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Effect of vitamin E and selenium supplementation on antioxidant status of male buffalo (*Bubalus bubalis*) calves*

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ABSTRACT

An experiment was conducted on 20 male buffalo calves (average body weight 75.30±2.20 kg) to study the effect of supplemental vitamin E and/or selenium on their antioxidant enzyme status. Group I served as control (without any supplementation), groups II, III and IV were supplemented with 0.3 mg selenium, 300 IU of DL-α-tocopheryl acetate, and both 300 IU DL-α-tocopheryl acetate and 0.3 mg selenium, 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 activity of antioxidant enzymes: catalase, super oxide dismutase (SOD), lipid peroxidase (LPO) and glutathione peroxidase (GSH-Px). Concentration of glutathione (GSH) was also measured in blood. Results revealed that the activity of erythrocyte LPO, catalase, and SOD did not differ (P>0.05) among the different treatments. Similarly, the concentration of GSH was also alike in 4 groups. However, erythrocyte GSH-Px activity and plasma Se concentration were found to be significantly higher in all the three supplemented groups as compared to control. The α-tocopherol concentration in plasma was found to be significantly higher in group III and IV as compared to group I and II. It may be concluded that vitamin E and Se supplementation increased the plasma level of these micronutrients and improved the antioxidant status of male buffalo calves in terms of erythrocyte GSH-Px activity.

KEY WORDS: vitamin E, selenium, antioxidant enzymes, buffalo calves

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INTRODUCTION

Under normal circumstances, oxidation and production of free radicals are an integral part of animal and human metabolism. Oxygen is the ultimate electron receptor in a closely linked electron flow system that produces energy in the form of ATP. However, when the electron flow becomes uncoupled, it leads to production of free radicals (Papas, 1996). Free radicals thus produced are destroyed by glutathione peroxidase, a selenium containing enzyme, whereas, vitamin E is a non-enzyme scavenger of free radicals. These two micronutrients act synergistically and protect the tissues against oxidative damage. Vitamin E reacts with peroxide radicals produced from polyunsaturated fatty acids in membrane phospholipids or lipoproteins to yield a stable hydroperoxide. The antioxidant activity of vitamin E in preventing lipid oxidation may be one of the mechanisms by which vitamin E enhances immunity (Chew, 1996). NRC (1989) has recommended a dietary level of 0.3 mg selenium and 15-40 mg vitamin E/kg DM for growing cattle. However, research suggests that relatively higher levels of vitamin E supplementation may improve calf performance, which may be due to enhanced immunity (Samanta et al., 2006; Shinde et al., 2007). 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 the fact that very limited information is available on the requirement and role of Se and vitamin E on antioxidant status of buffaloes, the present research work was planned in male buffalo calves to evaluate the effect of vitamin E and Se supplementation on the antioxidant status of male buffalo calves.

MATERIAL AND METHOD

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 offered to buffalo calves, % DM basis

Ingredients	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
Calcium	1.19	0.67
Phosphorus	0.96	0.07
α-Tocopherol, mg kg^{-1}	13.15	2.00
Selenium, mg kg^{-1}	0.17	0.13

Estimation of vitamins and selenium in feeds and plasma

The concentration of α-tocopherol in concentrate mixture and wheat straw offered to the experimental calves was estimated by using HPLC *(*McMurray et al.,1980). The α-tocopherol concentration in plasma was determined by the method of Milne and Botnen (1986) using High Performance Liqid Chromatography (HPLC, Shimadju, Japan). 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.

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

Processing of blood samples and preparation of erythrocyte pellet

Five ml of whole blood was collected into sterilized micro-centrifuge tube containing 0.75 ml of acid citrate dextrose (ACD; citric acid 8.0 g: sodium citrate 22.0 g and dextrose 25.0 g and volume made to 1l in distilled water) as anticoagulant. The samples collected over ice were brought to the laboratory immediately for further processing as described in subsequent sections.

The blood samples were centrifuged at 3000 rpm for 10 min at 4^{o}C and plasma and buffy coat were separated. The plasma was collected in plastic vials and kept at -40^{o}C until further analysis. The resulting erythrocyte pellet was washed thrice with phosphate buffer saline (PBS; disodium hydrogen phosphate 13.65 g, sodium dihydrogen phosphate 2.43 g and sodium chloride 10 g dissolved in 800 ml distilled water, pH adjusted to 7.4 and volume made to 1l) (Yagi et al., 1989). RBC diluted to 1:1 in PBS was used for the estimation of reduced glutathione and haemoglobin. For the estimation of catalase, super oxide dismutase (SOD), lipid peroxidase (LPO), and glutathione peroxidase (GSH-Px), 1 ml of the 1: 1 diluted RBCs in PBS were mixed with 9 ml distilled water to prepare a haemolyzate of 1: 20 dilution.

Estimation of antioxidant enzymes

The lipid peroxide level in the RBC haemolyzate was determined by the method of Placer et al. (1966), wherein; the concentration of malonaldialdehyde (MDA) in nmol of MDA/mg haemoglobin was calculated using the extinction coefficient of 1.56×10^{8}/M/cm (Utley et al., 1967). This was also expressed in nmol of MDA per mg haemoglobin. GSH concentration was estimated by the 5, 5′ dithiobis (2-nitrobenzoic acid) (DTNB) method (Prins and Loos, 1969). Catalase was assayed in erythrocytes by the spectrophotometer (Bergmeyer, 1983). Super oxide dismutase (SOD) activity of RBC haemolyzate samples was measured using nitro blue tetrazolium as a substrate after suitable dilution according to the method of Marklund and Marklund (1974) with certain modifications as suggested by Minami and Yoshikawa (1979). Glutathione peroxidase (GSH-Px) activity was determined by the method of Paglia and Valentine (1967).

Statistical analysis

Data were subjected to the test of significance between the different groups of buffalo calves using two way analysis of variance techniques (Snedecor and Cochran, 1980) and means were compared using Duncan's multiple range test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The concentration of α-tocopherol and Se in the blood plasma of buffalo calves of different groups and at different period intervals have been presented in Table 2.

Table 2. Plasma vitamin E and selenium status of buffalo calves

Group	Period, days					Mean	SEM	P value		
	0	45*	90***	135***	180***			G	P	G×P
α-Tocopherol, ppm										
I	1.76	1.78^{A}	1.98^{A}	2.88^{A}	3.68^{A}	2.42^{A}	0.22	0.001	0.001	0.005
II	1.71	2.25^{AB}	2.36^{A}	2.91^{A}	3.90^{A}	2.63^{A}	0.18			
III	1.70	4.11^{B}	4.92^{B}	5.38^{B}	5.90^{B}	4.40^{B}	0.35			
IV	1.61	4.09^{B}	4.58^{B}	5.29^{B}	5.56^{B}	4.22^{B}	0.35			
Selenium, ppb		***	***	***	***					
I	148	162^{A}	161^{A}	174^{A}	184^{A}	166^{A}	4.92	0.001	0.001	0.001
II	146	201^{AB}	283^{B}	319^{B}	329^{B}	256^{B}	15.28			
III	135	174^{AB}	191^{A}	193^{A}	207^{A}	180^{A}	6.89			
IV	153	236^{B}	304^{B}	352^{B}	366^{B}	282^{B}	16.58			

*P<0.05, ***P<0.001; ^{A,B} means bearing different superscripts in the same column differ significantly

The overall mean α-tocopherol concentration in plasma was 2.42, 2.63, 4.40 and 4.22 μg/ml in group I, II, III and IV, respectively. Statistical analysis of the data revealed significantly (P<0.001) higher concentrations of α-tocopherol in group III and IV, which were supplemented with vitamin E and vitamin E+Se, respectively, as compared to group I (control) and group II (supplemented with Se). The mean concentration of α-tocopherol at different periods also differed significantly (P<0.001). There was also a significant (P<0.005) group×period interaction observed among different groups and at different periods. On 45th day the mean α-tocopherol concentration was found to be significantly (P<0.05) higher in group III and IV as compared to group I. However, on 90, 135 and 180th days the mean vitamin E concentration was significantly (P<0.001) higher in group III and IV as compared to group I and II. Similar to our findings earlier research workers *(*Walsh et al., 1993; Cusack et al., 2005; Samanta et al., 2006; Rajeesh et al., 2008), also reported that vitamin E supplementation in the diet of calves caused a significant increase in their plasma α-tocopherol concentrations. However, Se supplementation had no effect on the plasma tocopherol concentration in Holstein calves *(*Weiss et al., 1990) and in male buffalo calves *(*Mudgal, 2005). The overall mean Se levels (ppb) in plasma of buffalo calves were 166, 256, 180 and 282, in groups I, II, III and IV, respectively. Statistical analysis revealed that the Se levels were significantly (P<0.001) lowest in group I, and highest in group IV. Period wise comparison of the mean Se levels also showed a significant difference (P<0.001)

at different period intervals. There was also a significant (P<0.001) group×period interaction observed regarding the blood plasma Se level. At 45th, 90th and 180th days, group II and group IV had significantly (P<0.001) higher levels of Se as compared to group I and III, indicating that the plasma Se levels in the groups supplemented with Se was increased. Similar to our results various researchers reported that supplemental Se increased the blood levels of Se in young cattle bulls (Pherson and Johnson, 1985), Holstein cows (Weiss et al., 1990) and in Hereford cows (Rowntree et al., 2004). The activity of different antioxidant enzymes, i.e. lipid peroxidase (LPO), catalase, super oxide dismutase (SOD) and glutathione peroxidase (GSH-Px), and the concentration of reduced glutathione (GSH), in the RBC of buffalo calves in different groups, estimated at different time intervals have been presented in Table 3. The mean values of LPO in

Table 3. Antioxidant enzyme status of buffalo calves in different groups

Group	Period, day					Mean	SEM	P value		
	0	45	90	135	180			G	P	G×P
LPO, μmol MDA formed/mg haemoglobin										
I	2.54	2.52	3.16	3.43	3.19	2.97	0.10	0.69	0.001	0.23
II	2.62	2.99	3.19	3.23	3.27	3.06	0.08			
III	2.62	2.64	3.16	3.31	3.52	3.05	0.09			
IV	2.89	2.60	3.07	3.16	3.55	3.06	0.08			
GSH, μmol/mg haemoglobin										
I	6.89	6.78	7.50	7.90	8.02	7.42	0.24	0.74	0.05	0.39
II	7.52	6.96	6.13	7.22	7.44	7.06	0.22			
III	6.09	7.50	7.18	7.74	7.35	7.17	0.27			
IV	6.95	6.12	7.75	7.62	8.21	7.33	0.29			
Catalase, unit/mg haemoglobin										
I	1.56	1.91	1.67	1.73	1.55	1.68	0.06	0.11	0.01	0.91
II	2.15	1.95	1.61	1.87	1.69	1.85	0.08			
III	1.87	1.77	1.31	1.72	1.54	1.64	0.10			
IV	1.81	1.73	1.23	1.56	1.55	1.58	0.09			
SOD, unit/mg haemoglobin										
I	22.91	24.43	22.32	23.66	25.96	23.86	0.44	0.70	0.001	0.26
II	24.95	23.93	22.81	21.94	26.55	24.04	0.46			
III	23.29	25.52	22.99	19.90	26.26	23.59	0.55			
IV	22.78	23.77	23.05	21.83	25.69	23.42	0.51			
GSH-Px, μmole NADPH oxidized/g haemoglobin/min										
		***	***	***	***					
I	16.99	15.29^{A}	16.75^{A}	16.79^{A}	12.06^{A}	15.58^{A}	0.50	0.001	0.001	0.001
II	15.37	19.66^{AB}	21.81^{B}	24.31^{B}	19.17^{B}	20.07^{BC}	0.71			
III	19.24	16.88^{AB}	19.36^{AB}	21.78^{AB}	17.59^{B}	18.97^{B}	0.50			
IV	18.50	20.95^{B}	21.99^{B}	23.88^{B}	20.06^{B}	21.08^{C}	0.54			

*** $P<0.001^{A,B,C}$ means bearing different superscripts in the same column differ significantly
LPO - lipid peroxidase; GSH - glutathione; SOD - super oxide dismutase; GSH-Px - glutathione peroxidase

erythrocytes were having an increasing trend with the advancement of experimental feeding, the values being significantly ($P<0.001$) lower at zero day, where as significantly ($P<0.001$) higher values were noted at the end of experimental feeding, i.e. at 180th day. But the mean values of LPO in different groups were found to be statistically comparable ($P>0.05$). Contrary to our results, crossbred calves supplemented with sodium selenite (0.25 mg/kg body weight/day) for 6 weeks had higher activity of LPO in treatment group as compared to control (Kaur et al., 2003). On the other hand, decreased ($P<0.05$) activity of LPO was observed in male buffalo calves which were intramuscularly injected with vitamin E and Se (250 and 7.5 mg, respectively) at weekly intervals for one month (Sandhu and Sangha, 2003).

The GSH concentration in different groups was also found to be comparable ($P>0.05$), the mean values being 7.42, 7.06, 7.17 and 7.33, in group I, II, III and IV, respectively. However, the GSH concentration at different time intervals differed significantly ($P<0.05$). Similar to our findings, supplementation of Se in male buffalo calves had no effect on their erythrocyte GSH levels (Walsh et al., 1993). However, contrary to these, it was reported that crossbred calves fed sodium selenite (0.25 mg/kg body weight/day) for 6 weeks had higher concentration of GSH in treatment group (Kaur et al., 2003). The GSH concentration in the tissue of crossbred calves was also not affected due to Se supplementation (Walsh et al., 1993).

Likewise, the catalase activity of erythrocytes also did not differ ($P>0.05$) among the different treatment groups. However, the catalase activity at different periods differed significantly ($P<0.01$). Similar to our findings, previous researchers (Sandhu and Sangha, 2003) reported that intramuscular injection of 250 mg vitamin E and 7.5 mg Se weekly for one month had no effect on their erythrocyte catalase activity.

The mean SOD enzyme activity was 23.86, 24.04, 23.59 and 23.42 U/mg Hb, in groups I, II, III and IV, respectively, and it did not differ among groups. When SOD activity at different periods was compared, it was found to be significantly ($P<0.001$) lower on 135th day at 21.83 U/mg Hb and highest on 180 days at 26.55 U/mg Hb. Similar to our findings, supplemental Se (0.438 ppm) was found to have no effect on the tissue SOD activity of crossbred calves (Walsh et al., 1993) and on the erythrocyte SOD activity of male buffalo calves (Mudgal,2005). Contrary to our findings, buffalo calves that were injected with 250 mg vitamin E and 7.5 mg Se weekly for one month had shown a decreased erythrocyte SOD activity (Sandhu and Sangha, 2003). The overall mean RBC GSH-Px activity was 15.58, 20.07, 18.97 and 21.08 µmole NADPH oxidized/g Hb/min, in groups I, II, III and IV, respectively. Statistical analysis of the data revealed that the mean RBC GSH-Px activity was significantly ($P<0.001$) lower in group I as compared to the other three groups. The overall mean RBC GSH-Px activity at different time intervals

also revealed significant differences ($P<0.001$). The group x period interaction was also found to be statistically significant ($P<0.001$). The mean RBC GSH-Px activity at 45th day was found to be significantly ($P<0.001$) lower in group I (15.29) as compared to group IV (20.95), where as on 90th and 135th days the mean RBC GSH-Px activity was significantly ($P<0.001$) higher in group II and IV as compared to group I. But on 180th day the GSH-Px activity was significantly ($P<0.001$) higher in all the supplemented groups (group II, III and IV) as compared to control (group I). Thus, the above results indicated that supplementation of either Se or vitamin E or both significantly improved the RBC GSH-Px activity. Similar to our results, a significant increase in RBC GSH-Px activity due to Se supplementation was reported in buffalo calves (Mudgal, 2005), in crossbred calves (Walsh et al., 1993) and in female white tailed deer (Brady, 1978). Buffalo calves that were injected with 250 mg vitamin E and 7.5 mg Se at weekly intervals for one month also had a significant increase in their erythrocyte GSH-Px activity (Sandhu and Sangha, 2003). A positive linear relationship ($r=0.958$) between blood GSH-Px activity and blood Se concentrations was also reported in dairy cattle (Scholz and Hutchinson, 1979).

CONCLUSIONS

It may be concluded that supplementation of vitamin E and Se increased the levels of these micronutrients in the plasma of male buffalo calves, whereby improving their antioxidant status (in terms of GSH-Px), as compared to the control (unsupplemented) buffalo calves.

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