Effect of Dietary Enrichment with Copper and Zinc Along with Additional Energy during Periparturient Period on Ovarian Steroids and Reproductive Performance in the Crossbred Cattle

B. Balamurugan1*, S. Mehrotra1, A. Bhimte2, M. Ramamoorthy1, A. Khatti1, V.P. Maurya2, G. Singh2 and M.C. Pathak2

1Division of Animal Reproduction; ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly-243122, Uttar Pradesh, India
2Division of Physiology and Climatology; ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly-243122, Uttar Pradesh, India

*Corresponding author

ABSTRACT

The study was conducted to evaluate the effect of copper and zinc along with additional energy on steroid hormone and reproductive performance during periparturient period in crossbred cattle. Advanced pregnant crossbred cows (n=20) of 2 to 4 parity and lactation yield of >10L/day were divided into two equal groups (n=10 cows/group). In Group I the cows were supplemented with copper (15.7/kg DM), zinc (22 mg/kg DM) and increased energy allowance in the form of 20% additional concentrate, Group II cows were fed with only basal diet. Copper and zinc were supplemented with wheat flour bolus from 4 before to 8 week after calving whereas energy allowance was increased from 2 to 8 week after calving on daily basis to individual animal. Blood samples were collected to determine estradiol (E2) and progesterone (P4) in the serum using RIA. Postpartum reproductive performance was assessed in terms of calving to conception interval, pregnancy rate and number of service per conception. Serum E2 increased significantly at 1st week of prepartum, day of calving, 1, 2, 3, 6, 7 and 8 weeks postpartum in Group I as compared to Group II. Further, Serum P4 level increased significantly from 2nd week of prepartum, 4, 5, 6, and 7 weeks postpartum in Group I than that of Group II. Marked improvement in the fertility was recorded in terms of reduced calving to conception interval, service per conception.

Keywords
Copper, Zinc, Ovarian steroids, Reproductive performance, Periparturient cattle

Introduction

Transition or periparturient period is defined as the transition from pregnancy to parturition and lactation, which falls in the window of three or four weeks before to three or four weeks after parturition (Bell, 1995). Feeding during this period is most significant as it affects the reproductive performance of dairy animals. In order to resume normal fertility after calving, optimum balance of energy, protein, trance minerals and vitamins must be essential during prepartum and postpartum period. During transition period dairy cows are undergoes physiological challenges to the homeostatic mechanisms leads to increased...
oxidative stress and negative energy balance (NEBAL), which affects reproductive hormones and ultimately results in impaired fertility Butler (2003). Reports have shown that there is a depression in the blood levels of micro minerals and vitamins around the periparturient period Meglia et al., (2001). Trace minerals such as Cu and Zn play an important role in dairy cow immune function, growth Enjalbert et al., (2006) and fertility Rabiee et al., (2010). Feeding of Zn, Cu and vit E have improved the performance of dairy cattle through early occurrence of postpartum estrus, of reduced calving to conception interval and service per conception De et al., (2014). Endocrine balances are required to support normal cyclicity and gradual restoration of fertility, so impaired fertility is associated with alteration in reproductive steroids in dairy cows Erb et al., (1976). Energy status of dairy cow has variable effect over profile of reproductive steroids Butler (2000). Profile of reproductive steroids helps to evaluate reproductive status of a cow (Horan, 2005; Sartoni, 2004). Therefore present investigation was designed to assess the effect of Cu and Zn along with additional energy supplementation on ovarian steroids and reproductive performance in crossbred cow.

**Materials and Methods**

**Experimental animals**

The experiment involved 20 apparently healthy advanced pregnant crossbred cows (Haryana × Holstein Friesian/Brown Swiss/Jersey) maintained at cattle and buffalo farm of Livestock Production and Management Section, Indian Veterinary Research Institute, Izatnagar. These animals were selected on the basis of their parity (second to fourth) and milk yield (>10L/day) and maintained under isomanagerial conditions.

**Experimental design**

The animals were divided into two groups (n=10) viz., treated (group I) and control (group II). Each cow was given access to green fodder, concentrate and water ad-libitum.

Animals of group I were supplemented with Copper sulphate and Zinc sulphate (CDH, India) at a dose rate of 15.7mg/kg DM and 22 mg/kg DM, NRC (2001), respectively and increased energy allowance in the form of 20% additional concentrate. Group II cows were fed with only basal diet without any supplementation.

Cu and Zn were supplemented with wheat flour in the form of bolus from 4 weeks before to 8 weeks after calving whereas energy allowance was increased from 2 to 8 week after calving on daily basis to individual animal.

**Blood sampling**

Blood samples from experimental animals were collected by jugular vein puncture aseptically using 18-G needle in sterilized vacutainers (heparinized or clot activators) at weekly interval from -4 to 8 weeks of calving.

Schedule for blood sampling was based on expected date of calving. The experiment was approved by the Institute Animal Ethics Committee.

**Estimation of estradiol and Progesterone**

Serum was separated by centrifugation at 800xg for 10 min and stored at -80°C until analysis. Progesterone and estradiol-17β concentrations in the serum were estimated by Radio Immuno Assay (RIA) using standard diagnostic kits (Immunotech, France). The radio activity was measured in Berthold multicrystal Gamma counter, LB2103.
Reproductive performance

All the cows were observed till day 150 postpartum to record the calving to conception interval and number of service per conception.

Statistical analysis

Data were first checked by Shapiro-Wilk test for adherence to a normal distribution. Time series or longitudinal data for estradiol and progesterone were analyzed using GLM repeated measure ANOVA. Data were presented as Mean ± SE. Calving to conception interval and Service per conception by independent ‘t’ test and pregnancy rate (%) was analyzed by Fisher’s exact Chi-square test.

Significance was set at 95%. GLM was done with SPSS software (IBM® SPSS® statistics, version 20.0) while Chi-square test was done with Graph Pad prism version 6.

Results and Discussion

Group I had significantly (P<0.05) higher concentration of serum estradiol at one week before calving, on the day of calving and persist up to 2,3,6,7 and 8 week postpartum compared to group II (Figure 1), which could be due to follicular activity during the early postpartum period. The trend of serum estradiol in both the groups is dependable with the report of Batra et al., (1982), who reported similar trend of serum estradiol increased from one month prepartum and showed peak level at parturition, thereafter decreased at one day postpartum and remained at basal level until first postpartum estrus Corah et al., (1974) reported non-significant effect of energy level on plasma estrogen at transition period in cows. Similar finding was obtained by Khatti et al., (2017) with supplementation of high energy diet with Vitamin E and Se in the serum of crossbred cows.

Serum progesterone was significantly (P<0.05) high at 2 and 3 week prepartum in group I as compared to group II. Similarly group I had significantly (P<0.05) high level of progesterone from 4 to 7 weeks of postpartum than that of group II.

The pattern of serum progesterone in both the groups concurred with the reports of Smith et al., (1973), who reported a steady decreased in the progesterone till day 2 before calving with a sharp decline on the day of parturition (0.6 ng/mL) and basal level during the early postpartum period in the cow. Similarly Batra et al., (1982) also reported that the progesterone concentration declined gradually and an abrupt fall was observed 1-2 days before calving in buffaloes.

Similarly, Bahga and Ganwar (1988) reported that progesterone concentration remained at basal levels from day 5 to 30 postpartum. In support of present results, Kamada and Hodate (1998) found that Se supplementation increased plasma progesterone concentration by 22% as compared to control in the postpartum cows which was attributed to the antioxidant effects and decreased amount of lipid peroxides (LPO) in the cells Atilia et al., (2015). However, Khatti et al., (2017) reported no marked effect of high energy diet supplemented with Vitamin E and Se on serum progesterone concentration during periparturient period. Further Ganie et al., (2014) also observed that Se supplementation had no effect on serum profile of reproductive steroids in buffalo heifers (Fig. 2).
Fig. 1 Effect of dietary enrichment with copper and zinc along with additional energy on serum estradiol concentration in the transition cows

![Graph showing serum estradiol concentration](image1)

Fig. 2 Effect of dietary enrichment with copper and zinc along with additional energy on serum progesterone concentration in the transition cows

![Graph showing serum progesterone concentration](image2)

Table 1 Reproductive data of both the groups

| Reproductive variable          | Group I        | Group II       |
|-------------------------------|----------------|----------------|
| Service period (in days)      | 90.2±11.04     | 85.87±7.83     |
| Overall service per conception| 1.2            | 1.62           |
Effect of energy and antioxidants status on reproductive steroids showed inconsistent findings in previous studies and it might be because of multifactorial influence such as body condition, inheritance, nutrition as well as endocrine factors.

Reproductive data of both the groups has been depicted in table 1. Service period in group I cows were not differ statistically with group II cows (90.2 ±11.04 days Vs 85.87±7.83 days). Service per conception also recorded in group I and group II (1.2 Vs 1.62). It is evident from the results that dietary enrichment with copper and zinc along with additional energy improved the postpartum reproductive performance in treated group as compared to control.

High yielding dairy cows had NEBAL that causes impaired reproductive performance by predisposing the cow to anoestrus Butler (2005), reproductive failure Butler (2003).

Energy limitation decreases the pulse frequency of LH; as a consequence, dominant follicle fails to ovulate Butler (2003). Increasing the glucogenic nutrient availability improves the energy balance resulting in better reproductive performance in dairy cows Knegsel et al., (2007). Supplementation of vitamin E and Se in late gestation has shown to improve the fertility in the buffalo and cow Mavi et al., (2006); Khatti et al., (2017). Vitamin E and Zn supplementation significantly (P<0.05) decreased the days open and took lesser service per conception in cows De et al., (2014, Campbell et al., (1998). In the present study, a significant improvement in the reproductive indices could be attributed predominantly to the additional energy and antioxidants supplementation in the ration of crossbred cows, which has its implications in optimal transition cow management practices. However, the individual contribution of additional energy vis-à-vis Cu and Zn, supplementation on postpartum reproductive performance could not be elucidated.

Dietary enrichment with copper and zinc along with additional energy during transition period had significant effect on ovarian steroids followed by improved the postpartum fertility in terms of service period and service per conception in crossbred cows.

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