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Effect of immunomodulatory diet on haematological indices in crossbred cows during transition period at Southern districts of Tamil Nadu

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Abstract
This study was conducted in 36 multiparous Cross Bred Jersey cows at Tirunelveli and Thoothukudi districts of Tamil Nadu state in India from 4 weeks prepartum to 4 weeks postpartum with the aim to evaluate the effect of an immunomodulatory diet on haematological parameters. Animals were randomly divided into three groups on the basis of feeding viz. Group I (n=12), as control, Group II (n=12), was fed with conventional balanced feed and Group III (n=12), was fed with an immunomodulatory diet with flax seed and the inclusion of organic minerals and supplements. Blood was collected on day -28, -21, -14, -7 prepartum; day 0 parturition; day 7, 14, 21 and 28 postpartum and subjected to haematological analysis and Hb (g/dl), PCV (%), TEC (10^6/µl), TLC (10^3/µl) and Platelets (x/µl) were estimated. This study revealed that Hb (g/dl) PCV (%), TEC (10^6/µl), TLC (10^3/µl) during prepartum, parturition and postpartum periods between day -28 prepartum to 28 postpartum in Group III was significantly higher ($p<0.05$) than Group II and Group I and Platelets count (x/µl) was significantly higher in Group III only during parturition than Group II and Group I.

Keywords: PUFA, haematological parameters, immunomodulation, postpartum, chelated mineral, transition period, mixture, flaxseed

Introduction

The last three weeks of pregnancy are characterized by the rapid development of foetus, synthesis of colostrum, development of mammary gland and metabolic adjustments favouring these physiological changes along with reduced dry matter and energy intake. The first three weeks post calving are characterized by onset of lactogenesis and increased nutrient loss through milk (Lean *et al.*, 2013) [19].

During the transition period, a critical change occurs in haemogram as dairy cows undergo drastic and tremendous metabolic adaptations from late gestation to early lactation postpartum. These changes are exquisitely controlled by hormonal changes to support the sudden changes in physiological state of lactation (Grummer, 1995) [20].

In recent years, dairy cow research has been more focused on roles of polyunsaturated fatty acids (PUFAs) in physiological processes such as cellular membrane integrity, lipid metabolism, energy partitioning, hormonal pathways, oxidative stress, inflammatory pathways and immune response (Moallem 2018; Bionaz *et al.*, 2020) [18, 2].

However, due to high rumen hydrogenation of the PUFA C18:2 n-6 (70%) and C18:3 n-3 (85%), makes oral supplementation of PUFA-rich fats an inefficient mechanism to enhance the PUFA concentrations of bovine milk (Juchem, 2007) [16]. Further, PUFA which escapes rumen fermentation without being hydrogenated and absorbed post-ruminally, can enhance the general health and immunity of dairy cows (Juchem *et al.*, 2010) [15].

Somkuwar *et al.* (2011) [14] reported that organic minerals are indicated during periparturient period during stress (calving transport, dramatic changes) in dairy cattle. Kantwa *et al.* (2022) [13] concluded that the supplementation of the chelated mineral mixture to the lactating buffaloes increases the milk yield, improves reproductive performance, cost of milk production and consequently improves economic conditions of livestock farmers.

Hence this study aimed to study the effect of immunomodulatory feed rich in flaxseed and chelated mineral mixture on the haematological parameters during transition period in dairy cows.

Materials and Methods
Location and Ethics
The study was conducted at the villages of Tirunelveli (8.7139° N, 77.7567° E) and Thoothukudi (8.7642° N, 78.1348° E) districts and Veterinary College and Research Institute, Tirunelveli (8.7288° N, 77.7061° E) of Tamil Nadu, India during the period from January 2022 to August 2023 under All India Coordinated Research Project (AICRP-Sc. No. 24014).

In the present study, ethics were followed to reduce animal suffering and adherence to best veterinary practices has been followed as per the permission accorded to the 50th Institutional Animal Ethics Committee (Tamil Nadu Veterinary and Animal Sciences University) with approval no. 02/LA/IAEC/2022.

Animals, Feeding, and Management
Apparently healthy, pregnant, multiparous Jersey crossbred cows weighed 348±28 kg (300-375 kg) maintained in a semi-intensive farming system were utilized for this study. Thirty-six number of eight months pregnant Jersey crossbred cows were selected based on per rectal examination.

After screening and selection, the crossbred cows were randomly allotted into three groups viz. Group I, II and III each comprises of 12 crossbred cows

Group I cows were fed with locally available feed (Control). Group II and Group III cows were fed with 3 kg of conventional balanced feed (T_1) and Immunomodulatory feed (T_2) enriched with flax seed and chelated mineral mixture, respectively (Table 1) from day 28 peri partum to day of calving (-28 to Day 0). After calving, 2 kg of feed up to 5 litre of milk yield and an additional 500 gms were fed per one litre of milk over and above 5 litre of milk yield up to day 45 post-partum (day 0 to 45 post-partum). The conventional and immunomodulatory feeds were procured from the Department of Animal Nutrition, Veterinary College and Research Institute, Tirunelveli as per the requirement

Apparently healthy, eight months pregnant, thirty-six number of multiparous Jersey Cross Bred cows weighed 348±28 kg maintained in a semi-extensive farming system were randomly divided into three groups viz. Group I (C) was control animals fed by the feed available locally by farmers, Group II and III were fed with 60% (3 kg) conventional balanced feed (T_1) and immunomodulatory feed with flax seed and the inclusion of organic minerals and supplements (T_2) (Table 1) respectively, prepared at Department of Animal Nutrition, Veterinary College and Research Institute, Tirunelveli (TANUVAS) and 40% of roughages and green fodder. After calving Group II and III were fed with an additional 500gm of feed per litre of milk yield over and above 5 litre up to day 45 post-partum.

Experiment Procedure
The blood samples were collected at fortnightly intervals from four weeks before the predicted calving date until four weeks after calving (day -28, -21, -14, -7, 0, 7, 14, 21 and 28, considering day 0 as the day of parturition). Approximately, 3 ml of blood was collected aseptically by jugular venipuncture from each crossbred cow, transferred to vacutainer with anticoagulant was utilized for haematological analysis.

The haematological parameters including Total Erythrocyte Count (TEC), Packed Cell Volume (PCV), Haemoglobin (Hb), Total Leukocyte Count (TLC) and Platelet Count (PLT) were evaluated from the collected blood in an EDTA vial using automatic haematology analyser (Mindray, BC-2800).

Table 1: Constituents and composition of the diets fed (each Kg) to cows during the trial

Feed Ingredients	T_1 (gm)	T_2 (gm)
Maize	230	230
Broken Rice	100	100
GNC	110	110
Coconut oil cake	120	0
Flax Seed	0	120
Sunflower oil cake	120	120
De Oiled Rice Bran	200	200
Wheat Bran	80	80
Calcite	28	27
Chelated Mineral Mixture*	0	2
Ultra TM	1	0
Salt	10	10
Soda bicarbonate	1	1
CP %	19.2	19.2
Ether Extract %	1.42	5.26
TDN %	73	74
Ca %	1.17	1.15
P %	0.71	0.72

*Chelated Mineral Mixture (Nutritional Value per Kg): Vitamin A: 7,00,000 IU, Vitamin D3: 70,000IU, Vitamin E: 250mg, Cobalt: 150 mg, Copper: 1200 mg, Iodine: 325 mg, Iron: 1500 mg, Magnesium: 6000 mg, Potassium:100 mg, Sodium: 5.9 mg, Manganese: 1500 mg, Sulphur: 0.72%, Zinc: 9600 mg, DL Methionine: 1000 mg, Calcium: 25.5% and Phosphorus : 12.75%.

Statistical Analysis
All the data were analysed by using the Software SPSS version 22.

Results and Discussion
Haemoglobin
The Hb concentration (g/dl) in Group I (control) crossbred cows was significantly ($p<0.05$) decreased from day -28 prepartum to day 0 parturition followed by a significant ($p<0.05$) increase up to day 28 postpartum. Group II (T_1) crossbred cows, a significant increase ($p<0.05$) in Hb concentration from day -28 to -7 prepartum followed by a significant ($p<0.05$) decrease on day 0 parturition and a non-significant increase up to day 28 postpartum. Group III (T_2) crossbred cows, Hb concentration was increased significantly ($p<0.05$) from day -28 to -7 prepartum followed by a significant ($p<0.05$) decrease on day 0 parturition and significant ($p<0.05$) increase up to day 28 postpartum (Figure 1). The present findings are in agreement with Kour *et al.* (2023) [12] and Gavan *et al.* (2010) [11] who reported that mean Hb concentration was significantly ($p<0.05$) decreased at pre-calving to calving and increased significantly ($p<0.05$) at post calving in Holstein cows.

The Hb concentration (g/dl) during prepartum, parturition and postpartum periods between day -21 prepartum to +28 postpartum were significantly ($p<0.05$) higher in Group III (T_2) than Group II (T_1) and Group I (control) crossbred cows and Group II (T_1) cows had significantly ($p<0.05$) higher Hb concentration (g/dl) than Group I (control) crossbred cows. However, no significant difference was observed on day -28 prepartum between all the groups.

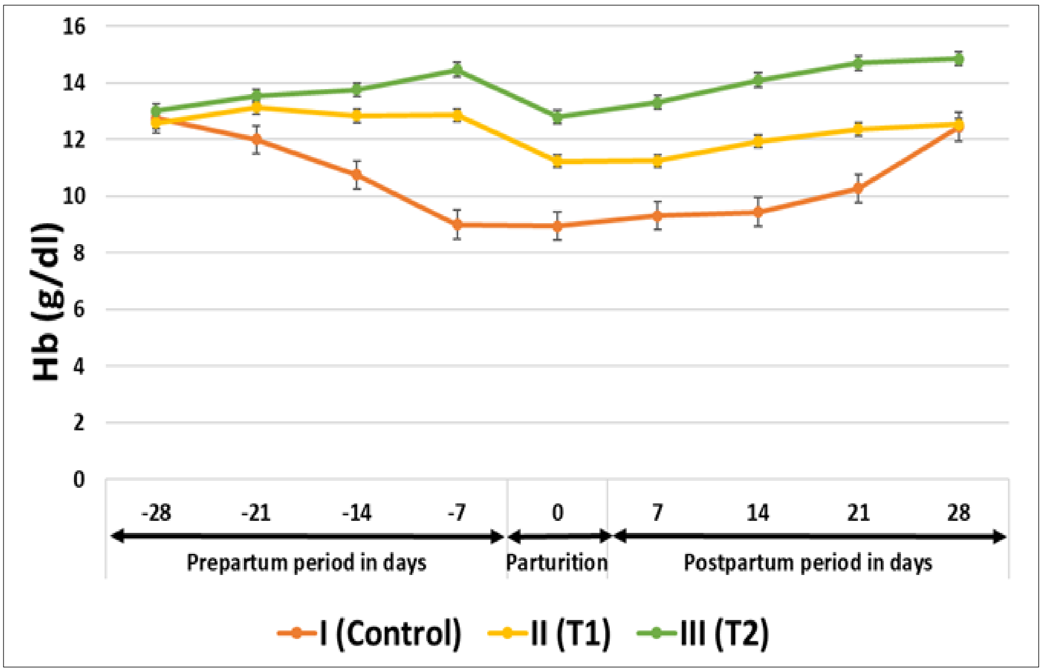

Fig 1: Mean (± SE) haemoglobin in unorganized (Control), conventional (T_1) and immunomodulatory (T_2) feeding groups of transient crossbred cows

This decreased concentration of Hb from the prepartum to parturition might be due to the dilution of blood attributed by the increase of plasma volume and the increased Hb concentration from parturition to postpartum might be due to an increased rate of erythropoiesis for increased Hb requirement of mammary glands for milk synthesis and the concomitant rise in blood flow to mammary glands and parenchyma (Kumar and Pachauri, 2000) [17].

The increase in Hb concentration by feeding flaxseed might be due to the stimulation of immune processes by adding fatty acids reducing susceptibility to the development of inflammatory processes during transition period, which in turn increases erythropoiesis (El-Diahy *et al.* 2016) [6] and chelated minerals mixture increases the availability of Fe and Cu which boosts haemoglobin production (Ahirwar *et al.* 2023) [8].

Total Erythrocyte Count (TEC)

The TEC (10^6/µl) in Group I (control) crossbred cows was decreased significantly (p<0.05) from day -28 prepartum up to day 0 parturition followed by a significant (p<0.05) increase up to day 28 postpartum. Group II (T_1) crossbred cows, TEC (10^6/µl) significantly decreased (p<0.05) from day -28 prepartum to day 0 parturition and a significant (p<0.05) increase up to day 28 postpartum. Group III (T_2) crossbred cows, TEC (10^6/µl) was significantly increased (p<0.05) from day -28 to -7 prepartum followed by a significant decrease (p<0.05) on day 0 parturition and significant increase (p<0.05) up to day 28 postpartum (Figure 2). The present findings are in agreement with Kour *et al.* (2023) [12] and Gavan *et al.* (2010) [11] who reported that mean TEC was significantly (p<0.05) decreased at pre-calving to calving and increased significantly (p<0.05) at post calving in Holstein cows.

The TEC (10^6/µl) during prepartum, parturition and postpartum periods between day -21 prepartum to +28 postpartum were significantly (p<0.05) higher in Group III (T_2) crossbred cows than Group II (T_1) and Group I (control) crossbred cows and Group II (T_1) had significantly (p<0.05) higher TEC (10^6/µl) than Group I (control) crossbred cows. However, no significant difference was observed on day -28 prepartum between all the groups.

The decreased TEC during prepartum might be due to due to deficiency of iron and increased cortisol and estrogen level during parturition resulting in immunosuppression which leads to inhibition of erythropoiesis in bone marrow (Gavan *et al.*, 2010; Elshahawy and Abdullaziz, 2017) [11, 3]. However, the elevation of TEC during postpartum period might be due to erythrocytosis at the time of uterine involution and haemoconcentration because of the reduction in water intake, increased milk production and spleen contraction (Van Soest and Blosser, 1954; Wiss and Wardrop, 2010) [9, 10].

The increase in TEC by feeding flaxseed might be due to the stimulation of immune and anti-oxidant responses which increases erythropoiesis and slows down the destruction of erythrocytes (El-Diahy *et al.* 2016) and chelated minerals mixture increases the availability of Fe and Cu which enhances the uptake of folic acid and stimulates the formation of RBCs (Ahirwar *et al.* 2023) [8].

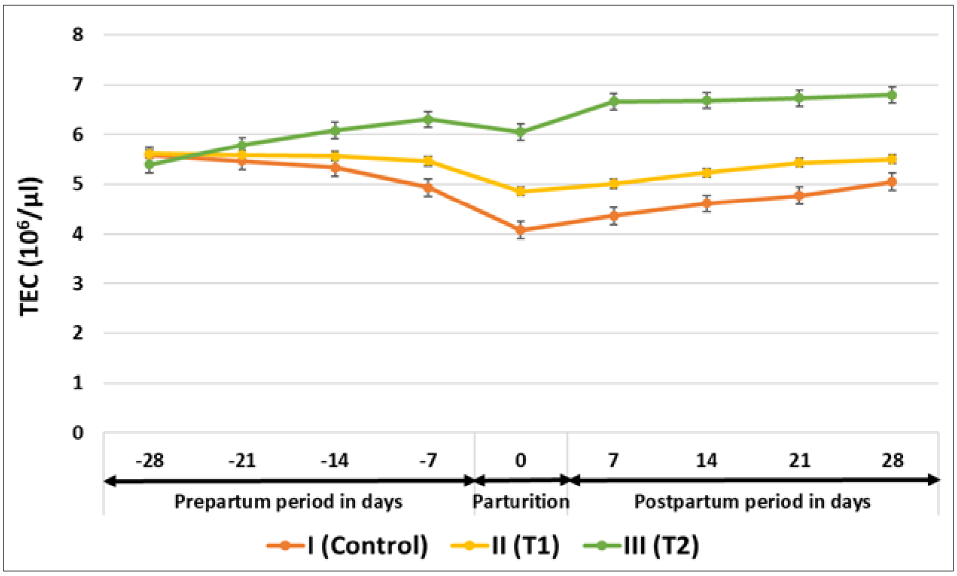

Fig 2: Mean (± SE) TEC in unorganized (Control), conventional (T_1) and immunomodulatory (T_2) feeding groups of transient crossbred cows

Total Leucocyte Count (TLC)

The TLC (10^3/µl) in Group I (control) crossbred cows was significantly decreased (p<0.05) from day -28 prepartum up to day 0 parturition followed by a significant (p<0.05) increase up to day 28 postpartum. Group II (T_1) crossbred cows, a significant increase (p<0.05) in TLC (10^3/µl) from day -28 to -21 prepartum and decreased significantly (p<0.05) up to day 0 parturition and a significant (p<0.05) increase up to day 28 postpartum. Group III (T_2) crossbred cows, TLC (10^3/µl) was significantly increased (p<0.05) from day -28 prepartum to day 0 parturition followed by a no significant difference up to day 28 postpartum (Figure 3).

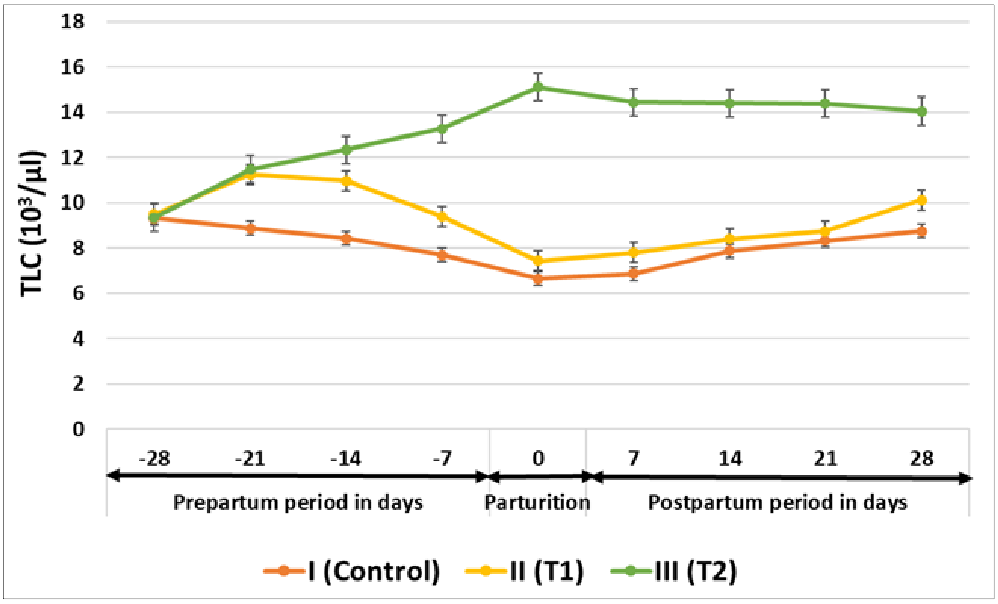

Fig 3: Mean (± SE) TLC in unorganized (Control), conventional (T_1) and immunomodulatory (T_2) feeding groups of transient crossbred cows

These findings are in concurrence with Elshahawy and Abdullaziz (2017) [3] who reported significant (p<0.05) decrease in WBC from prepartum to parturition and increased non-significantly during postpartum in Friesian dairy cows.

The TLC (10^3/µl) during prepartum, parturition and postpartum periods between day -21 prepartum to +28 postpartum were significantly (p<0.05) higher in Group III (T_2) than Group II (T_1) and Group I (control) crossbred cows and Group II (T_1) had significantly (p<0.05) higher TLC (10^3/µl) than Group I (control) crossbred cows. However, no significant difference was observed on day -28 prepartum between the groups.

This increase in TLC in group III might be due to PUFA enhancing the lymphocyte population in response to the influx of leucocytes towards the uterine lumen and mammary glands (El-Diahy *et al.*, 2016) [6] and increased peripheral blood mononuclear cells proliferative response (Lessard *et al.*, 2004).

Packed Cell Volume (PCV)

The packed cell volume (%) in Group I (control) crossbred cows was decreased significantly (p<0.05) from day -28 prepartum to day 0 parturition followed by significant increase (p<0.05) up to day 28 postpartum. Group II (T_1) crossbred cows, a significant (p<0.05) increase in PCV (%) on day 0 parturition followed by a significant (p<0.05) decrease up to day 28 postpartum and no significant difference was observed from day -28 to -7 prepartum. Group III (T_2) crossbred cows, PCV (%) was significantly increased ($p<0.05$) from -28 prepartum to day 0 parturition followed by a significant decrease ($p<0.05$) up to day 28 postpartum (Figure 4). These findings were in agreement with Elshahawy and Abdullaziz (2017) [3] reported a significant ($p<0.05$) decrease in mean PCV from prepartum to parturition, and a significant ($p<0.05$) increase postpartum in Friesian dairy cows. The decrease in PCV percent from -28 prepartum to parturition in group I was due to diminished immunological status leads to suppression of erythropoiesis from bone marrow attributed to anaemia during pregnancy and parturition followed by an increase in PCV percent after calving due to prolonged refusal to drink water, loss of foetal fluid and an increase in milk yield in dairy cows (Joshi *et al.*, 2018).

The PCV (%) during prepartum, parturition and postpartum periods were significantly ($p<0.05$) higher in Group III (T_2) than Group II (T_1) and Group I (control) crossbred cows from day -14 prepartum to 28 postpartum and Group II (T_1) had significantly ($p<0.05$) higher PCV (%) than Group I (control) crossbred cows from day -7 prepartum to 14 postpartum.

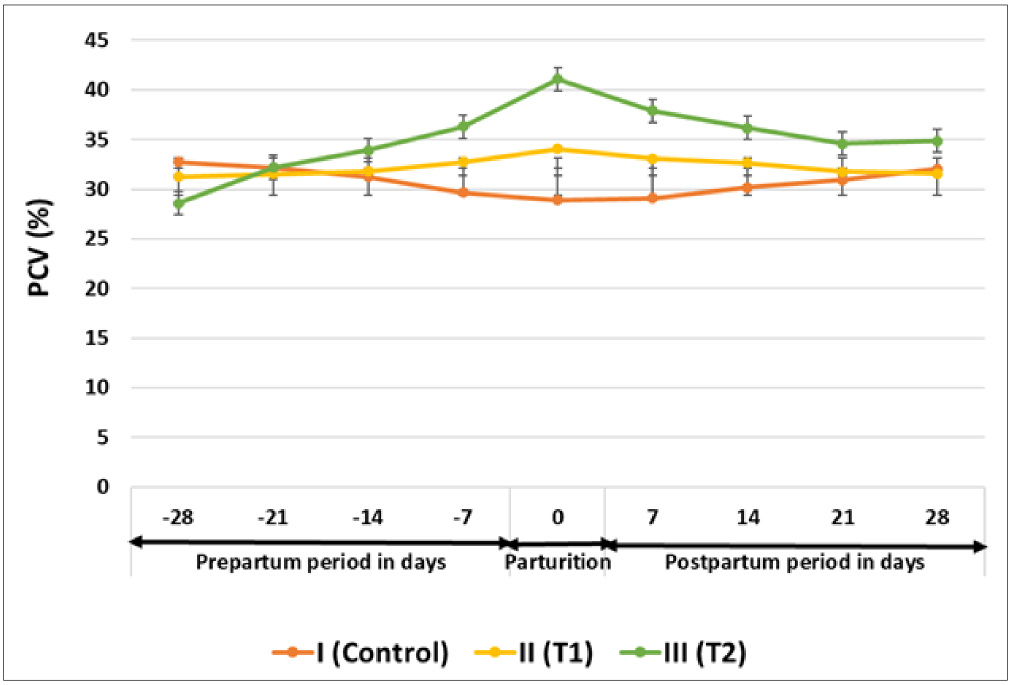

Fig 4: Mean (± SE) PCV in unorganized (Control), conventional (T_1) and immunomodulatory (T_2) feeding groups of transient crossbred cows

However, no significant difference was observed on day -28 prepartum between the groups. This increase in PCV by feeding flaxseed might be due to the immunomodulation response which increases erythropoiesis in bone marrow and increases in dry matter intake postpartum dairy cows (El-Diahy *et al.* 2016) [6].

Platelets Count

The platelet count (x/µl) in Group I (control) crossbred cows was non-significantly decreased from day -28 prepartum up to day 0 parturition followed by a non-significant increase up to day 28 postpartum. Group II (T_1) crossbred cows, a non-significant increase in platelet count (x/µl) from day -28 to -7 prepartum followed by a non-significant decrease on day 0 parturition and a non-significant increase up to day 28 postpartum. Group III (T_2) crossbred cows, platelet count (x/µl) was non-significantly increased from day -28 prepartum to day 0 parturition followed by a non-significant decrease up to day 28 postpartum (Figure 5).

The platelet count (x/µl) was significantly ($p<0.05$) higher on day 0 parturition in Group III (T_2) than Group II (T_1) and Group I (control) crossbred cows and no significant difference was observed from day -28 to -7 prepartum and day 7 to 28 postpartum among all the groups. Group II (T_1) cows, platelet count (x/µl) was significantly ($p<0.05$) higher on day 0 parturition than Group I and no significant difference was observed on the remaining days among both groups. The decrease in platelets count in Group I cows prepartum to calving might be due to an increased in concentration of pro-inflammatory cytokines (like TBF-alpha and interferons) which inhibits erythropoiesis by the action on erythroid precursors in the bone marrow (Chikazawa and Dunning, 2016) and postpartum increase in platelets count might be due to possible haemoconcentration because of the reduction in dry matter intake, water intake and galactopoiesis (Van Soest and Blosser, 1954) [9].

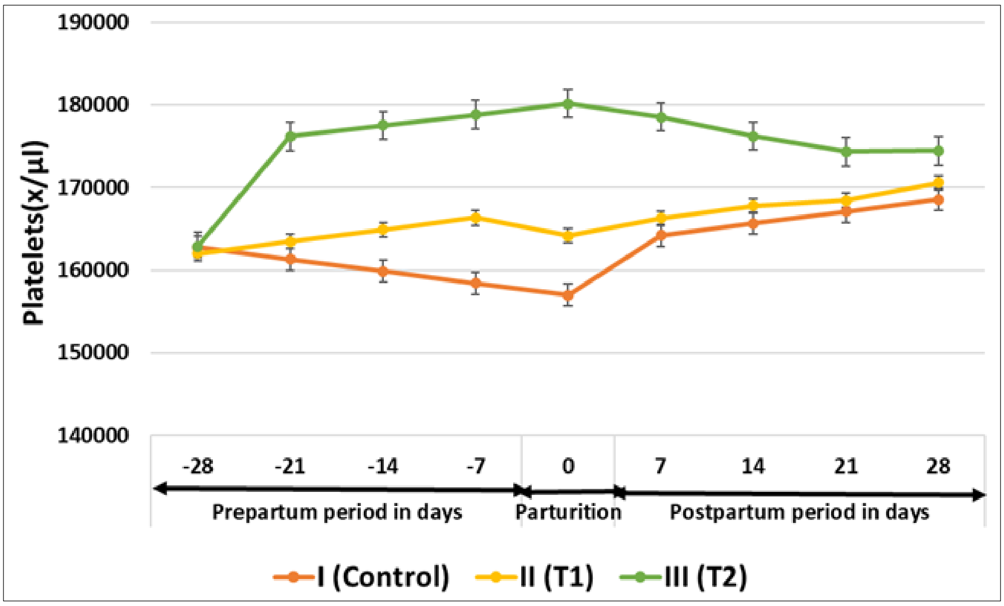

Fig 5: Mean (± SE) platelets count in unorganized (Control), conventional (T_1) and immunomodulatory (T_2) feeding groups of transient crossbred cows

This increase in platelet count by feeding flaxseed might be due to the activation of COX-2 pathway which stimulates the activating platelet factors and thromboxane A2 which leads to thrombocytosis (Sordillo *et al.*, 2009) [4].

Conclusion

Dietary Flaxseed and chelated mineral mixture supplemented immunomodulatory diet modulates the haematological indices. Feeding PUFA-rich immunomodulatory diets and altering concentration of fatty acids to reproductive tissues may be a novel strategy to integrate nutrition and reproductive management for improving animal productivity.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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