Haryana Vet. (April, 2021) **60(SI)**, 51-54

Research Article

EFFECT OF DIETARY ENRICHMENT WITH HIGH ENERGY DIET AND ADDITIONAL SUPPLEMENTATION OF TRACE MINERALS, VITAMIN DURING THE PERIPARTURIENT PERIOD IN CROSSBRED COWS

B. BALAMURUGAN*, S. MEHROTRA[1], T. VELIGATLA[2], M. RAMAMOORTHY[1], M. GOPI[3], V.P. MAURYA[2], G. SINGH[2] and K. NARAYANAN[1]
Department of Veterinary Gynaecology and Obstetrics, BHU-Faculty of Veterinary and Animal Science, RGSC, Barkachha, Mirzapur-231001, India
[1]Division of Animal Reproduction, [2]Division of Physiology and Climatology,
[3]Division of Avian Physiology and Reproduction, ICAR-Indian Veterinary Research Institute, Bareilly-243122, India

Received: 22.06.2020; Accepted: 18.07.2020

ABSTRACT

The study was to asses effect of 20% additional concentrate in diet alone or supplemented with copper (Cu), zinc (Zn), selenium (Se) and vitamin E (Vit. E) during transition period on immunity and reproductive performance in crossbred cows. Crossbred cows (n= 30) of 2-4 parity in advance pregnancy in last trimester and lactation yield of more than 10 L/day were divided randomly into three equal groups. In Group I, the cows were fed diet supplemented with Cu, Zn, Se, Vit. E and 20% additional concentrate. Group II cows were provided 20% additional concentrate only. Group III (control) cows were fed with only basal diet without supplementation of minerals, vitamin and extra concentrate. Blood samples were collected at weekly interval from 4 weeks pre to 8 weeks after calving. Immunity was assessed by estimating phagocytic activity (PA) and lymphocyte proliferation assay (LPA). Results indicated increased phagocytic activity from 3 to 8 week postpartum ($P < 0.05$) while LPA showed difference ($P < 0.05$) at 0, +3 and +6 to +8 weeks in group I than that of group III. Further, Group II showed significantly ($P<0.05$) higher LPA value from 3 to 8 weeks postpartum as compared to Group I and III. Group I and II Cows had a significantly ($P<0.05$) higher PA of granulocytes and LPA as compared to group III. Occurrence of postpartum estrus was shorter in group II ($P<0.05$) with higher conception rate ($P<0.05$).

Keywords: Immunity, Periparturient cows, Reproductive performance, Trace minerals, Vitamins

In the modern dairy herds, high yielding dairy cows experience physiological, metabolic and oxidative stress during their periparturient period. During this period, there will be a transition from the demands of fetal growth, parturition, lactation subsequent to different reproductive events. The periparturient period is characterized by vibrant changes in the loss of appetite, negative energy balance, change in hormone profiles (Balamurugan *et al.*, 2018a), micronutrient deficiencies (Bhimte *et al.*, 2018), immune compromisation and oxidative stress (Khatti *et al.*, 2017). These factors can disrupt homeostasis not only in bodily organs, but also at the tissue, cellular and molecular level. This contributes to diseases that commonly affect cows during the periparturient period, including delayed uterine involution, retained placenta, metritis, mastitis and ketosis to decreased reproductive performance (Sobiech *et al.*, 2015; Balamurugan *et al.*, 2018b). Stresses to cows during periparturient period affects the immunity and make the animals more vulnerable to infectious diseases (Maurya, 2011). It may be partly due to a decrease in plasma concentrations of antioxidant micronutrients such as Copper (Cu), Selenium (Se), Zinc (Zn) and vitamin E observed at this time (Balamurugan *et al.*, 2017). Altered populations and function of mononuclear leukocytes have been observed in cows during the periparturient period (Aleri *et al.*, 2016).

Various nutritional interventions are used in periparturient dairy animals as an additional rations to minimize stress due to gestation, calving and early lactation. Vitamin E and vitamin A have been shown to enhance lymphocytic activity (De *et al.*, 2015). Zn and Cu are the component of superoxide dismutase (SOD) which scavenges superoxide, one of the components of ROS in the immune cells. During periparturient period, the protective effects of vitamin E and Se on animal health may be due to their role in the reduction of plasma cortisol level (Dimri *et al.*, 2010). Dairy cows require Zn, Cu and Se to maintain antioxidant activity of their immune system. Considering these facts, the present study was designed to investigate the effect of 20% extra concentrate diet with additional supplementation of Vitamin E, Cu, Zn and Se on immunity and reproductive indices in periparturient crossbred cows under Indian conditions.

MATERIALS AND METHODS

Experimental animals: Study was conducted in 30 apparently healthy pregnant crossbred cows (Hariana × Holstein Friesian/Brown Swiss/Jersey) in their advance pregnancy (near to term of pregnancy) maintained at cattle and buffalo farm of Livestock Production and Management Section, Indian Veterinary Research Institute, Izatnagar. These experimental animals were selected on the basis of parity (second to fourth) and milk yield (>10L/day) and maintained under iso-managerial condition.

Experimental design: The experimental animals were divided into three groups (n= 10 cows/group), Group I cows were fed 20% additional concentrate supplemented with Cu (copper sulphate, CDH, India), Zn (zinc sulphate,

*Corresponding author: balavet07@gmail.com

CDH, India), Se (Sodium Selenite, CDH, India) and Vit E (DL-α-tocopherol acetate CDH, India) orally at the dose rate of 15.7 mg/ kg DM, 22 mg/ kg DM, 0.3 mg/ kg DM, and 80 IU/kg DM, respectively from four week before to eight weeks of calving (where 0 is day of anticipated date of calving). Group II cows were provided 20% additional concentrate only. Group III cows were fed with only basal diet without any supplementation which served as control. Cu, Zn, Se and Vitamin 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 veni puncture aseptically using 18-G needle in sterilized vacutainers (clot activators) at weekly interval from -4 to 8 weeks of calving. Heparinized vacutainer was used for isolation of peripheral blood mononuclear cells (PBMC) and granulocytes.

Estimation of immunity indices : Phagocytic activity (PA) and lymphocyte proliferation assay (LPA) were done according to Dang *et al.* (2013) with some modifications. Granulocytes were isolated by GranulosepTM GSM-1119 (Hisep, Himedia, India) and lymphocyte separation media (LSM) Histopaque-1077(MP Biomedicals, India). After separation of cells (centrifugation 1000 × g for 30 min), cells were washed with cold phosphate buffer saline and suspended in RPMI-1640 media supplemented with 10% foetal calf serum. Then, diluted cell suspension (200µL per well) in triplicate was placed in a 96-well flat-bottomed tissue culture plate. The cells were allowed to proliferate with zymosan @ 650 µg/mL (MP Biomedicals, India) and nitroblue tetrazolium @ 250 µg/mL (NBT, MP Biomedicals, India) added to the cell suspension and incubated at 38.5 °C in a humidified CO_2 incubator (95% air and 5% CO_2) for 4 h. The colour change was measured at 540 nm using ELISA reader (Thermo Scientific, Multiskan Go, USA).

PBMC was isolated with the help of Histopaque-1077 (MP Biomedicals, India) through centrifugation (3000 × g for 30 min); then cells were washed with cold phosphate buffer saline (PBS, MP Biomedicals, India). Thereafter, cells were treated with concanavalin A (50 µg/mL) in RPMI-1640 media supplemented with 10% foetal calf serum in 38.5 °C; 5% CO_2 for 48–72 h. Formazan crystals were made by adding MTT (Methyl thiazolyldiphenyl terazolium bromide, Biomedicals, India) dye and the absorbance was measured at 503 and 630 nm in an ELISA reader after 4 hrs of incubation.

Measurement of postpartum reproductive performance: Oestrus detection was done twice a day by using trained teaser bull. The experimental cows exhibiting estrus were inseminated using frozen semen. All the non-return cows were subjected to pregnancy diagnosis by transrectal palpation at 60 to 65 days of post AI. First postpartum estrus and pregnancy rate were recorded.

Statistical analysis: Time serious data for variables such as phagocytic activity and lymphocyte proliferation activity were analysed by using GLM repeat measure ANOVA. Kaplan-Meier survival analysis was used to see the interval from calving to first postpartum estrus. Fisher's exact chi-square test was done to determine the difference between treatment and conception. Metric variables are presented as Mean±SE. Significance was set at 95%. Statistical analysis was done with SPSS software version 16.0.

RESULTS AND DISCUSSION

Immune function

Phagocytic activity: The trend of phagocytic activity was comparable in all the groups during prepartum period (Fig. 1). However, group I and II had significantly ($P<0.05$) higher phagocytic activity from 3 to 8 weeks postpartum than that of group III. Further group I had significantly ($P<0.05$) higher phagocytic activity (PA) of granulocytes from 3 to 8 weeks postpartum than that of group II. Phagocytic activity increased significantly on the day of calving and decreased during the first two weeks of calving in group I and II as compared to group III; thereafter, it increased till 8 weeks postpartum. This observation is in accordance with Khatti *et al.* (2017) who reported the more phagocytic activity following supplementation of high energy diet with Vit E and Se in the crossbred cow. Similar trend was observed following supplementation of high energy diet with Cu and Zn in the crossbred dairy cows (Balamurugan *et al.*, 2019). De *et al.*

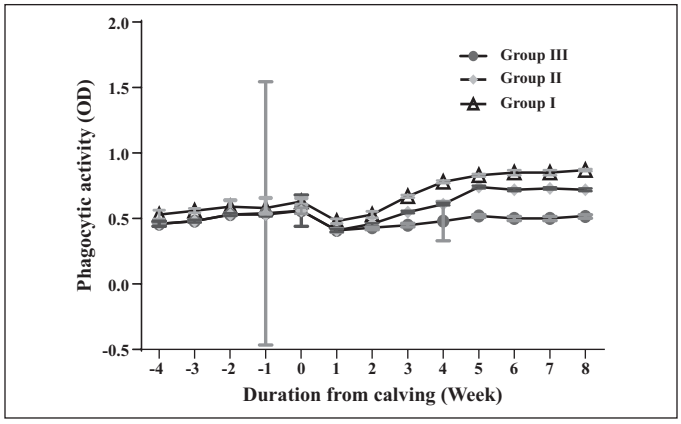

Fig. 1. Effect of extra diet with supplementation of trace minerals, vitamin on phagocytic activity (PA) during the periparturient period in the crossbred cows.

(2015) reported a positive effect of Cu and Zn on phagocytosis in periparturient crossbred cows. Dang *et al.* (2013) reported suppression in the PA of neutrophils on day 3 of postpartum that was overcome after two weeks of postpartum in the Sahiwal cows supplemented with Cu, Zn and Vit E. In contrast, subcutaneous injection of Zn, Se, Mn and Cu administered on day 230 and 260 of gestation as well as day 35 postpartum did not improve the phagocytic function of PMN cells; but decreased the NEB (Machado *et al.*, 2014). The reason behind the decrease in the leukocytic function during first two weeks of postpartum in group II could not be explained convincingly. A significantly high PA on the day of parturition might be due to some unknown neuroendocrine changes and down regulation of glucocorticoid receptors (De *et al*., 2015).

Lymphocyte proliferation assay: The mean stimulation index of lymphocytes was significantly (P<0.05) higher at 0, +3 and +6 to +8 weeks in group I as compared to group III (Fig. 2). Further, Group II showed significantly (P<0.05) higher LPA value from +3 to +8 weeks postpartum than that of group III. LPA value increased significantly (P<0.05) on the day of calving and decreased during the first two weeks of calving in group I and II as compared to group III; thereafter, they increased till 8 week postpartum. Similar trend was observed following supplementation of high energy diet with Vit. E and Se in the periparturient crossbred cow (Khatti *et al.*, 2017). Supplementation of Cu and Zn during the transition period to the basal diet improved the functions of lymphocytes in the crossbred dairy cows (De *et al.*, 2015). The reason behind the decrease in the leukocytic function during first two weeks of postpartum in group I could not be explained convincingly. However, a lower leukocytic function during the early postpartum is consistent with the hypothesis of weakened immunity due to oxidative and lactational stress (De *et al.*, 2015).

The effect of additional concentrate in diet with supplementation of Cu, Zn, Se and Vitamin E, during the periparturient on variables of reproductive performance in crossbred cows is presented in Table 1. The mean interval from calving to occurrence of first postpartum oestrus was shorter by 21.1 and 14 days in group I and group II, respectively than that of group III cows. A log rank test revealed a statistically significant (P<0.05) difference in the occurrence of postpartum estrus (Fig. 3). Similarly, conception rate was increased by 38.47% and 16.24% in group I and II, respectively as compared to group III (Table 1). A significant improvement in the conception rate in group I and II could be attributed chiefly to the additional energy to overcome the NEBL that decreases the LH pulse

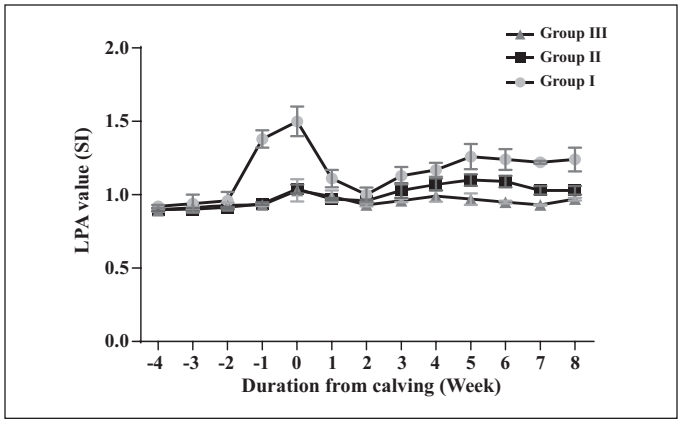

Fig. 2. Effect of 20% additional concentrate with supplementation of trace minerals, vitamin on lymphocyte proliferation assay (LPA) during the periparturient period in the crossbred cows.

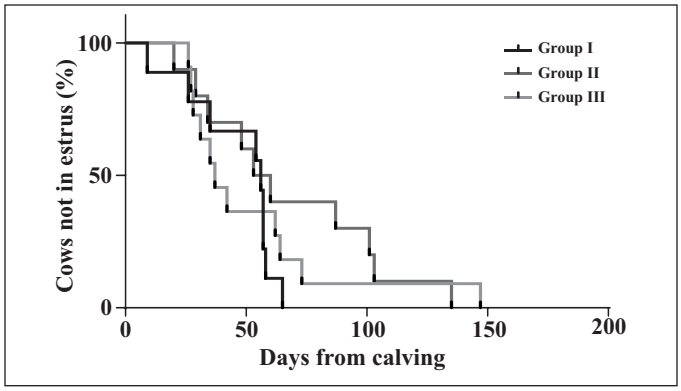

Fig. 3. Kaplan-Meier survival analysis showing the occurrence of first postpartum oestrus (%) over a period of 150 days from calving in the crossbred cow

Table 1

Effect of 20% additional concentrate in diet with supplementation of trace minerals, vitamin on the postpartum reproductive variables in the crossbred cows

Variable	Group I	Group II	Group III
Interval from calving to occurrence of first postpartum estrus (day)	45.9[c]	53.0[b]	67.0[a]
Overall Conception rate (%)	100[a]	77.77[b]	61.53[c]

frequency resulting in acyclicity (Balamurugan *et al.*, 2019). Increasing the dietary energy during prepartum period resulted in early ovulation during postpartum and was attributed to an increase in the plasma insulin, IGF-1 and leptin (Cavestany *et al.*, 2009). In the present study, a significant improvement in the reproductive indices could be attributed predominantly to the additional energy, trace minerals and vitamin supplementation in the ration of crossbred cows, which has its implications in optimal transition cow management practices. However, the individual contribution of additional energy in comparison with Cu, Zn, Se and Vit. E supplementation on postpartum reproductive performance could not be elucidated.

CONCLUSION

Supplementing diet with 20 % additional concentrate along with trace minerals (Cu, Zn and Se) and vitamin (Vit. E) cows during periparturient period in crossbred cows significantly improved the immunity level and postpartum reproductive performance as occurrence of postpartum estrus and conception rate.

ACKNOWLEDGEMENT

The authors thank The Director, Indian Veterinary Research Institute (ICAR-IVRI) for facilitating the work.

REFERENCES

Aleri, J., Hine, B., Pyman, M., Mansell, P., Wales, W., Mallard, B. and Fisher, A. (2016). Assessing adaptive immune response phenotypes in Australian Holstein-Friesian heifers in a pasture-based production system. *J. Anim. Sci.* **93**: 3713 -3721.

Balamurugan, B., Mehrotra, S., Tejaswi, V., Bhimte, A., Ramamoorthy, M., Maurya, V.P. and Singh, G. (2018a). 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. *Int. J. Liv. Res*. **8**: 97-104.

Balamurugan, B., Mehrotra, S., Bhimte, A., Ramamoorthy, M., Khatti, A., Maurya, V.P., Singh, G. and Pathak, M.C. (2018b). 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. *Int. J. Curr. Microbiol. App. Sci*. **7**: 654-660.

Balamurugan, B., Ramamoorthy, M., Mandal, R.S.K., Keerthana, J., Gopalakrishnan, G., Kavya, K., Kharayat, N., Chaudhary, G.R. and Katiyar, R. (2017). Mineral an important nutrient for efficient reproductive health in dairy cattle. *Int. J. Environ. Sci. Technol*. **6**: 694-701.

Balamurugan, B., Mehrotra, S., Veligatla, T., Jena, D., Ramamoorthy, M., Gopi, M., Maurya, V.P., Singh, G., Kumar, H. and Narayanan, K. (2019). Effect of high-energy diet supplemented with additional copper and zinc during the transition period on oxidative stress and postpartum reproductive performance in the crossbred cows. *Biol. Rhy. Res*. **50(6)**: doi:10.1080/09291016.2019.1628399.

Bhimte, A., Singh, L.K., Balamurugan, B., Lakhani, N., Maurya, V.P., Bharti. M.K., Kumar, A., Singh, G. and Sarkar., M. (2018). Amelioration of transition stress by supplementation of antioxidant (Vitamin E), trace minerals (Selenium, Copper, Zinc) with increased energy allowance in crossbred cows. *J. Pharmacog. Phytochem.* **6**: 1449-1454.

Cavestany, D., Kulcsár, M., Crespi, D., Chilliard, Y., La Manna, A., Balogh, O., Keresztes, M., Delavaud, C., Huszenicza, G. and Meikle, A. (2009). Effect of prepartum energetic supplementation on productive and reproductive characteristics, and metabolic and hormonal profiles in dairy cows under grazing conditions. *Reprod. Domest. Anim.* **44**: 663-671.

Dang, A.K., Prasad, S., De, K., Pal, S., Mukherjee, J. and Sandeep, I.V.R. (2013). Effect of supplementation of vitamin E, copper and zinc on the *in vitro* phagocytic activity and lymphocyte proliferation index of peripartum Sahiwal (*Bos indicus*) cows. *J. Anim. Physiol. Anim. Nutr*. **97**: 315-321.

De, K., Pal, S., Mukherjee, J., Prasad, S. and Dang, A.K. (2015). Effect of in vitro copper and zinc supplementation on neutrophil phagocytic activity and lymphocytic proliferation response of transition dairy cows. *Agri Res*. **4**: 388-395.

Dimri, U., Ranjan, R., Sharma, M.C. and Varshney, V.P. (2010). Effect of vitamin E and selenium supplementation on oxidative stress indices and cortisol level in blood in water buffaloes during pregnancy and early postpartum period. *Trop. Anim. Health Prod.* **42**: 405-410.

Khatti, A., Mehrota, S., Patel, P.K., Singh, G., Maurya, V.P., Mehla, A.S., Chaudhari, R.K., Das, G.K., Singh, M. and Sarkar, M. (2017). Supplementation of Vitamin E, Selenium and increased energy allowance mitigates the transition stress and improves postpartum reproductive performance in the crossbred cow. *Theriogenology*. **104**: 142-148.

Machado, V.S., Oikonomou, G., Lima, S.F., Bicalho, M.L.S., Kacar, C., Foditch, C., Felippe, M.J., Gilbert, R.O. and Bicalho, R.C. (2014). The effect of injectable traces minerals (Selenium, Copper, Zinc and Manganese) on peripheral leukocyte activity and serum superoxide dismutase activity of lactating Holstein cows. *Vet. J*. **200**: 299-304.

Maurya, P. (2011). Leptin level in relation to immunity, energy metabolites and cellular adaptations during dry period and early lactation in crossbred cows. MVSc thesis submitted to NDRI, Karnal, India.

Sobiech, P., Rypu, K., Kotwica, B.W. and Michalski, S. (2015). Usefulness of calcium-magnesium products in parturient paresis in HF cows. *J. Elementol*. **15**: 693-704.