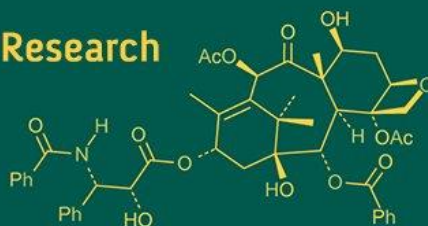


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Effect of organic zinc, vitamin A and vitamin E supplementation on morphometric changes, NEFA and BHBA during the transition period of crossbred COWS

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Abstract

Two experiments were conducted to evaluate the effects of different zinc sources and the combined supplementation of zinc with vitamins A and E on morphometric and metabolic responses in Holstein crossbred cows during the transition period. A total of forty cows (530±30 kg body weight) were studied over 150 days including one month prepartum and four months postpartum.

In the first experiment cows were randomly assigned to four dietary treatments: basal diet without supplemental zinc (BD), zinc sulfate (Zn_s), zinc glycinate (Zn_g) and zinc methionate (Zn_m). Diets were formulated to meet ICAR-2013^[6] nutrient requirements and supplements were provided according to NRC-2001^[8] recommendations. Results showed significantly higher ($p<0.05$) body weight and body condition score (BCS) in cows fed Zn_m and Zn_g. Additionally, Zn_m supplementation resulted in significantly lower ($p<0.05$) plasma non-esterified fatty acids (NEFA) and β -hydroxybutyrate (BHBA) concentrations compared to other groups.

In the second experiment, the best-performing organic zinc source (Zn_m) was combined with either vitamin A (Zn_{mA}) or vitamin E (Zn_{mE}) and compared with Zn_s. While body weight changes did not differ significantly ($p>0.05$), BCS was significantly higher ($p<0.05$) in Zn_{mE} followed by Zn_{mA} and Zn_s. NEFA concentrations were significantly lower ($p<0.05$) in Zn_{mE}, whereas BHBA levels showed no significant differences among treatments.

The findings indicate that zinc methionate particularly when combined with vitamin E improves morphometric traits and metabolic status by alleviating transition stress in crossbred dairy cows.

Keywords: Organic zinc, vitamins a and e, BCS, transition period

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. The period is characterised by negative energy balance, fat mobilisation, and elevation of circulating non-esterified fatty acids and ketone bodies (Seifi, et al. 2007)^[11]. The degree of changes in body reserves of a cow as assessed by periodical body condition score (BCS) and body weight changes during this period are the effective management tools. Besides BCS and body weight, NEFA and BHBA concentrations in the serum also act as internal indicators (metabolic) during transition period. 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. There is pressing need to optimize the feeding strategies and managerial aspects during this period. There was 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. Most of the fat soluble antioxidant vitamins such as retinol, α -tocopherol and β -carotene decrease at the time of parturition are reported to be associated with several health problems (Weiss, 1990)^[12]. Besides zinc as the important trace mineral supplementation, other micronutrients especially fat-soluble vitamins.

i.e., vitamins A and E are gaining importance in supplementation during the transition phase of the cows. With above overview, the present investigation was carried out by conducting two experiments by supplementing different sources (forms) of zinc and (or) zinc with vitamin A and E in the conventional rations.

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 were selected from farmers of nearby villages of Korutla, Jagityal district located in central Telangana and the town lies at 18.8°N latitude and 78.93°E longitude and rises to an average height of 258 m above the sea level. The animals were 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.

The proximate analysis of feeds was performed as per the procedures described by AOAC (2019) [2].

Mineral estimation:

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 using Air/Acetylene gas. 0.5g of sample was taken in a conical flask and 10 ml of nitric acid (concentrated) was added and

kept overnight. The next day, 10 ml nitric acid was added again to the same conical flask along with and 2 to 3ml of perchloric acid. The sample was digested by using hot plate until it developed the colour less solution or 1 to 2 ml of solution was remained in conical flask. The digested sample was cooled and added 10 ml of 2N HCl. The digested solution was filtered by using 42 no. filter paper and volume was made to 50 ml by adding double distilled water. The mineral concentration was recorded by using atomic absorption spectrophotometer (AAS).

The Tri-acid digestion method is the mostly used procedure for serum minerals like Cu, Zn, Fe, Mn, Co, Mg estimations by AAS. Reagents used are of tri-acid mixture: 9:3:1 Nitric acid. Perchloric acid, Sulfuric acid. 1ml serum was taken in 100 ml conical flask and 10ml of tri-acid mixture was added. It developed pale yellow color. The sample was digested by using hot plate at 60°C until colorless or 1 ml sample remained in the conical flask. Cooled the sample and made volume to 25 ml by using double distilled water. Read the sample by using AAS for minerals.

Experimental Rations and Feeding Regimen

Conventional practice of feeding concentrates and roughages separately was followed throughout the experiment. Chopped green super Napier (*Pennisetum purpureum*) (around 25 kg) and paddy straw (around 5 kg) were used as sources of roughage for feeding of experimental animals in all the groups. 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 during the dry period as mentioned below

Treatment group	Rations offered
BD	Basal diet without Zn supplementation
Zn _i	Basal diet + inorganic Zn (Zinc sulphate) (1303.75 gm/10 kg mineral mixture)
Zn _g	Basal diet + Zinc glycinate (1354 gm/10 kg mineral mixture)
Zn _m	Basal diet + Zinc methionate (2195 gm/10 kg mineral mixture)

Four different rations were fed to four treatment groups during the lactation period as mentioned below

Treatment group	Rations offered
BD	Basal diet without Zn supplementation
Zn _i	Basal diet + inorganic Zn (Zinc sulphate) (933.75 gm/10 kg mineral mixture)
Zn _g	Basal diet + Zinc glycinate (970 gm/10 kg mineral mixture)
Zn _m	Basal diet + Zinc methionate (1570 gm/10 kg mineral mixture)

*All the forms of Zn were supplemented as per NRC (2001) [8] nutrient requirements for dairy cattle. During the lactation period zinc requirement was 1261 mg/day/animal in high yielders and during the late gestation additional zinc requirement of 274 mg/day/animal was supplemented.

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 at 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.

Addition of different zinc sources to the mineral mixture

All the cows were fed basal diet (roughages and concentrates) in accordance with the ICAR (2013) [6]

nutrient requirements for the experimental period of 150 days i.e., 1-month before calving to 4-months after calving. Fifty grams of mineral mixture per day per animal was fed individually. Zinc was supplemented to the mineral mixture prepared, as per NRC (2001) [8] nutrient requirements for dairy cattle in different rations of experiment viz. in the basal diet (BD) no zinc was added, in the Zn_i diet- the added zinc sulphate (27 % available) was procured from Venvet Chemicals Private Limited (Msquare, Patrikanagar, No. 1, Madhapur, Hyderabad), Zn_g diet- the added zinc glycinate (26% available) was procured from Avitech (GP-37, Udyog Vihar, Sector-18, Gurgaon, Haryana, India) and Zn_m ration- the added zinc methionate (16% available) was procured from Novus company (Ground Floor, No.20, 2nd Chithirai Street Noombal, Vellappanchavadi, Chennai, Tamil Nadu).

Body Weight Recording The animals were weighed before start of the experiment, after calving (on the day of calving) and at monthly interval using the Minnesota formula.

$$W = LG^2/600$$

L is length of the animal from point of shoulder to point of buttocks in inches.

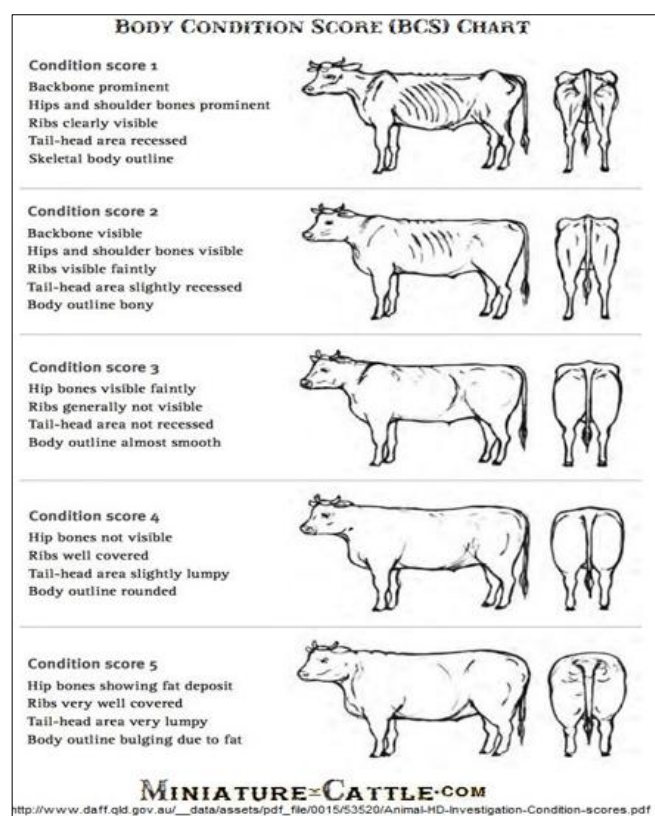
G is the chest girth of the animal in inches and measured with the tape.

The weight of the animal is expressed in kilograms.

Body Condition Scoring (BCS)

The BCS of the animals was recorded five times during experiment period, first at start of the experiment (pregnant stage of the animal), second at the time of calving and thereafter at monthly intervals. The score for individual animal was given with visual appraisal based on observing five important points as short ribs, backbone, hook and pin bones, thurl (area over pelvis) and tail head as per Homestead and miniature cattle directory (Ferguson *et al.*, 1994) [5].

Fig1: Body Condition Score Chart



Results and Discussion:

The results obtained in these two experiments are presented below in the tables showing concentration of serum zinc, serum vitamin A and E, Body weight changes, BCS changes, NEFA and BHBA concentration in the serum.

The present study demonstrated significant alterations in metabolic and physiological parameters of crossbred transition cows in response to zinc supplementation from different sources, with or without vitamins A and E. The decline in plasma zinc concentration observed on the day of calving may be attributed to redistribution of zinc towards fetal requirements and colostrum synthesis. Similar observations were reported Patel *et al.* (2017) [10] on periparturient dairy animals, suggesting a transient shift in mineral metabolism during parturition. The higher serum

zinc concentrations observed in cows supplemented with organic zinc sources, particularly zinc methionine and zinc glycinate, indicate superior bioavailability compared to inorganic zinc, thereby enhancing systemic zinc status.

Body weight changes across the transition period followed a typical pattern, with maximum values before calving and a decline during early lactation. This reduction is primarily associated with negative energy balance (NEB), reduced dry matter intake, and increased energy demands for milk production. However, cows supplemented with organic zinc sources exhibited relatively higher body weights, suggesting improved nutrient utilization and metabolic efficiency. These findings are consistent with earlier reports indicating that organic trace minerals support better growth performance and reduce body weight loss during early lactation Antari *et al.*, 2021) [1]. Body condition score (BCS) declined during the postpartum period, reflecting mobilization of body reserves to meet energy demands. The lack of significant differences in BCS among treatment groups suggests that while zinc supplementation improves metabolic parameters, its direct influence on body reserve mobilization may be limited. Nevertheless, the observed trend aligns with previous studies Chandra *et al.* (2013) [4] indicating that BCS changes during transition are largely governed by energy balance rather than mineral supplementation alone. Non-esterified fatty acids (NEFA), an important indicator of lipid mobilization, were significantly elevated around calving, confirming intensified fat mobilization during this period. The reduced NEFA concentrations in zinc-supplemented groups, especially with zinc methionine, indicate improved energy balance and reduced lipolysis. This suggests that organic zinc supplementation may enhance metabolic adaptation by improving feed intake and insulin sensitivity. These are in accordance with Chandra *et al.* (2013) [4]. Elevated NEFA levels have been associated with increased risk of metabolic disorders; hence, their reduction in supplemented groups indicates better health status and metabolic stability. Similarly, beta-hydroxybutyric acid (BHBA) concentrations increased after calving, reflecting enhanced ketogenesis due to NEB. However, lower BHBA levels in zinc-supplemented groups suggest improved hepatic metabolism and reduced ketone body formation. This may be linked to improved insulin activity, which promotes lipogenesis and inhibits excessive fat mobilization (Barletta *et al.*, 2017) [3]. The observed relationship between NEFA and BHBA further supports the role of energy balance in regulating metabolic health during the transition period. The decrease in serum vitamin A Oliveira *et al.* (2015) [9] and E Maurya *et al.* (2014) [7] concentrations at calving can be explained by their transfer into colostrum, which is essential for neonatal immunity. Supplementation of these vitamins resulted in higher circulating levels, indicating effective dietary absorption and utilization. Their antioxidant properties likely contributed to reduced oxidative stress and improved immune function, thereby supporting overall metabolic health during the transition period. The combined supplementation of zinc methionine with vitamins A and E showed synergistic effects in improving body weight, reducing NEFA and BHBA concentrations, and enhancing metabolic stability. These findings suggest that integration of organic trace minerals with antioxidant vitamins can effectively mitigate the adverse effects of NEB and oxidative stress during the transition period. Overall, the

study highlights the importance of mineral form and vitamin supplementation in modulating metabolic responses in transition dairy cows. The improved performance observed

with organic zinc sources, particularly zinc methionine, underscores their potential role in enhancing productivity and health during this critical physiological phase.

Table 1: Serum zinc concentration ($\mu\text{moles/litre}$) in crossbred dairy cows

Day	Zn concentration ($\mu\text{moles/litres}$)				Mean $\pm$ SEM	P Value		
	BD	Zn _i	Zn _g	Zn _m		D	P	D*P
-15	1.71	1.72	1.73	1.76	1.73 ^c $\pm$ 0.13	0.0001	0.0001	0.924
0	0.87	0.94	1.16	1.39	1.09 ^b $\pm$ 0.09			
15	0.73	0.81	0.98	1.09	0.90 ^{ab} $\pm$ 0.11			
30	0.69	0.75	0.83	0.92	0.79 ^a $\pm$ 0.12			
60	0.54	0.65	0.72	0.82	0.68 ^a $\pm$ 0.15			
Mean $\pm$ SEM	0.91 ^A $\pm$ 0.13	0.97 ^{AB} $\pm$ 0.12	1.08 ^B $\pm$ 0.14	1.19 ^{BC} $\pm$ 0.16				

^{ABC} 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_i: Basal diet + inorganic Zn (Zinc sulphate)

Zn_g: Basal diet + Zinc glycinate, Zn_m: Basal diet + Zinc methionate

Table 2: Body weight (kg) of crossbred cows fed conventional rations supplemented with various forms of zinc at different time intervals

Day	Body weight (kg)				Mean $\pm$ SEM	P Value		
	BD	Zn _i	Zn _g	Zn _m		D	P	D*P
Prepartum	531.17	535.56	568.00	562.18	549.23 ^c $\pm$ 7.53	0.0001	0.0001	0.924
Day of Calving	510.77	529.23	551.73	553.46	536.30 ^b $\pm$ 7.87			
1 st month	496.49	519.12	542.32	543.82	525.44 ^{ab} $\pm$ 8.02			
2 nd month	486.30	512.02	531.12	536.51	516.49 ^a $\pm$ 7.65			
3 rd month	482.73	510.04	537.73	546.87	518.34 ^a $\pm$ 8.39			
Mean $\pm$ SEM	501.49 ^A $\pm$ 6.61	521.19 ^B $\pm$ 8.88	546.18 ^C $\pm$ 8.09	548.57 ^C $\pm$ 7.97				

Table 3: BCS in crossbred cows at different time intervals with supplementation of different sources of zinc in conventional rations

Day	Body condition score				Mean $\pm$ SEM	P Value		
	BD	Zn _i	Zn _g	Zn _m		D	P	D*P
Prepartum	3.48	3.63	3.76	3.65	3.63 ^d $\pm$ 0.06	0.0001	0.0001	0.405
Day of calving	3.16	3.29	3.47	3.44	3.34 ^c $\pm$ 0.05			
1 st month	3.09	3.20	3.40	3.39	3.27 ^{ab} $\pm$ 0.04			
2 nd month	3.11	3.18	3.37	3.35	3.25 ^a $\pm$ 0.04			
3 rd month	3.17	3.24	3.38	3.51	3.32 ^{bc} $\pm$ 0.03			
Mean $\pm$ SEM	3.20 ^A $\pm$ 0.03	3.30 ^A $\pm$ 0.04	3.47 ^B $\pm$ 0.04	3.46 ^B $\pm$ 0.05				

Table 4: NEFA (mmol/L) in crossbred cows at different time intervals with supplementation of different sources of zinc in conventional rations

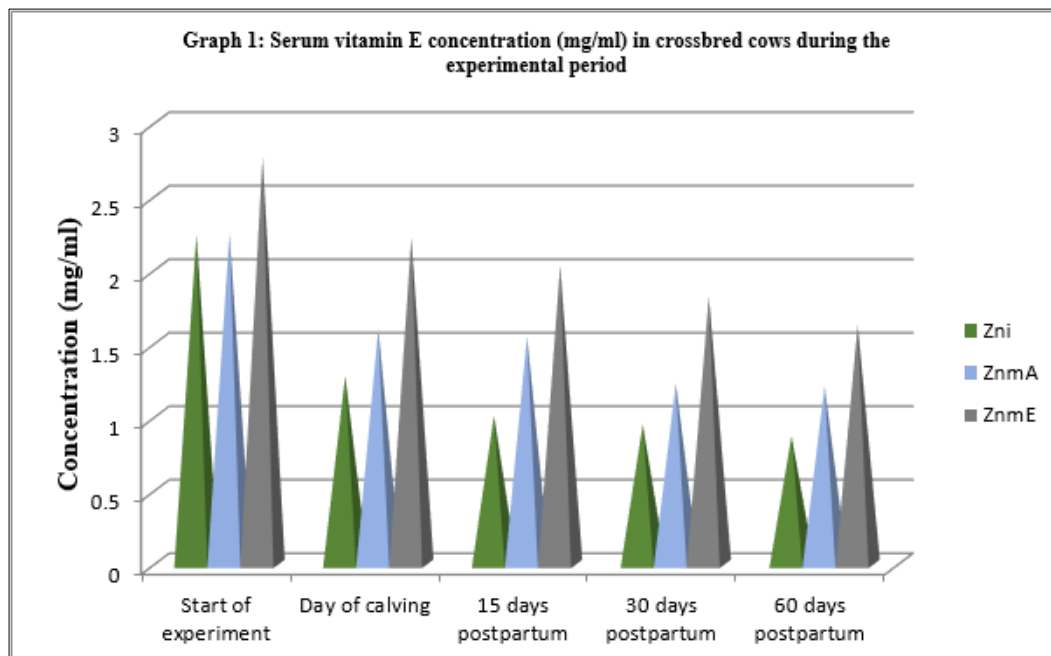
Day	NEFA (mmol/L)				Mean $\pm$ SEM	P Value		
	BD	Zn _i	Zn _g	Zn _m		D	P	D*P
-15	0.54 ^v	0.56 ^v	0.58 ^w	0.58 ^w	0.56 ^a $\pm$ 0.01	0.0001	0.0001	0.0001
0	0.80 ^y	0.69 ^x	0.78 ^y	0.76 ^y	0.76 ^d $\pm$ 0.02			
15	0.75 ^{xy}	0.66 ^w	0.70 ^x	0.66 ^x	0.69 ^c $\pm$ 0.02			
30	0.72 ^{wxy}	0.62 ^{vw}	0.59 ^w	0.57 ^w	0.62 ^b $\pm$ 0.02			
60	0.67 ^w	0.61 ^x	0.52 ^v	0.52 ^v	0.58 ^a $\pm$ 0.02			
Mean $\pm$ SEM	0.70 ^B $\pm$ 0.02	0.63 ^A $\pm$ 0.02	0.63 ^A $\pm$ 0.01	0.62 ^A $\pm$ 0.02				

Table 5: BHBA (mmol/L) in crossbred cows at different time intervals with supplementation of different sources of zinc in conventional rations

Day	BHBA (mmol/L)				Mean $\pm$ SEM	P Value		
	BD	Zn _i	Zn _g	Zn _m		D	P	D*P
-15	0.54	0.55 ^{vw}	0.59 ^x	0.60 ^x	0.57 ^c $\pm$ 0.02	0.002	0.0001	0.0001
0	0.62	0.64 ^x	0.60 ^x	0.57 ^y	0.60 ^d $\pm$ 0.02			
15	0.60	0.59 ^{wx}	0.52 ^w	0.58 ^x	0.57 ^c $\pm$ 0.02			
30	0.57	0.54 ^{vw}	0.47 ^v	0.45 ^w	0.50 ^b $\pm$ 0.02			
60	0.56	0.49 ^v	0.45 ^v	0.43 ^v	0.49 ^a $\pm$ 0.02			
Mean $\pm$ SEM	0.58 ^B $\pm$ 0.02	0.56 ^B $\pm$ 0.02	0.53 ^A $\pm$ 0.01	0.52 ^A $\pm$ 0.01				

Table 6: Serum vitamin A concentration ($\mu\text{g/ml}$) in crossbred cows during the experimental period

Day	Serum vitamin A concentration ($\mu\text{g/ml}$)				P Value		
	Zn _i	Zn _{mA}	Zn _{mE}	Mean $\pm$ SEM	D	P	D*P
Start of experiment	0.79	0.87	0.80	0.82 ^d $\pm$ 0.11	0.048	0.043	0.216
Day of calving	0.55	0.75	0.60	0.63 ^c $\pm$ 0.13			
15 days postpartum	0.32	0.65	0.55	0.50 ^{bc} $\pm$ 0.09			
30 days postpartum	0.23	0.62	0.42	0.42 ^b $\pm$ 0.14			
60 days postpartum	0.22	0.55	0.38	0.38 ^a $\pm$ 0.10			
Mean $\pm$ SEM	0.42 ^A $\pm$ 0.07	0.68 ^C $\pm$ 0.09	0.55 ^B $\pm$ 0.06				

**Table 7:** Body weight (kg) in crossbred cows at different time intervals with supplementation of zinc methionate with vitamins A and E in conventional rations

Day	Body weight (kg)			Mean $\pm$ SEM	P Value		
	Zn _i	Zn _{mA}	Zn _{mE}		D	P	D*P
Prepartum	571.25	561.73	567.80	566.92 ^c $\pm$ 6.47	0.732	0.0001	0.968
Day of calving	541.78	540.08	543.32	541.73 ^a $\pm$ 6.76			
1 st month	546.31	547.42	547.58	547.10 ^a $\pm$ 6.73			
2 nd month	549.34	552.65	555.66	552.55 ^{ab} $\pm$ 7.03			
3 rd month	553.29	563.61	564.59	560.50 ^{bc} $\pm$ 6.84			
Mean $\pm$ SEM	552.39 $\pm$ 4.42	553.10 $\pm$ 9.99	555.79 $\pm$ 5.90				

Table 8: Body condition score in crossbred cows at different time intervals with supplementation of zinc methionate with vitamins A and E in conventional rations

Day	Body condition score			Mean $\pm$ SEM	P Value		
	Zn _i	Zn _{mA}	Zn _{mE}		D	P	D*P
Prepartum	3.49	3.46	3.61	3.52 ^d $\pm$ 0.04	0.0001	0.0001	0.931
Day of calving	3.15	3.20	3.36	3.23 ^a $\pm$ 0.03			
1 st month	3.25	3.29	3.43	3.32 ^b $\pm$ 0.03			
2 nd month	3.27	3.34	3.52	3.38 ^{bc} $\pm$ 0.03			
3 rd month	3.34	3.36	3.54	3.41 ^c $\pm$ 0.04			
Mean $\pm$ SEM	3.30 ^A $\pm$ 0.02	3.33 ^A $\pm$ 0.02	3.49 ^B $\pm$ 0.06				

Table 9: Non-esterified fatty acids (mmol/L) in crossbred cows at different time intervals with supplementation of zinc methionate with vitamins A and E in conventional rations

Day	NEFA (mmol/L)			Mean $\pm$ SEM	P Value		
	Zn _i	Zn _{mA}	Zn _{mE}		D	P	D*P
-15	0.54	0.57	0.55	0.55 ^a $\pm$ 0.02	0.0001	0.0001	0.092
0	0.82	0.76	0.70	0.76 ^d $\pm$ 0.03			
15	0.75	0.70	0.64	0.70 ^c $\pm$ 0.02			
30	0.70	0.66	0.59	0.65 ^b $\pm$ 0.04			
60	0.59	0.60	0.56	0.58 ^a $\pm$ 0.03			
Mean $\pm$ SEM	0.68 ^B $\pm$ 0.02	0.66 ^B $\pm$ 0.03	0.61 ^A $\pm$ 0.02				

Table 10: Beta hydroxy butyric acid (BHBA) (mmol/L) in crossbred cows at different time intervals with supplementation of zinc methionate with vitamins A and E in conventional rations

Day	BHBA (mmol/L)			Mean±SEM	P Value		
	Zn _i	Zn _{mA}	Zn _{mE}		D	P	D*P
-15	0.59	0.60	0.63	0.61 ^a ±0.02	0.796	0.0001	0.398
0	0.71	0.68	0.66	0.69 ^d ±0.02			
15	0.68	0.67	0.66	0.67 ^{cd} ±0.02			
30	0.66	0.65	0.64	0.65 ^{bc} ±0.02			
60	0.62	0.63	0.64	0.63 ^{ab} ±0.02			
Mean±SEM	0.65±0.01	0.65±0.02	0.65±0.02				

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