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Effect of zinc and vitamin E supplementation on hormones and blood biochemicals in peri-partum Sahiwal cows

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ABSTRACT

Thirty-two advanced pregnant multiparous Sahiwal cows were used to study the effect of additional zinc (Zn) and vitamin E (VE) supplementation on hormonal and biochemical changes. Cows were randomly assigned to four groups and fed a basal diet of compounded concentrate, berseem fodder, and wheat straw in a ratio of 60:20:20. The groups were: (1) the basal diet with no supplement (control treatment); (2) the basal diet supplemented with 60 mg/kg DM/cow daily of Zn (Zn treatment); (3) the basal diet supplemented with 1000 IU/cow daily of vitamin E (VE treatment); and (4) the basal diet supplemented with a combination of 60 mg Zn/kg DM/cow and 1000 IU vitamin E/cow/d (Zn + VE treatment). Blood samples were collected on −60, −45, −30, −15, −7, −3, 0, 3, 7, 15, 30, 45, 60, 90, and 120 d in relation to expected date of calving and were analyzed for endocrine variables and biochemical changes. Plasma concentrations of leptin, insulin, insulin like growth factor-1 (IGF-1), triidothyronine (T3), and tetraiodothyronine (T4) were decreased toward calving and observed lowest ($P < 0.05$) on 3 d post-partum. However, plasma levels of growth hormone (GH) and cortisol increased toward calving and were found highest ($P < 0.05$) on 3 d post-partum. Pre-partum concentrations of leptin and IGF-1 were higher ($P < 0.05$) than its respective concentration observed during post-partum. Post-partum concentrations of GH and cortisol were higher ($P < 0.05$) than its respective pre-partum concentration. Pre-partum concentrations of urea, triglycerides, Zn, and VE were higher ($P < 0.05$) and total cholesterol and HDL cholesterol were lower than its values observed in post-partum among all the groups. Treatments had significant ($P < 0.05$) effect on plasma hormonal levels and levels of Zn and VE but no effect on biochemical attributes. Cows fed on diet supplemented with Zn + VE had highest ($P < 0.05$) pre as well as post-calving concentrations of leptin (6.38 vs 5.01 ng/ml), insulin (1.39 vs 1.33 ng/ml), GH (9.29 vs 13.72 ng/ml), IGF-1 (14.55 vs 12.59 nmol/l), T3 (1.45 vs 1.40 ng/ml), T4 (32.44 vs 31.79 ng/ml) whereas as lowest concentration of cortisol hormone (3.05 vs 3.44 ng/ml). Cows supplemented with combination of Zn and VE showed minimum decline in plasma concentration of leptin, insulin, GH, IGF-1, T3, and T4, and minimum increase in cortisol concentration. In conclusion, dairy cows around parturition faces various endocrine and biochemical alterations and supplementation of Zn in combination with VE can ameliorate adverse effect of calving stress by maintaining circulatory concentration of hormone and biochemicals towards the basal levels.

1. Introduction

The period of peri-partum between late pregnancy and early lactation presents huge metabolic challenges to the dairy cow. The dramatic increased demand for nutrients such as glucose, amino acids and fatty acids for the onset of lactation in transition cows is often accompanied by a decrease in voluntary feed intake that causes a negative energy balance (NEB) [1]. To meet their energy demand, the cow mobilises its body energy reserves which is reflected from altered levels of metabolic hormones and blood biochemicals [2]. Altered concentrations of blood metabolites and metabolic hormones during this period resulting in diverse risk of metabolic and production related diseases [3]. Key adaptation during periparturient period includes increased synthesis and secretion of growth hormone and decreased responsiveness of white adipose tissues to the insulin [4]. Synthesis of leptin in bovine white adipose tissues during peri-partum period is negatively correlated

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with growth hormone [5].

Provision of adequate Zn and VE supplementation during the peripartum period may be used as a strategy to not only enhance the cow's immunity against disease but also maintain milk quality and production [6]. Zn is an essential trace element for various physiological functions that influence the growth, health and reproduction in different ways. It has been shown to influence blood metabolites and hormones at several levels, including hormone secretion and activity and binding to the target tissue [7]. Specificity protein 1 (Sp1) is a Zn finger protein involved in the stimulation of leptin transcription by glucose and insulin [8]. Zn is required for the activity of the enzyme, 5′- deiodinase, which converts hormone T4 to T3 [9]. Zn deficiency may also be associated with GH resistance and reduced IGF-1, although the mechanisms of each of these are unknown [10].

Vitamin E is a potent lipid soluble antioxidant in biological systems with the ability to directly quench free radicals therefore, prevents oxidative damage of white adipose tissues responsible for leptin production [11]. VE is required for preventing lipid mobilization from body reserves during condition of NEB [12]. Functioning of the pituitary-thyroid system has been shown to be slowed down in animals fed with low tocopherol containing diets for a long period of time [13]. Prepartum supplementation of VE might have reduced the reactive oxygen metabolites (ROM) production, leading to reductions in oxidative stress and cortisol concentrations [14].

Numerous studies have documented that plasma VE concentration decrease gradually throughout the pre-partum period, reach the lowest values around calving and then increase gradually after calving [15]. Simultaneously, stress can also cause a rapid redistribution of Zn out of extracellular fluids causing concentrations of Zn in serum to fall into the "deficient" range even when dietary Zn is adequate [16]. Therefore, extra supplementation of Zn and VE during peri-parturient period in dairy animals is warranted. Keeping these points in view, the present study was designed to investigate the effect of supplementation of Zn and VE on metabolic changes in periparturient Sahiwal cows.

2. Materials and methods

Animal care procedures were approved and conducted under the established standard of the Institutional Animal Ethics Committee, constituted as per the article number 13 of the Committee for the Purpose of Control and Supervision of Experiments on Animals rules laid down by the Government of India.

2.1. Animals, feeding, and experimental design

Thirty-two multiparous (4 ± 1 parity) advanced pregnant clinically healthy Sahiwal cows (*Bos indicus*) were selected from Livestock Research Centre of National Dairy Research Institute, Karnal, Haryana. Selected cows were assigned randomly to four treatments (8 cows per each treatment) stratified by body weight (470 ± 28 kg), parity and lactational yield (2800 ± 92 kg/lactation). Experimental cows were monitored from 2 months before (−60 d) to 4 months after (120 d) expected date of calving. Cows were fed a total mixed ration (TMR) based diet containing (per kg DM) 600 g of compounded concentrate, 200 g berseem fodder, and 200 g of wheat straw to meet their nutrient requirements as per NRC [17] recommendations. Compounded concentrate consisted (g/kg DM) of 472 g grounded yellow maize grain, 262 g decorticated ground nut cake, 107 g wheat bran, 138 g de-oiled rice bran, 17 g mineral mixture and vitamins premix, and 5 g salt. The ingredient and nutrient composition of TMR offered during experimental period is presented in Table 1.

Cows were fed their diets without any supplements (control treatment) or supplemented with 60 mg Zn/kg DM/cow (Feed grade $ZnSO_4 \cdot 7H_2O$, Luancheng Terife Agricultural Materials, Shijiazhuang, Hebei Province, China) (Zn treatment) or supplemented with 1000 IU VE/cow/d (DL-alpha-tocopheryl acetate, Xi'an Healthful Biotechnology, Hi-New Zone, Xi'an, China) (VE treatment) or supplemented with a combination of 60 mg Zn/kg DM/cow and 1000 IU VE/cow/d (Zn + VE treatment). Basal diet supplied 49 mg of Zn/kg DM and 211 IU VE, respectively. The TMR was prepared daily by hand mixing and offered twice a d at 09:00 h and 18:00 h. The supplements were delivered in 100 g compounded concentrate DM to individual cows, once daily, before the morning feeding at 08: 00 h to ensure the full dose of treatment was received. Remaining portion of compounded concentrate was incorporated in TMR.

Table 1
Ingredient and nutrient compositions of TMR fed during the experimental period.

Attributes	Content (g/kg DM or as mentioned)
ingredient composition	
Berseem fodder	200
Wheat straw	200
Ground yellow maize	283
Groundnut cake	157
Wheat bran	64
Rice bran	83
Mineral mixture and vitamins premix[1]	10
Salt	3
Zinc sulphate heptahydrate[2]	2
DL-alpha-tocopheryl acetate[2]	4
Chemical composition	
Dry matter	756
Organic matter	856
Crude protein	175
Crude fibre	291
Total ash	95
NDF	386
ADF	259
Calcium	10
Phosphorus	4.1
Magnesium	2.3
Manganese, mg/kg	63.83
Copper, mg/kg	23.22
Zinc, mg/kg	49.00
DL-alpha-tocopheryl acetate, IU	211

[1] Premix composition per kilogram: vitamin A 500,000 IU, vitamin D3 10,000 IU, vitamin E 100 mg, Ca 190,000, P 90,000, Na 50,000, Cu 300 mg, Fe 3000 mg,Mn 2000 mg, I 100 mg, Co 100 mg, Se 1 mg, Mg 19,000 mg, and BHT antioxidant 3000 mg.
[2] Supplemental zinc sulphate heptahydrate (0.20%) and DL-alph-tocopheryl acetate (0.40%) were substituted for ground yellow maize to provide 60 mg/kg Zn and 1000IU Vitamin E.

2.2. Sampling and laboratory analyses

Samples of TMR were taken weekly, composited at the end of experiment, dried at 65 °C in a forced air oven for 48 h and stored for further analyses. Stored dried samples of TMR were analyzed for DM (Method 973.18c), CP (Method 4.2.08), EE (Method 920.85) and total ash (Method 923.03) [18]. The methods of Van Soest et al. [19] were used for NDF, ADF, and ADL determination. Content of VE in TMR was estimated by HPLC (Waters HPLC, Model 510, Milford, MA, USA) [20]. Content of Zn, Cu, Mn, and Fe in TMR were analyzed by using Atomic Absorption Spectrophotometer (AAS, Model Z-5000, Hitachi High-Technologies Corporation, Tokyo, Japan).

Peripheral blood samples were collected in heparinized vacuutainer tubes (BD Franklin, USA) by venipuncture of anterior vena cava on d 60, 45, 30, 15, 7, and 3 prior to expected date of calving, 0 d (day of calving) and on 3, 7, 15, 30, 45, 60, 90, and 120 d post-calving. Blood samples were centrifuged at 1200*g* at 4 °C for 20 min. Serum was separated into 2 ml eppendorf tubes and frozen at −20 °C for analyses of hormone and biochemical attributes.

Leptin concentration in plasma was measured by "Bovine Leptin ELISA kit" (Cusabio Biotech Co., Ltd. China). The sensitivity of the assay was 1.56 pg/ml (intra and inter assay coefficients of variation were 4.25% and 8.05%, respectively). Plasma concentration of insulin