Original Research

Effect of High Energy Diet with Additional Supplementation of Copper, Zinc, Selenium and Vit. E during the Transition Period on Ovarian Steroids and Reproductive Performance in the Crossbred Cows

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Rec. Date:	Apr 14, 2018 06:25
Accept Date:	Jun 02, 2018 12:01
DOI	10.5455/ijlr.20180414062519

Abstract

The study was conducted with the objective to study the effect of high energy diet supplemented with copper (Cu), zinc (Zn), selenium (Se) and Vit E on ovarian steroids and reproductive performance during transition period in crossbred cattle. Periparturient crossbred cows (n= 20) between 2nd to 4th parity with lactation yield of >10 L/day were divided into two equal groups. Cows in Group I were given high energy diet in the form of 20% additional concentrate supplemented with Cu (15.7mg/kg DM), Zn (22 mg/kg DM) Se (0.3 mg/kg DM) and vit E (80 IU/kg DM). Whereas, Group II cows were fed basal diet kept as control. Blood samples were to determine estradiol (E_2) and progesterone (P_4) in the serum using RIA. Postpartum reproductive performance was assessed in terms of resumption of postpartum estrus, calving to conception interval, pregnancy rate and service per conception. Serum E_2 increased significantly from -1 to +8 weeks in Group I as compared to Group II. Further, serum P_4 level increased significantly from +1, +2 and +6 weeks in Group I in comparison with Group II. Marked improvement in the fertility was recorded in terms of resumption of postpartum estrus, reduced calving to conception interval, service per conception and higher pregnancy rate.

Key words: Estradiol, Progesterone, Periparturient Cow, Reproductive Performance

How to cite: Balachandran, B., Mehrotra, S., Veligatla, T., Bhimte, A., Muthu, R., Maurya, V., & Singh, G. (2018). Effect of High Energy Diet with Additional Supplementation of Copper, Zinc, Selenium and Vit. E during the Transition Period on Ovarian Steroids and Reproductive Performance in the Crossbred Cows. International Journal of Livestock Research, 8(10), 97-104. doi: 10.5455/ijlr.20180414062519

Introduction

Livestock play a major role in the agricultural sector in developing nations. Optimal health in the transition cow is the key to success of the subsequent lactation, optimum production and reproduction from today's modern dairy cows (Mulligan *et al.,* 2006). The transition period is defined as the transition from pregnancy to parturition and lactation, which extend from the last three or four weeks before to the first three or four weeks after parturition (Bell, 1995). Dairy cattle defense mechanisms are compromised during the transition period due to increased nutrient demands, oxidative stress and dysfunction of the immune response that result in increased incidence of infectious diseases and metabolic disorders especially during the first month of lactation (Abd Ellah *et al*., 2016; Sordillo *et al*., 2007; LeBlanc *et al*., 2006).Transition period in dairy cows is characterized by 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 postpartum (Butler, 2003). During the transition period requirement of oxygen level gets increased due to the increased metabolic demand resulting in production of reactive oxygen species (Sodillo, 2005) which affects physiological processes linked to reproduction (Agarwal and Allamaneni, 2004) and synthesis of reproductive steroids, because ovarian steroidogenic tissues are much sensitive to free radical damage (Carlson *et al.,* 1993). Further, a continuous depletion of endogenous antioxidant molecules and or deficiency during this period results in decreased availability of the endogenous antioxidant defenses to counter the increased oxidant produced leading to oxidative stress and finally impaired reproductive performance in the transition cows (Sodillo, 2005). Trace minerals such as Zn, Cu and Se are part of enzymes and some vitamins (Vit. E & Vit. C) which are key components of the antioxidant system (Weiss, 2009). 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, Zn and Se play an important role in dairy cow immune function (Balamurugan *et al.,* 2017), growth (Enjalbert *et al*., 2006) and fertility (Rabiee *et al*., 2010). Feeding of Zn, Cu and Se have improved the performance of dairy cattle through reducing days to first service and enhancing fertility rates (Rabiee *et al*., 2010) besides lowering the incidence of periparturient diseases (Defrain *et al.,* 2004). Endocrine balances are required to support normal cyclicity and gradual restoration of fertility postpartum (Erb *et al*., 1971), 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). Keeping in view the paucity of literature on examining the additive or possible synergistic actions of supplemented micro minerals with extra energy during periparturient period, the present investigation was designed to assess the effect of Cu, Zn, Se, Vit. E and extra energy on ovarian steroids and reproductive performance in crossbred cattle.

Materials and Methods

  DOI 10.5455/ijlr.20180414062519

Experimental Design

The experiment animals comprised of 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. 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, zinc sulphate, sodium selenite and Vit. E DL-α-tocopherol acetate (CDH, India) at a dose rate of 15.7mg/kg, 22 mg/kg, 0.3 mg/kg and 80 IU/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, Zn, Se and Vit. E 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 were collected by jugular venipuncture aseptically using 18-G needle in sterilized vacutainers (clot activators) at weekly interval from -4 to 8 weeks of calving. Schedule for supplementation and 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 800×g 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 occurrence of post partum estrus, calving to conception interval, pregnancy rate 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. Occurrence of postpartum estrus, service period and Service per conception by independent 't' test and pregnancy rate (%) was analyzed by Fisher's exact Chi-square test.

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

Results and Discussion

Though the concentration of serum estradiol was high in Group I-4 to -1 week before calving, a significant ($P<0.05$) increase was seen at calving and persisted till 8 week post calving (Fig. 1), which could be due to follicular activity during the early postpartum period. The trend of serum estradiol in both the groups is consistent with the report of Henricks *et al.* (1972), who reported that total estrogen increased from 14 days before calving and showed peak at calving in cows. Smith *et al.*, 1973 and Corah *et al.*, 1974, demonstrated that estradiol increased linearly 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 supplemented with Vit. E and Se in the serum of crossbred cows.

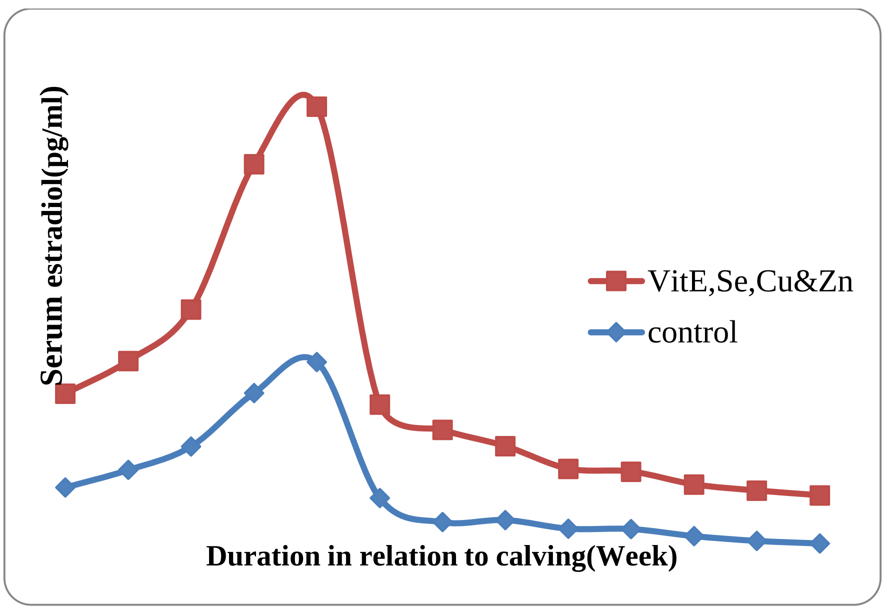

Fig. 1: Effect of high energy diet supplemented with Cu, Zn, Se and Vit. E on serum estrasiol concentration in the periparturient cows

Serum progesterone was significantly ($P<0.05$) high during the first six weeks of postpartum in group I as compared to group II. However, during the antepartum period, the level of serum progesterone showed non-significant difference between the groups (Fig. 2). 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

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early postpartum period in the cow. Similarly, Bahga and Ganwar (1988) reported that progesterone concentration remained at basal levels from day 5 to 30 postpartum.

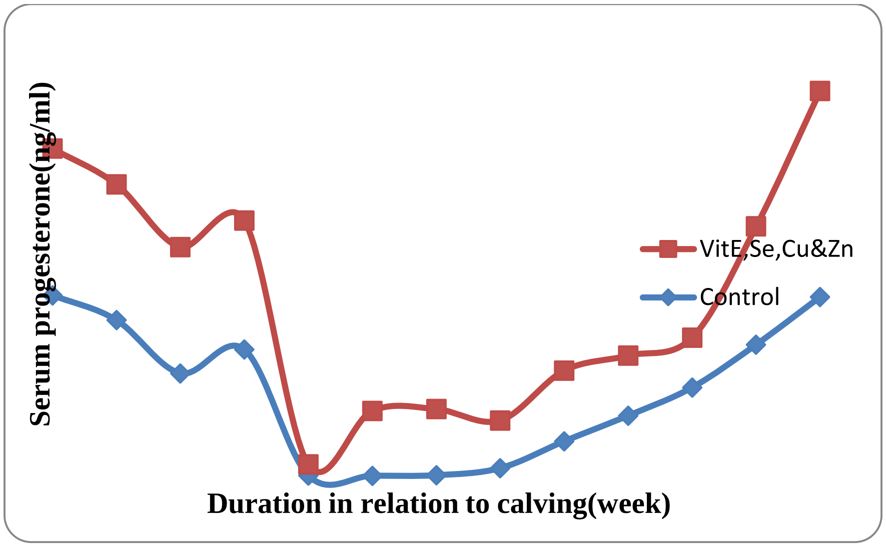

Fig. 2: Effect of high energy diet supplemented with Cu, Zn, Se and Vit. E on serum progesterone concentration in the periparturient cows

In support of our 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. Effect of antioxidants and energy 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. Occurrence of postpartum estrus was noticed significantly ($P<0.05$) earlier as compared to control animals (53.8 days Vs 67.0 days). Similarly service period in group I cows was significantly ($P<0.05$) shorter than that of control (68.5 daysVs85.87 days). Pregnancy rate was 100% in treated animals as compared to 61.53% in control, which also differ significantly ($P<0.001$). Accordingly service per conception also recorded significantly ($P<0.05$) low in group I animals. It is evident from the results that high energy supplemented with trace minerals and Vit. E significantly 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 anesrus (Butler, 2005), reproductive failure (Butler, 2003). Energy limitation decreases the pulse

 DOI 10.5455/ijlr.20180414062519

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 (Van Knegsel *et al.,* 2007).

Table 1: Effect of high energy diet supplementation with Cu, Zn, Se and Vit. E on Postpartum reproductive variables

Reproductive Variable	Group I	Group II	P Value
Occurrence of Post partum estrus (Day)	53.8±9.23	67±11.93	(P<0.05)
Service period (in days)	68.5±5.54	85.87±13.01	(P<0.05)
Service per conception	1	1.62	(P<0.05)
Pregnancy rate (%)	100	61.53	(P<0.001)

Supplementation of Vit. 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). 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, Zn, Se and Vit. E supplementation on postpartum reproductive performance could not be elucidated.

Conclusion

Supplementing diet with high energy along with trace minerals (Cu, Zn, Se) and Vit. E cows during transition period in crossbred cows significantly improved the postpartum fertility in terms of occurrence of postpartum estrus, service period, pregnancy rate and service per conception

Acknowledgments

The study was supported by ICAR-IVRI vide project # IVRI/P&C/15-18/006 which was granted to Dr.Vijai Prakash Maurya. The authors thank the Director, IVRI for funding support. The authors acknowledge Mr. M. C. Pathak, Dr. K. Narayanan, Dr. Himani, Dr. Gopi and farm employees at LPM section, IVRI for their meticulous support.

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 DOI 10.5455/ijlr.20180414062519