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Amelioration of transition stress by supplementation of antioxidant (Vitamin E), trace minerals (Selenium, copper, zinc) with increased energy allowance in crossbred cows

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Abstract
The experiment was conducted to investigate the effect of antioxidant (vitamin E (Vit E)) and trace minerals (selenium (Se), copper (Cu), zinc (Zn)), high energy diet supplementation and its effect on milk yield (MY) and biochemical parameters in crossbred cows During the transition period. For this, advanced pregnant crossbred cows (n = 20) were selected. Treatment(TRT) (n=10) group was supplemented with Vit E, Se, Cu and Zn incorporated in wheat flour bolus from -4 to 8 week of calving and were provided with 20% additional concentrate from 2 to 8 week of calving. Control (CON) group was given only basal diet without any supplementation. MY of each animal was recorded. Blood samples were collected on weekly interval from -4 to 8 week of calving. MY was higher ($p<0.01$) in the TRT versus CON. Some biochemical parameters ($p<0.05$) levels were also found significantly elevated and some significantly decrease ($p<0.05$) in the TRT versus CON group.

Keywords: Transition cows, Milk yield, Plasma metabolites, Liver enzymes, Antioxidants, Trace elements

1. Introduction

The transition period in dairy cows (3 week before and 3 week after parturition) represents a time of immense physiological stress for animals. The animal undergoes major physiological, nutrition, metabolic, endocrine and immunological changes to shift from non-lactating to lactating state [1]. A further challenge is that Dry matter intake (DMI) during the transition period is generally insufficient to meet the energy requirement for lactation and maintenance [2]. During the transition period, prepartum fetal growth and postpartum milk production increases the total energy demand on the animal. With the onset of lactation, additional metabolic activity of mammary gland increases by fourfold and mostly major nutrient (protein, fat and carbohydrate) is partitioned towards mammary gland to sustain copious milk secretions. The requirement of milk production during the onset of lactation increase dramatically that forces the cow to utilize nutrient (especially energy and protein) from body reserves that will results in to negative energy balance (NEB). Prepartum vitamin and mineral supplementation minimize the NEB by increase in DMI and decrease in the incidence of postpartum subclinical and clinical mastitis in the supplemented groups [3], because these micronutrients perform essential and specific roles for the performance of basic physiological processes, and their deficiency or excess adversely affects animal well-being [4], fertility and production. Both trace minerals and vitamin E are required to optimize the effectiveness of neutrophils when attacking and destroying invading bacteria. Cu is important to the antioxidant system because it is part of the copper-zinc superoxide dismutase enzyme. This enzyme converts toxic superoxide radicals to hydrogen peroxide in the cell. Thus Zn may impact immune function because of its essential role in the cell replication and proliferation. Zinc is also required for the synthesis of metallothionein, a metal binding protein that may scavenge hydroxide radicals [5]. Most of biological parameters affected during the transition phase due to decrease food intake under the influence of many type of stress that will increase reactive oxygen species and lead to the oxidative stress condition it resulted in to animal prone for many type of infection.

The demand for energy and protein increases dramatically with metabolic adaptation to lactation [6]. Tissue mobilization begins prepartum, increase protein catabolism and liver activity for energy, nutrient supply in negative energy balance condition. NEB increases activity of different liver enzyme which causes liver damage that will detrimental to the animal well being and production aspect. The aim of this study was to investigate the effects of Vit E and Trace mineral (Cu, Zn and Se) supplementation with extra energy allowance from 2nd week of calving on blood metabolites and milk production during the transition period in crossbred dairy cows.

2. Method and materials

All the experiments, procedures and protocols on animals were conducted following the approval of the Ethics Committee, Indian Veterinary Research Institute.

2.1 Experimental animals

The experiment involved twenty apparently healthy advanced pregnant crossbred cattle (Haryana/ Holstein Friesian/ Brown Swiss/Jersey) maintained at cattle and buffalo farm of Livestock Production and Management Section, Indian Veterinary Research Institute, Izatnagar. The institute is located at an altitude of 564 feet above the mean sea level, at latitude of 28-N and a longitude of 79- E. The experimental cows were in second to fourth parity with milk yield more than 10 L/day in their last lactation. Animals were maintained under isomanagerial conditions of housing, feeding and milking.

2.1.1 Experimental design

The experimental crossbred cows were divided into two groups (n = 10 cows/group), viz., treated (TRT) and control (CON). Each cow was given access to fodder and water ad-libitum. Animals of TRT group were supplemented with vitamin E (DL-a-tocopherol acetate, CDH, India), Selenium (Sodium selenite, CDH, India), Copper (Copper sulfate, CDH, India) and Zinc (Zinc sulfate, CDH, India) per oral at the dose rate of 80 IU/kg DMI, 0.3 mg/kg DMI, 15.7 mg per kgDMI and 22 mg per kgDMI [7] respectively in addition to standard feeding practices from -28 days of pre-partum till 56 days of lactation. These animals starting 20% extra energy (concentrate mixture) from 2 weeks after calving upto 8 weeks after calving. Cows in the TRT group were given wheat flour bolus, which was used As a vehicle to deliver Vitamin E and trace minerals. Cows in the TRT received increased energy in the form of 20% additional concentrate mixture from 2 to 8 weeks postpartum. However, CON group was given the basal diet. According to the farm policy, the experimental herd was examined twice a month by a team of specialists in veterinary medicine, gynaecology and surgery to record the occurrence of acidosis and/or laminitis due to possible carbohydrate overload in the TRT.

2.1.2 Milk sample Analyses

For milk sample analysis Milk samples were collected twice a day during morning (6.00AM) and evening (6.00PM). Milk production was recorded daily.

2.1.3 Analyses of biochemical metabolites

Blood samples were scheduled to be collected before feeding from jugular vein using Serum Collection tube (BD vaccutainer, USA) containing heparin at 13 different time points of transition period. Sampling weeks include -4,-3,-2,-1 before expected date of calving, 0 day (Parturition day) and +1,+2,+3,+4,+5,+6,+7,+8 after calving. samples were brought to the laboratory in chilled iceboxes soon after collection, for serum preparation, the blood was incubated 30 minutes by 30 ^{0}C and then centrifuged at 2500 rpm for 30 minutes at room temperature. Blood serum was stored at -80 ^{0}C until analysis. Concentrations of total protein (TP), albumin (ALB), albumin: globulin ratio, total Blood urea nitrogen (BUN), liver enzyme aspartate aminotransferase (AST), alanine aminotransferase (ALT) were estimated using commercial kits (Span Cogent diagnostic kits, India) with standard method using double beam UV-Visible Spectrophotometer (Electronics Corporation of India Ltd., India).

2.1.4 Statistical analysis

All experimental data are shown as mean ± SEM. The statistical significance of differences in mean were assessed using the software SPSS.60 by Repeated measure two way ANOVA mixed model. Differences were considered significant if $P<0.05$.

3. Results and Discussion

The present study shows how trace minerals and Vit E supplementation affect the various Biochemical parameters and milk yield. Overall value of milk yield (fig.1) was significantly higher ($p<0.01$) in the TRT than that of CON. This was attributed to increase in DMI and decrease in the incidence of subclinical and clinical mastitis in TRT as compare to CON. In dairy animals milk yield is directly affected by animal health status, health of the mammary gland, environmental condition, and DMI [8]. Supplementation of trace mineral and vitamin maintain the udder health and prevent from mastitis and other infections to the udder. In the influence of Cu, Zn, Se and Vit E biological action that improve the antioxidant defense system activity. Superoxide dismutase enzyme contain Cu it prevent from toxic superoxide converted it in to ordinary oxygen or H_2O_2 by dismutation, glutathione peroxides enzyme contain Se it maintains the intracellular redox status, preserve the sulfhydryl group of protein which play an important role in muscle protein, Zn play important role in mammary cell division and protein synthesis and it increase the membrane integrity of udder and teat epithelial tissue [9]. Vit E act as natural antioxidant, they all are prevent from oxidative damage and maintain the health of the mammary gland and increase the milk production. Reactive oxygen species (ROS) cause cell damage and influence the action of immune cell resulting in increased mastitis risk [10, 11]. Postpartum as mammary gland demand increases for the lactation, extra energy ration with supplementation prevent from NEB to treated animals.

In our study number of mastitis cases decrease in the TRT as compare to CON and milk production significantly increase ($p<0.01$) in the TRT but in other studies, Chawla and Kaur [3], Maurya [12] showed overall milk yield increase ($p<0.05$) in the TRT group (Vitamin E +Zn). Griffiths *et al.* [13] supplementing cows with CTM (providing daily 360 mg Zn, 200 mg Mn, 125 mg Cu as amino acid complexes and 12 mg cobalt (Co) from Co glucoheptonate) resulted in a 6.3% increase in the milk production. In Weiss and Spears [14] and Bourne *et al.* [15] study there was no significant difference on milk production in Vit E supplemented group. Moeini *et al.* [16] was found that milk production increased in the supplemented group (double injection of Vit E 1000 IU and selenium 10 mg) group till 8 weeks of lactation but in our study milk production increase till 13 weeks. Lacetera *et al.*

[17] found only 10% increase in milk production in Vit E and Se treated group.

Total protein (Fig.2) concentration was significantly ($P<0.05$) lower in the CON. The significant difference between both groups of animal was observed at prepartum, calving and post partum. Albumin concentration (Fig.3) varied significantly ($P<0.05$) between the groups. Lower concentration was found in the control animal before few weeks of calving, at calving and 4th week onwards of the experimental period. The albumin globulin ratio (Fig.4) varied significantly ($P<0.05$) between the groups. Postpartum albumin and globulin ratio was higher in the supplemented group than that of control. The change in the level of total serum protein is due to the reduced feed intake before calving which causes different stress which resulted in oxidative stress condition. Postpartum NEB increases utilization of protein as amino acids from muscle or different body tissues for complete the increased energy requirement of the mammary gland for growth and milk production in high yielding dairy cows. The level could also be influenced by partitioning of nutrient and protein towards fetal growth and utilization of amino acids from the maternal circulation for protein synthesis in the fetal muscles. Ca linked to the albumin, Ca level decreased in last gestation period due to the involvement in fetal survival and formation of colostrum so albumin concentration decrease 1 week before calving, at calving and post partum in CON cows compare to TRT. Trace minerals (Se, Zn, and Cu) actively participate in mitigation of oxidative stress and minimize the protein catabolism from muscles. CON animal showed more protein catabolism during 5th week onwards due to utilize more energy and major nutrient for the increased the milk production. Postpartum 5th week onward energy partitioning and protein catabolism increases in both group for milk production, but it is less pronounced in TRT, due to the combined effect of 20% extra energy with trace minerals and antioxidants supplementation and they enhance the protein and energy metabolism.

In our study CON showed significant decrease ($p<0.05$) level of TP and ALB at prepartum, calving and postpartum but in others study, Tothova *et al.* [18] observed no significant difference in serum TP and ALB levels at prepartum and calving between both group. Ucar *et al.* [19] stated that ALB levels remained unchanged in cows receiving mineral solution. Avci and Kizil [20] noted that the intrapartum TP and ALB values were low in cows receiving trace elements. Omur *et al.* [21] observed TP and ALB concentration remained unchanged in the treatment group.

BUN concentration (Fig.5) was higher ($p<0.05$) in the supplemented group than that of control. The decline in BUN level before parturition due to the reductions in DMI. The decreased level of plasma BUN level in CON is probably associated with decline in the protein intake before calving. Due to the decrease in protein intake it results in to decrease in glomerular filtration rate (GFR) caused by the calving stress [22], it affect the protein metabolism in body. Elitok *et al.* [23] found low levels of urea in postpartum cows given a birth recently and considered that this situation resulted from the decrease in protein anabolism caused by fat infiltration during that period. Decreases urea in postpartum period in the CON resulted from the increased liver activity in NEB condition for milk production cause the liver damage which decreases the anabolism of protein. Postpartum protein anabolism increase in influence of 20% extra energy with the supplementation of minerals and vitamin from 2nd week onwards in the TRT. According to Omur *et al.* [21] the treatment cows showed no any change in urea levels in postpartum periods. Abdalla *et al.* [24] reported that the significant difference ($p<0.05$) between treated and control group observed only at calving.

The AST and ALT enzyme activities presented in Fig.6 and 7 respectively were significant increase ($p<0.05$) in the CON. Increase activity of transaminase enzymes in CON could be associated with reduced DMI around calving and post partum increased energy demand by mammary gland for milk production resulted in to NEB condition of cows that may lead to alteration of normal functions of liver [25]. AST and ALT is proposed to be the common parameters for the detection and diagnosis of liver damage [26]. The AST level increased ($p<0.05$) in the CON before 1 week of calving and postpartum till 21 days, increased transaminase enzyme activity indicate the stress and damage status that occur in the body [27].

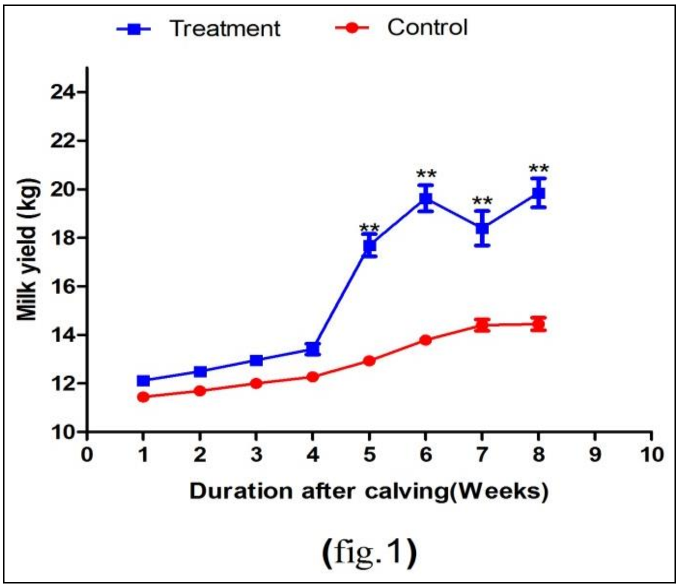
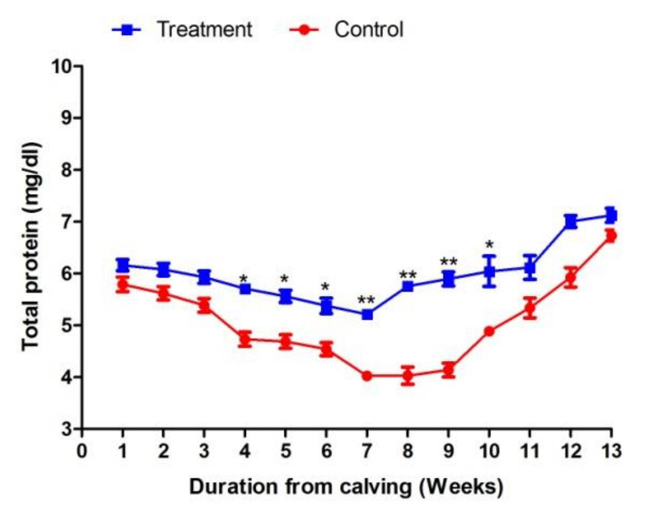
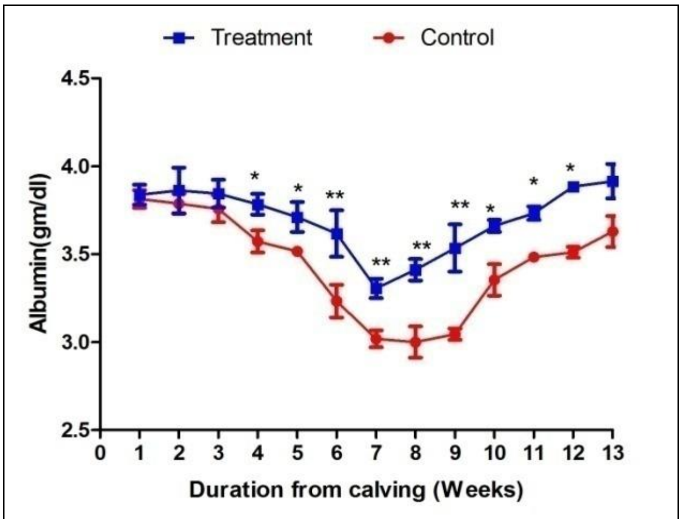


Fig 1: Effect of Vit E and Trace minerals (Se, Cu, Zn) supplementation with additional energy on the milk production during the transition period in crossbred cow (Mean±SE). Single and double asterisk represent significance at 5% and 1%, respectively.



(fig.2)



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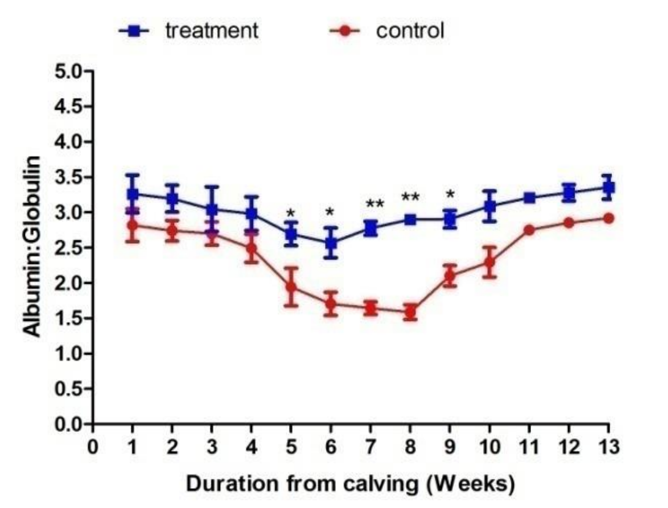

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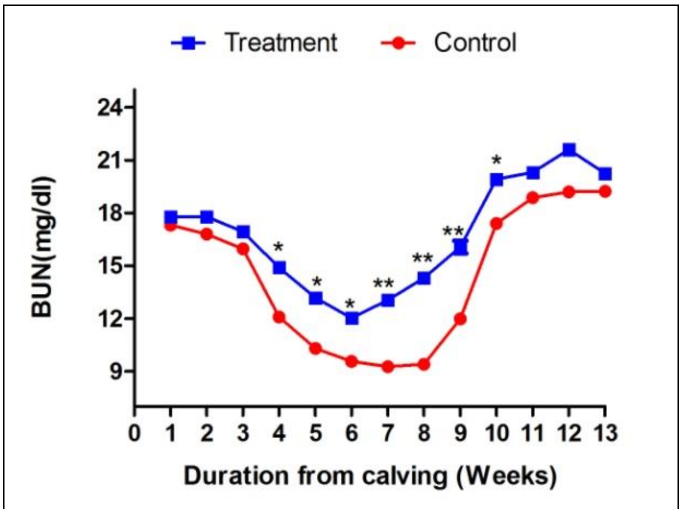

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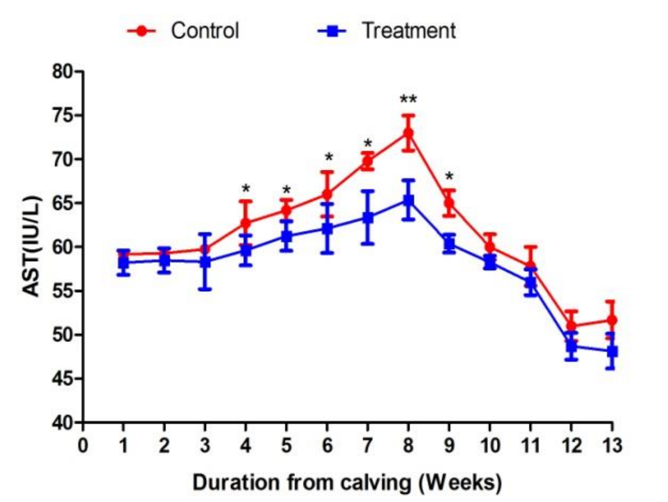
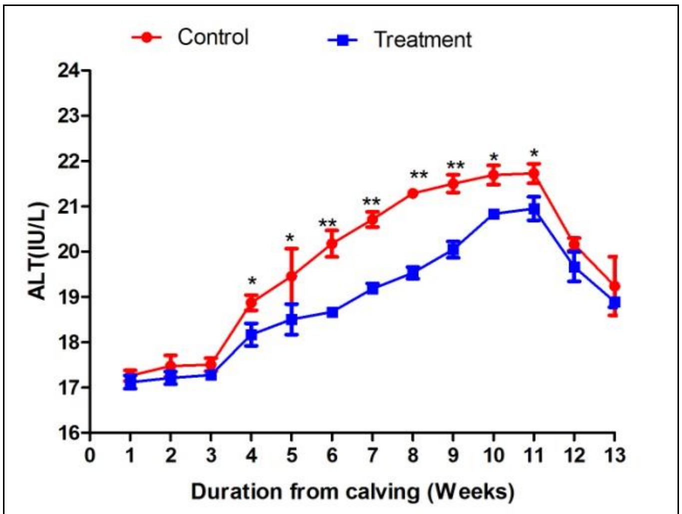


(fig.6)



(fig.7)

Fig 2-7: Effect of Vit E and Trace minerals (Se, Cu, Zn) supplementation with additional energy on the serum metabolites of (2) total protein, (3) total albumin, (4) albumin: globulin (AP; G), (5) BUN (Blood urea nitrogen), (6) AST (Aspartate Aminotransferase), (7) Alanine Aminotransferase (ALT) during the transition period in crossbred cow (Mean±SE). Single and double asterisk represent significance at 5% and 1%, respectively.

Changes in blood concentrations of AST enzyme activity may all reflect alterations in liver function associated with fat accumulation due to the increase non esterified fatty acids (NEFA) level after calving [28]. Protein catabolism increases creatinine kinase (CK) activity that may lead to increase AST activity [22]. We observed that AST and ALT activity increase prepartum and post partum in both group but it significantly increase in the CON. In TRT due to the influence of extra energy ration from 2nd week onwards of the calving with supplementation improve the liver function and minimize the activity of transaminase enzymes. Omur *et al*. [21] observed that the AST levels in both groups were higher in the parturient period as compared to the prepartum period. Although Hafez *et al*. [29] stated that the AST and ALT levels are higher in the pregnant animals than non-pregnant animals, and Bobe *et al*. [30] and Bogin *et al*. [31] showed the AST and ALT levels were higher in the postpartum period as compared to the dry period. Ucar *et al*. [19] emphasized that in cows receiving mineral solution, the ALT levels remained the same, while the AST tended to increase. It indicating that during postpartum this increase resulted from the deficiency in liver functions developed due to the NEB in that period.

4. Conclusion

Supplementation of vitamin E and trace mineral along with increasing energy content during the transition period in the cross breed cows mitigates the oxidative stress, reduce the DMI depression, improve the liver function by minimizing the negative energy balance condition, and improve the milk production.

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6. Conflict of Interest

Authors declare that they have no conflict of interest.

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