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TV Chaitanya Kumar

Assistant Professor, Department
of Veterinary Biochemistry
College of Veterinary Science,
Sri Venkateswara Veterinary
University, Tirupati, Andhra
Pradesh, India

Y Adithyananth

Department of Veterinary
Medicine, College of Veterinary
Science, Sri Venkateswara
Veterinary University, Tirupati,
Andhra Pradesh, India

K Rajesh

Department of Veterinary
Medicine, College of Veterinary
Science, Sri Venkateswara
Veterinary University, Tirupati,
Andhra Pradesh, India

B Shobha Mani

Director of Extension,
Sri Venkateswara Veterinary
University, Tirupati,
Andhra Pradesh, India

Corresponding Author:

TV Chaitanya Kumar

Assistant Professor, Department
of Veterinary Biochemistry
College of Veterinary Science,
Sri Venkateswara Veterinary
University, Tirupati,
Andhra Pradesh, India

Role of Vitamin E and selenium treatment on hematobiochemical changes during transition period in dairy cows

TV Chaitanya Kumar, Y Adithyananth, K Rajesh and B Shobha Mani

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Abstract

The transition period (three weeks prepartum to three weeks postpartum) in dairy cattle is marked by metabolic stress, oxidative imbalance, and alterations in mineral and hematological parameters. The present study evaluated hematobiochemical changes in oxidative stress-affected crossbred dairy cows and assessed the effect of Vitamin E and selenium supplementation. Thirty animals were divided into three groups: Group I (normal control), Group II (oxidative stress-affected, untreated), and Group III (oxidative stress-affected, treated with Vitamin E + selenium). Blood samples were analyzed for calcium, phosphorus, magnesium, and hematological indices including hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), and differential leukocyte count (DLC).

Serum calcium (mg/dL, mean $\pm$ SD) at three weeks prepartum was 10.0 ± 0.87 (control), 7.04 ± 0.57 (untreated), and 7.63 ± 1.01 (treated), with postpartum levels of 9.71 ± 0.6 , 7.42 ± 0.3 , and 8.44 ± 0.48 , respectively. Calcium decreased significantly ($p \leq 0.05$) in untreated animals, while treatment improved levels near normal. Magnesium (2.5 ± 0.27 to 2.46 ± 0.34 mg/dL in controls) and phosphorus (6.44 ± 0.82 to 6.71 ± 0.52 mg/dL) remained stable across groups.

Hematological indices remained largely within physiological limits. Mean Hb ranged from 8.93 ± 1.25 to 9.03 ± 0.82 g/dL, PCV from $27.8 \pm 2.94\%$ to $28.4 \pm 2.17\%$, and TEC from $5.4 \pm 0.32 \times 10^6/\mu\text{L}$ to $5.62 \pm 0.20 \times 10^6/\mu\text{L}$ in controls, showing minimal variation. TLC values were higher in oxidative stress groups (9570 ± 1322 cells/ μL in untreated vs. 8210 ± 647 cells/ μL in control), indicating immune activation. Lymphocytes increased significantly in the treated group postpartum ($72.4 \pm 2.76\%$) compared with controls ($65.3 \pm 1.83\%$), while neutrophils were elevated in untreated cows ($29.7 \pm 2.26\%$) suggestive of inflammation.

In conclusion, oxidative stress significantly lowered calcium and altered leukocyte dynamics during the transition period. Vitamin E and selenium supplementation improved calcium homeostasis, maintained erythrocyte integrity, and enhanced lymphocyte response, highlighting their efficacy in mitigating oxidative and metabolic stress in dairy cattle.

Keywords: Transition period, oxidative stress, calcium, vitamin e, selenium, hematology, dairy cows

Introduction

Transition period spanning from three weeks prepartum to three weeks postpartum is associated with alterations in macro-mineral levels due to intense metabolism and their secretion into fluids like milk. In particular, calcium, phosphorus, and magnesium are essential for neuromuscular function, metabolism, and immune competence. Hypocalcaemia, both clinical and subclinical, is common in early lactation owing to the sudden demand for calcium for colostrum and milk synthesis [1]. Low calcium levels are linked to increased risk of RFM, mastitis, and metritis due to impaired muscle contractility and reduced immune cell function [2]. Intestinal calcium absorption and Parathyroid Hormone (PTH) response is impaired by oxidative stress, further exacerbating hypocalcaemia [3]. Magnesium plays a crucial role in calcium homeostasis and energy metabolism, and imbalances may meddle with calcium regulation and immune function. Similarly, phosphorus is vital for ATP production and bone metabolism.

Though the levels stay relatively stable during the transition period, phosphorus interacts with calcium and magnesium and contributes to overall metabolic balance [4].

Likewise, the biochemical markers of oxidative stress, haematological parameters also offer valuable insights into the physiological and immunological status of dairy cattle during the period of transition. Parameters such as the haemoglobin (Hb), the Packed Cell Volume (PCV), the Total Erythrocyte Count (TEC), the Total Leukocyte Count (TLC), and the Differential Leukocyte Count (DLC) are sensitive to both oxidative damage and immune challenges. For example, increased neutrophil counts postpartum are characteristically associated with inflammatory and infectious conditions, while lymphocyte counts may reflect the immune modulatory effects of antioxidants [5, 6]. Oxidative stress can also lead to erythrocyte membrane damage and reduced erythrocyte lifespan, as observed in untreated animals with elevated MDA levels [7]. Hence, studying hematobiochemical changes during transition period is of greatest importance in relation to oxidative stress. Therefore, present study is aimed at studying hematobiochemical changes in animals during transition period under oxidative and to study how antioxidants like Vitamin E and selenium treatment could deal with such changes.

Materials and Methods

Experimental Animals

A total of 30 animals were selected for present study from the animals that has been presented to clinical complex, college of veterinary science, Tirupati and local dispensaries. Group I (control group) included ten animals which are physiologically normal, Group II and Group III included 10 animals each which has shown oxidative stress evident from elevated MDA levels presented in our previous work. Group III (treated oxidative stress group) animals have received Vit E and Selenium treatment (E CARE SE), whereas group II (untreated oxidative stress group) animals remain untreated.

Blood sampling

Blood samples were collected from the selected cattle during the period of study. Blood was collected from jugular vein into both K2 EDTA and clot activator vials. After collection, blood in EDTA vials was used for hematological analysis. The blood collected in clot activator vials was centrifuged at 2500 rpm for 5 min to separate serum and it was stored at -20, °C till further analysis.

Hematology

Hematological parameters such as haemoglobin, packed cell volume, total erythrocyte count, total leukocyte count, and differential leukocyte count were estimated as per the standard procedures [8].

Hb (gm%) was estimated by acid hematin method, PCV (%) was estimated by microhematocrit method, TEC ($10^6/\mu\text{l}$) and TLC ($10^3/\mu\text{l}$) were carried out by haemocytometer method. Blood smear was stained with Leishman's method and DLC was carried out by battlement method.

Serum biochemical parameters (Calcium, Phosphorus and Magnesium) were estimated by semi auto analyzer using the Assay kits and techniques. Serum biochemistry of all the parameters under study was performed with MISPAVIVA chemistry analyzer, AGAPPE DIAGNOSTICS Ltd. by using the kits supplied by ERBA diagnostics (Mannheim, Germany) and BEACON diagnostics Pvt. Ltd. (Gujarat) as per the

manufacturer instructions.

Statistical Analysis

The observed data of the present study was analyzed by two-way Anova using Graph Pad Prism version 8.4.3 to study the significant difference in the means of all parameters in between the groups and in across the weeks under study and the results were interpreted.

Results

Calcium, magnesium and phosphorous estimations during the transition period in cross bred dairy cattle (N=10)

Calcium, Magnesium and Phosphorous levels estimated during the transition period in animals during the study period are presented in terms of mean values along with standard deviation values in Table 1 and Graphical presentation in Figure 1, 2 and 3 for Calcium, Magnesium and Phosphorous respectively.

Calcium Levels (mg/dl)

Calcium levels (mg/dL, mean $\pm$ SD) were 10 ± 0.87 , 7.04 ± 0.57 , and 7.63 ± 1.01 at 3 weeks pre-calving, and 9.71 ± 0.6 , 7.42 ± 0.3 , and 8.44 ± 0.48 at 3 weeks post-calving for the normal control, untreated, and treated groups, respectively. Within groups, the control group remained stable, the untreated group showed a significant drop at 1-week post-calving ($p\leq0.05$), and the treated group remained stable but lower than control. Between groups, the control group had significantly higher calcium than the untreated group, which was significantly lower than the treated group throughout the transition period ($p\leq0.05$).

Magnesium Levels (mg/dl)

Magnesium levels (mg/dL, mean $\pm$ SD) were 2.5 ± 0.27 , 2.19 ± 0.29 , and 2.26 ± 0.4 at 3 weeks pre-calving, and 2.46 ± 0.34 , 2.32 ± 0.19 , and 2.3 ± 0.27 at 3 weeks post-calving for the normal control, untreated, and treated groups, respectively. Within groups, magnesium levels remained stable with no significant changes throughout the study ($p>0.05$). Between groups, the control group was significantly higher than the untreated group at 3 weeks pre-calving ($p\leq0.05$), while other weeks showed no significant differences.

Phosphorous Levels (mg/dl)

Phosphorous levels (mg/dL, mean $\pm$ SD) were 6.44 ± 0.82 , 5.42 ± 0.74 , and 5.13 ± 0.62 at 3 weeks pre-calving, and 6.71 ± 0.52 , 4.93 ± 0.57 , and 5.05 ± 0.49 at 3 weeks post-calving for the normal control, untreated, and treated groups, respectively. Within groups, levels were stable in control and treated groups ($p>0.05$), while the untreated group showed a significant increase postpartum ($p\leq0.05$). Between groups, the control group had significantly higher phosphorous than both untreated and treated groups throughout the transition period ($p\leq0.05$).

Hematological Parameters

Hemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC) and Total Leukocyte Count (TLC) levels were estimated during the transition period in animals during the study period are presented in terms of mean values along with standard deviation values in Table 2 and Graphical presentation in Figure 4, 5, 6 and 7 for Haemoglobin, PCV, TEC and TLC respectively.

Haemoglobin (Hb) Levels

Haemoglobin (Hb) levels (g/dL) in the normal control, untreated, and treated groups were 8.93 ± 1.25 , 9.42 ± 1.05 , and 9.82 ± 0.82 at 3 weeks pre-calving, and 9.03 ± 0.82 , 9.27 ± 0.95 , and 9.91 ± 0.73 at 3 weeks post-calving, respectively. Across the transition period, Hb values showed no significant variation within groups or between groups ($p > 0.05$), indicating stable haemoglobin levels regardless of treatment or time.

Packed Cell Volume (PCV) Levels

PCV levels (% Mean $\pm$ SD) were 27.8 ± 2.94 , 27.5 ± 2.17 , and 27.6 ± 1.97 at 3 weeks pre-calving, and 28.4 ± 2.17 , 26.9 ± 1.37 , and 28.9 ± 1.36 at 3 weeks post-calving for the normal control, untreated, and treated groups, respectively. Within groups, PCV values showed no significant variation during the study, except for a significant decrease in the untreated group at 3 weeks post-calving ($p \leq 0.05$). Between groups, no significant differences were observed throughout, except at 3 weeks post-calving, where the treated group had significantly higher PCV than the untreated group ($p \leq 0.05$).

Total Erythrocyte Count (TEC) Levels

TEC levels ($\times 10^6/\mu\text{L}$, mean $\pm$ SD) were 5.4 ± 0.32 , 5.28 ± 0.23 , and 5.13 ± 0.24 at 3 weeks pre-calving, and 5.62 ± 0.20 , 5.2 ± 0.12 , and 5.42 ± 0.19 at 3 weeks post-calving for the normal control, untreated, and treated groups, respectively. Within groups, no significant changes were observed during the study ($p > 0.05$). Between groups, TEC in the treated group was significantly lower than the control group at 3 weeks pre-calving ($p \leq 0.05$) but significantly higher than the untreated group during all subsequent weeks ($p \leq 0.05$).

Total Leukocyte Count (TLC) Levels

TLC levels (cells/ μL , Mean $\pm$ SD) were 8210 ± 647 , 9570 ± 1322 , and 8680 ± 645 at 3 weeks pre-calving, and 8300 ± 377 , 9290 ± 1103 , and 9054 ± 1010 at 3 weeks post-calving for the normal control, untreated, and treated groups, respectively. Within groups, no significant variations were observed during the study ($p > 0.05$). Between groups, the untreated group showed significantly higher TLC than the control throughout ($p \leq 0.05$), while the treated group had significantly higher values than the control at most weeks but generally lower than the untreated group ($p \leq 0.05$).

Differential Leukocyte Counts

Differential Leukocyte Count (Lymphocytes, Neutrophils, Eosinophils and Monocytes) levels were estimated during the transition period in animals during the study period are presented in terms of mean values along with standard deviation values in Table 3 and Graphical presentation in Figure 8, 9, 10 and 11 for Lymphocytes, Neutrophils, Eosinophils and Monocytes respectively.

Lymphocyte Counts

Lymphocyte counts (% Mean $\pm$ SD) were 66.5 ± 4.3 , 65.1 ± 3.51 , and 69.9 ± 3.6 at 3 weeks pre-calving, and 65.3 ± 1.83 , 64.9 ± 2.38 , and 72.4 ± 2.76 at 3 weeks post-calving for the normal control, untreated, and treated groups, respectively. Within groups, lymphocyte counts showed no significant variation during the study ($p > 0.05$). Between groups, the treated group exhibited significantly higher lymphocyte counts than the control and untreated groups at two weeks pre-calving and at all post-calving weeks ($p \leq 0.05$).

Neutrophil Counts

Neutrophil counts (% Mean $\pm$ SD) were 28.3 ± 4.27 , 31.8 ± 4.21 , and 26.2 ± 2.82 at 3 weeks pre-calving, and 29.4 ± 3.66 , 29.7 ± 2.26 , and 23.4 ± 3.5 at 3 weeks post-calving for the normal control, untreated, and treated groups, respectively. Within groups, no significant changes were observed during the study ($p > 0.05$). Between groups, the treated group consistently showed significantly lower neutrophil counts than the untreated group, particularly during the postpartum period ($p \leq 0.05$).

Eosinophil Counts

Eosinophil counts (% Mean $\pm$ SD) were 4.0 ± 1.6 , 2.2 ± 1.6 , and 3.2 ± 0.9 at 3 weeks pre-calving, and 4.1 ± 1.5 , 3.9 ± 2.0 , and 2.6 ± 1.1 at 3 weeks post-calving for the normal control, untreated, and treated groups, respectively. Within groups, no significant variations were observed throughout the study ($p > 0.05$). Between groups, the treated group showed significantly lower eosinophil counts than the control and untreated groups at 3- and 2-weeks pre-calving ($p \leq 0.05$).

Monocyte Counts

Monocyte counts (% Mean $\pm$ SD) were 1.3 ± 0.9 , 1.0 ± 0.7 , and 0.7 ± 0.7 at 3 weeks pre-calving, and 1.2 ± 1.0 , 1.5 ± 0.8 , and 1.3 ± 0.7 at 3 weeks post-calving for the normal control, untreated, and treated groups, respectively. Within groups, monocyte counts remained stable with no significant changes throughout the study ($p > 0.05$). Between groups, no significant differences were observed at any time point.

Discussion

Calcium, Phosphorous and Magnesium

Serum calcium levels (mg/dl) in the all the three groups under study varied significantly. Serum calcium levels in the oxidative stress groups were significantly low in levels when compared to normal control group at three weeks prepartum, this observed decrease in serum calcium levels in oxidative stress group could be because of compromised intestinal calcium absorption which would be triggered by oxidative stress [3]. Further, the calcium levels have remained lower in untreated oxidative stress group during postpartum, but in treated group their levels have significantly increased by three weeks after calving and was comparable with normal control group, although their levels were lower than the normal serum levels. However, the improved calcium levels can be ascertained to the improved levels of antioxidant enzymes in the treated oxidative stress group (Group III), and these increased levels antioxidant enzymes in gut would have helped in mitigating oxidative stress and may have promoted intestinal calcium absorption [3]. Further the levels of both Phosphorus and Magnesium have remained unaltered during all the weeks under study in the three groups of study and was in their normal range. This observed pattern was in accordance with some of the similar studies [9, 10].

Haematological Parameters

Haemoglobin PCV and TEC

The levels of haemoglobin, PCV and TEC in all the three groups under study observed to be in normal reference range during the entire period of study. However, their levels have non-significantly increased as the pregnancy advanced in all the three groups. These results were in concordance with other studies where in the levels of Hb, PCV and TEC has increased non-significantly by partum [11]. Further, this

increasing trend of Hb, TEC and PCV has continued in normal control and treated oxidative stress group but not in untreated oxidative stress group. In untreated oxidative stress group, TEC has significantly decreased by three weeks postpartum when compared to treated (Vit E + selenium) oxidative stress group, this could be because of the membrane damage caused by the erythrolysis due to persisting oxidative damage [7]. However, Vit E and selenium treatment was able to prevent such damage in Group III.

TLC and DLC

TLC counts in all the three groups under study has increased non significantly from three weeks Prepartum to partum and there on decreased by 3 weeks postpartum. This observed

trend is in accordance with various other similar studies in related species [12, 13]. However, both the oxidative stress groups when compared to normal control group has shown significantly higher levels of TLC during the entire period of study. This could be due to increased count of lymphocytes as well as neutrophils observed in both the groups postpartum. The observed significant higher levels of average neutrophils count in untreated group when compared to treated group could be because of infectious diseases and subclinical infections during transition period in these animals. Whereas the observed significantly higher levels of lymphocytes in treated group postpartum when compared to normal control and untreated group could be due to the proliferative effect of Vitamin E on lymphocytes [14, 15].

Table 1: Calcium, magnesium and phosphorous estimations during the transition period in cross bred dairy cattle (N=10), (Mean $\pm$ SD)

Transition Period	Calcium (mg/dl)			Magnesium (mg/dl)			Phosphorous (mg/dl)		
	Normal control group	Untreated group	Treated group	Normal control group	Untreated group	Treated group	Normal control group	Untreated group	Treated group
Before 3 weeks	10 $\pm$ 0.87 ^{a,p}	7.04 $\pm$ 0.57 ^{b,pqr}	7.63 $\pm$ 1.01 ^{b,p}	2.5 $\pm$ 0.27 ^{a,p}	2.19 $\pm$ 0.29 ^{b,p}	2.26 $\pm$ 0.4 ^{ab,p}	6.44 $\pm$ 0.82 ^{a,p}	5.42 $\pm$ 0.74 ^{b,pq}	5.13 $\pm$ 0.62 ^{b,p}
Before 2 weeks	9.83 $\pm$ 0.91 ^{a,pq}	7.09 $\pm$ 0.49 ^{b,pqr}	7.81 $\pm$ 1.03 ^{c,p}	2.45 $\pm$ 0.26 ^{a,p}	2.21 $\pm$ 0.29 ^{a,p}	2.27 $\pm$ 0.29 ^{a,p}	6.66 $\pm$ 0.79 ^{a,p}	5.27 $\pm$ 0.67 ^{b,pr}	5.01 $\pm$ 0.53 ^{b,p}
Before 1 week	9.6 $\pm$ 0.57 ^{a,pq}	7.05 $\pm$ 0.25 ^{b,pqr}	8.13 $\pm$ 1.03 ^{c,p}	2.36 $\pm$ 0.22 ^{a,p}	2.14 $\pm$ 0.15 ^{a,p}	2.29 $\pm$ 0.3 ^{a,p}	6.46 $\pm$ 0.61 ^{a,p}	4.95 $\pm$ 0.74 ^{b,ps}	4.87 $\pm$ 0.53 ^{b,p}
After 1 week	9.06 $\pm$ 0.52 ^{a,q}	6.46 $\pm$ 0.5 ^{b,q}	8.1 $\pm$ 0.68 ^{c,p}	2.21 $\pm$ 0.21 ^{a,p}	2.03 $\pm$ 0.13 ^{a,p}	2.15 $\pm$ 0.23 ^{a,p}	6.18 $\pm$ 0.41 ^{a,p}	3.92 $\pm$ 0.54 ^{b,t}	4.55 $\pm$ 0.68 ^{b,p}
After 2 weeks	9.47 $\pm$ 0.55 ^{a,pq}	7.09 $\pm$ 0.25 ^{b,pqr}	8.2 $\pm$ 0.61 ^{c,p}	2.41 $\pm$ 0.15 ^{a,p}	2.27 $\pm$ 0.27 ^{a,p}	2.23 $\pm$ 0.16 ^{a,p}	6.28 $\pm$ 0.81 ^{a,p}	4.43 $\pm$ 0.45 ^{b,ts}	4.72 $\pm$ 0.59 ^{b,p}
After 3 weeks	9.71 $\pm$ 0.6 ^{a,pq}	7.42 $\pm$ 0.3 ^{b,pr}	8.44 $\pm$ 0.48 ^{a,p}	2.46 $\pm$ 0.34 ^{a,p}	2.32 $\pm$ 0.19 ^{a,p}	2.3 $\pm$ 0.27 ^{a,p}	6.71 $\pm$ 0.52 ^{a,p}	4.93 $\pm$ 0.57 ^{b,qrs}	5.05 $\pm$ 0.49 ^{b,p}

Table 2: Haemoglobin, Packed Cell Volume (PCV), Total Erythrocyte Count (TEC) and Total Leukocyte Count (TLC) estimations during the transition period in cross bred dairy cattle (N=10), (Mean $\pm$ SD)

Transition period	Haemoglobin (gm percent)			Packed Cell Volume (PCV) (Percent)			Total Erythrocyte Count (TEC) (Millions / Microlitre)			Total Leukocyte Count (TLC) (Count/Microlitre)		
	Normal control group	Untreated group	Treated group	Normal control group	Untreated group	Treated group	Normal control group	Untreated group	Treated group	Normal control group	Untreated group	Treated group
Before 3 weeks	8.93 $\pm$ 1.25 ^{a,p}	9.42 $\pm$ 1.05 ^{a,p}	9.82 $\pm$ 0.82 ^{a,p}	27.8 $\pm$ 2.94 ^{a,p}	27.5 $\pm$ 2.17 ^{a,pqr}	27.6 $\pm$ 1.97 ^{a,p}	5.4 $\pm$ 0.32 ^{a,p}	5.28 $\pm$ 0.23 ^{a,p}	5.13 $\pm$ 0.24 ^{b,p}	8210 $\pm$ 647 ^{a,p}	9570 $\pm$ 1322 ^{b,p}	8680 $\pm$ 645 ^{b,p}
Before 2 weeks	8.89 $\pm$ 1.23 ^{a,p}	9.25 $\pm$ 0.98 ^{a,p}	9.86 $\pm$ 1.07 ^{a,p}	27.2 $\pm$ 3.04 ^{a,p}	27.3 $\pm$ 1.76 ^{a,pqr}	29.3 $\pm$ 2.41 ^{a,p}	5.47 $\pm$ 0.34 ^{ab,p}	5.2 $\pm$ 0.19 ^{a,p}	5.24 $\pm$ 0.31 ^{b,p}	8200 $\pm$ 579 ^{a,p}	9620 $\pm$ 1358 ^{b,p}	8950 $\pm$ 498 ^{b,p}
Before 1 week	9.24 $\pm$ 1.05 ^{a,p}	9.58 $\pm$ 0.96 ^{a,p}	10.03 $\pm$ 0.64 ^{a,p}	27.1 $\pm$ 2.8 ^{a,p}	28.1 $\pm$ 1.73 ^{a,pqr}	29.7 $\pm$ 1.84 ^{a,p}	5.55 $\pm$ 0.21 ^{a,p}	5.07 $\pm$ 0.16 ^{b,p}	5.31 $\pm$ 1.94 ^{a,p}	8380 $\pm$ 346 ^{a,p}	9680 $\pm$ 1274 ^{b,p}	8940 $\pm$ 1002 ^{b,p}
After 1 week	9.02 $\pm$ 0.9 ^{a,p}	9.08 $\pm$ 0.95 ^{a,p}	9.31 $\pm$ 0.86 ^{b,p}	27.7 $\pm$ 2.8 ^{a,p}	29.6 $\pm$ 1.35 ^{a,p}	29.34 $\pm$ 2.37 ^{a,p}	5.56 $\pm$ 0.28 ^{a,p}	4.99 $\pm$ 0.19 ^{b,p}	5.09 $\pm$ 0.25 ^{a,p}	8490 $\pm$ 412 ^{a,p}	9630 $\pm$ 1166 ^{b,p}	9540 $\pm$ 954 ^{ab,p}
After 2 weeks	9.02 $\pm$ 0.84 ^{a,p}	9.09 $\pm$ 0.97 ^{a,p}	9.87 $\pm$ 0.67 ^{a,p}	28.1 $\pm$ 2.55 ^{a,p}	28.7 $\pm$ 1.34 ^{a,pqr}	29.01 $\pm$ 1.99 ^{a,p}	5.54 $\pm$ 0.24 ^{a,p}	5.12 $\pm$ 0.2 ^{b,p}	5.31 $\pm$ 0.28 ^{a,p}	8340 $\pm$ 384 ^{a,p}	9490 $\pm$ 1158 ^{b,p}	8950 $\pm$ 523 ^{ab,p}
After 3 weeks	9.03 $\pm$ 0.82 ^{a,p}	9.27 $\pm$ 0.95 ^{a,p}	9.91 $\pm$ 0.73 ^{a,p}	28.4 $\pm$ 2.17 ^{ab,p}	26.9 $\pm$ 1.37 ^{a,r}	28.9 $\pm$ 1.36 ^{b,p}	5.62 $\pm$ 0.20 ^{ab,p}	5.2 $\pm$ 0.12 ^{a,p}	5.42 $\pm$ 0.19 ^{b,p}	8300 $\pm$ 377 ^{a,p}	9290 $\pm$ 1103 ^{b,p}	9054 $\pm$ 1010 ^{b,p}

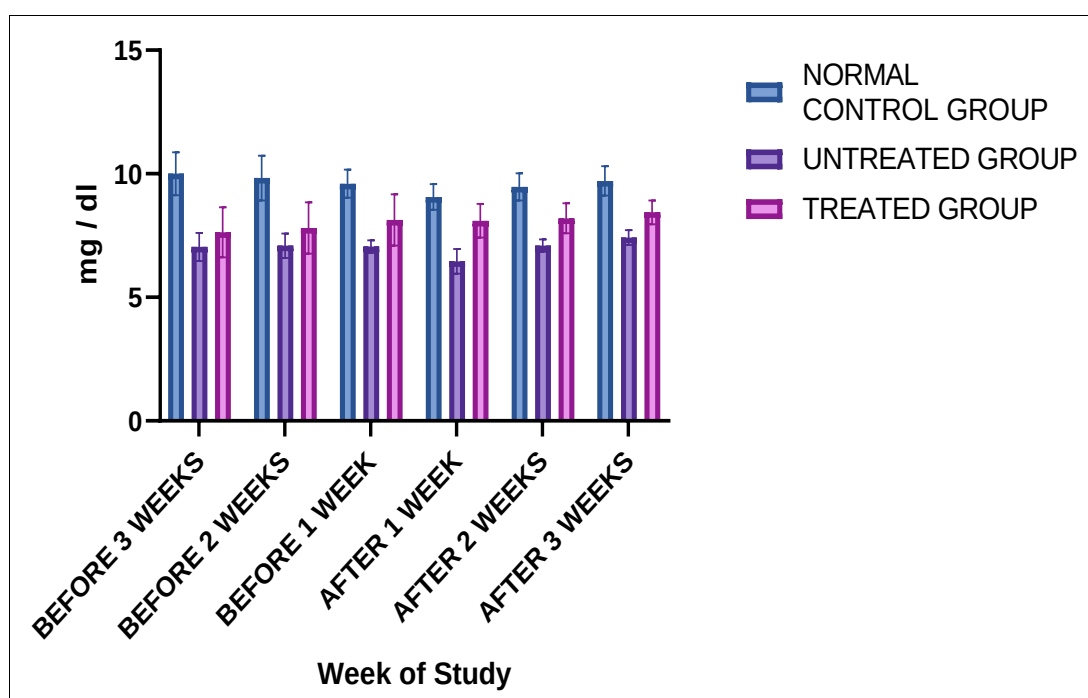
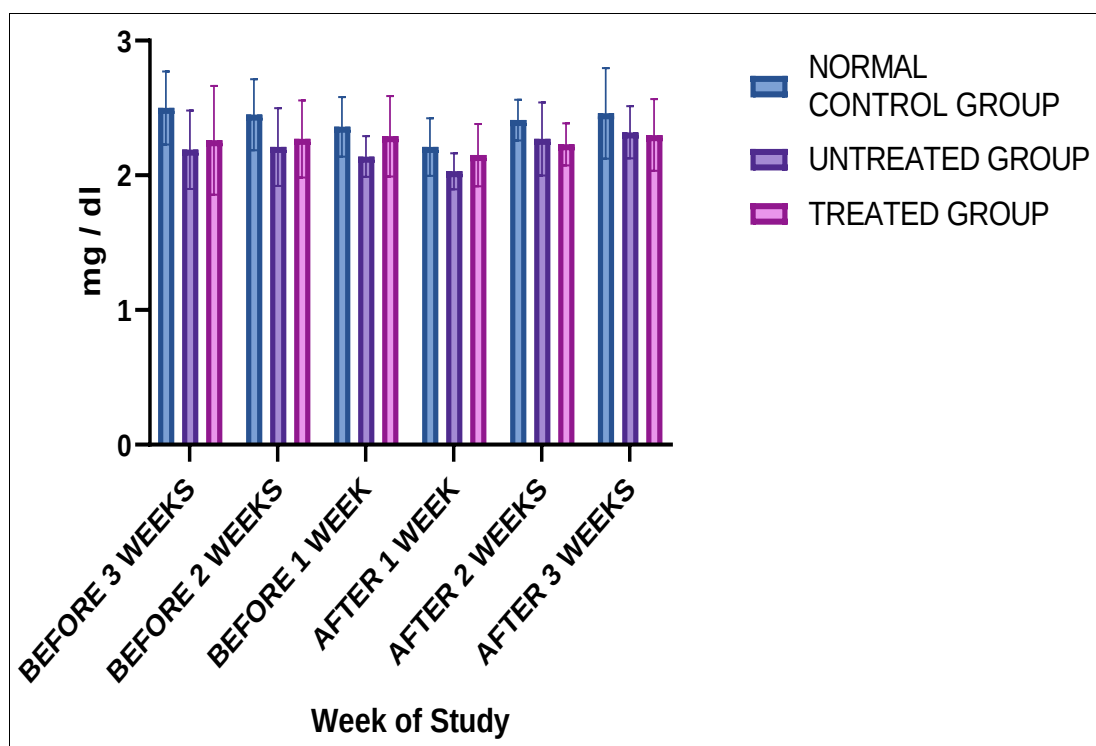
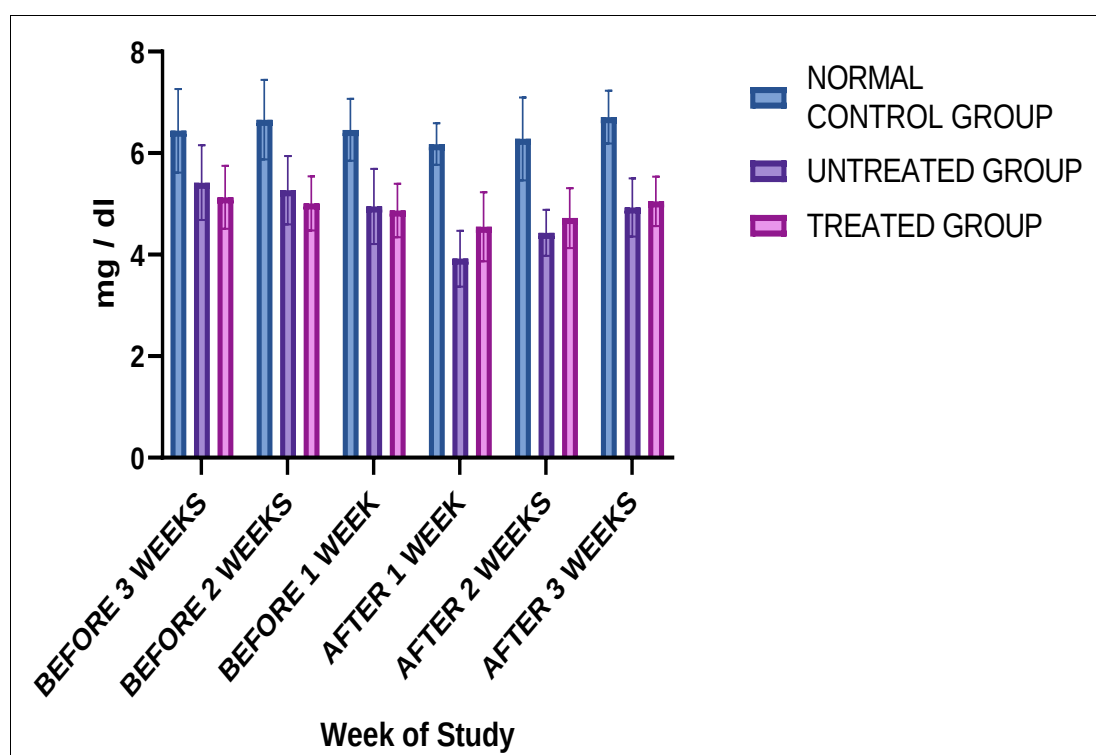


Fig 1: Calcium estimation during the transition period in cross bred dairy cattle (N=10), (Mean $\pm$ SD)

Table 3: Differential Leukocyte Count (Lymphocytes, Neutrophils, Eosinophils and Monocytes) estimations during the transition period in cross bred dairy cattle (N=10), (Mean $\pm$ SD)

Transition Period	Lymphocytes (Count/100)			Neutrophils (Count/100)			Eosinophils (Count/100)			Monocytes (Count/100)		
	Normal control group	Untreated group	Treated group	Normal control group	Untreated group	Treated group	Normal control group	Untreated group	Treated group	Normal control group	Untreated group	Treated Group
Before 3 weeks	66.5 $\pm$ 4.3 ^{a,p}	65.1 $\pm$ 3.51 ^{a,p}	69.9 $\pm$ 3.6 ^{a,p}	28.3 $\pm$ 4.27 ^{ab,p}	31.8 $\pm$ 4.21 ^{a,p}	26.2 $\pm$ 2.82 ^{b,p}	4 $\pm$ 1.6 ^{a,p}	2.2 $\pm$ 1.6 ^{b,p}	3.2 $\pm$ 0.9 ^{ab,p}	1.3 $\pm$ 0.9 ^{a,p}	1 $\pm$ 0.7 ^{a,p}	0.7 $\pm$ 0.7 ^{a,p}
Before 2 weeks	65.5 $\pm$ 4.8 ^{a,p}	68.2 $\pm$ 8.04 ^{ab,p}	72.7 $\pm$ 5.62 ^{b,p}	28.9 $\pm$ 4.61 ^{a,p}	28.2 $\pm$ 8.12 ^{a,p}	24.4 $\pm$ 7.04 ^{a,p}	3.6 $\pm$ 2.2 ^{a,p}	2.6 $\pm$ 1.2 ^{ab,p}	1.8 $\pm$ 1.3 ^{b,p}	2 $\pm$ 1.6 ^{a,p}	1 $\pm$ 1.6 ^{a,p}	1.2 $\pm$ 1.8 ^{a,p}
Before 1 week	66 $\pm$ 4.45 ^{a,p}	66.1 $\pm$ 7.78 ^{a,p}	68.5 $\pm$ 8.15 ^{a,p}	29.4 $\pm$ 3.98 ^{a,p}	29.5 $\pm$ 7.09 ^{a,p}	26.7 $\pm$ 8.31 ^{a,p}	2.9 $\pm$ 1.7 ^{a,p}	2.8 $\pm$ 1.0 ^{a,p}	2.9 $\pm$ 1.3 ^{a,p}	1.7 $\pm$ 1.6 ^{a,p}	1.1 $\pm$ 1.2 ^{a,p}	2 $\pm$ 1.4 ^{a,p}
After 1 week	66.1 $\pm$ 3.3 ^{a,p}	65.6 $\pm$ 5.56 ^{a,p}	71.6 $\pm$ 3.5 ^{b,p}	29.4 $\pm$ 3.41 ^{ab,p}	30.1 $\pm$ 5.22 ^{a,p}	24.8 $\pm$ 2.39 ^{b,p}	3.3 $\pm$ 0.9 ^{a,p}	3.3 $\pm$ 1.5 ^{a,p}	3 $\pm$ 1.2 ^{a,p}	1.2 $\pm$ 1 ^{a,p}	1 $\pm$ 0.9 ^{a,p}	0.5 $\pm$ 0.7 ^{a,p}
After 2 weeks	65.9 $\pm$ 2.77 ^{a,p}	64.9 $\pm$ 2.56 ^{a,p}	74.5 $\pm$ 4.62 ^{b,p}	29.6 $\pm$ 3.27 ^{a,p}	30.7 $\pm$ 2.91 ^{a,p}	22 $\pm$ 5.64 ^{b,p}	3.3 $\pm$ 1.3 ^{a,p}	3.3 $\pm$ 1.2 ^{a,p}	2.4 $\pm$ 0.8 ^{a,p}	1.1 $\pm$ 1 ^{a,p}	1.1 $\pm$ 0.6 ^{a,p}	1 $\pm$ 1.6 ^{a,p}
After 3 weeks	65.3 $\pm$ 1.83 ^{a,p}	64.9 $\pm$ 2.38 ^{a,p}	72.4 $\pm$ 2.76 ^{b,p}	29.4 $\pm$ 3.66 ^{a,p}	29.7 $\pm$ 2.26 ^{a,p}	23.4 $\pm$ 3.5 ^{b,p}	4.1 $\pm$ 1.5 ^{a,p}	3.9 $\pm$ 2.0 ^{a,p}	2.6 $\pm$ 1.1 ^{a,p}	1.2 $\pm$ 1 ^{a,p}	1.5 $\pm$ 0.8 ^{a,p}	1.3 $\pm$ 0.7 ^{a,p}

**Fig 2:** Magnesium estimation during the transition period in cross bred dairy Cattle (N=10), (Mean $\pm$ SD)**Fig 3:** Phosphorous estimation during the transition period in cross bred dairy cattle (N=10), (Mean $\pm$ SD)

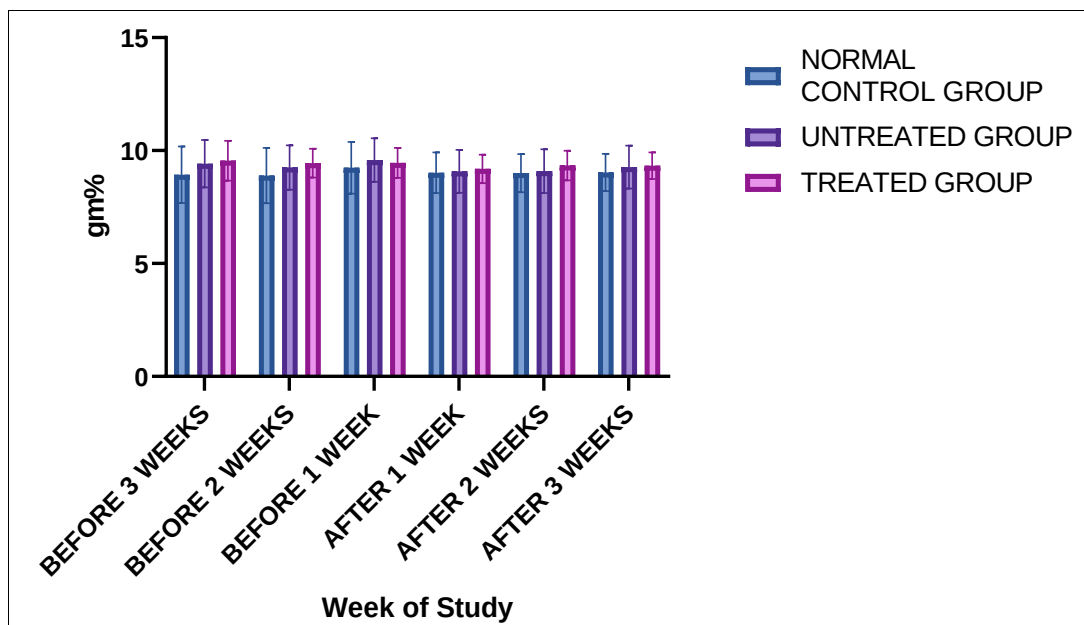


Fig 4: Haemoglobin estimation during the transition period in cross bred dairy cattle (N=10), (Mean $\pm$ SD)

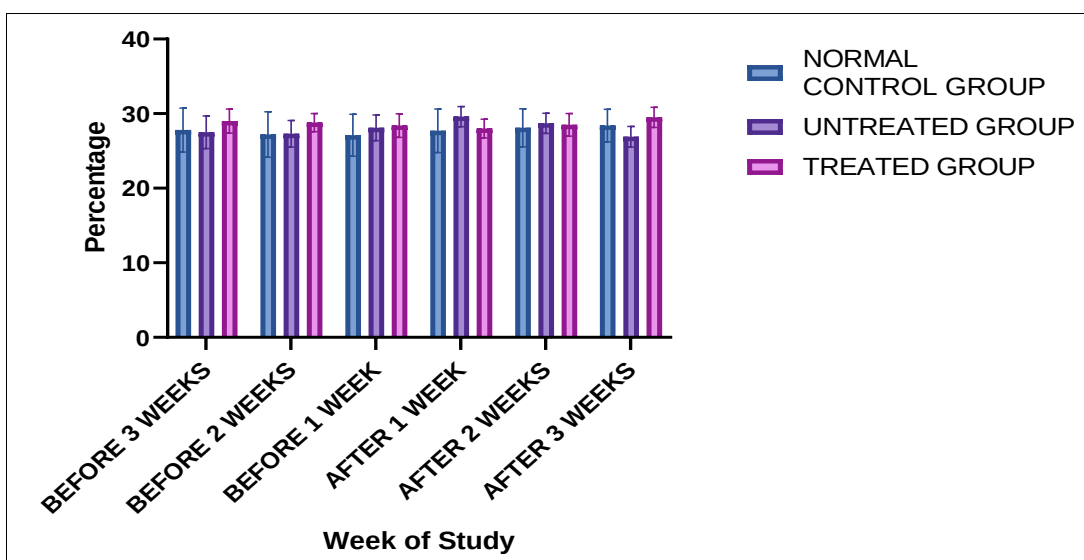


Fig 5: Packed Cell Volume (PCV) estimations during the transition period in cross bred dairy Cattle (N=10), (Mean $\pm$ SD)

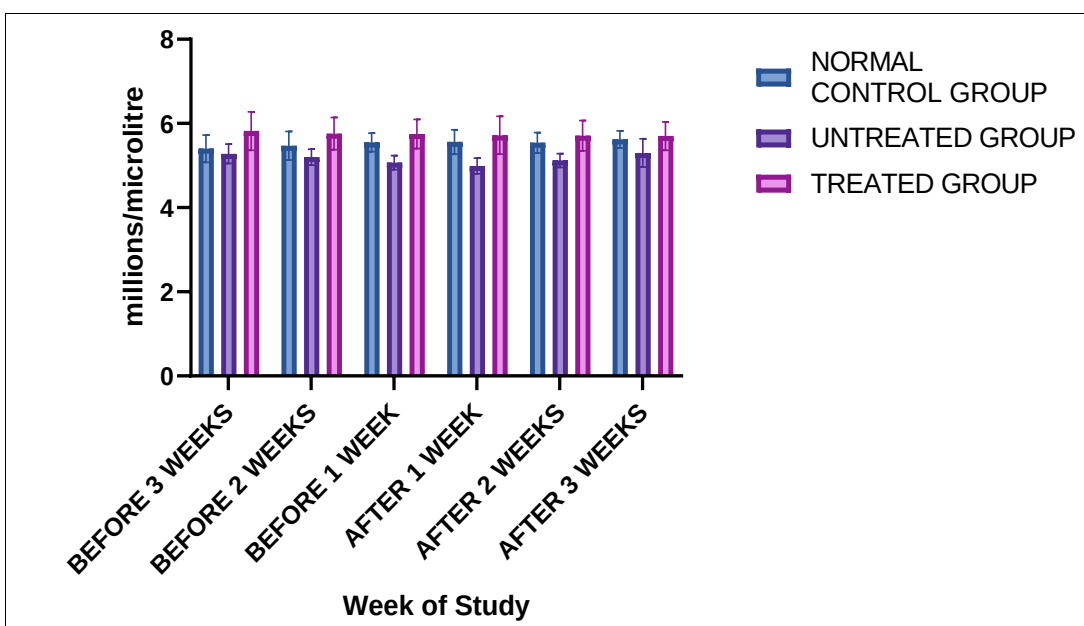


Fig 6: Total Erythrocyte Count (TEC) estimation during the Transition period in cross bred dairy cattle (N=10), (Mean $\pm$ SD)

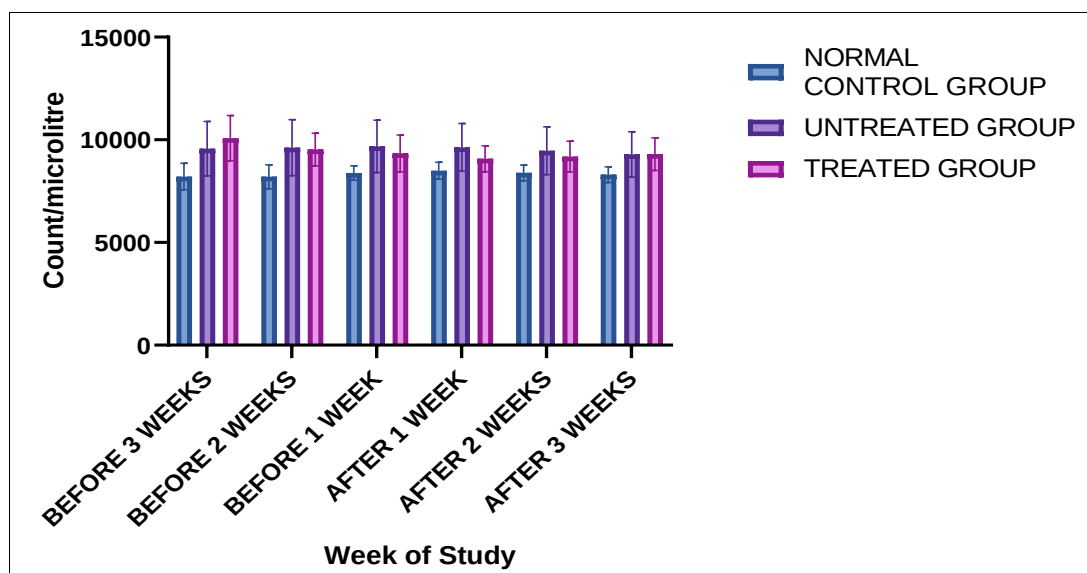


Fig 7: Total Leukocyte Count (TLC) estimation during the transition period in cross bred dairy cattle (N=10), (Mean $\pm$ SD)

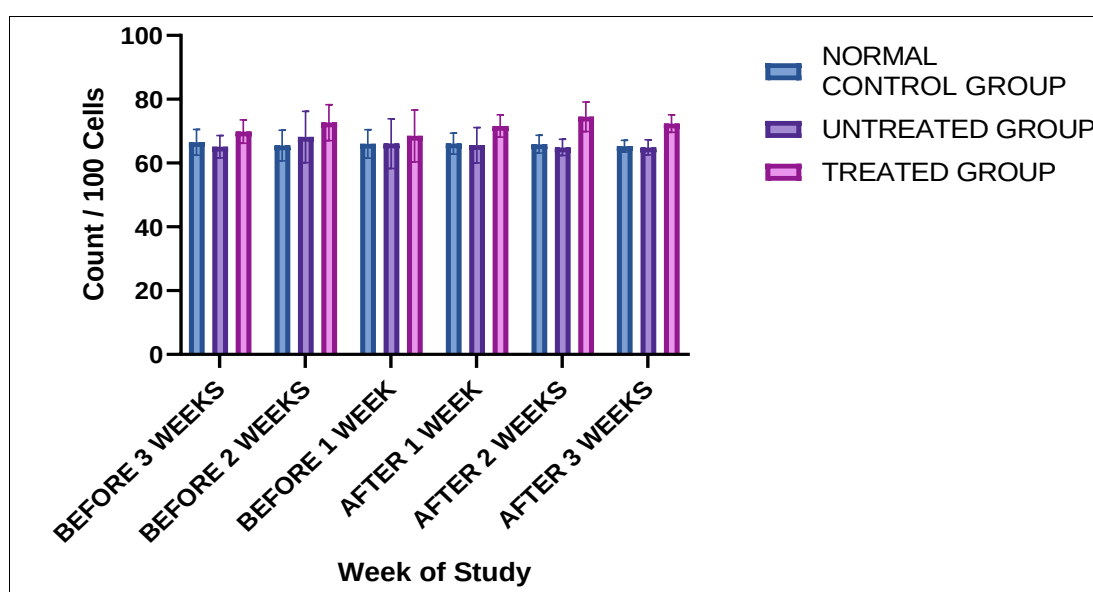


Fig 8: Differential Leukocyte Count-Lymphocytes estimation during the Transition period in cross bred dairy cattle (N=10), (Mean $\pm$ SD)

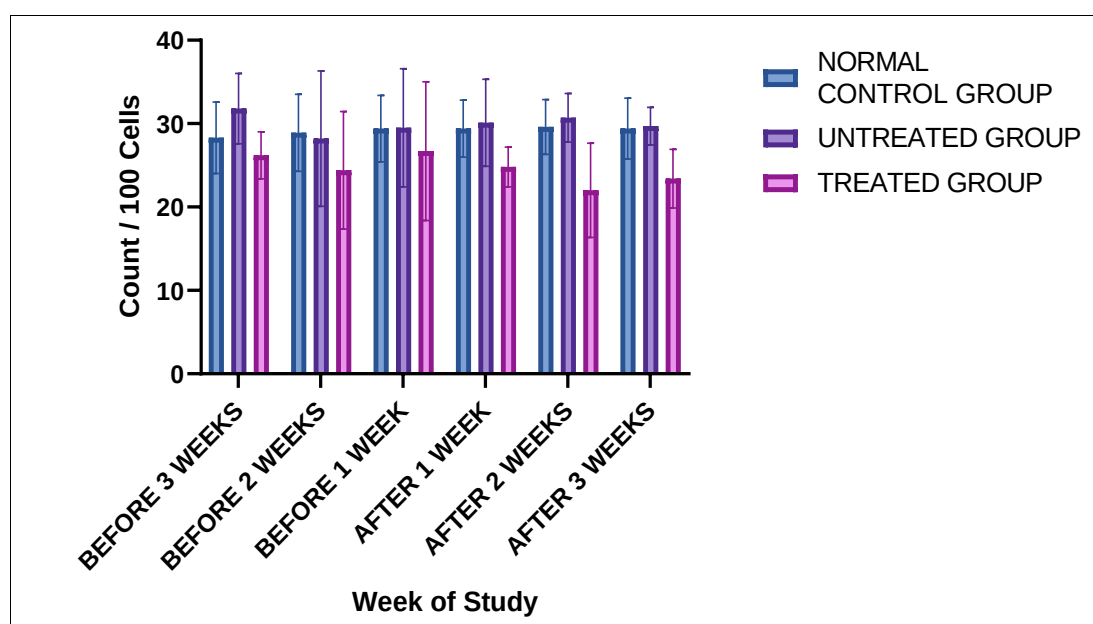


Fig 9: Differential Leukocyte Count-Neutrophils estimation during the Transition period in cross bred dairy cattle (N=10), (Mean $\pm$ SD)

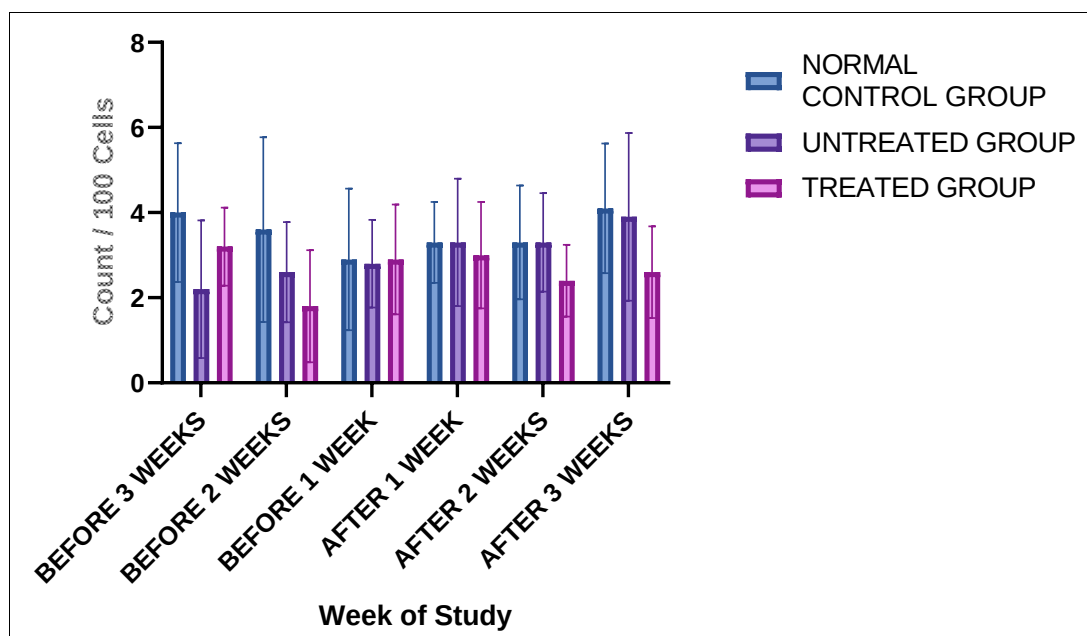


Fig 10: Differential Leukocyte Count-Eosinophils estimation during the transition period in cross bred dairy cattle (N=10), (Mean $\pm$ SD)

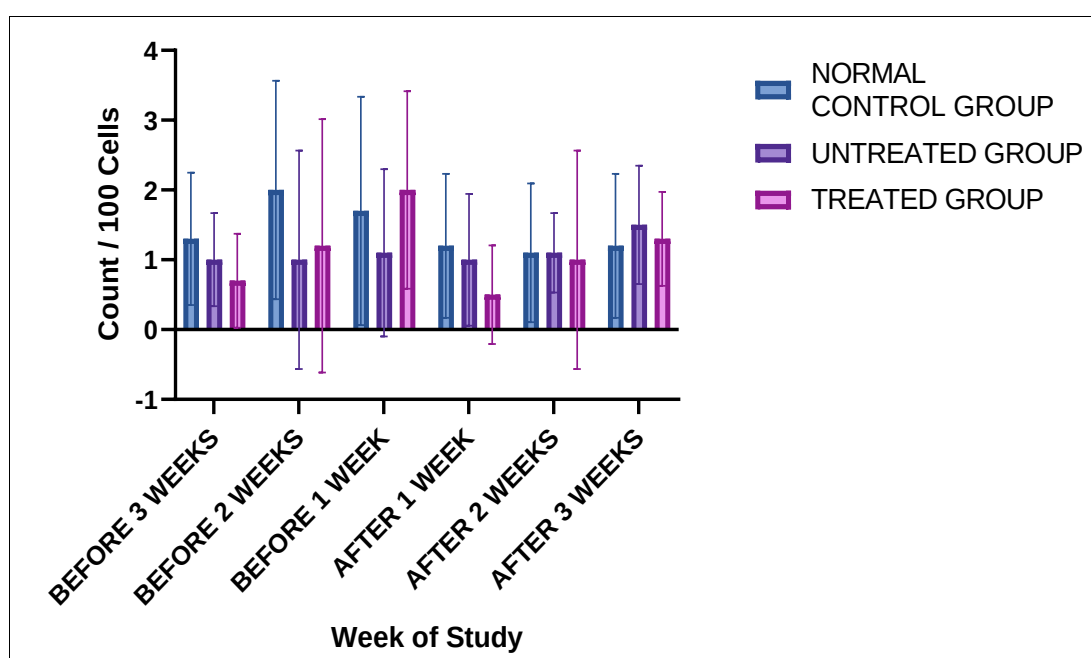


Fig 11: Differential Leukocyte Count-Monocytes estimation during the Transition period in cross bred dairy cattle (N=10), (Mean $\pm$ SD)

Conclusion

There was no pronounced effect of oxidative stress and metabolic stress on haematological parameters in any of these groups except that the membrane damage induced erythrolysis and decreased TLC count in untreated group, which was reverted by Vitamin E and selenium treatment in group III.

Conflict of Interest

Not available

Financial Support

Not available

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