EFFECT OF VITAMIN E AND SELENIUM ON IMMUNITY IN NEWBORN JERSEY AND BUFFALO CALVES

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Summary

Effect of vitamin E and selenium supplementation on immunity was studied in newborn Jersey and buffalo calves. The supplement contained 500 mg vitamin E and 200 μg selenium; and was fed daily from birth to day 30. Differences in weight gain, total leucocytic count, differential leucocytic count, antibody titre and susceptibility to disease were found to be nonsignificant between supplemented and control calves during the study period of 3 months. Vitamin E seemed to enhance the recovery from disease in buffalo calves. Buffalo calves were found to be more sensitive to selenium toxicity than Jersey calves.
(Key Words: Vitamin E, Selenium, Immunity, Calves, Buffalo)

Introduction

Vitamin E alone or in combination with selenium is known to stimulate immune system in young and adult animals (Sheffy and Schultz, 1979; Tengerdy, 1980). Increased humoral antibody titres have been reported following vitamin E supplementation in mice (Heinzerling, 1974[b]), rabbits (Solano, 1957), chickens (Tengerdy and Brown, 1977) and sheep (Tengerdy et al., 1983). Selenium and vitamin E act synergistically to stimulate antibody production. Increased protection has also been observed with vitamin E supplementation in chicken and turkeys against *E. coli* infection and in mice against *Diplococcus pneumoniae* (Heinzerling et al., 1974[a,b]).

Newborn calves respond poorly to foreign antigens due to unprimed state of their immune system and depend upon passive transfer of antibodies and lymphocytes from dams through colostrum to resist against the invading pathogens. First month of calf's age is critical and our previous study indicated 27.9 to 47.6 percent of total first year deaths during this period (Afzal et al., 1983). This study was designed to determine the immunostimulatory effect of vitamin E and selenium supplementation in newborn calves for resistance against natural infections.

Materials and Methods

Experimental animal and vitamin E-selenium supplement

Calves (18 Jersey and 10 buffalo) born at Animal Sciences Institute Farm between October 1986 and April 1987 were included in the study. Jersey and buffalo calves were separately divided at random in two groups with equal number of animals.

One group from each Jersey and buffalo calves was fed 5.0 g of vitamin E-selenium supplement per head per day from birth to day 30. The supplement contained vit E 500 mg, selenium 200 μg, BHT 500 mg, vit A 25000 I.U and vit D_3 6250 I.U per 5 g with limestone as a carrier.

First two buffalo calves fed on the vit E-selenium supplement showed signs of selenium toxicity and died. The buffalo calves were then fed 2.5 g of vitamin E daily till day 30 (5.0 g Rovimix E-50SD, kindly gifted by Messers. F. Haffmann-LaRoche & Co. Switzerland).

Leucocyte counts

Citrated blood samples were collected from each calf at birth, weekly intervals for first six weeks and thereafter, fortnightly. Total leucocytic count was done using hemocytometer and differential leucocytic count was done after staining the

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non-citrated blood smear with Wright's stain (Benjamin, 1978).

Immunological studies

To study the antibody production response, each calf was vaccinated with 1.0 ml of *Pasteurella multocida* alum-precipitated bacterin (Veterinary Research Institute, Lahore) on day 15 of birth. Booster dose was given at 5 weeks age. Pre-and post-vaccination serum samples were collected and stored at -20°C till antibody titration. Antibody titration was carried out using indirect enzyme-linked immunosorbent assay (ELISA).

ELISA antigen: *P. multocida* Robert's type 1 (Kindly supplied by Veterinary Research Institute, Lahore) was grown in tryptose broth. Twenty-four hour culture was centrifuged at 4000 X g for 30 minutes and pellet suspended in sterile normal saline. The suspension was sonicated (Ultrasonic Ltd., USA) for 15 minutes. The sonicate was centrifuged at 4000 X g for 30 minutes and supernatent used as antigen.

ELISA protocol: U-shaped, 96-well microtitre plates (Flow laboratories, Irvine, Scotland) were sensitized with 100 µl of antigen in carbonate buffer (pH 9.6) for 18 hours at room temperature. Plates were washed once with phosphate buffered saline having 0.05% Tween – 20 (PBS – Tween 20). Wells were blocked with 5% chicken serum in PBS-Tween 20 for one hour at 37°C.

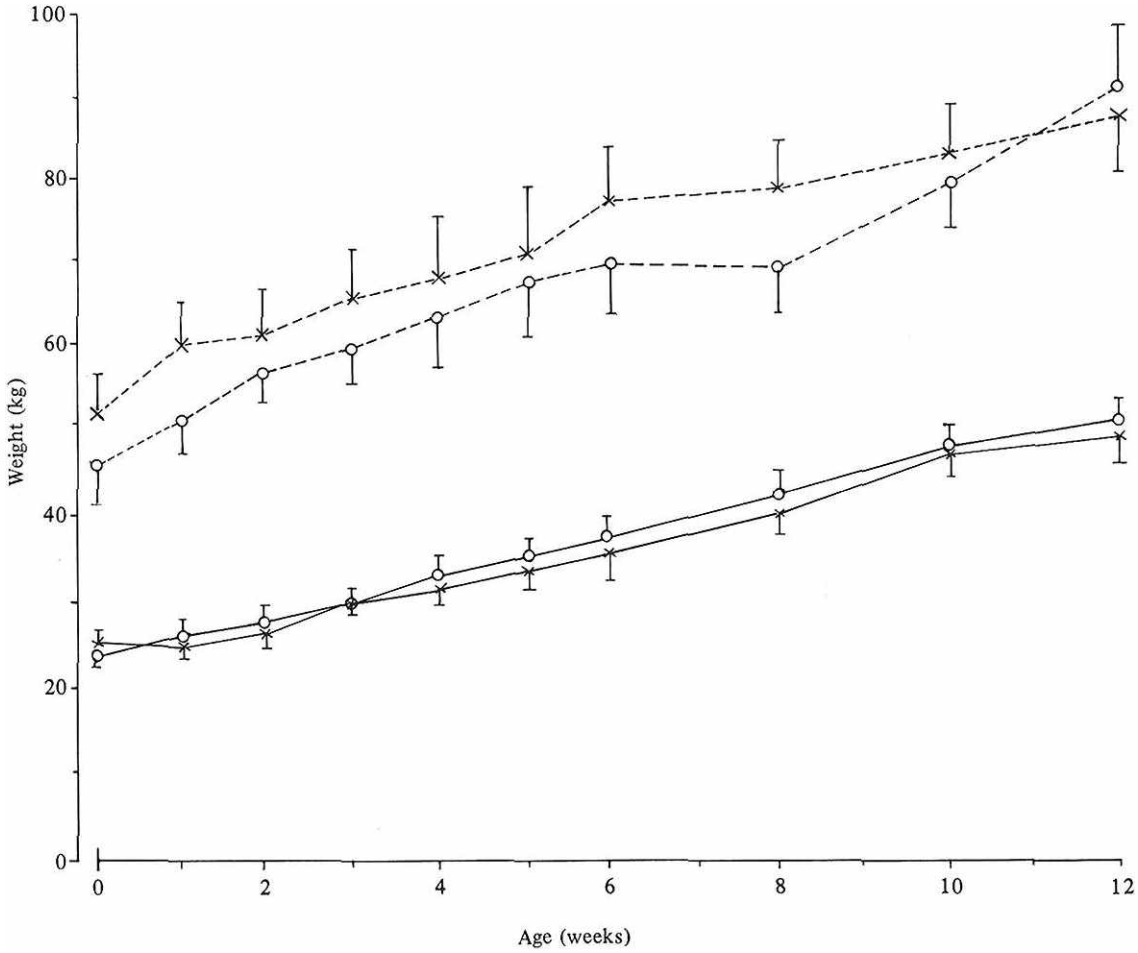

Figure 1. Effect of vitamin E-selenium supplementation on body weight of Jersey and buffalo calves. Solid lines depict Jersey calves while dotted lines indicate buffalo calves. O indicates vitamin E-selenium supplemented group and X denotes control group.

Plates were washed 3 X and 50 µl of test serum in quadruplicate (diluted 1:10 in PBS-Tween 20) was added. The plates were incubated at 37°C for 2 hours. After washing 5 X, 50 µl of 1:1000 diluted *staphylococcal protein Aperoxidase conjugated* (Sigma Chemical Co. St. Lois, Missouri, USA) was added and the plates incubated for two hours at 37°C. Plates were washed 5 X and 50 µl of substrate (0-phenylenediamine dihydrochloride + 0.01% hydrogen peroxide) was added. The plates were allowed to develop in dark for 30 minutes. Colour development was stopped with 2N sulphuric acid and plates were read in ELISA reader at 490 nm.

Statistical analysis

Comparisons between treatment and control groups were made using oneway analysis of variance.

Results and Discussion

Vit E-selenium or vit E supplement had a non-significant effect on body weight gain in newborn buffalo and cow calves (figure 1). Buffalo calves were born heavier than Jersey calves and net body weight gain was also higher in buffalo than Jersey calves in the study period. This could be attributed to the species difference. Previous studies have also shown that buffalo calves have a higher daily weight gain than dairy cattle (Alvi, 1984).

Number of total leucocytes in the peripheral blood of Jersey and buffalo calves were not affected by vit E-selenium supplementation (figure 2 and 3). Leucocyte numbers almost remained similar during first 3 months of calf age. Leucocyte numbers in the peripheral blood of buffalo calves were greater than in Jersey calves. This seems to be a species difference. Average number of leucocytes ranged from 4.87 ± 0.69 to 7.46 ± 0.71 thousands

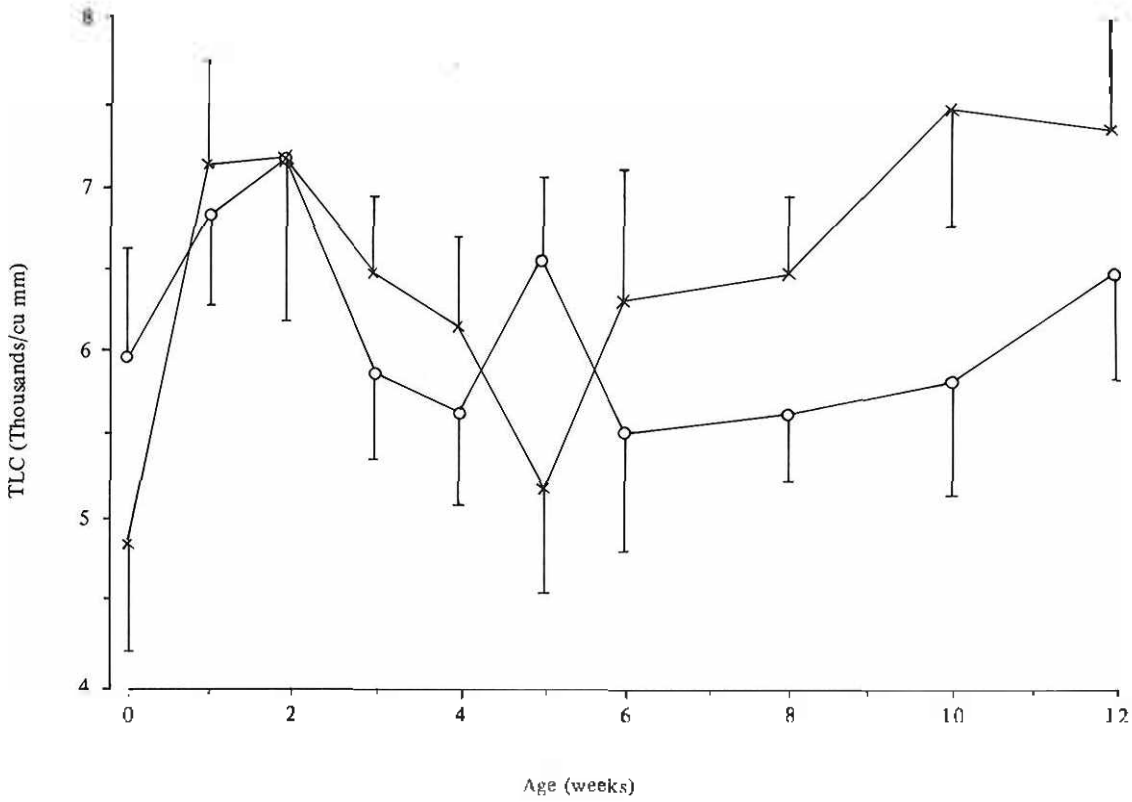

Figure 2. Total leucocytic count in vitamin E-selenium fed and control Jersey calves. O indicates vitamin E-selenium supplemented group and X denotes control group.

per cubic mm in Jersey and 7.67 ± 1.46 to 12.63 ± 1.67 thousands per cubic mm in buffalo calves. Similar leucocyte numbers in sucklers have been reported by other workers (Irfan, 1967; Karam et al., 1979; Thangraj et al., 1979).

Neutrophil and lymphocyte pattern during first 3 months of age was similar in cattle and buffalo calves (figure 4 and 5). At birth neutrophils were highest in proportion among leucocytes, decreased upto 6-8 weeks and then remained constant. Lymphocyte pattern was opposite to neutrophil pattern. Lymphocyte proportion increased after birth and became constant at 6-8 weeks. Vit E-selenium supplement did not have a significant effect on the proportions of neutrophils and lymphocytes in Jersey and buffalo calves. Neutrophils act as primary defence line of the body and are quick to respond against foreign material. Thus it seems that higher neutrophil proportions at birth will be needed to safeguard against potential pathogens. At this time, lymphocytes although fully functional in bovines (Schultz, 1973) respond poorly due to unprimed nature of their immune response and have a long lag phase. With time, as a result of exposure to a number of antigens, lymphocytes proliferate and their proportion is increased.

Antibodies against *P. multocida* were not detected in Jersey calves following first vaccination at 2 weeks age. However, increase in antibody titre was observed following booster dose at 5 weeks age (figure 6). Higher antibody titres were seen in vit E-selenium fed calves than control animals after the booster dose, although these differences were statistically nonsignificant. This finding documents the previous studies that vita-

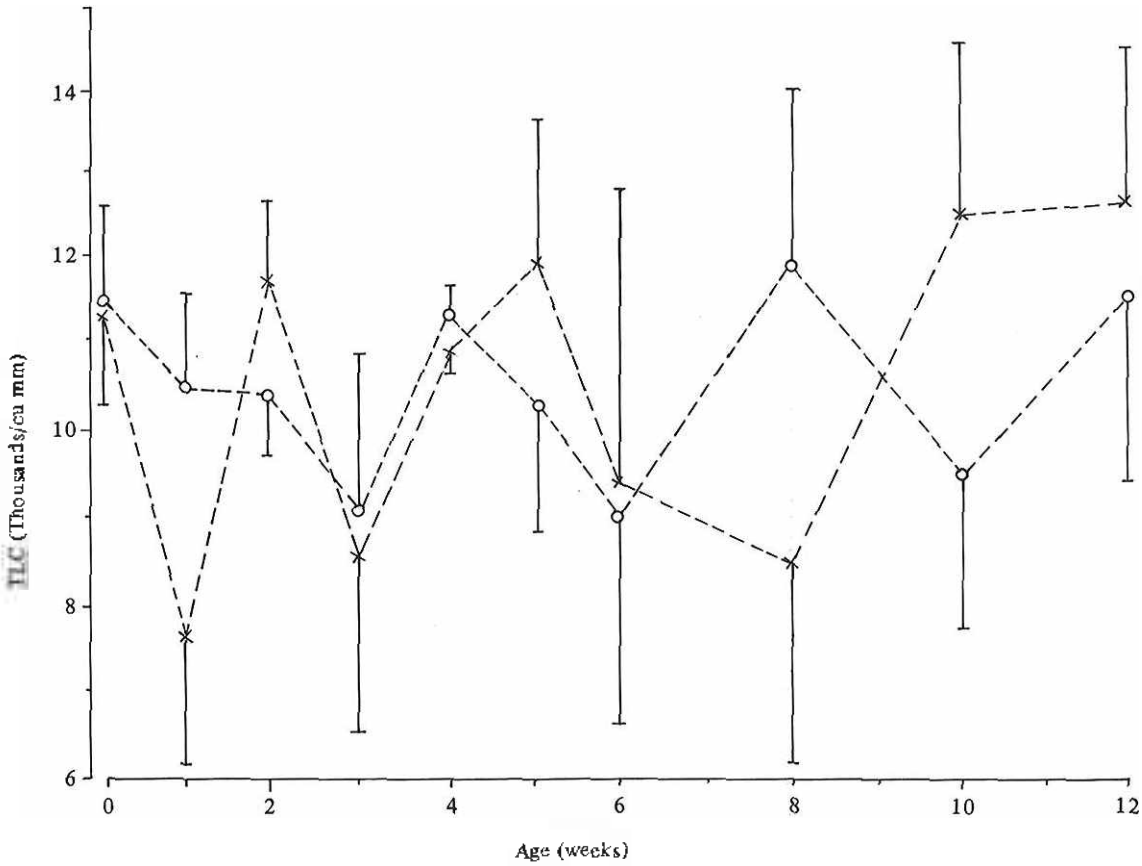

Figure 3. Total leucocytic count in vitamin E fed and control buffalo calves. O indicates vitamin E supplemented group and X denotes control group.

min E supplementation produces higher antibody levels in laboratory animals and sheep (Solano, 1975; Heinzerling et al., 1974[a,b]; Tengerdy and Brown, 1977; Tengerdy et al., 1983; Afzal et al., 1984). Vitamin E-selenium supplement was withdrawn after 30 days of birth, thus marked rise in antibody titres as reported in previous studies has not been observed in this study.

Disease status of the animals has been shown in table 1. Diarrhoea was frequently seen in Jersey calves. This was traced to a feeding error. It was observed that these calves were fed on milk stored at room temperature for 6 hours prior to feeding. Number of days animals had diarrhoea did not differ significantly among vit E-selenium fed and control groups. Two buffalo calves in the control group died while no death was recorded in the buffalo calves after vit E-selenium supplement was replaced with vit E alone. Two buffalo calves developed symptoms of oral necrobacillosis during the experiment. The calf in vit E group recovered from the infection while the control calf died.

TABLE 1. DISEASE STATUS OF EXPERIMENTAL CALVES

	Number of calves		
	Total	Had diarrhoea	Died
Jersey calves			
Vit E-Selenium group	9	9	Nil
Control group	9	8	Nil
Buffalo calves			
Vit E-Selenium group	5	3	2[a]
Control group	5	1	2

[a]Died of selenium poisoning.

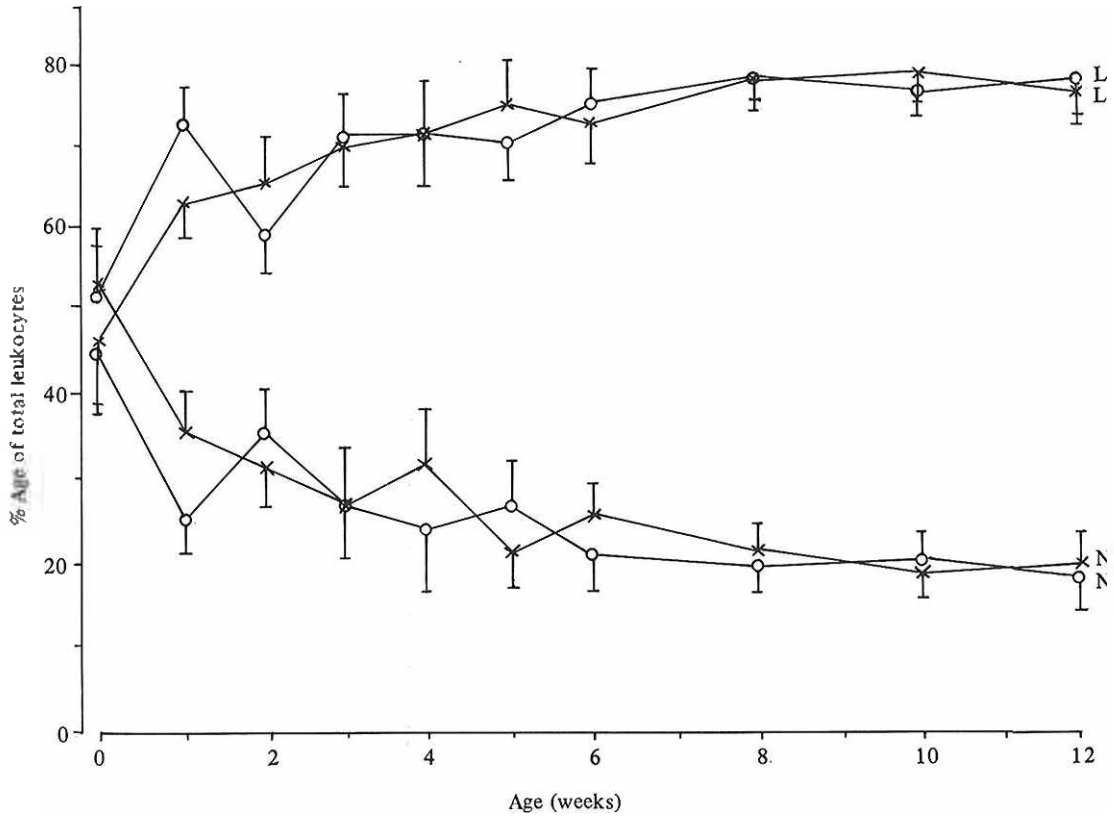

Figure 4. Peripheral blood lymphocytes and neutrophils in vitamin E-selenium fed and control Jersey calves. O indicates vitamin E-selenium supplemented group and X denotes control group.

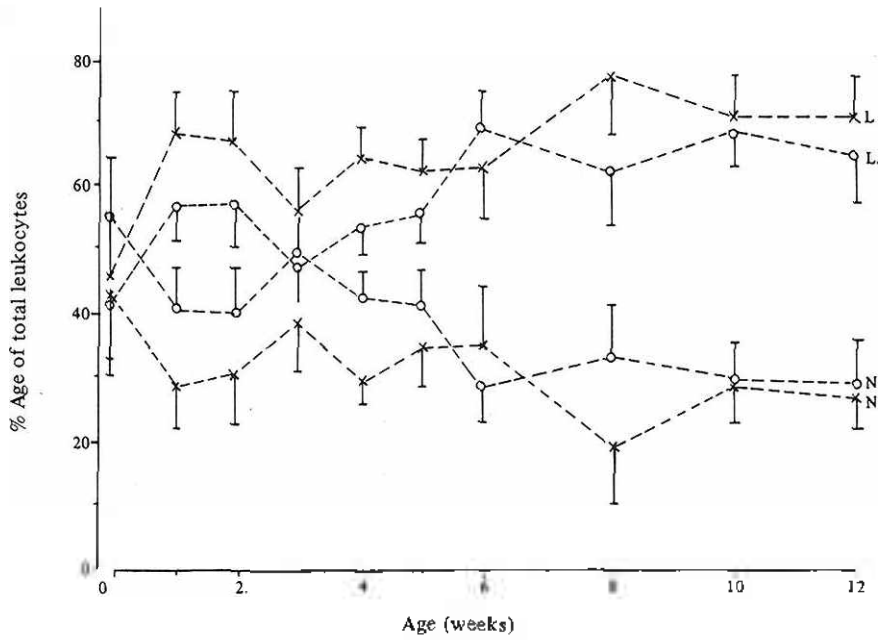

Figure 5. Peripheral blood lymphocytes and neutrophils in vitamin E fed and control buffalo calves. O indicates vitamin E supplemented group and X denotes control group.

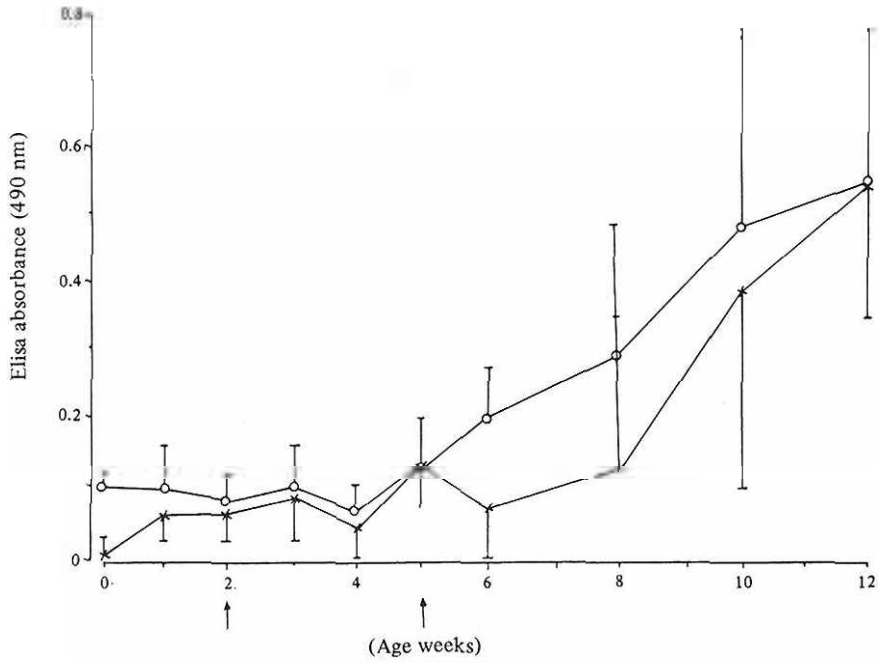

Figure 6. Antibody titre of vitamin E-selenium fed and control Jersey calves after vaccination with *P. multocida* bacterin. O indicates vitamin E-selenium supplemented group and X denotes control group.

Vitamin E seemed to enhance recovery from disease in buffalo-calves. Since sample size in this study was small, further studies will be needed to validate this hypothesis.

Buffalo calves seemed to be more sensitive to selenium poisoning than Jersey calves. Selenium fed daily for 30 days did not have any ill effect on Jersey calves but caused toxicity and death in buffalo calves which were heavier than Jersey calves. Toxicity symptoms appeared after 25-30 days of supplement feeding. Alopecia was the main clinical symptom. On postmortem, liver showed discolouration. Increased fibrosis in portal areas of liver was seen on histological examination indicating selenium toxicity. The experiment was not designed to study the sensitivity of Jersey and buffalo-calves to selenium but it seemed an important observation. Higher incidence and severe lesions of Deg-Nala disease which is suspected of being caused by selenium toxicity (Arora et al., 1975) have also been observed in buffaloes than cattle (Irfan, 1971). Further studies will be needed to determine the toxic limits of selenium for buffaloes.

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