was taken in a conical flask and added 10 ml of nitric acid (concentrated) and keep it the overnight. Next day 10 ml nitric acid added again and 2 to 3ml of perchloric acid to it. Digested the sample by using hot plate until gives the colorless solution or 1 to 2ml of solution in conical flask remains. Cooled the digested sample and added 10ml of 2N HCl. Filtered the digested solution by using 42 no. filter paper. By adding double distilled water made volume to 50ml. Read the sample by using atomic absorption spectrophotometer (AAS) for mineral estimation.

$$\text{Ppm} = \frac{\text{Reading X Dilution (50 ml)}}{\text{Sample weight}}$$

### Tri-acid digestion

The Tri-acid digestion method is the most frequently used procedure for serum minerals like Cu, Zn, Fe, Mn, Co, Mg estimations by atomic absorption spectrophotometer by using Air/Acetylene gas. 1 ml serum was taken in 100 ml conical flask and added 10 ml of tri-acid mixture. It gives pale yellow color. Digested the sample by using hot plate at 60 °C until colorless or 1ml sample remains in conical flask. Cooled the sample and made volume to 25 ml by using double distilled water. Read the sample by using AAS for minerals.

$$\text{Ppm (mg/1000 ml)} = \text{reading X 25 (25 ml Dilution)}.$$

## Results

**Table 1:** Zinc concentration in different feed ingredients offered to the animals during experimental period

Ingredient	Zn (mg/kg)
Super Napier green fodder	10.52
Paddy straw	34.36
Maize grain	21.58
Wheat bran	69.41
Cotton seed cake (un decarticated)	55.39
Moong dal chunni	32.16

\*Each value is an average of triplicate analysis

**Table 2:** Serum zinc concentration ( $\mu$ moles/litres) of crossbred transition cows fed conventional rations with supplementation of different sources of zinc and at different time intervals

Day	Zn concentration ( $\mu$ moles/litres)				Mean $\pm$ SEM	P-Value		
	BD	Zn <sub>i</sub>	Zn <sub>g</sub>	Zn <sub>m</sub>		D	P	D*P
-15	1.71	1.72	1.73	1.76	1.73 <sup>c</sup> $\pm$ 0.13	0.0001	0.001	0.924
0	0.87	0.94	1.16	1.39	1.09 <sup>b</sup> $\pm$ 0.09			
15	0.73	0.81	0.98	1.09	0.90 <sup>ab</sup> $\pm$ 0.11			
30	0.69	0.75	0.83	0.92	0.79 <sup>a</sup> $\pm$ 0.12			
60	0.54	0.65	0.72	0.82	0.68 <sup>a</sup> $\pm$ 0.15			
Mean $\pm$ S.Em	0.91 <sup>A</sup> $\pm$ 0.13	0.97 <sup>AB</sup> $\pm$ 0.12	1.08 <sup>B</sup> $\pm$ 0.14	1.19 <sup>BC</sup> $\pm$ 0.16				

<sup>ABC</sup> Means with different superscripts in a row differ significantly:  $p < 0.05$

\*Each value is an average of ten observations

P-Value: Probability value SEM: Standard Error Mean

BD: Basal diet without Zn supplementation, Zn<sub>i</sub>: Basal diet + inorganic Zn (Zinc sulphate)

Zn<sub>g</sub>: Basal diet + Zinc glycinate, Zn<sub>m</sub>: Basal diet + Zinc methionate

The serum zinc concentration ( $\mu$ moles/liter) of crossbred transition cows fed conventional rations with supplementation of different sources of zinc and at different time intervals is presented in Table 1. The mean values of serum zinc concentration ( $\mu$ moles/ liter) in crossbred transition cows for BD, Zn<sub>i</sub>, Zn<sub>g</sub> and Zn<sub>m</sub> fed rations was 0.91, 0.97, 1.08 and 1.19, respectively and the values were significantly ( $p < 0.05$ ) different among the groups. The higher zinc concentration ( $\mu$ moles/liter) was recorded in Zn<sub>m</sub> ration. Comparable values were observed in Zn<sub>g</sub> ration. The lowest zinc concentration ( $\mu$ moles/liter) was recorded in BD ration which was comparable with Zn<sub>i</sub> ration.

The mean values for serum zinc concentration ( $\mu$ moles/liter) during prepartum (15 days before the start of trial), on the day of calving, 1<sup>st</sup> month, 2<sup>nd</sup> month and 3<sup>rd</sup> month post-partum was 1.73, 1.09, 0.90, 0.79 and 0.68, respectively and were significantly ( $p < 0.05$ ) different. Significantly higher ( $p < 0.05$ ) serum zinc concentration ( $\mu$ moles/liter) was recorded 15 days prepartum and lower ( $p < 0.05$ ) zinc concentration ( $\mu$ moles/liter) was observed on 30 and 60 days postpartum. The medium comparable values were observed on the day of calving, 15 and 30days postpartum. The mean values of reproductive parameter in crossbred transition cows were recorded for first heat after calving were 61.80, 55.50, 52.30 and 51.80 for BD, Zn<sub>i</sub>, Zn<sub>g</sub> and Zn<sub>m</sub>, respectively. Significantly decreased ( $p < 0.05$ ) anoestrus period was observed in zinc methionate (Zn<sub>m</sub>) supplemented group (10 days decreased) when compared to BD group.

## Discussion

Plasma zinc concentration decreased on the day of calving in the current study might be due to redistribution of zinc in various tissues and this is in agreement with other experiments conducted by Maurya *et al.* (2014) [8] and Chandra *et al.* (2014) [3] in Karan Fries cows and buffaloes, respectively. The mean values of serum zinc concentration ( $\mu$ moles/liter) in crossbred transition cows for BD, Zn<sub>i</sub>, Zn<sub>g</sub>

and Zn<sub>m</sub> fed rations was 0.91, 0.97, 1.08 and 1.19, respectively. Mean serum zinc concentration was 6.59%, 18.68%, 30.76% higher in CB cows fed Zn<sub>i</sub>, Zn<sub>g</sub> and Zn<sub>m</sub> fed cows, respectively when compared to BD ration fed cows. This might be due to more bioavailability of zinc in organic sources than inorganic and without zinc supplementation. These results are in agreement with the Cope *et al.* (2009) [5]. Patel *et al.* (2017) [12] also reported similar results in cows with regard to serum zinc concentrations during transition period. In agreement with the present study results, decreased first heat after calving with the supplementation of zinc at various levels in Karan Fries cows (Patel *et al.*, 2017) [12] and Balamurugan *et al.* (2021) [2] stated that, the supplementation of trace minerals had shown reduced number of days for coming to heat after calving in crossbred cows.

## Conclusion

The animals in the organic zinc treated group have shown the decreased anoestrus period after calving compared to inorganic zinc and animals without zinc. The decrease in the anoestrus period might be due to more bioavailability of zinc in the organic sources that had shown its effect in ameliorating the transition stress and improve reproductive performance.

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