

# **Effect of Transition Period and Supplementation of Vitamins and Selenium on Physiological Responses of Crossbred Dairy Cows**

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## **ABSTRACT**

The dairy industry in Sudan is based on native breeds, crossbreeds and pure exogenous imported cattle. Development of effective management strategies to alleviate the adverse effects of physiological status and impacts of thermal and nutritional stresses requires basic and applied research work. In dairy cows, the periparturient period presents major physiological challenges as it is affected by metabolic stressors and changes in endocrine status and immune function which result in increased risk of diseases. The objective of this study was to evaluate the physiological changes which occur during the periparturient period in crossbred dairy cows. It was, also, intended to provide information regarding the potentials of supplementation with vitamins and selenium during the transition period. Twenty-four clinically healthy cows were assigned to 3 groups of 8 cows each, a control group and two treated groups, which received parenterally vitamin E and selenium (Vit. E + Se) or multivitamins: A, D and E (MY). The cows were monitored for 8 weeks before and 4 weeks after calving. The postpartum decrease in body weight of cows was more pronounced in the control group. The calf birth weight was significantly higher in the supplemented groups. PCV and Hb concentration decreased during the periparturient period; cows supplemented with Vit E+ Se had higher PCV values during the transition period. The total leukocyte count (TLC), neutrophil and eosinophil ratios increased, whereas lymphocyte ratio decreased at parturition and postpartum period. Supplementation with vitamins and Se decreased TLC, neutrophil and eosinophil ratios and increased lymphocyte ratio. Plasma total protein, albumin and urea levels decreased at parturition, the decrease was more pronounced in the control group. The triglyceride and cholesterol levels decreased at parturition and postpartum period with no effect of supplementation. Plasma Na increased at parturition and decreased postpartum in all experimental groups. Plasma P decreased at parturition and increased postpartum. Plasma Mg decreased at parturition and postpartum in all experimental groups. The activity of transferase enzymes (AST and ALT) increased at parturition with no effect of supplementation. Plasma insulin decreased at parturition in all experimental groups. The information generated could be used in monitoring the nutritional status and health of dairy cows. Antioxidants, including vitamins and Se, could be used to confer protection and enhance immunity of cows, particularly during the transition period, characterized by metabolic stress and immunosuppression.

**Key words:** Dairy cows; transition period; vitamins; selenium

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## INTRODUCTION

The transition from late pregnancy to early lactation constitutes a critical period in dairy cow production cycle due to marked endocrine and metabolic changes that accompany late pregnancy, parturition and initiation of lactation (Grummer, 1993; Kehrli *et al.*, 1998; Smith and Risco, 2005; Seifi *et al.*, 2007). The physiological and nutritional changes in periparturient period could induce clinical and subclinical ailments and reproductive disorders (Duffield *et al.*, 2003; Block, 2010). The demand for energy, proteins and minerals increases dramatically with advance of pregnancy (Goff and Horst, 1997), tissue mobilization begins prepartum with the commonly observed feed intake depression during the last 3 weeks of gestation (Short *et al.*, 1990; Bertics *et al.*, 1992; Grummer *et al.*, 1995). Dairy cows may experience negative energy balance (NEB) after parturition, which is considered as metabolic adaptation to lactation that could be independent of the genetic potential of cows for milk yield (Mouffok *et al.*, 2011). During the periparturient period, adequate micronutrient intake is particularly important in order to prevent diseases around calving. As the type and quality of the dry period diet can vary considerably, it can be difficult to predict the need for extra supplementation (Meglia *et al.*, 2004). Natural and synthetic antioxidants in the feed as well as optimal levels of minerals, principally Se, help to maintain the levels of endogenous antioxidants in tissues (Pavlata *et al.*, 2004; Calamari *et al.*, 2011). The aim of this study was to investigate the changes which occur in physiological responses during the transition period in crossbred cows under tropical conditions. It was, also, intended to evaluate the effects of vitamins and Se supplementation on blood constituents, metabolites, minerals and enzymes during the transition period in crossbred dairy cows.

## MATERIALS AND METHODS

Twenty-four clinically healthy dairy cows were used. The animals were monitored for two months before and one month after calving. The cows were in their second to sixth lactation. Artificial insemination (AI) was used to induce pregnancy. The cows calved during the period November 2010 to February 2011 and were dried off 3 weeks before the predicted day of parturition. The cows were assigned to 3 groups of 8 cows each, a control group (C group) and two treated groups which received vitamin E and Selenium (Vit.E+Se group) or multivitamins: A, D, and E (MV group). The protocol of administration of supplements was at -3, -1, +1 and +3 weeks relative to parturition. The dose of vitamin E and Se received intramuscularly was 10 ml (contained 1000 mg vitamin- E- acetate and 10 mg sodium selenite) (alfasan, Woerden – Holland). The dose of multivitamins received intramuscularly was 10 ml (contained 1,000,000 IU vitamin A, 500,000 IU vitamin D<sub>3</sub> and 500 mg vitamin E (Supers Diana, Spain).

## **Blood collection and analysis**

Ten ml blood were collected from the jugular vein at 08.00 -09.00 a.m. at time points -8 , -3, -2, -1 weeks before predicted calving, at parturition (0) and + 1, +2, +3 and +4 weeks postcalving. The blood was collected into test tubes containing anticoagulant K<sub>2</sub>EDTA to determine haematological parameters. Plasma obtained by centrifugation of blood samples was used for determination of the concentrations of metabolites, minerals, osmolality, enzymes and hormones. Haematological parameters were measured using the methods described by Jain (1986). Plasma metabolites and hormone were determined using kits according to the instructions of the manufacturers. The concentration of Na was determined by flame photometry technique as described by Wootton (1974). Plasma total osmolality was determined by freezing point depression using a cryoscopic osmometer (Osmomat 030, Gonotec-Germany).

## **Statistical analysis**

The data obtained were used to compute the mean values  $\pm$  standard deviation. The analyses were performed using two way ANOVA with one repeated measure factor procedures of STATA8 system (1998) to evaluate the haematological, biochemical and hormonal responses to supplementation of vitamins and Se during the transition period. Mean values were, also, calculated from weekly values of different experimental groups in three phases: prepartum, at parturition and postpartum..

## **RESULTS AND DISCUSSION**

The results presented in Table 1 indicate that the mean BW of cows of control and supplemented groups showed significant decrease postpartum ( $P \leq 0.01$ ); the decrease was more pronounced in the control group. The postpartum weight loss is mainly due to the foetus, placenta and foetal fluids beside decrease in food intake. Marked change in BW in Holstein and Jersey cows was attributed to mobilization of body stores around parturition (Curetzy *et al.*, 2006). The mean birth weight of calves was significantly ( $P \leq 0.05$ ) greater in supplemented groups compared to the control group. These responses may be attributed to improvement of the nutritional status of cows and uterine health. The study of Lekatz *et al.* (2011) indicated that adequate Se supplementation increased muscle amino acids in ewes and foetus and increased liver glycogen in foetus during mid and late pregnancy.

The general pattern of PCV and Hb concentration values presented in Figs. 1 and 2, respectively indicates a decrease at parturition and postpartum period. PCV values for Vit E+Se group showed non-significant decrease between the periods. This pattern of response may be associated with decrease in feed intake during prepartum period. PCV

values, also, may decline in transition cows due to protein catabolism and provision of amino acids for milk yield and gluconeogenesis (Kida, 2002). Positive correlation of body condition score (BCS) with erythron parameters revealed that higher BCS would generally result in higher erythron values and lower condition score cows might experience more challenges to avoid anaemia during the transition period (Rafia *et al.*, 2012). The response to Vit+Se could be attributed to enhancement of metabolic activity with Se supplementation which may increase erythropoiesis. Selenoproteins are involved in thyroid activity, antioxidant activity and erythrocyte stability, mainly by their role as a part of enzymes (Surai, 2006). An increase in total erythrocyte count and osmotic resistance of erythrocyte has been reported in sheep supplemented Se yeast (Faixova *et al.*, 2007)

For all groups, the TLC values increased significantly ( $P \leq 0.01$ ) at parturition and at +1, +2 + 3 and +4 weeks postpartum compared to prepartum values (Fig. 3). The supplemented groups showed significantly ( $P \leq 0.05$ ) lower TLC compared to the control group at calving and at +3 and +4 weeks, respectively. Leukocytosis could be associated with gynaecological alterations during this critical period; higher TLC in the control group of cows at and postpartum period is indicative of susceptibility to infections. The values reported in Fig. 4 show that there was increase in neutrophil ratio in all experimental groups at parturition that was more pronounced in the control group. However, Vit E+Se group did not show any significant difference during the experimental period. The supplemented groups had significantly ( $P \leq 0.05$ ) lower neutrophil ratio compared to the control group at -2, 0 and +2 weeks. This response may be associated with the stress pattern of parturition associated with alterations in cellular immunity. Fig. 5 indicates that there was a decline in lymphocyte ratio at parturition in all the groups; the drop was more pronounced in the control group. The lymphocytes migration manner could be different compared to neutrophils; a hypercortisolaemia at calving may not affect the adhesion molecules of lymphocytes, and accordingly lymphocytes can migrate into the tissues (Alon *et al.*, 1995).

Fig. 6a shows that in control and supplemented groups, the monocyte ratio was higher at calving. At parturition, Vit E+Se group had significantly ( $P \leq 0.01$ ) higher monocyte ratio compared to control and MV group. The ratios of eosinophil in Fig.7 showed non-significant decrease at parturition in all the groups. Postpartum, eosinophil ratios showed a slight decrease in supplemented groups compared to respective prepartum values. Vit E+Se group showed significantly ( $P \leq 0.05$ ) lower values of eosinophil compared to control and MV group at week 3 postpartum. However, MV group showed significantly ( $P \leq 0.05$ ) lower eosinophil ratio at +4 week postpartum compared to control and Vit E + Se groups. Monocytosis and eosinopenia may occur when cows are under stress (El-Goul *et al.*, 2000). An improvement in blood neutrophil phagocytosis and killing activities in dairy cows supplemented organic Se was reported by Silvestre *et al.* (2007). A relationship between Se status of the cow around parturition

and the function of the immune system and disease resistance was suggested (Smith *et al.*, 1997). Supplementation of vitamins E and A throughout the dry period was associated with decline of incidence of mastitis (Smith *et al.*, 1984) and retained placenta (LaBlank *et al.*, 2004). Politis *et al.* (1996) suggested that supplementation of vitamin E improved the production of IL-1 and antigen expression by blood monocytes. The decrease in the ratio of eosinophils in both supplemented groups after parturition (Fig. 7a and b) may be related to the role of vitamins and Se in reducing the risk of disease and infection.

The control group showed significantly ( $P \leq 0.01$ ) lower total protein and albumin values at parturition compared to values measured pre- and postpartum (Figs. 8 and 9). There was a decrease in total protein and albumin levels at parturition in all experimental groups with no significant differences between periods in VitE+Se group. This pattern may be associated with the decrease in feed intake prepartum. During late gestation, a decrease in serum protein level could, also, be influenced by increased foetal growth, and especially the utilization of amino acids from the maternal circulation for protein synthesis in the foetal muscles (Antunovic *et al.*, 2002). A decrease in albumin level at parturition associated with decrease in Ca concentrations was attributed to the fact that one fraction of the total pool of Ca is linked to albumin and depends partly on its concentration (Goff, 2000; Seifi *et al.*, 2005). The increase in plasma total protein level in Vit E+Se supplemented cows may be attributed to the role of Se in decreased protein catabolism in muscle and an increase in essential and non-essential amino acids (Laktaz *et al.*, 2010; 2011); the regulation and synthesis of proteins in different organs and tissues are highly dependent on Se supply. The urea concentration decreased at parturition in all the groups (Figs. 10a and b). Fig. 10b shows significantly ( $P \leq 0.05$ ) lower urea concentration in control group at parturition. There was no significant difference among periods in supplemented groups. The decline in urea level at parturition may reflect reductions in DMI. The higher plasma urea level in supplemented groups is probably associated with relatively improved appetite and increased food intake and metabolic activity during transition period. Calamari *et al.* (2011) suggested enhancement of energy and protein metabolism in Vit.E+Se supplemented cows during oxidative stress.

For all experimental groups, there was decrease in cholesterol and triglyceride level at calving and postpartum (Figs. 11 and 12). This response could be associated with negative energy balance (NEB) in this period. The decrease in cholesterol concentration during the last stage of pregnancy is, also, likely due to the increased requirement of foetal tissue. However, higher plasma triglycerides in prepartum crossbred cows were attributed to decrease in their catabolism and milk production (Marcos *et al.*, 1990). The significant decrease in plasma triglyceride after parturition (Fig. 12) is attributed to various factors. Effective use of triglyceride for milk production may decrease the triglyceride level. This could be interpreted as the effect of increased lipolysis which is regulated by hormones (Holtenius and Hjort, 1990). The marked postpartum decrease in

plasma triglyceride in this study may, also, be associated with decreased insulin level (Fig. 19). This is combined with a decrease in sensitivity of adipose tissue to insulin and increase in somatotropin may reduce the synthesis of triglycerides in adipose tissue and favour their mobilization during the transition period (Lanna and Bauman, 1999).

Fig. 13 shows an increase in Na concentration in all the groups at parturition; the increase was pronounced ( $P \leq 0.05$ ) in vitE+ Se group. The postpartum decline in Na level may be associated with loss of Na in colostrum and milk. The plasma osmolality may be influenced by concentration of Na and other electrolytes around parturition. The decline in plasma osmolality in early lactation (Fig.16) may be associated with secretion of Na into colostrum and concomitant decrease in the concentration of total protein reported during the transition period. However, Chaiyabutr *et al.* (2000a) reported that the plasma osmolality remained unchanged throughout late pregnancy and lactation, suggesting that homeostasis was maintained during this period. Fig. 14 shows that P concentration was higher during prepartum period, decreased slightly around calving and then increased at +4 week postpartum in the control and supplemented groups. The decrease in plasma P level at calving could be related to the demand of colostrum and milk production; an inverse relationship between milk production and plasma P concentration was reported in lactating cows (Forar *et al.*, 1982; Kume *et al.*, 2003). An increase in P level during early lactation could, also, partly be related to relief of blood from haemodilution which usually occurs during pregnancy (Elnageeb and Abdelatif, 2010). Fig. 15 shows that there was a decrease in plasma Mg values around parturition in all the groups. Initiation of lactation and drainage of blood Mg into milk was suggested to be the main cause of decrease in blood Mg (Kume *et al.*, 1998). Kume and Tanabe (1993) reported that in HF cows, Mg level was the highest in colostrum at parturition and decreased rapidly by 24 hrs postpartum.

The AST and ALT enzyme activities presented in Figs.17 and 18 were non-significant increase at parturition in control and supplemented groups. The increase in the activity of transaminases around calving could be associated with negative energy balance status of cows. Transaminase enzyme activity reflects the stress and damage status that occur in the body (Kawashima *et al.*, 2007). The changes in the activities of these enzymes may, also, be related to reduce DMI around parturition that may lead to alteration of normal functions of the liver (Greenfield *et al.*, 2000). The rise in plasma AST activity around calving is attributable to liver damage as evidenced by increase in CK activity in Holstein cows (Elitok *et al.*, 2006). Fig. 19 indicates that for all the groups there was a slight decrease in plasma insulin level at parturition, then, increased at +3 weeks postpartum. This response may be associated with a low body score condition around calving. Sano *et al.* (1991) indicated that insulin uptake by the liver may change during lactation and pregnancy. The current pattern suggests that the physiological status might influence the plasma insulin level. The results in crossbred cows show an increase in insulin level in postpartum period (Figs. 19a and 19b). The plasma insulin level

increased during the onset of lactation compared to late pregnancy in HF cows and remained constant at a higher level during the lactating period (Chaiyabutr *et al.*, 2000b; Swali and Wathes, 2007); this response might be associated with changes in glucose turnover rate (Chaiyabutr *et al.*, 1998). A decrease in sensitivity of adipose tissue to insulin was, also, reported at the onset of lactation (Faulkner and Pollock, 1990).

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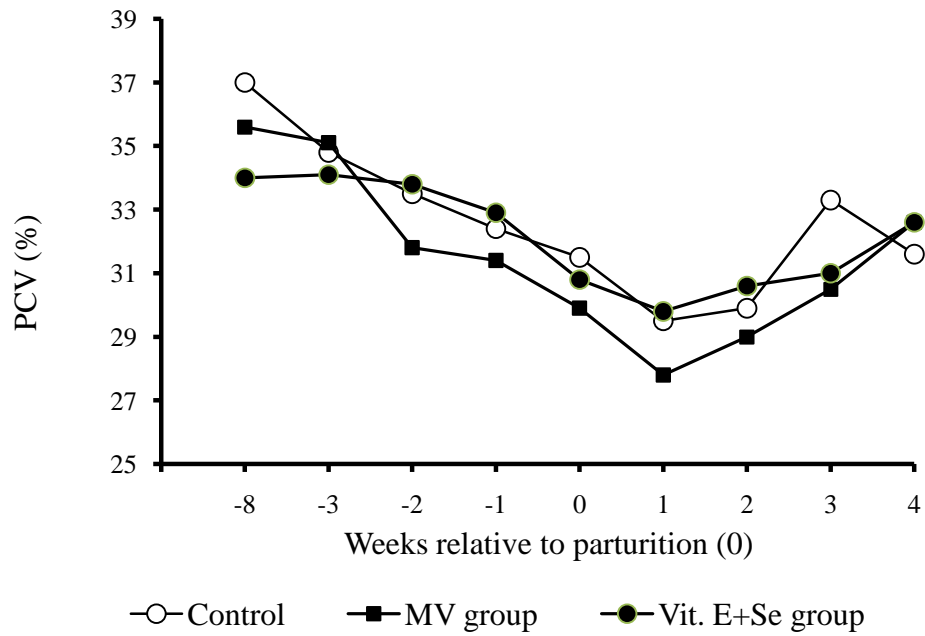
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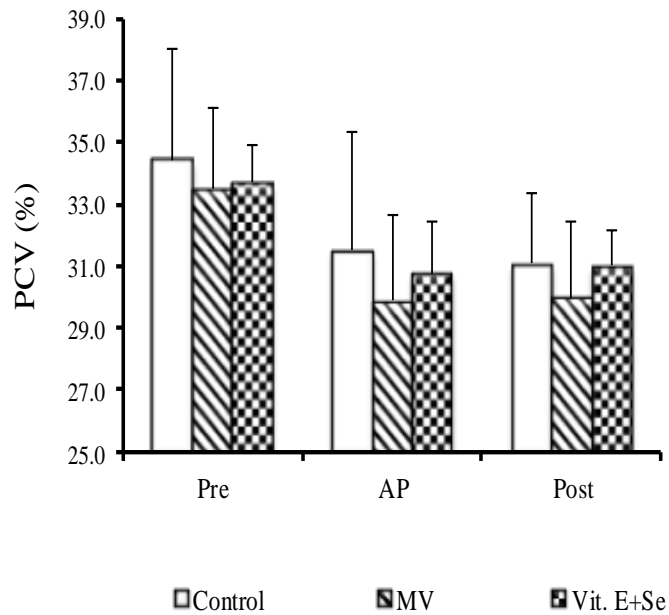
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**Table 1.** Effects of transition period and supplementation of vitamins and Se on mean body weight (BW) of cows and birth weight of calves.

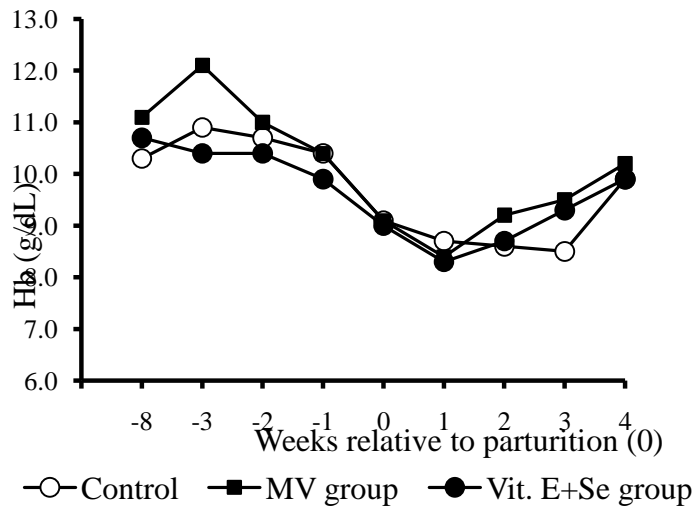
Groups	Cows BW				Calf birth Weight/kg	Placental and fluid W (%)
	Prepartum	Postpartum	Loss(%)	Loss(Kg)		
Control group	480.62 <sup>Aa</sup> ±40.04	409.4 <sup>Ba</sup> ±11.36	17.7	84.00	29.5 <sup>b</sup> ±2.45	65.30
Vit.E+Se group	470.0 <sup>Aa</sup> ±53.45	413.0 <sup>Ba</sup> ±55.06	12.3	57.0	35.88 <sup>a</sup> ± 2.75	37.74
MV group	486.88 <sup>Aa</sup> ±35.25	418.75 <sup>Ba</sup> ±41.81	14.0	68.13	35.0 <sup>a</sup> ±3.96	50.00
Mean±SD	479.3 <sup>A</sup> ±5.27	409.4 <sup>B</sup> ±11.36	14.70 ±2.67	69.71 ±13.57	33.43 ±4.20	51.01 ±13.81



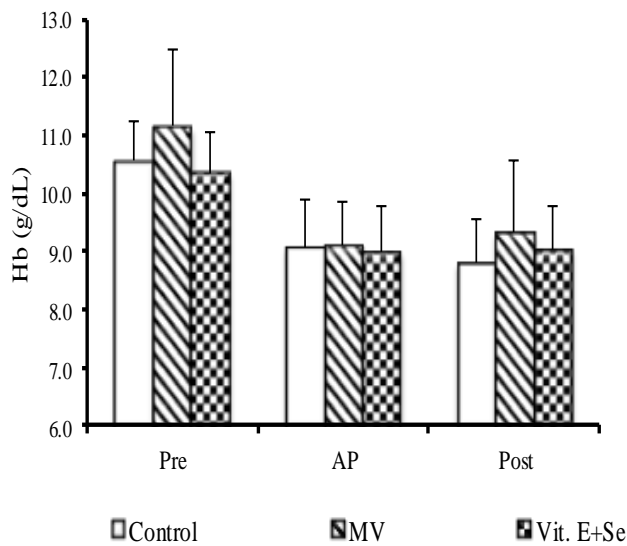
**Fig. 1 (a).** Effects of transition period and supplementation of multivitamins (MV) and vitamin E+ selenium(Vit.E+Se) on packed cell volume (PCV) in crossbred dairy cows .



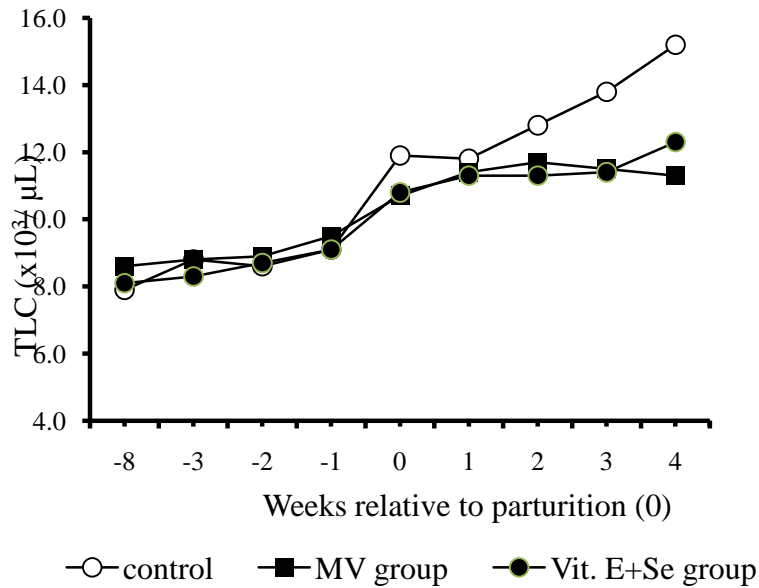
**Fig. 1(b).** Mean values of packed cell volume (PCV) at precalving (Pre), at parturition (AP) and postcalving (Post) as influenced by multivitamins (MV) and vitamin E+selenium (Vit.E+Se) supplementation in crossbred dairy cows.



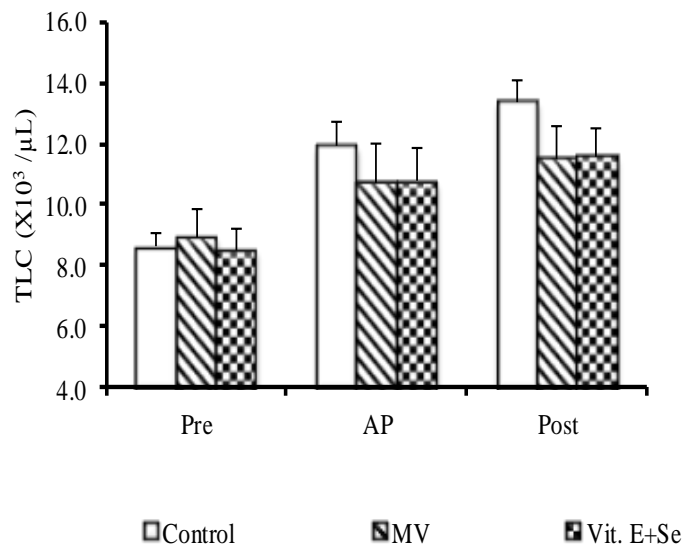
**Fig. 2(a).** Effects of transition period and supplementation of multivitamins (MV) and vitamin E+selenium (VitE+Se) on haemoglobin concentration (Hb) in crossbred dairy cows .



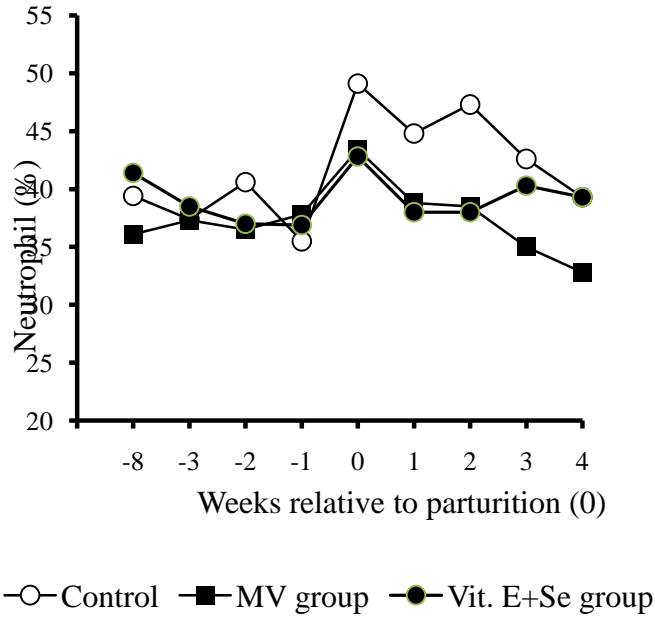
**Fig. 2(b).** Mean values of haemoglobin concentration (Hb) at precalving (Pre), at parturition (AP) and postcalving (Post) as influenced by multivitamins (MV) and vitamin E +selenium (Vit.E+Se) supplementation in crossbred dairy cows.



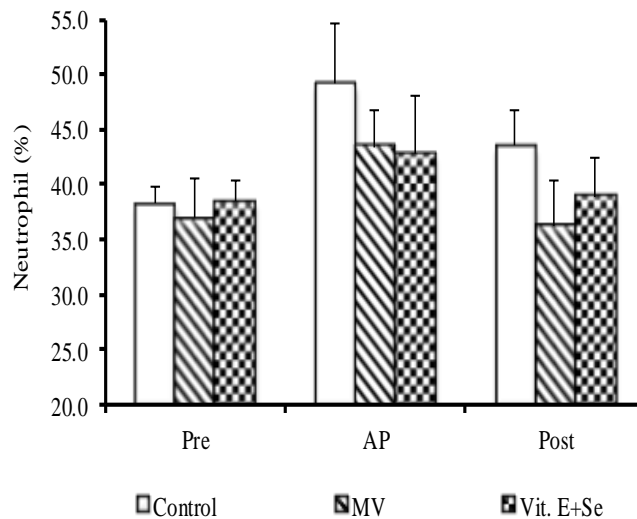
**Fig. 3(a).** Effects of transition period and multivitamins (MV) and vitamin E+selenium (Vit.E+Se) supplementation on total leukocyte count (TLC) in crossbred dairy cows .



**Fig. 3(b).** Mean values of total leukocyte count (TLC) at precalving (Pre) , at parturition (AP) and postcalving (Post) as influenced by multivitamins (MV) and vitamin E +selenium (Vit.E+Se) supplementation in crossbred dairy cows.

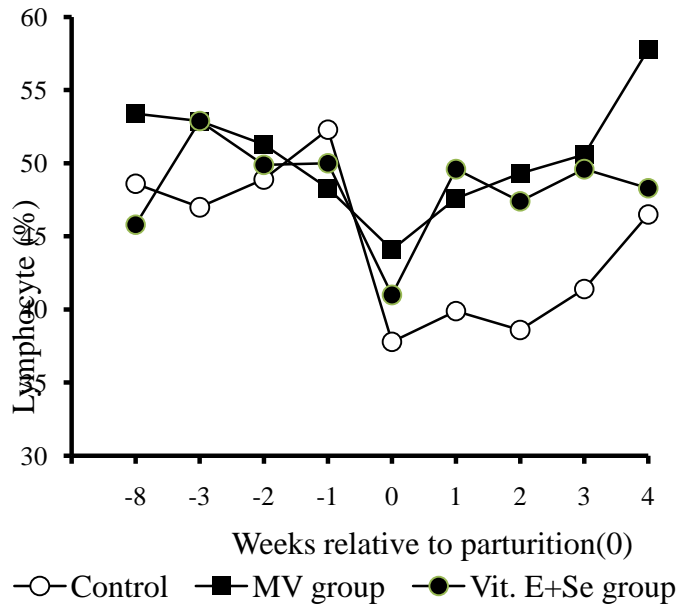


**Fig. 4 (a).** Effect of transition period and supplementation of multivitamins (MV) and vitamin E+ selenium (Vit.E+Se) on neutrophil ratio in crossbred dairy cows .

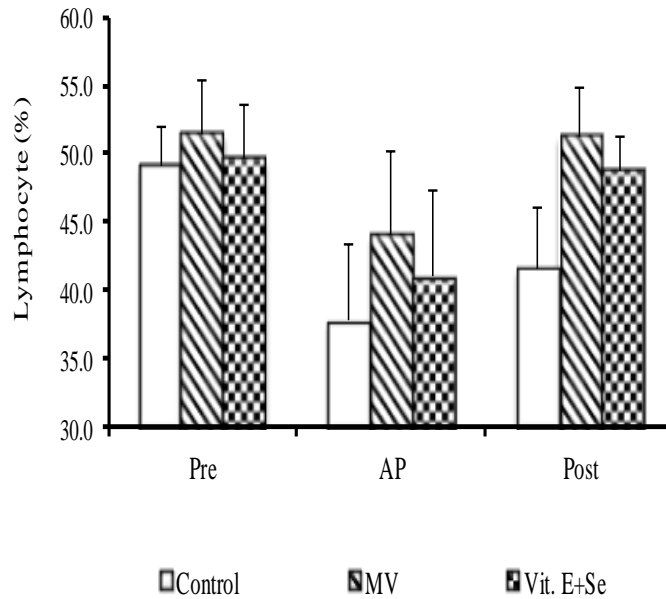


**Fig 4(b).** Mean values of neutrophil ratio at precalving (Pre) , at parturition (AP) and postcalving (Post) as influenced by multivitamins (MV) and vitamin E + selenium (Vit.E+Se) supplementation in crossbred dairy cows.



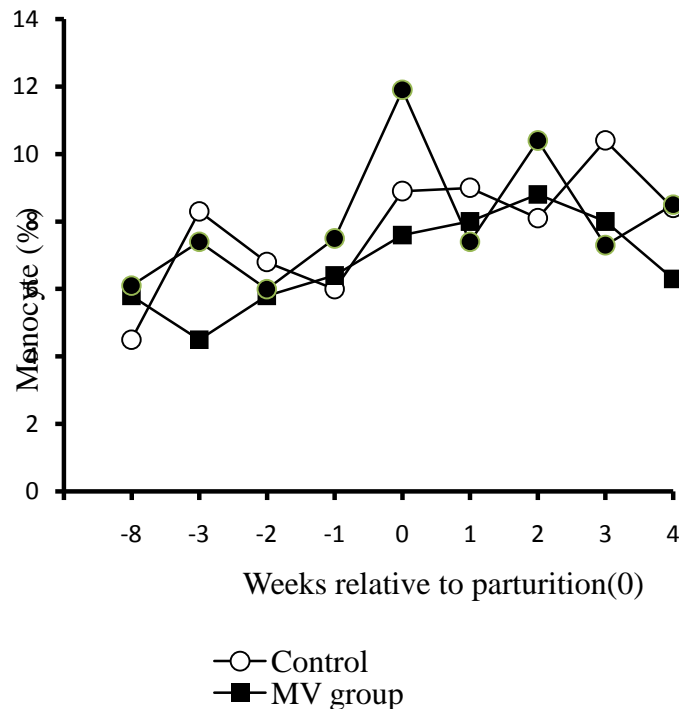


**Fig. 5 (a).** Effects of transition period and supplementation of multivitamins (MV) and vitamin E+selenium (Vit.E+Se) on lymphocyte ratio in crossbred dairy cows.

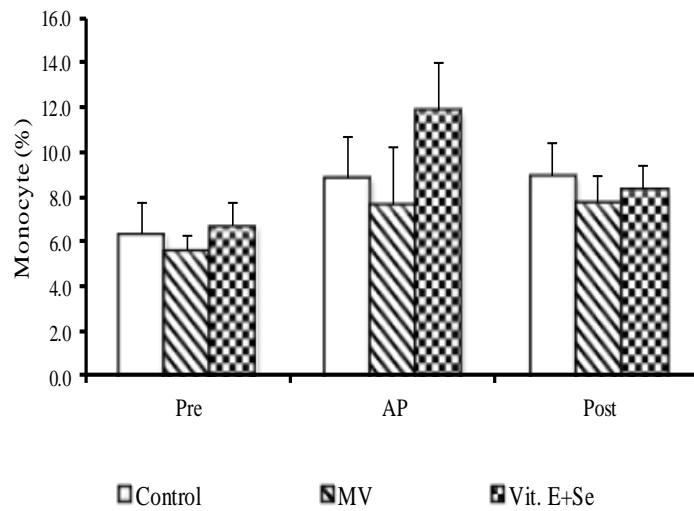


**Fig. 5(b).** Mean values of lymphocyte ratio at precalving (pre) , at parturition (AP) and postcalving (Post) as influenced by multivitamins (MV) and

vitamin E + selenium (Vit.E+Se) supplementation in crossbred dairy cows.

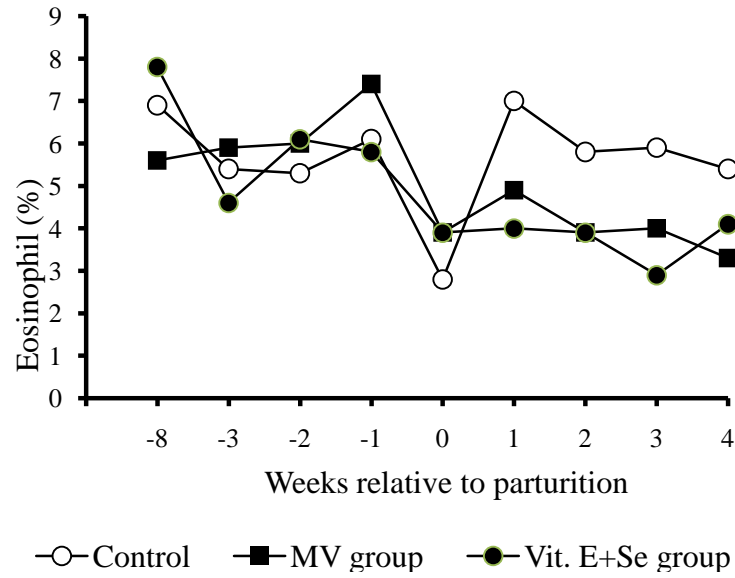


**Fig. 6(a).** Effects of transition period and supplementation of multivitamins (MV) and vitamin E+selenium (Vit.E+Se) on monocyte ratio in crossbred dairy cows .

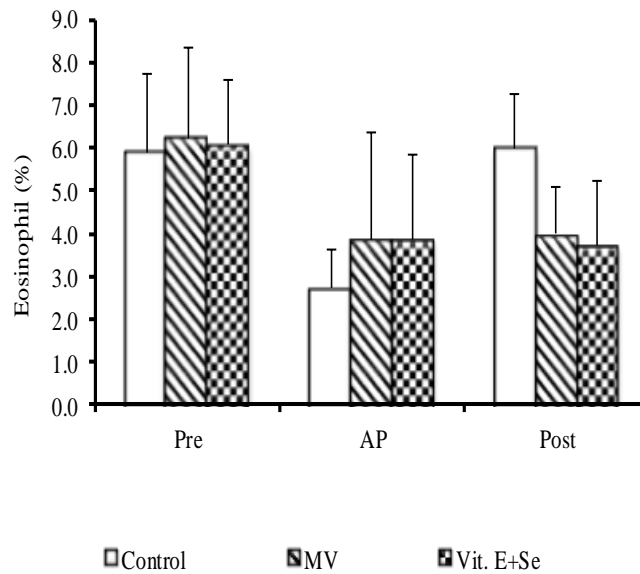


**Fig. 6 (b).** Mean values of monocyte ratio at precalving (Pre) , at parturition (AP) and postcalving (Post) as influenced by multivitamins (MV) and

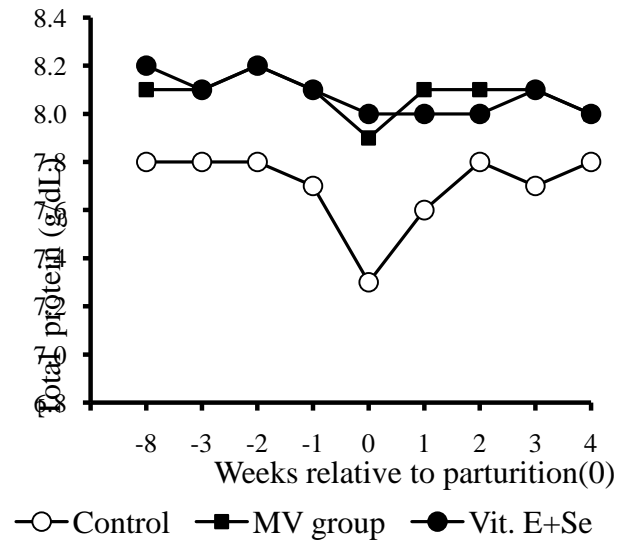
vitamin E + selenium (Vit.E+Se) supplementation in crossbred dairy cows.



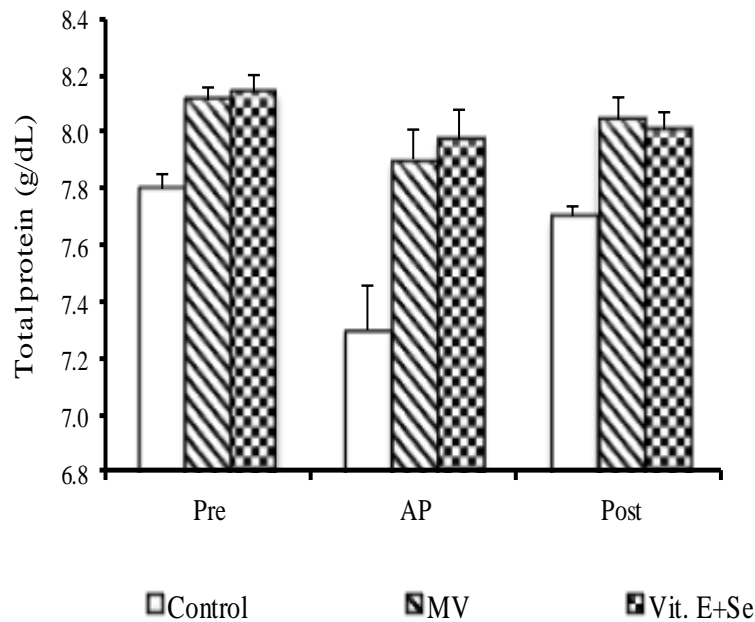
**Fig. 7(a).** Effects of transition period and supplementation of multivitamins (MV) and vitamin E+selenium (Vit.E+Se) on eosinophil ratio in crossbred dairy cows .



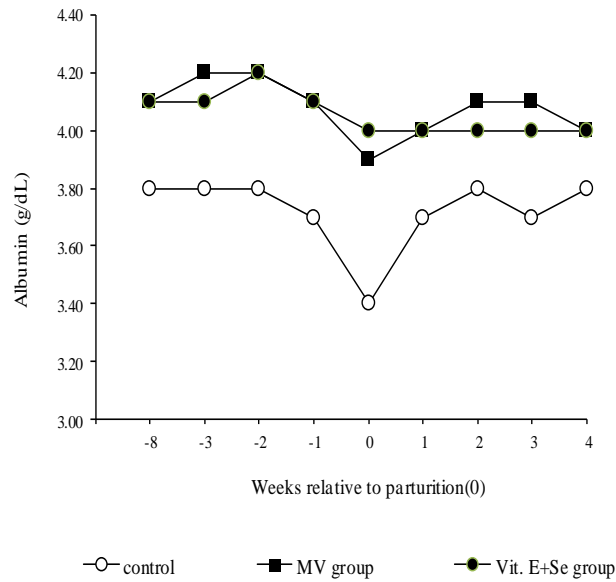
**Fig.7 (b).** Mean values of eosinophil ratio at precalving (Pre), at parturition (AP) and postcalving (Post) as influenced by multivitamins (MV) and vitamin E + selenium (Vit.E+Se) supplementation in crossbred dairy cows.



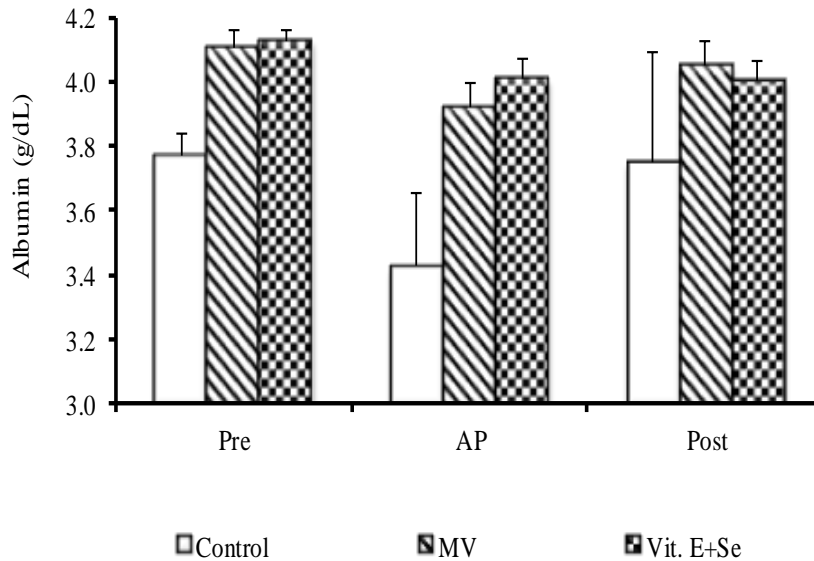
**Fig. 8(a).** Effect of transition period and supplementation of multivitamins (MV) and vitamin E+selenium (Vit.E+Se) on plasma total protein concentration in crossbred dairy cows.



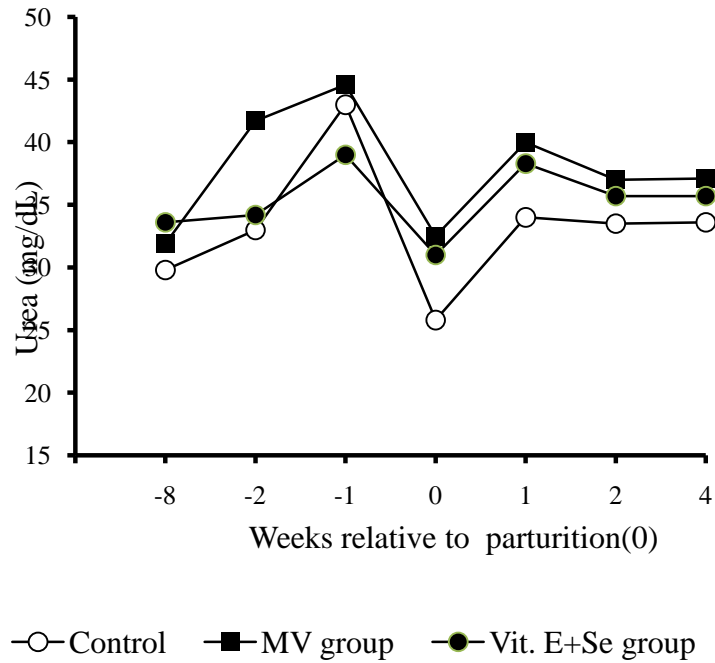
**Fig. 8(b).** Mean values of plasma total protein at precalving (Pre), at parturition (AP) and postcalving (Post) as influenced by multivitamins (MV) and vitamin E + selenium (Vit.E+Se) supplementation in crossbred dairy cows.



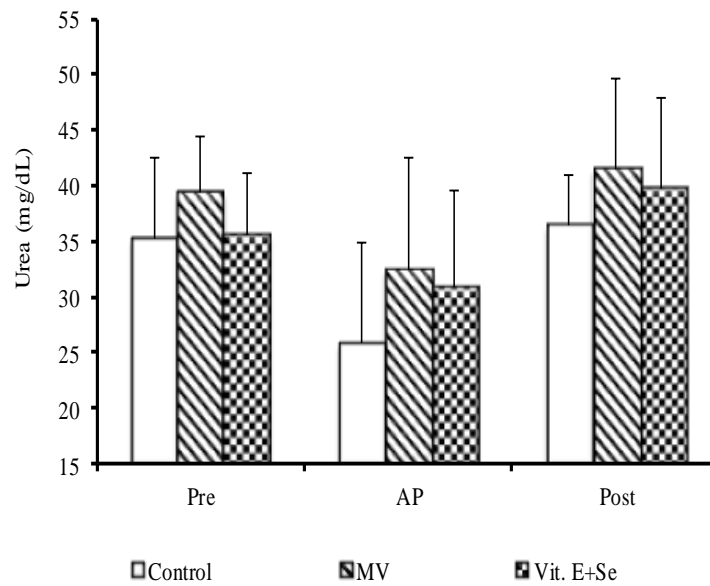
**Fig. 9(a).** Effect of transition period and supplementation of multivitamins (MV) and vitamin E+selenium (Vit.E+Se) on plasma albumin concentration in crossbred dairy cows .



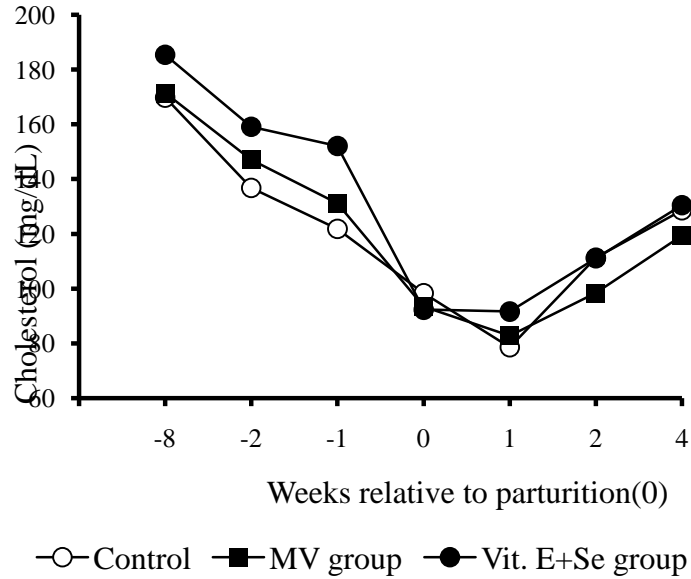
**Fig. 9(b).** Mean values of plasma albumin at precalving (Pre), at parturition (AP) and postcalving (Post) as influenced by multivitamins (MV) and vitamin E + selenium (Vit.E+Se) supplementation in crossbred dairy cows.



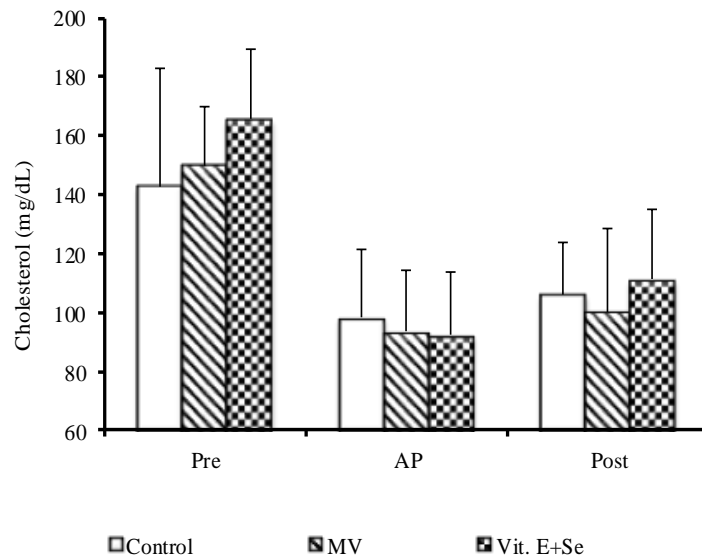
**Fig. 10(a).** Effect of transition period and supplementation of multivitamins (MV) and vitamin E+selenium (Vit.E+Se) on plasma urea concentration in crossbred dairy cows .



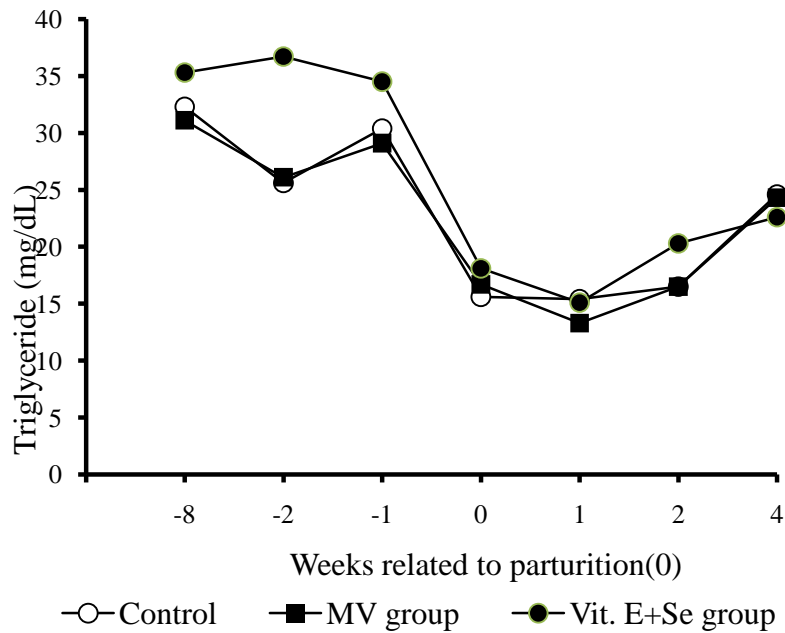
**Fig. 10(b).** Mean values of plasma urea concentration at precalving (Pre), at parturition (AP) and postcalving (Post) as influenced by multivitamins (MV) and vitamin E + selenium (Vit.E+Se) supplementation in crossbred dairy cows.



**Fig. 11(a).** Effect of transition period and supplementation of multivitamins (MV) and vitamin E + selenium (Vit.E+Se) on plasma cholesterol concentration in crossbred dairy cows.

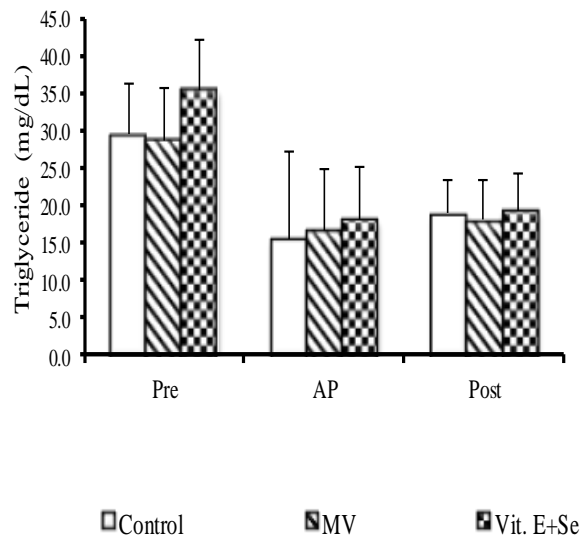


**Fig. 11(b).** Mean values of plasma cholesterol concentration at precalving (pre), at parturition (AP) and postcalving (Post) as influenced by multivitamins (MV) and vitamin E + selenium (Vit.E+Se) supplementation in crossbred dairy cows.

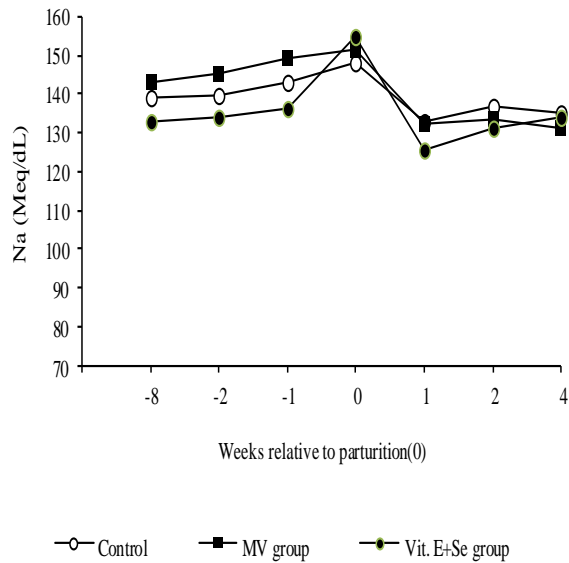


**Fig. 12(a).** Effect of transition period and supplementation of multivitamins (MV) and vitamin E + selenium (Vit.E+Se) on plasma triglyceride concentration in crossbred dairy cows.

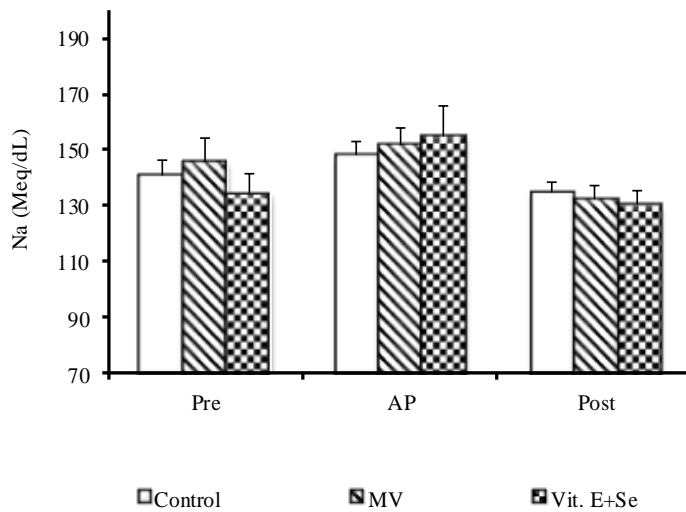




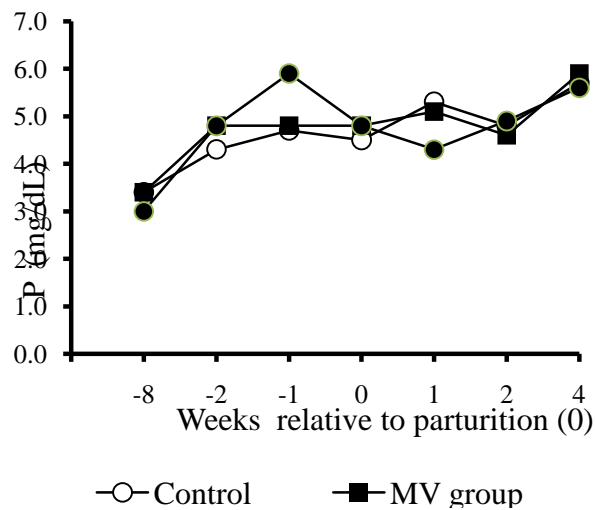
**Fig. 12(b).** Mean values of plasma triglyceride concentration at precalving (pre), at parturition (AP) and postcalving (Post) as influenced by multivitamins (MV) and vitamin E + selenium (Vit.E+Se) supplementation in crossbred dairy cows.



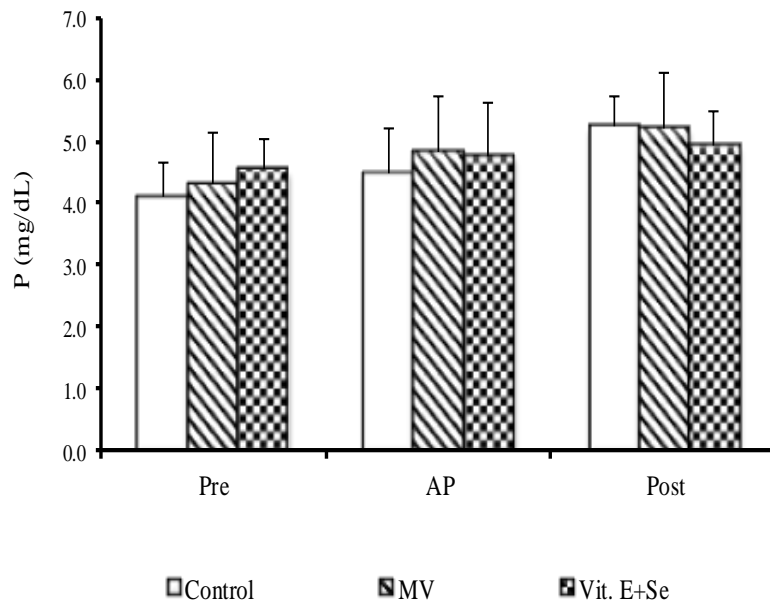
**Fig.13(a).** Effect of transition period and supplementation of multivitamins (MV) and vitamin E+selenium (Vit.E+Se) on plasma sodium(Na) in crossbred dairy cows .



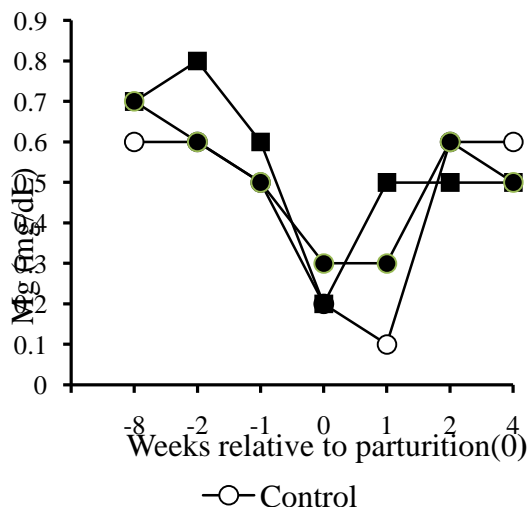
**Fig. 13(b).** Mean values of plasma sodium concentration (Na) precalving (Pre), at parturition (AP) and postcalving (Post) as influenced by multivitamins (MV) and vitamin E+selenium (Vit.E+Se) supplementation in crossbred dairy cows.



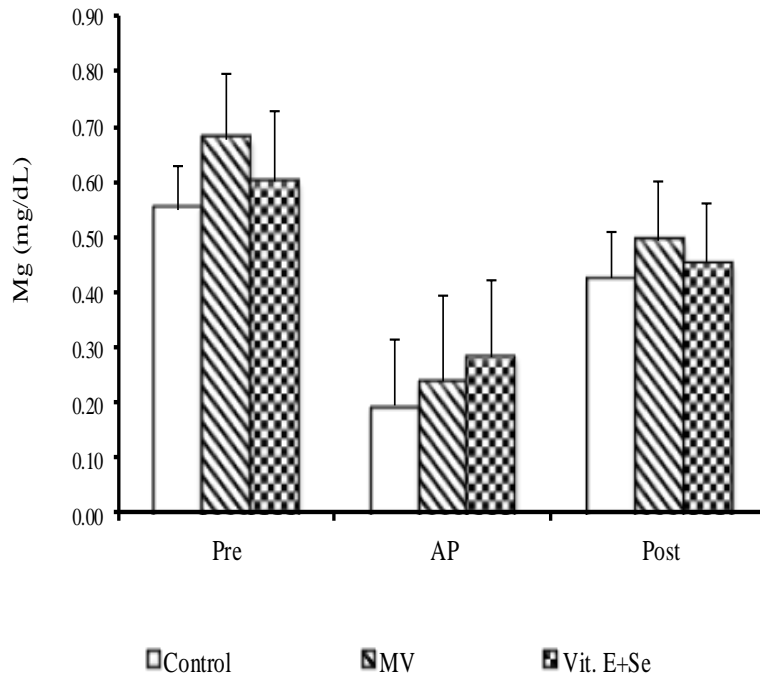
**Fig. 14(a).** Effect of transition period and supplementation of multivitamins (MV) and vitamin E+selenium (Vit.E+Se) on plasma phosphorus (P) concentration in crossbred dairy cows .



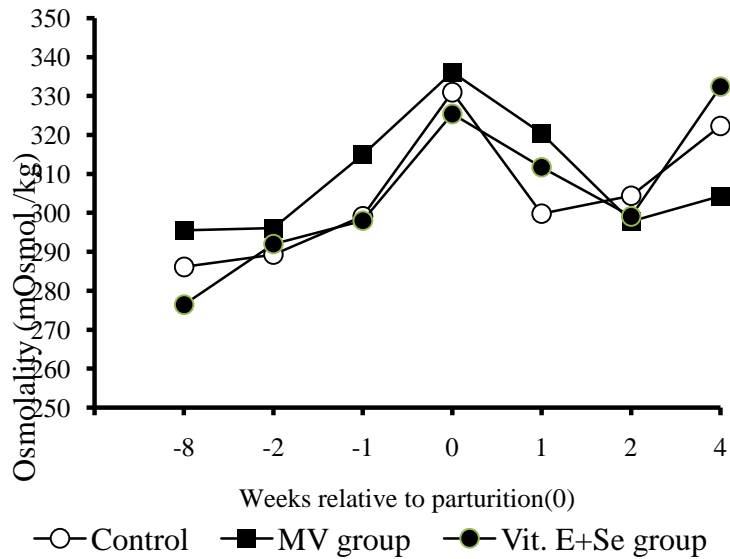
**Fig. 14(b).** Mean values of plasma phosphorus concentration (P) at precalving (Pre), parturition (AP) and postcalving (Post) as influenced by multivitamins (MV) and vitamin E+selenium supplementation in crossbred dairy cows.



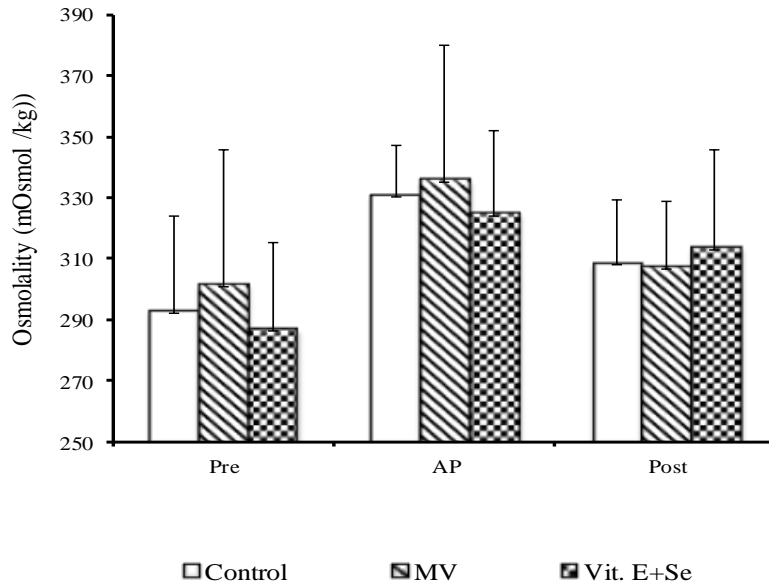
**Fig. 15(a).** Effect of transition period and supplementation of multivitamins (MV) and vitamin E+selenium (Vit.E+Se) on plasma magnesium (Mg) concentration in crossbred dairy cows .



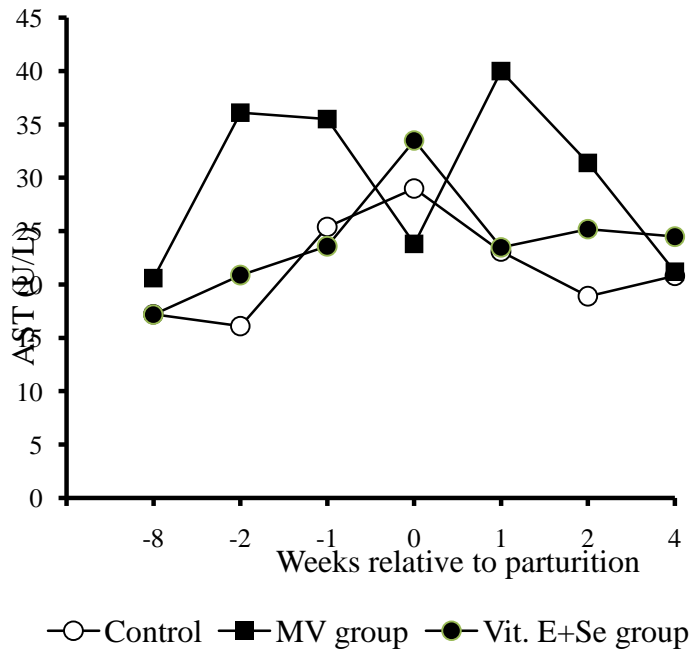
**Fig. 15(b).** Mean values of plasma magnesium concentration (Mg) at precalving (Pre), at parturition (AP) and postcalving (Post) as influenced by multivitamins (MV) and vitamin E+selenium supplementation in crossbred dairy cows.



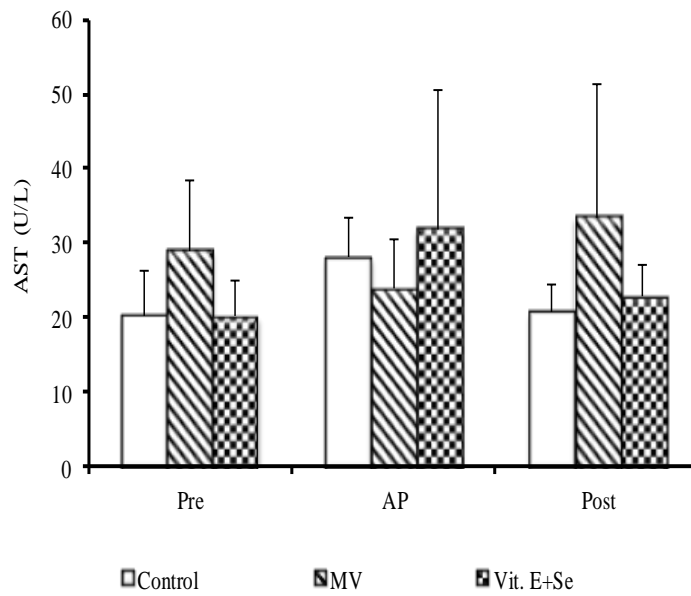
**Fig. 16(a).** Effect of transition period and supplementation of multivitamins (MV) and vitamin E+selenium(Vit.E+Se) on plasma osmolality in crossbred dairy cows .



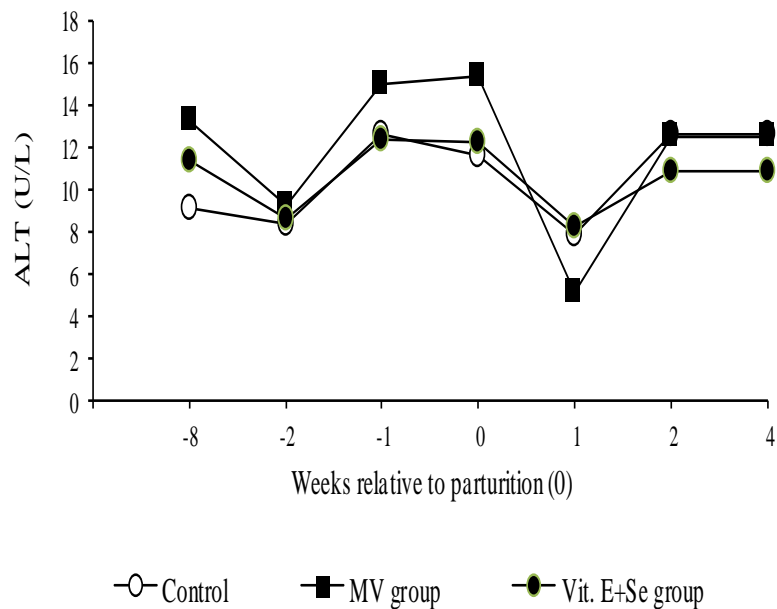
**Fig. 16(b).** Mean values of plasma osmolality at precalving (Pre), at parturition (AP) and postcalving (Post) as influenced by multivitamins (MV) and vitamin E+selenium (Vit.E+Se) supplementation in crossbred dairy cows



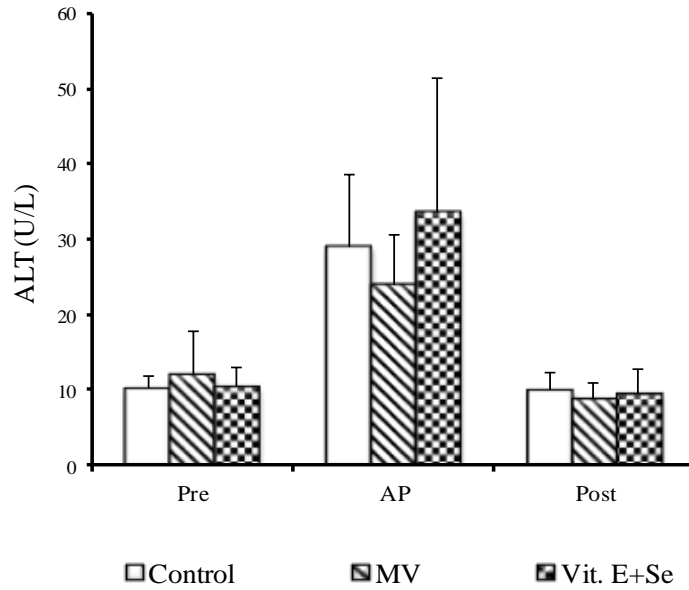
**Fig. 17(a).** Effect of transition period and supplementation of multivitamins (MV) and vitamin E+selenium (Vit.E+Se) on plasma aspartate aminotransferase (AST) activity in crossbred dairy cows .



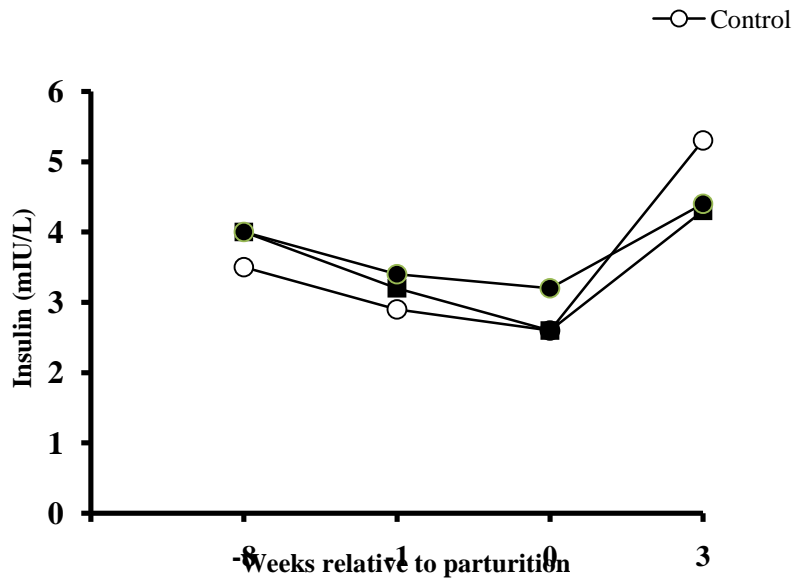
**Fig. 17(b).** Mean values of plasma aspartate aminotransferase (AST) activity at precalving (Pre), at parturition (AP) and postcalving (Post) as influenced by multivitamins (MV) and vitamin E+selenium (Vit.E+Se) supplementation in crossbred dairy cows.



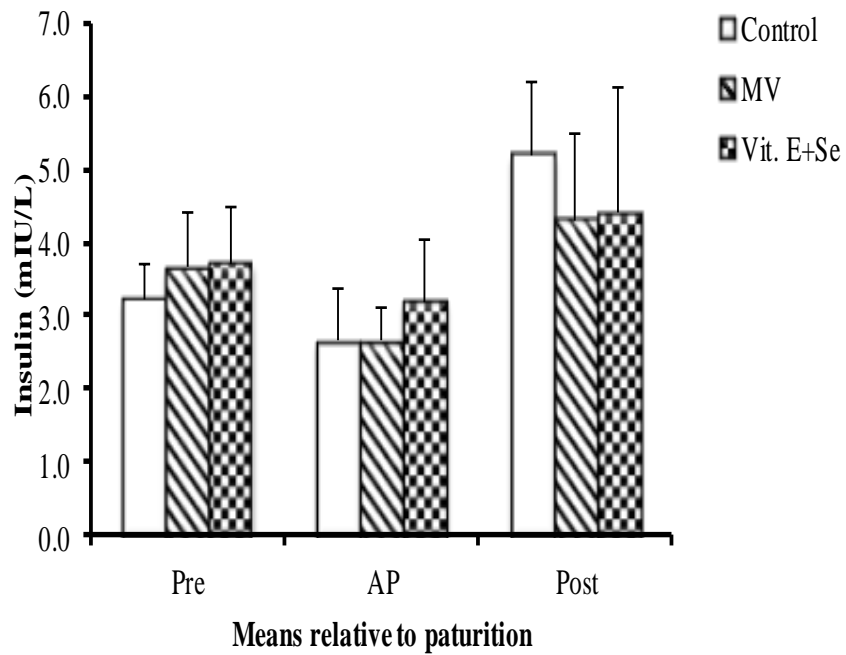
**Fig. 18(a).** Effect of transition period and supplementation of multivitamins (MV) and vitamin E+selenium (Vit.E+Se) on plasma alanin aminotransferase (ALT) activity in crossbred dairy cows .



**Fig. 18(b).** Mean values of plasma alanin aminotransferase (ALT) activity at precalving (Pre), at parturition (AP) and postcalving (Post) as influenced by multivitamins (MV) and vitamin E+selenium (Vit.E+Se) supplementation in crossbred dairy cows.



**Fig. (19a).** Effect of transition period and supplementation of multivitamins (MV) and vitamin E+selenium on plasma insulin level in crossbred dairy cows.



**Fig. (19b).** Mean values of plasma insulin level at precalving (Pre), parturition (AP) and postcalving (Post) as influenced by multivitamins (MV) and vitamin E+selenium supplementation in crossbred dairy cows.