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Effect of vitamin E and selenium supplementation on haematology, blood chemistry and thyroid hormones in male buffalo (*Bubalus bubalis*) calves*

P.L. Shinde, R.S. Dass[1] and A.K. Garg

Mineral and Vitamin Nutrition Laboratory,
Centre of Advanced Studies in Animal Nutrition,
Indian Veterinary Research institute
Izatnagar-243122 (UP), India

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ABSTRACT

An experiment was conducted on 20 male buffalo calves (average body weight 75.30±2.20 kg) to elucidate the effect of supplemental vitamin E and/or selenium (Se) on their haematology, blood metabolic profile, serum enzymes and thyroid hormones status. Group I served as control (without any supplementation), groups II, III and IV were supplemented with 0.3 mg Se, 300 IU of DL-α-tocopheryl acetate, and both 300 IU DL-α-tocopheryl acetate and 0.3 mg Se, respectively. Animals were fed on wheat straw and concentrate mixture to meet their nutrient requirements. Blood was collected at zero day and subsequently at 45, 90, 135 and 180 days and analysed for haematological parameters, blood chemistry and level of thyroid hormones. Results revealed no significant difference (P>0.05) in haematological parameters (haemoglobin, packed cell volume, red blood cell, white blood cell) due to the supplementation of vitamin E ,Se or both vitamin E + Se in buffalo calves. Similarly, the concentration of various blood biochemical components (glucose, total protein, albumin, globulin, urea, creatinine, total cholesterol, and triglycerides) except high density lipid (HDL) cholesterol was statistically similar in 4 groups. Results revealed significantly (P<0.01) higher concentration (mg/dl) of HDL cholesterol in buffalo calves given Se and Se + vitamin E as compared to control and vitamin E supplemented groups. There was no significant difference in serum enzymes (serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase, creatine kinase and lactate dehydrogenase) activities due to different treatments. Level of triiodothyronine was significantly (P<0.01) higher in buffalo calves supplemented with selenium, and vitamin E + Se, as compared to non-supplemented control group; whereas concentration of thyroxine (nmol/l) was statistically similar in 4 groups, without showing

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[1] Corresponding author: e-mail: rsd@ivri.up.nic.in

any effect of different treatments. It may be concluded that supplementation of Se and vitamin E in the diet of buffalo calves had no beneficial effect on blood haematology and blood chemistry except an increase in the serum levels of high density lipid cholesterol and triiodothyronine as compared to non-supplemented control buffalo calves.

KEY WORDS: vitamin E, selenium, serum enzymes, triiodothyronine, thyroxine, lipoprotein, buffalo calves

INTRODUCTION

The nutritional essentiality of selenium arose from the work of Patterson et al. (1957) in chickens. Subsequently, it was found that vitamin E and selenium are essential for proper health, immunity and reproductive functions of animals, and their inter-relationship became an active and rewarding field of research, as vitamin E and Se are the micronutrients that share a common biological function. Selenium is a component of glutathione peroxidase enzyme, which destroys free radicals in the cytoplasm, whereas, vitamin E is a non-enzyme scavenger of free radicals that functions as a specific lipid soluble antioxidant in cell membranes. Subsequently, it was confirmed that Se and vitamin E act synergistically and protect the tissues against oxidative damage. Both Se and vitamin E have been shown to improve immune responses (Shinde et al., 2007). Selenium is also a component of enzyme type I deiodinase that is required for the conversion of thyroxine (T_4) into more active tri-iodothyronine. NRC (1989) has recommended a dietary level of 0.3 ppm Se and 15-40 mg/kg DM of vitamin E for growing cattle. However, research suggests that relatively higher levels of vitamin E supplementation may improve calf performance (Reddy et al*.,* 1987), which may be due to enhanced immunity (Rajeesh et al., 2008). Selenium is required for the development and expression of non-specific, humoral and cell mediated immune responses. However, the mechanism by which Se affects the immune system is speculative. Probably, the effects of Se through glutathione peroxidase and on the cellular levels of reduced glutathione, as well as the ability of selenium to interact with cell membranes, represent the immune-enhancing role of Se. As such, there appears to be no standard report on the requirement of Se and vitamin E for buffaloes and often values recommended for cattle are used for buffaloes. Keeping in view that very little information is available on the requirement and role of vitamin E and Se on blood haematology, blood chemistry and thyroid hormones in buffaloes, the present research work was planned to evaluate the effect of vitamin E and Se supplementation on these aspects in male buffalo calves.

MATERIAL AND METHODS

Animals, feeding and management

To carry out this experiment, twenty male buffalo (*Bubalus bubalis*) calves were procured from the local market. These animals were maintained on the experimental diet comprising of concentrate mixture and wheat straw for a period of two months during which they were treated against ecto and endo parasites before the start of the experiment, and subsequently at regular intervals. All the calves were vaccinated against foot and mouth disease. The buffalo calves were distributed in four different groups of five animals in each on the basis of their body weights following complete randomized design. During the experimental period all the calves were kept in a well ventilated shed with individual feeding and watering arrangements. Calves in all the four groups were fed on wheat straw and concentrate mixture to meet their nutrient requirements for a daily weight gain of 500 g (Kearl, 1982). The concentrate mixture consisted of, %: crushed maize grain 25, soyabean meal 30, wheat bran 42, mineral mixture 2 and common salt 1. Treatments were: group I (control), without any supplementation, group II supplemented with 0.3 mg Se/kg DM from sodium selenite, group III supplemented with 300 IU DL-α-tocopheryl acetate (Impextraco, Belgium) and group IV supplemented with both 0.3 mg Se/kg DM and 300 IU DL-α-tocopheryl acetate, mixed well in the concentrate mixture. Available green fodder (2 kg) was given once a week to all the calves to meet their vitamin A requirement. Clean and fresh drinking water was provided twice a day to all the animals. The chemical composition of wheat straw and concentrate mixture is presented in Table 1. The crude protein content of the concentrate mixture and wheat straw was 20.4 and 3.6%, respectively, whereas the basal α-tocopherol and Se concentration were 13.15, 2.00 and 0.17 and 0.13 mg kg^{-1}, respectively.

Table 1. Chemical composition of feeds, % DM basis

Components	Concentrate mixture	Wheat straw
Organic matter	90.0	92.6
Crude protein	20.4	3.6
Ether extract	2.5	1.7
Neutral detergent fibre	41.5	82.3
Acid detergent fibre	13.2	56.7
Cellulose	9.5	48.6
Hemicelluloses	28.3	25.6
Ca	1.19	0.67
P	0.96	0.07
α-tocopherol, mg kg^{-1}	13.15	2.00
Se, mg kg^{-1}	0.17	0.13

Collection of blood

About 12 ml blood was collected from each calf through jugular venipuncture in the morning (before watering and feeding) at zero day and subsequently at 45 days interval. Out of 12 ml, 10 ml blood was collected into clean and dry test tube and kept in slanting position for 45 min for the separation of serum for blood chemistry and thyroid hormones. Remaining 2 ml was taken in another clean and dry ependroph tube (2 ml) containing anticoagulant (heparin) for the haematological studies. The blood samples were centrifuged at 3000 rpm for 10 min at 4°C and serum was separated. The serum was collected in plastic vials and kept at -40°C until further analysis.

Estimation of α-tocopherol in feeds

The concentration of α-tocopherol in concentrates mixture and wheat straw offered to the experimental calves was estimated by using HPLC. The α-tocopherol standard was procured from M/s Sigma (USA), and was diluted using ethanol (Merck, Germany). Methanol (HPLC grade) was used as a mobile phase to maintain a flow rate of 2 ml/min and α-tocopherol was detected at 292 nm.

Estimation of selenium in feeds

Selenium in feed samples was estimated by Atomic Absorbance Spectrophotometer (Model 4141, Electronic Corporation of India Ltd., Hyderabad, India) using a nitrous oxide-acetylene flame, nitrogen as inert gas and sodium borohydrate (0.6% w/v in 0.5% NaOH) as a reducing agent. Samples of feed were digested using double acid (HNO_3, $HClO_4$; 4:1) mixture and volume was made with double distilled water.

Estimation of proximate principles and fibre fractions in feed

Feed samples were analysed for proximate principles and fibre fractions using standard methods.

Estimation of haematological parameters

Blood kept for haemoglobin estimation was treated with Drabkin's solution. Ferricyanide from Drabkin's solution converted haemoglobin to methaemoglobin, which was further converted to cyanmethaemoglobin by the action of cyanide. The cyanmethaemoglobin has an absorbance which is proportional to the haemoglobin

concentration (g/100 ml blood). To estimate packed cell volume (PCV) Wintrobe tubes were filled with blood up to the ten mark and centrifuged at 3000 rpm for 30 min. PCV was expressed as percentage. Haemocytometer was used to determine the total leukocyte count (TLC) and total erythrocyte count (TEC) in blood. Blood was drawn up to the 0.5 mark of the WBC pipette followed by WBC diluting fluid up to 11 marks. The pipette was then rotated between the fingers for a few seconds in order to facilitate proper mixing of the contents. After few minutes, which allowed haemolysis of RBCs, the counting chamber was charged after discarding the first few drops of the diluted sample. Once the cells were settled down, WBCs were counted in the four large squares. This number multiplied by 50 gave the TLC (10^3 cells/ µl). Anticoagulated blood was drawn up to the 0.5 mark of the RBC pipette followed by RBC diluting fluid up to 101 marks. The pipette was then rotated between the fingers for a few seconds in order to facilitate proper mixing of the contents. After few minutes, the counting chamber was charged after discarding the first few drops of the diluted sample. Once the cells were settled down, RBCs were counted in the five squares. This number multiplied by 10^6 gave the TEC (10^6 cells/µl).

Estimation of serum biochemical components

Diagnostic kits manufactured by Span Diagnostic Limited, Surat (India), were used for the analysis of all the blood biochemical components. Serum total protein (TP) and albumin were estimated by biuret and BCG dye binding method (Dumas et al., 1971), whereas serum globulin was calculated by subtracting serum albumin from TP. Urea in serum was determined by diacetylmonooxime (DAM) method and serum creatinine was determined by the alkaline picrate method. High density lipoproteins were obtained in the supernatant after the centrifugation of the serum. Cholesterol was estimated according to Wybenga et al. (1970). The amount of triglycerides present in serum samples was estimated by following the enzymatic method as given by McGowan (1983).

Estimation of serum enzymes

Serum alkaline phosphatase (ALP) activity was estimated by the method of Kind and King (1954). Serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) activities in blood serum were determined (Reitman and Frankel, 1957). Serum lactate dehydrogenase (LDH) activity was estimated by the method of Wroblewski and Duean (1955), creatine kinase (CK) activity in blood serum by modified standard method according to the recommendations of European Committee for Clinical Laboratory Standards and of International Federation of Clinical Chemistry (Szasz et al., 1976).

Estimation of serum thyroid hormones

Estimation of triiodothyronine (T_3) and thyroxin (T_4), in blood serum samples was done by radioimmunoassay technique using an automatic gamma counter (Packard, USA, Model Cobra II). RIA kits for T_3 and T_4 were procured from M/s Immunotech Radiova (Czech Republic).

Statistical analysis

Data generated was analysed using SPSS (1996) by one way analysis of variance. Treatment means are presented along with standard errors of the mean (SEM). Individual animal was the experimental unit for analysis of all the parameters. When significant differences were noticed, means were separated by using Duncans test. The P-values compared the differences between different groups.

RESULTS

Results of haematological parameters studied in this experiment are presented in Table 2. The mean haemoglobin (Hb) values were in normal range and were found to be comparable ($P>0.05$) in all the four groups. It was found that the Hb values were significantly ($P<0.001$) lower at zero day as compared to other periods, followed by a significant ($P<0.001$) increase on 45 d; and on 90, 135 and 180 days Hb values were found to be comparable. The cumulative group mean PCV (%) values were found to be statistically ($P>0.05$) similar in all the four groups. The mean treatment and period RBC values (at 0, 45, 90, 135 and 180 d) were also statistically ($P>0.05$) similar in 4 groups. Results revealed no significant effect of treatment and time intervals on the total erythrocyte count and total leukocyte counts in blood of buffalo calves.

The data concerning blood glucose, total protein, albumin, globulin, albumin: globulin ratio, total cholesterol, HDL cholesterol, triglyceride, urea and creatinine are presented in Table 3. Results revealed no significant effect of treatments on blood glucose level. Serum glucose level was found to be significantly ($P<0.001$) higher at 45 and 90 days of experimental feeding as compared to 0, 135 and 180 d. The mean total protein (TP) values in groups I, II, III and IV were found to be statistically ($P>0.05$) comparable. Mean albumin concentrations were statistically ($P>0.05$) similar in 4 groups and the overall period means of serum albumin were found to be significantly ($P<0.001$) lower at zero day of experimental feeding at 2.47 mg/dl, followed by a significant ($P<0.001$) increase on 45 d, whereas it was highest (3.24 mg/dl) on 180 d of

Table 2. Haematological parameters of buffalo calves in different groups

Group	Blood collection, days						SEM	P value		
	0	45	90	135	180	Mean		G	P	G × P
Haemoglobin, g/dl										
I	9.78	12.00	12.30	11.46	11.87	11.48	0.25	0.95	0.000	0.18
II	10.18	11.16	11.27	11.60	12.18	11.28	0.18			
III	10.19	10.68	11.62	11.99	12.13	11.32	0.19			
IV	10.12	10.50	11.38	11.72	12.34	11.21	0.19			
Packed cell volume, %										
I	41.00	41.20	36.80	36.20	36.80	38.40	0.99	0.67	0.99	0.19
II	40.60	39.20	38.40	39.40	38.00	39.12	1.15			
III	36.00	38.40	38.20	37.20	40.20	38.00	0.73			
IV	35.60	37.40	41.60	43.20	39.80	39.52	0.87			
Total erythrocyte count,T/l										
I	6.81	6.80	6.83	6.53	6.53	6.70	0.15	0.33	0.22	0.74
II	6.85	6.79	6.77	7.27	7.41	7.02	0.11			
III	6.45	6.67	6.80	7.00	6.93	6.77	0.13			
IV	6.46	6.38	6.68	7.15	7.16	6.76	0.12			
Total leukocyte count, G/l										
I	8.32	7.56	7.00	6.17	6.25	7.06	0.22	0.21	0.000	0.25
II	8.14	7.37	7.61	7.00	7.05	7.43	0.23			
III	8.42	7.03	7.95	6.25	6.72	7.27	0.22			
IV	8.34	8.78	7.46	6.32	6.91	7.56	0.22			

G/l: Giga per liter blood; T/l: Tera per liter blood

experimental feeding. The mean serum globulin values were statistically (P>0.05) alike in all the 4 groups of calves. The mean albumin: globulin ratio in different treatments were also found to be statistically (P>0.05) alike. The mean serum total cholesterol concentration (mg/dl) were numerically higher in Se, and vitamin E + Se supplemented groups as compared to control and vitamin E supplemented groups, but did not reveal any statistical (P>0.05) differences. The mean HDL cholesterol values were found to be significantly (P<0.01) higher in group II (supplemented with 0.3 mg Se/kg DM) and group IV(supplemented with both Se 0.3 mg/kg DM intake and vitamin E 300 IU/head/day) as compared to group III (supplemented with vitamin E 300 IU/head/day) and group I (control). HDL cholesterol values at zero day of experimental feeding were significantly (P<0.001) lower followed by a significant increase on 45 and 135 d and significantly higher values were recorded on 180 days of experimental feeding. There was a significant (P<0.02)

Table 3. Blood biochemical profile of male buffalo calves in different groups

Group	Blood collection, ays					Mean	SEM	G	P	G×P
	0	45	90	135	180					
Glucose, mg/dl										
I	61.24	63.88	62.19	62.00	56.54	61.17	1.55	0.74	0.000	0.89
II	58.76	69.08	70.85	60.00	54.15	62.59	1.90			
III	57.66	66.17	66.67	55.90	55.17	60.32	1.92			
IV	58.09	64.28	68.36	62.19	58.98	62.38	1.74			
Total protein, g/dl										
I	5.89	7.22	7.62	7.78	7.96	7.29	0.19	0.73	0.000	0.12
II	6.22	6.85	7.02	7.81	7.97	7.17	0.18			
III	6.33	6.11	7.10	8.13	7.92	7.12	0.22			
IV	5.44	7.11	7.25	7.96	7.90	7.13	0.20			
Albumin, g/l										
I	2.30	2.79	2.87	3.04	3.46	2.89	0.10	0.93	0.000	0.23
II	2.64	2.61	3.08	3.25	3.12	2.94	0.07			
III	2.76	2.54	3.01	3.12	3.25	2.94	0.08			
IV	2.20	2.93	3.14	3.27	3.11	2.93	0.09			
Globulin, g/dl										
I	3.59	4.44	4.75	4.74	4.50	4.40	0.15	0.56	0.000	0.44
II	3.58	4.24	3.94	4.56	4.85	4.23	0.14			
III	3.58	3.57	4.09	5.00	4.66	4.18	0.18			
IV	3.24	4.17	4.12	4.69	4.79	4.20	0.13			
Albumin: globulin ratio										
I	0.67	0.64	0.62	0.65	0.78	0.67	0.03	0.51	0.47	0.47
II	0.76	0.63	0.80	0.71	0.65	0.71	0.03			
III	0.78	0.74	0.77	0.64	0.72	0.73	0.03			
IV	0.68	0.71	0.77	0.71	0.65	0.70	0.02			
Total cholesterol, mg/dl										
I	109.70	105.35	115.56	109.34	113.79	110.75	1.63	0.12	0.04	0.95
II	115.84	114.81	122.43	116.32	118.39	117.56	3.55			
III	103.76	109.70	121.16	113.33	111.72	111.94	3.02			
IV	103.22	120.10	129.84	121.15	118.99	118.66	2.72			
HDL cholesterol, mg/dl										
I	30.40	29.46	43.29*^{A}	47.68	46.4**^{A}	39.44*^{A}	1.87	0.01	0.000	0.02
II	30.10	35.79	50.00^{B}	47.34	58.77^{B}	44.40^{C}	2.49			
III	25.20	30.45	43.15^{A}	48.99	53.39^{AB}	40.23^{AB}	2.39			
IV	20.79	35.52	47.40^{AB}	52.22	59.16^{B}	43.02^{BC}	2.89			

continued on the page 249

Table 3. continued

Group	Blood collection, days					Mean	SEM	G	P	G×P
	0	45	90	135	180					
Triglyceride, mg/dl										
I	58.02	45.35	46.46	40.64	43.64	46.82	3.34	0.18	0.09	0.87
II	56.04	48.77	50.57	48.24	57.90	52.30	1.55			
III	54.42	47.29	50.96	54.01	55.87	52.51	1.91			
IV	55.62	46.53	50.30	55.36	57.08	52.98	1.7			
Urea, mg/dl										
I	29.55	32.74	37.30	37.41	36.19	34.64	0.87	0.20	0.000	0.70
II	32.78	35.72	37.81	42.55	36.70	37.11	1.02			
III	32.12	35.61	38.61	41.59	37.07	37.00	1.30			
IV	28.12	34.09	37.34	45.22	39.76	36.91	1.43			
Creatinine, mg/dl										
I	0.88	0.95	0.97	0.99	1.09	0.98	0.03	0.08	0.000	0.86
II	0.88	1.11	1.09	1.11	1.14	1.07	0.03			
III	0.80	1.13	1.04	1.15	1.13	1.05	0.04			
IV	0.90	1.13	1.03	1.11	1.13	1.06	0.03			

^{A,B,C} means bearing different superscripts in the same column differ significantly *P<0.05, *P<0.01

treatment x period interaction regarding HDL cholesterol values. At 90 days of experimental feeding the HDL values in group II were found to be significantly (P<0.05) higher as compared to group I and III, but were found to be comparable to that of group IV. Further, it was observed that at 180 d the HDL cholesterol values were significantly (P<0.01) higher in groups II and IV than control, but HDL cholesterol values of group II and IV were found to be comparable to that of group III. Although numerically higher triglyceride (mg/dl) values were noted in all the supplemented animals as compared to control, but on statistical comparison they were found to be similar. The overall mean values for serum urea and creatinine (mg/dl) did not differ (P>0.05) among different treatments and at different time intervals.

The results of the blood enzyme viz., serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), lactate dehydrogenase (LDH), creatine kinase (CK), and alkaline phosphatase (ALP) are presented in Table 4. The mean SGOT and SGPT, activities (unit/ml) did not reveal any statistical significant (P>0.05) differences due to treatments. The mean LDH and CK activities (IU/L) were also comparable (P>0.05) among the different groups. The ALP (KA units) values in groups I to IV did not reveal any statistically significant differences.

The levels of triiodothyronin (T_3) and thyroxine (T_4) in different groups as well as at different periods has been presented in Table 5. The serum T_3 levels (nmol/l) at zero day were found to significantly ($P<0.001$) lower at 1.15 nmol/l as compared to other periods showing a trend of a progressive increase towards the end of the experiment. The mean serum T_3 levels were found to be 1.50, 1.84, 1.66 and 1.92 nmol/l, respectively in groups I, II, III and IV. The statistical analysis revealed that the serum T_3 levels were significantly ($P<0.01$) higher in group II and IV as compared to control, whereas the T_3 levels between group I

Table 4. Serum enzyme profile of buffalo calves in different groups

Group	Blood collection, days						SEM	P value		
	0	45	90	135	180	mean		G	P	G × P
SGOT, Units/ml										
I	74.01	74.46	75.39	68.39	67.62	71.97	0.93	0.20	0.000	0.97
II	75.80	76.70	75.83	68.55	68.20	73.02	1.04			
III	73.22	73.76	75.79	65.69	66.00	70.89	1.17			
IV	76.56	78.22	75.55	66.43	67.69	72.89	1.23			
SGPT, Units/ml										
I	43.93	43.53	44.72	43.79	45.21	44.24	0.27	0.54	0.002	0.56
II	44.34	43.91	43.49	45.35	44.79	44.38	0.24			
III	45.08	43.88	43.94	45.38	45.43	44.74	0.31			
IV	43.98	43.11	44.46	45.24	45.01	44.36	0.25			
LDH, IU/l										
I	695.70	641.20	733.11	751.27	745.40	713.34	28.55	0.13	0.37	0.90
II	696.16	663.64	689.29	616.62	612.35	655.61	18.38			
III	696.77	642.80	687.00	629.44	602.73	651.75	17.22			
IV	691.28	633.41	667.00	652.96	623.03	653.53	16.06			
CK, IU/l										
I	96.58	110.78	109.21	110.84	96.72	104.83	6.71	0.84	0.05	0.99
II	104.29	115.08	107.65	108.65	94.72	106.08	4.77			
III	98.49	110.86	101.19	103.21	90.74	100.90	4.78			
IV	104.16	109.06	107.05	94.41	85.27	99.99	4.63			
ALP, KA units										
I	18.73	17.38	22.22	26.53	24.19	21.81	1.51	0.89	0.001	0.99
II	18.43	17.71	20.47	27.50	28.92	22.61	2.21			
III	17.93	17.62	23.39	33.17	26.45	23.71	2.71			
IV	18.95	16.75	24.27	31.58	27.16	23.74	1.91			

SGOT - serum glutamate oxaloacetate transaminase; SGPT - serum glutamate pyruvate transaminase; LDH - lactate dehydrogenase; CK - creatine kinase; ALP - alkaline phosphatase

and III were found to be comparable. The overall mean values of T_4 (nmol/l) were 39.69, 42.86, 40.16 and 44.14, in groups I, II, III and IV, respectively. The statistical analysis revealed a non-significant difference (P>0.05) when different groups were compared. When different periods were compared the mean T_4 value was found to be significantly (P<0.001) lower on zero day as compared to other periods, while the highest values were observed on 180 d. The T_4: T_3 ratio were found to be comparable (P>0.05) among the different treatments as well as in different periods.

Table 5. Thyroid hormone profile of buffalo calves in different groups

Group	Period, days					Mean	SEM	G	P	G × P
	0	45	90	135	180					
T_3, nmol/l										
I	1.08	1.15	1.67	1.75	1.85	1.50^{A}	0.09	0.01	0.000	0.96
II							0.09			
	1.18	1.82	1.97	2.04	2.19	1.84^{BC}				
III							0.10			
	1.16	1.48	1.84	1.83	1.97	1.66^{AB}				
IV	1.17	1.95	2.05	2.25	2.16	1.92^{C}	0.13			
T_4, nmol/l										
I	30.97	36.19	38.67	47.48	45.12	39.69	1.83	0.14	0.000	0.89
II	31.23	38.97	41.50	47.69	54.90	42.86	2.56			
III	29.31	34.52	38.76	46.46	51.72	40.16	2.01			
IV	33.82	41.00	48.17	47.04	50.67	44.14	1.60			
T_4,T_3 ratio										
I	29.73	35.86	26.42	29.02	24.44	29.09	2.41	0.34	0.51	0.91
II	29.55	21.95	21.03	23.42	25.36	24.26	1.51			
III	28.33	25.87	24.14	26.97	27.49	26.56	2.12			
IV	30.91	21.94	25.99	23.04	23.80	25.14	1.43			

$^{A, B, C}$ means bearing different superscripts in the same column differ significantly (P<0.01)
T_3 - triiodothyronine; T_4 - thyroxine

DISCUSSION

Results indicated that the supplementation of Se, vitamin E or both had no effect on the haematological parameters of buffalo calves. Similar results were reported earlier by Bednarek et al. (1996), who observed that the erythrocytes count, haemoglobin and PCV values of the vitamin E and Se supplemented calves were similar to that of control group. Contrary to the above findings, Qureshi et al. (2001) reported significantly (P<0.05) higher haemoglobin concentration, RBC count and PCV values in buffaloes supplemented with vitamin E and selenium.

The overall mean values of serum glucose (mg/dl) were found to be similar (P>0.05) in the four groups and were within the normal range, indicating that the supplementation of Se, vitamin E, or both had no effect on the serum glucose level. Similar results were reported by Cipriano et al. (1982), who did not find any significant (P>0.05) effect of supplemental vitamin E (500 mg dl-alpha tocopheryl acetate per day) on blood glucose level in calves. However, Nayyar et al. (2003) reported significantly (P<0.01) higher level of blood glucose in anoestrous buffalo heifers supplemented with vitamin E + Se as compared to control group. Contrary to above, Singh et al. (2002) observed low blood glucose concentration in buffalo calves fed wheat straw containing high Se (8.54 ppm).

The mean total protein (TP) values in groups I, II, III and IV were found to be statistically (P>0.05) comparable. Similar to our findings, Cipriano et al. (1982) and Reddy et al. (1987) did not observe any significant effect of supplemental vitamin E on the serum total protein values in calves. Similar finding were reported by Arthur et al. (1988), where in they did not find any effect of Se supplementation on plasma albumin levels in Friesian steers. Similarly, Reddy et al. (1987) also did not observe any effect of supplemental vitamin E on the serum albumin indicating that supplemental vitamin E, Se or both had no effect on the serum total protein levels of buffalo calves. The mean serum globulin and albumin: globulin ratio in different treatments were also found to be statistically (P>0.05) alike in four groups without showing any effect of Se, vitamin E or Se + vitamin E supplementation. Similarly, Reddy et al. (1987) also did not find any effect of graded levels of vitamin E supplementation on serum globulin and A: G ratio. Similarly, Arthur et al. (1988) in Friesian steers and Singh et al. (2002) in buffalo calves reported that Se supplementation (0.1 and 8.54 ppm, respectively), had no effect on the serum globulin and A: G ratio.

The mean values of total cholesterol, although numerically higher in Se and vitamin E + Se supplemented groups as compared to control and vitamin E supplemented groups, but did not reveal any statistical (P>0.05) differences. Similarly, Njeru et al. (1995) reported that graded levels of vitamin E supplementation had no effect on the cholesterol levels of yearling cattle. Similarly, Arthur et al. (1988) in Friesian steers and Singh et al. (2002) in buffalo calves reported that Se supplementation at a level of 0.1 and 8.54 ppm, respectively, had no effect on the blood cholesterol levels. Contrary to our findings, Nayyar et al. (2003) reported that supplementation of vitamin E (500 IU) and vitamin E + Se (500 IU vitamin E + 2 mg Se) per head per day to anoestrous buffalo heifers caused a significant (P<0.01) increase in the levels of cholesterol, as compared to unsuppleemnted anoestrous buffalo heifers. The mean HDL cholesterol values were found to be significantly (P<0.01) higher in group II (supplemented with 0.3 mg Se/kg DM) and group IV (supplemented with both Se 0.3 mg/kg DM intake and vitamin E 300 IU/head/day) as compared to group

III (supplemented with vitamin E 300 IU/head/day) and group I (control).These results showed that Se and vitamin E supplementation increased the serum HDL cholesterol levels and the increase was more pronounced due to Se supplementation as compared to vitamin E supplementation. Similarly, Mudgal et al. (2008) also reported that Se supplementation in buffalo calves significantly ($P<0.05$) increased their HDL cholesterol levels. The reason for an increase in the serum HDL cholesterol values due to Se and vitamin E supplementation may be due to the fact that Se is essential to maintain the integrity of pancreas and hence efficient digestion and absorption of fats, whereas regarding vitamin E, it is transported though circulation as components of lipoproteins, and cattle maintain majority of their serum vitamin E in the HDL fraction.

The overall mean triglyceride values (mg/dl) though numerically higher in all the supplemented animals as compared to control, but on statistical comparison they were found to be similar. Similar to our findings, Njeru et al. (1995) reported that supplementation of graded levels of vitamin E to yearling cattle had no effect on the serum triglyceride concentration. Contrarily, Wojcicki et al. (1991) reported that supplementation of vitamin E partially reduced the serum triglyceride levels of adult rabbits which were fed a high fat diet.

Non-significant ($P>0.05$) variation in serum urea and creatinine in 4 groups of buffalo calves was similar to the findings of Reddy et al. (1987) who did not notice any effect on serum urea and creatinine values of calves, supplemented with graded levels of vitamin E. Cipriano et al. (1982) also did not observe any significant differences ($P>0.05$) in the serum creatinine levels of calves that were fed a control, vitamin E supplemented (500 mg/day) or vitamin E deficient diet. Mudgal et al. (2008) also reported that supplementation of Se to buffalo calves had no effect on their serum urea and creatinine levels. Since serum urea and creatinine are indicators of the normal physiological status and N metabolism of the animals, hence it can be concluded that supplementation of Se, vitamin E or both had no effect on the nitrogen metabolism of buffalo calves.

Non-significant variation in the activities of SGOT and SGPT were similar to earlier findings of Samanta and Dass (2007) and Mudgal et al. (2008), who did not find any effect of supplemental vitamin E and Se on the activity of these enzymes in crossbred and buffalo calves, respectively. Contrary to present findings, Kursa and Kroupova (1976) reported significantly higher activity of SGOT and SGPT in serum of calves given extra vitamin E (15-30 mg/kg) in milk replacer. Regarding Se supplementation, Singh et al. (2002) observed that buffalo calves fed high Se (8.54 ppm) had higher activity of plasma SGOT and SGPT. The levels of vitamin E and Se that were present in the basal diet used in this experiment may be sufficient to maintain the normal enzyme activities of SGOT and SGPT and hence supplemental vitamin E and Se did not show any effect on the SGOT and SGPT activities in buffalo calves.

LDH isoenzyme profiles were the first to be used in veterinary medicine to detect organ injury, whereas CK have also been used as a sensitive indicator to detect muscle damage in clinical cases. Elevated levels of LDH and CK are suggestive of a tentative diagnosis of vitamin E and/or Se deficiency. Supplementation of vitamin E and Se were found to decrease the activity of these enzymes in buffalo calves (Amer et al., 1986); but, in the present experiment, it was found that supplemental vitamin E and/or Se had no effect on the serum activities of LDH and CK, which might be due to the fact that the levels of these micronutrients in the basal diet was sufficient enough to maintain the normal activities of these enzymes. The ALP (KA units) values observed in this study were not affected by treatments, and Hatfield et al. (2002) also did not find any effect of supplemental vitamin E (330 IU) in ewes on their serum ALP activity. Contrary to this, Kursa and Kroupova (1979) reported higher ALP activity in calves supplemented with vitamin E as compared with control calves. With regards to Se supplementation, Mudgal et al. (2008) did not find any effect of supplemental Se (0.3 ppm) on the ALP activity of buffalo calves. Contrary to these, Singh et al. (2002) reported higher ALP activity in buffalo calves fed wheat straw containing 8.54 ppm Se, where as Arthur et al. (1988) reported a decrease in ALP activity in Friesian steers fed a Se deficient diet (0.015 ppm).

Results indicated that buffalo calves that were supplemented with Se and vitamin E+Se, had significantly ($P<0.01$) higher levels of T_3 as compared to control. Similarly, Nayyar et al. (2003) also reported that buffalo heifers that were supplemented with vitamin E and vitamin E + Se had significantly ($P<0.01$) higher levels of T_3 as compared to unsuppleemnted control animals and the increase in the levels of T_3 was reported to be due to supplementation of Se. Arthur et al. (1988) reported that the peripheral concentration of T_3 was reduced and that of T_4 was increased in calves that were fed a synthetic diet that was deficient in Se as compared to the calves that were fed the same diet supplemented with Se. Non-significant variation in the concentration of serum T_4 and T_3: T_4 ratio observed in the present study was similar to the findings of Donald et al. (1994) in ewes supplemented with different levels of Se (0-8 mg) as an aqueous solution of sodium selenite. This may be due to the fact that type I iodothyronine-5´-deiodinase is a Se dependent enzyme, which is responsible for the deiodination of T_4 to T_3.

CONCLUSIONS

It may be concluded that supplementation of selenium and vitamin E in the diet of buffalo calves had no beneficial effect on blood haematology and blood chemistry except an increase in the serum levels of high density lipid cholesterol and triiodothyronine as compared to non-supplemented control buffalo calves.

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