

## Effect of Dietary Vitamin E Supplementation on Serum $\alpha$ -Tocopherol and Immune Status of Crossbred Calves

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**ABSTRACT :** An experiment was conducted with twenty crossbred male calves (7-15 days old) divided into 4 different experimental groups on the basis of body weights to study the effect of vitamin E supplementation on the serum  $\alpha$ -tocopherol concentration and immune response of the calves. All the calves were fed on milk and calf starter up to 13 weeks and afterwards, they were fed on concentrate mixture and oat hay up to 32 weeks of age. In addition, the calves in groups I, II, III and IV were supplemented with 0, 125, 250 and 500 IU feed grade DL- $\alpha$ -tocopheryl acetate, respectively. Blood samples were collected at 0 day and subsequently at 1, 2, 3, 4, 6 and 8 months of age to monitor the serum  $\alpha$ -tocopherol concentration in crossbred calves. After 24 weeks of experimental feeding, 4 animals from each group were intramuscularly inoculated with single dose (3 ml) of *Haemorrhagic septicaemia* (*Pasteurella multocida* P52 strain) oil adjuvant vaccine. The cumulative group mean serum  $\alpha$ -tocopherol concentration ( $\mu\text{g}/100\text{ ml}$ ) was 88.12, 210.11, 235.21 and 294.02 in-groups I, II, III and IV, respectively and differed significantly ( $p < 0.001$ ) among the four groups. Lymphocyte stimulation indices (LSI) did not differ among the groups significantly. The pooled mean ELISA antibody titer against *Pasteurella multocida* (P52 strain) was 788.02, 926.85, 1,214.00 and 1,109.51 for group I, II, III and IV, respectively, which indicated higher antibody titer in groups supplemented with vitamin E as compared to the control group. It may be concluded that vitamin E supplementation increased the  $\alpha$ -tocopherol concentration in serum and dietary supplementation of vitamin E at higher level has a humoral immune enhancing effect against killed bacterial antigen. (*Asian-Aust. J. Anim. Sci.* 2006. Vol 19, No. 4 : 500-506)

**Key Words :** Vitamin E, Serum  $\alpha$ -Tocopherol, Crossbred Calves, Immune Response, ELISA, LTT

### INTRODUCTION

Nutrition plays a significant role in the development and function of the immune system (Biesel, 1982; Sheffy and Wilson, 1982). Continued high morbidity and mortality of calves warrant research emphasis on optimizing the immune response and necessitate improving the understanding of the role of nutrition. In this context, vitamin E, the most effective chain breaking, lipid soluble antioxidant in the biological membrane of all cells, has recently shown potential beneficial effect on immune system. Newborn animals usually have a low blood tocopherol, probably because of poor placental transfer (Paulson et al., 1968). Calves on low vitamin E diets are likely to become deficient within first few weeks, unless supplemented (Siddons and Mills, 1981). Serum  $\alpha$ -tocopherol concentration in calves fed on diets supplemented with  $\alpha$ -tocopherol increased steadily (Reddy et al., 1986; Hill, 1987; Walsh et al., 1993; Kahlon and Raj Vir Singh, 2004). NRC (2001) has increased the requirement of vitamin E for calves by 25% i.e. from 40 IU/kg of dietary DM to 50 IU/kg. This increase is based on

the fact that although 40 IU vitamin E/kg DM is adequate to prevent classical signs of vitamin E deficiency, such as muscular dystrophy or retardation of growth of calves in controlled system, but calves under conditions of stress, which is common in practice, might require higher dose of vitamin E to augment the immune system. With respect to immunocompetency, higher levels of vitamin E in the diet of calves have been found to influence both cellular (Cipriano et al., 1982) and humoral immune responses positively (Reddy et al., 1985). The protective effects of vitamin E on animal health may be involved with its role in reduction of glucocorticoids, which are known to be immunosuppressor (Orth, 1992). Objectives of the present experiment were to study the effect of graded levels of supplemental vitamin E on the  $\alpha$ -tocopherol concentration in blood serum and humoral and cell mediated immune response of male crossbred cattle calves.

### MATERIALS AND METHODS

#### Animal's management and feeding

Twenty male crossbred (*Bos indicus* × *Bos Taurus*) calves (7-15 days old, 22.7 kg average body weight) were procured on staggered basis as and when they were born from Livestock Production Research Unit of Indian Veterinary Research Institute, Izatnagar and distributed randomly in four different experimental groups on the basis of their body weight. During the experiment, the calves

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were kept in well-ventilated shed with individual feeding and watering arrangements. Crossbred calves in 4 groups were fed on milk and concentrate mixture up to 91 days of their age, afterwards they were fed on concentrate mixture and oat hay in the ratio of 1:1. Concentrate mixture comprised of crushed maize, soybean meal, wheat bran, mineral mixture and common salt in the ratio of 40:30:27:2:1. Rovimix was added in the concentrate mixture at 20g/q to fulfill vitamin A and D requirements. Calves were fed to meet their nutrient (DM and CP) requirements as per NRC (1989). In addition, the calves in group I, II, III and IV were supplemented with 0, 125, 250 and 500 IU feed grade DL- $\alpha$ -tocopheryl acetate, respectively. This feeding practice lasted for 8 months.

### Blood collection and vaccination of animals

About 10 ml blood was collected from the jugular vein for collection of serum from each calf at the beginning and subsequently at 1, 2, 3, 4, 6 and 8 months after experimental feeding. Serum samples were preserved in deep freezer (-20°C) in sterile plastic vials till the completion of biochemical analysis. At 24 weeks of experimental feeding 4 animals from each group were intramuscularly inoculated with single dose (3 ml) of Haemorrhagic septicaemia oil adjuvant vaccine and blood was collected at different days post vaccination to study the immune response.

### Analytical techniques

*Estimation of  $\alpha$ -tocopherol in serum* : Alpha-tocopherol in blood serum was estimated by high performance liquid chromatography (HPLC) as per the method given by McMurray and Blanchflower (1979) with minor modification. A STR ODS II (150 mm $\times$ 4.00 mm ID) reverse phase column and flow rate of 2 ml/min and methanol/water (95:5) mobile phase were used with fluorescence excitation at 296 nm and emission at 330 nm. Blood serum (0.5 ml) was pipette into a glass tube, and then 1 ml ethanol (90% ethanol containing 3% ascorbic acid) was added with mixing to precipitate protein followed by 2 ml of n-hexane. Then, the mixture was shaken vigorously for 60 sec on a vortex mixer and then the tube was kept in ice containing common salt for some time until the lower portion was freeze, then the upper hexane layer was decanted in a clean test tube. The process was repeated thrice. Then the combined hexane phase was dried in a vacuum oven at 70°C under nitrogen atmosphere. The residue was dissolved in appropriate quantity of n-hexane. 20  $\mu$ l of this dissolved residue was injected to HPLC after filtering it through 0.22- $\mu$  filter paper.

### Immunological response studies

*Preparation of experimental HS oil adjuvant vaccine* : The experimental vaccine batch was prepared in the Division of Biological Standardization, IVRI, Izatnagar.

*Pasteurella multocida* P<sub>52</sub> (Type Culture Lab., IVRI) was grown in six Roux flasks containing approximately 125 ml Brain Heart Infusion agar (Hi-Media, India). After incubation for 18 h at 37°C, the growth from each flask was harvested in 10-15 ml of sterilized 0.15 M NaCl containing 0.5% (v/v) formalin using sterilized glass beads. Purity of the harvest from each bottle was checked under microscope in wet film and after Gram staining. The pure harvests were pooled and kept at 37°C for 24 h. The pure pooled harvest of P<sub>52</sub> cells in formal saline were filtered through sterilized pad of muslin cloth and the filtrate was centrifuged at 5,000 rpm (Remi centrifuge, India) for 15 minutes. The pellet was washed three times in formal saline by centrifugation. Finally, the washed sediment was re-suspended in formal saline and matched with Brown's opacity tube no. 7. This suspension was finally mixed with oil adjuvant as per the method described by Bain et al. (1982). Placing a drop of emulsion on cold-water surface checked stability of the water in oil emulsion. The vaccine was stored at 4°C until used. Sterility and safety of the experimental vaccine was tested as per the procedure laid down in schedule F1 of Drug and Cosmetics Act of Govt. of India (1979).

### Humoral immune response by enzyme linked immunosorbent assay (ELISA)

After 24 weeks of experimental feeding, 4 animals from each group were intramuscularly inoculated with single dose (3 ml) of *Haemorrhagic septicaemia* oil adjuvant vaccine. The blood samples were collected at 0, 14, 28, 42 and 56 days of post vaccination (DPV) for determination of humoral immune response. About 10 ml of blood was collected in clean-labeled tubes and the harvested serum was carefully transferred to the clean-labeled plastic vials and stored in deep freeze for further analysis by ELISA.

### Preparation of sonicated antigen for ELISA

Roux flask containing Brain Heart Infusion (Hi-Media) was inoculated with pure nutrient broth culture of *Pasteurella multocida* P<sub>52</sub> and incubated at 37°C for 18 h. The culture was harvested with NSS and sterilized glass beads. Purity of harvested culture was confirmed by Gram's staining technique and the contents were centrifuged at 5,000 rpm for 30 min. The sediments were washed twice with NSS and finally suspended in NSS to a density match with Brown's opacity tube no.7. The antigen was disrupted by 100-watt ultrasonic disintegrator at 6 $\mu$  amplitude peak to peak for 30 min and used for ELISA (Almeida et al., 1979). The protein concentration was measured as per the method of Lowry et al. (1951) and the concentration of protein used for ELISA was 10  $\mu$ g/50  $\mu$ g of the sonicated antigen.

### Dilution of test sera samples

A final 1:50 dilution of serum sample was obtained by adding 5  $\mu$ l serum to 195  $\mu$ l of dilution buffer in each well

**Table 1.** Effect of graded levels of vitamin E supplementation on serum  $\alpha$ -tocopherol concentration ( $\mu\text{g}/\text{dl}$ ) in crossbred calves

Attributes	I	II	III	IV	Pooled *** mean $\pm$ SE
0 day	68.14 $\pm$ 4.70	69.79 $\pm$ 7.95	70.25 $\pm$ 23.55	68.35 $\pm$ 20.87	69.13 <sup>P</sup> $\pm$ 7.34
1st month**	103.39 <sup>a</sup> $\pm$ 23.87	240.82 <sup>b</sup> $\pm$ 30.44	289.59 <sup>b</sup> $\pm$ 47.09	397.81 <sup>c</sup> $\pm$ 36.63	257.90 <sup>st</sup> $\pm$ 31.59
2nd month**	92.00 <sup>a</sup> $\pm$ 15.41	310.77 <sup>b</sup> $\pm$ 42.93	347.22 <sup>b</sup> $\pm$ 35.02	448.18 <sup>c</sup> $\pm$ 28.72	299.54 <sup>t</sup> $\pm$ 36.50
3rd month**	72.64 <sup>a</sup> $\pm$ 9.54	265.84 <sup>b</sup> $\pm$ 39.00	244.39 <sup>b</sup> $\pm$ 30.40	334.14 <sup>b</sup> $\pm$ 40.88	229.25 <sup>rs</sup> $\pm$ 28.78
4th month**	74.13 <sup>a</sup> $\pm$ 15.03	245.44 <sup>b</sup> $\pm$ 9.16	277.375 <sup>b</sup> $\pm$ 16.88	272.37 <sup>b</sup> $\pm$ 20.03	217.43 <sup>rs</sup> $\pm$ 22.71
6th month**	107.28 <sup>a</sup> $\pm$ 6.11	200.54 <sup>ab</sup> $\pm$ 38.81	221.68 <sup>ab</sup> $\pm$ 34.04	306.71 <sup>b</sup> $\pm$ 50.18	209.05 <sup>r</sup> $\pm$ 24.42
8th month**	99.26 <sup>a</sup> $\pm$ 24.26	137.59 <sup>ab</sup> $\pm$ 11.18	195.56 <sup>ab</sup> $\pm$ 37.95	230.94 <sup>b</sup> $\pm$ 48.93	165.84 <sup>q</sup> $\pm$ 19.99
Pooled***	88.12 <sup>a</sup> $\pm$ 6.03	210.11 <sup>b</sup> $\pm$ 17.62	235.21 <sup>b</sup> $\pm$ 19.22	294.02 <sup>c</sup> $\pm$ 25.25	
Mean $\pm$ SE					

\*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

a, b, c, d Means bearing different superscripts in a row differ significantly.

p, q, r, s, t Means bearing different superscripts in a column differ significantly.

of an uncoated protein binding 96 well micro titer plate. The diluted sera samples were allowed to equilibrate in dilution buffer for 5 min prior to transferring to antigen coated ELISA plate.

#### Preparation of controls

The positive control serum (against *P. multocida*) and negative control serum of calves were diluted in dilution buffer to get a dilution of 1:100. Normal non-immunized calf serum was taken as negative control.

#### Test proper

For the test, the procedure laid down by Almeida et al. (1979) for ELISA was followed. Polystyrene plates (Griener, Germany) wells were covered individually with 50  $\mu\text{l}$  of *P. multocida* (P52 strain) antigen containing approximately 10  $\mu\text{g}$  protein. Then 50  $\mu\text{l}$  of coating buffer (pH 9.6) was added to each well and the plates were incubated at 4°C in a refrigerator for 24 h. Excess buffer solution from the well was discarded by inversion and 50  $\mu\text{l}$  of methyl alcohol was added to each well and incubated at 37°C for 2 h for drying. The antigen-coated plates were washed twice with washing solution. 50  $\mu\text{l}$  of serum dilution buffer was added to each well with the exception of first three and last three wells of the first and sixth row. 50  $\mu\text{l}$  of unknown diluted (1:50) serum was added to each corresponding marked well of antigen coated test plate producing a final dilution of 1:100. The plate was then incubated at 37°C for 2 h. The liquid was tapped out from each well and then each well was washed with washing solution. A soaking time of 3 min was allowed and the procedure was repeated twice. 100  $\mu\text{l}$  of suitably diluted conjugate (antibovine HRPO conjugate, procured from National Institute of Immunology, New Delhi) was then dispensed into each assay well and the plate was incubated for 90 min at 37°C. Again the plate was washed twice with washing solution. 100  $\mu\text{l}$  of substrate solution was then pipette into each test well and the plate was incubated at room temperature for 20 min. Later 100  $\mu\text{l}$

of reaction stopping solution was added to each well. The ELISA plate was read with the ELISA reader (Electronic Corporation of India, Hyderabad, India) at 492 nm and then titer was calculated.

#### Assessment of cellular immunity by lymphocyte transformation test (LTT)

After 7 and 28 days post-vaccination (DPV) of *Haemorrhagic septicaemia* oil adjuvant vaccine, 5 ml of blood was collected from two calves in each group by jugular vein puncture in a syringe containing 20 units/ml heparin and processed further for assessing the cell mediated immune response. Two ml of blood was diluted with an equal volume of sterile PBS and layered on Ficoll-Paque (Amersham Pharmacia Biotech AB; Uppsala, Swedon). The diluted blood was centrifuged at 400 g for 20 min at room temperature (RT). The cellular band at the interface was collected and washed twice with serum-free RPMI-1640. The viability and enumeration of obtained lymphocytes was carried out by trypan blue dye exclusion test. After enumeration, the viable lymphocyte concentration was adjusted to  $2 \times 10^5$  cells per ml. This purified lymphocyte suspension was used for measuring cell mediated immunity (CMI) response as per the procedure of Mosmann (1983). The assay was carried out in 96 well flat-bottomed tissue culture polystyrene plates. Purified lymphocyte suspension containing  $2 \times 10^5$  cells was dispensed into each well and two hundred microlitre of RPMI-1640 containing 5  $\mu\text{g}/\text{ml}$  of 3-[4,5-dimethyl thiazole-2-yl-2, 5 diphenyl tetrazolium bromide] (MTT) (10 mg/ml) was added to each well and plates. After 72 h of incubation plates were centrifuged at 1,000 g for 10 min at RT. The supernatant was removed carefully and 175  $\mu\text{l}$  of 1N HCl in isopropanol was added into each well to dissolve the formazan crystals. The absorbance was read at 550 nm in computerized spectrophotometer (Microscan, India).

#### Statistical analysis

Data were subjected to a test of significance between

**Table 2.** Effect of vitamin E supplementation on mean ELISA antibody titer in crossbred calves

Attributes	0 DPV	14 DPV	28 DPV	42 DPV	56 DPV	Pooled mean±SE
I	0.00±0.00	930.13±59.22	1,142.85±178.14	989.74±154.12	877.40±314.80	788.02±260.55
II	0.00±0.00	889.87±116.43	1,585.90±54.11	1379.58±178.54	778.89±157.14	926.85±302.68
III	0.00±0.00	1292.93±317.17	1,946.63±418.59	1570.12±228.02	1,260.31±407.22	1,214.00±436.87
IV	0.00±0.00	942.40±89.06	1,878.66±213.65	1402.27±126.56	1,324.25±62.64	1,109.51±340.50
Pooled**	0.00 <sup>a</sup> ±0.00	1,013.83 <sup>ab</sup> ±179.28	1,638.51 <sup>d</sup> ±279.00	1335.43 <sup>cd</sup> ±191.82	1,060.21 <sup>bc</sup> ±271.11	
Mean±SE						

\*\* p<0.01.

<sup>a, b, c, d</sup> Means bearing different superscripts in a row differ significantly.

DPV: Days post-vaccination.

the levels of  $\alpha$ -tocopherol supplementation to calves and blood serum levels of  $\alpha$ -tocopherol in different groups of calves using two way analysis of variance technique (Snedecor and Cochran, (1980) and means were compared using Duncan's multiple range test (Steel and Torrie, 1980).

## RESULTS AND DISCUSSION

### Serum $\alpha$ -tocopherol

Results revealed that supplementation of vitamin E in the diet of young calves enhanced the blood serum level significantly ( $p<0.001$ ) and also there was significant difference ( $p<0.001$ ) in serum  $\alpha$ -tocopherol concentrations at different ages of calves (Table 1). The cumulative period means of serum  $\alpha$ -tocopherol concentration ( $\mu\text{g}/100\text{ ml}$ ) were 69.13±7.34, 257.90±31.59, 299.54±36.50, 229.25±28.78, 217.43±22.71, 209.05±24.42 and 165.84±19.99 at 0, 1, 2, 3, 4, 6 and 8 months of age. The cumulative group mean serum  $\alpha$ -tocopherol concentration was significantly ( $p<0.001$ ) different among the groups. The values were 88.12±6.03, 210.11±17.62, 235.21±19.22 and 294.02±25.25 in-group I, II, III and IV, respectively. The concentrations of serum  $\alpha$ -tocopherol were more than double in calves supplemented with 125, 250 and 500 IU vitamin E as compared to control group calves. Serum concentration of  $\alpha$ -tocopherol reached their respective peaks at 4 to 8 weeks of age and then declined gradually. This may have been due to increasing body weight and decreased absorption post-weaning (Alderson et al., 1971). Similar findings were also reported by Reddy et al. (1987) in cattle calves. Coelho (1991) found a high correlation between plasma and liver levels of  $\alpha$ -tocopherol and between dietary  $\alpha$ -tocopherol and plasma levels. Since vitamin E is not stored for a long time in the body, plasma or serum tocopherol levels can be used to determine the vitamin E status of cattle. Rapid increase in plasma  $\alpha$ -tocopherol levels in cattle calf has been found after vitamin E supplementation (Hill, 1987; Hill et al., 1990, 1993; Chatterjee et al., 2003).

### Humoral immune response

The mean serum antibody titers at different days post

vaccination (DPV) are presented in Table 2. No significant difference among various treatment groups was observed though the calves supplemented with various levels of vitamin E showed comparatively high ELISA antibody titers than the control group. The pooled mean serum antibody titers were 788.02±260.55, 926.85±302.68, 1,214.00±436.87 and 1,109.51±340.50 for group I, II, III and IV, respectively. The pooled mean serum antibody titers at 0, 14, 28, 42 and 56 DPI were 0.00±0.00, 1,013.83±179.28, 1,638.51±279.00, 1,335.43±191.82 and 1,060.21±271.11, respectively. The pooled mean antibody titer at 28 DPI was highest in all groups, which gradually decreased in all the groups. The effect of vitamin E on humoral immune response to killed whole cell *Pasteurella multocida* P52 oil adjuvant vaccine was investigated for the purpose to study the immunomodulating effect of DL- $\alpha$ -tocopheryl acetate when administered as a dietary supplement in crossbred calves. Rise in mean ELISA antibody titer was observed from 14 days post inoculation in all the groups which peaked on 28 days post inoculation. This finding is in agreement with the findings of Pati (1994), Mondal (1996) and Rawat and Jaiswal (2004) who have also found the maximum ELISA titers of OAV vaccinated calves and rabbits between 3<sup>rd</sup> and 5<sup>th</sup> week of vaccination. In the present study group II, III and IV receiving 125, 250 and 500 IU of DL- $\alpha$ -tocopheryl acetate showed a comparatively higher antibody titer than the control group, given no supplemental vitamin E, indicating that dietary supplementation of vitamin E at higher level has a humoral immune enhancing effect against killed bacterial antigen. Segagni (1955) observed that vitamin E supplemented rabbits produced antibodies earlier to typhoid vaccine, staphylococcus and O-streptolysin than those fed control diets, without observable difference in peak antibody levels between the groups. Contrary to above Franchini et al. (1991) and Muneer et al. (1994) did not observe any significant beneficial effect of vitamin E on humoral immune response against *E. coli* in poultry and *Pasteurella multocida* type 6B immunization in buffalo calves. However, significant immunoenhancing effect of vitamin E has been reported against *Brucella ovis* (Afzal et al., 1984;

**Table 3.** Effect of graded levels of vitamin E supplementation on lymphocyte stimulation indices with PHA-P mitogen in crossbred calves

Days	Groups			
	I	II	III	IV
7 DPV	1.18±0.03	1.17±0.07	1.20±0.03	1.37±0.03
28 DPV	1.18±0.01	1.21±0.13	1.19±0.06	1.26±0.06

DPI: Days post Vaccination.

Tengerdy et al., 1991), *Clostridium perfringens* type D vaccine in sheep (Tengerdy et al., 1983) and *Mycoplasma mycoides* subsp. *capri* in rabbit (Rana and Srivastava, 1999) and *P. multocida* whole cell vaccine (Rawat and Jaiswal, 2004) when administered as a locally concentrated preparation in the microenvironment of water-in-oil depot of injected vaccine. It has been reported that vitamin E in water-in-oil (w/o) formulation increases antibody response due to mitogenic stimulation of T and B cells, and due to stimulation of phagocytosis by PMN consequent with inhibiting effect of prostaglandin biosynthesis (Panganmala and Cornwel, 1982). In addition, the immunostimulatory effect of vitamin E is also related to its antioxidant property. Tengerdy et al. (1983) observed that vitamin E in oil adjuvant enhanced ELISA antibody titer against *Clostridium perfringens* type C and D toxins much more than dietary supplementation. In water-in-oil (w/o) depots, vitamin E protects cell membranes of immunocompetent cells (ICCs) against oxidative damage resulting in optimal antigenic presentation. Mechanism of immunoenhancing effect of vitamin E administered orally or locally is probably due to higher blastogenic response of lymphocytes. Heinzerling (1974) observed that in vitamin E supplemented mice there was an increased accumulation of [3H] labelled DL- $\alpha$ -tocopheryl acetate in the thymus and spleen. Further, it was reported that rapid removal of labeled tocopherol nicotinate from the blood stream was accompanied by rapid deposition in the spleen, lungs and liver, suggesting that the vitamin undergoes phagocytosis by the cells of the reticuloendothelial system (Spratt and Kratzing, 1971; Frigg and Gallo-Torres, 1977). The eventual effect of these changes is higher humoral and cell mediated immune response against different bacterial and viral antigen as has been observed in the present study. Reddy et al. (1987) also reported that there was no significant difference in mean serum anti BHV-1 antibody titers among different levels of vitamin E supplemented calves but calves given supplemental vitamin E showed a trend towards higher IgG response to vaccination at 7 weeks of age. Similar findings were also reported by Rivera et al. (2002) in cattle.

#### Cell mediated immune response

Mean lymphocyte stimulation indices (LSI) are presented in Table 3. There was no significant difference

( $p > 0.05$ ) in mean LSI of different treatment groups at 7 DPI and 28 DPI. At 7 DPI the mean LSI to PHA-P was  $1.18 \pm 0.03$ ,  $1.17 \pm 0.07$ ,  $1.20 \pm 0.03$  and  $1.37 \pm 0.03$  in group I, II, III and IV, respectively. The mean LSI in group I, II, III and IV were  $1.18 \pm 0.01$ ,  $1.21 \pm 0.13$ ,  $1.19 \pm 0.06$  and  $1.26 \pm 0.06$ , respectively at 28 DPI. Similar findings were reported by Cuesta et al. (1996). Pehrson et al. (1991) observed no significant difference in mean LSI against leucoagglutinin, pokeweed and concanavalin in calves supplemented with 200-600 mg vitamin E. Similarly, Cipriano et al. (1982) did not find any significant difference in mean LSI in calves supplemented with 1 g of DL- $\alpha$ -tocopheryl acetate/day when compared to control calves. Reddy et al. (1986) reported that *in vitro* addition of vitamin E to lymphocyte cultures did not increase phytohaemagglutinin-induced blastogenesis. Contrary to the above results, Reddy et al. (1987) reported that overall mean lymphocyte blastogenic responses to various T-cell mitogen (PHA, concanavalin, pokeweed) and B cell mitogen (lipopolysaccharide) were higher in vitamin E supplemented calves than in control calves. Corwin and Gordon (1982) reported that lymphocytes from mice supplemented with 5 mg vitamin E/100 g diet showed a 2.5 times increased response to mitogen but supplementation with 50 mg vitamin E/100 g diet increased the response by more than eight folds. Larsen and Tollersrud (1981) also observed increased response to PHA with lymphocytes obtained from pigs supplemented with various amounts of  $\alpha$ -tocopherol and sodium selenite for a period of 12 weeks. Since cell mediated immunity does not play any significant role in protection against extra-cellular bacterial pathogen the blastogenic response of lymphocytes against P52 antigen was not undertaken (Bain et al., 1982; Tizzard, 1995).

#### CONCLUSION

It may be concluded that supplementation of graded levels of vitamin E in the diet of crossbred calves increased the  $\alpha$ -tocopherol concentration in blood serum and also enhanced the humoral immune response of crossbred calves against *P. multocida* (P<sub>52</sub>) whole cell antigen.

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