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Effect of vitamin E and selenium yeast supplementation on growth and antioxidant status in male goat kids

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ABSTRACT

An experiment was conducted on 24 male goat kids (6.80±0.20 kg ABW) to elucidate the effect of supplemental vitamin E and/or selenium yeast on their growth, serum antioxidant enzymes, selenium and α- tocopherol status. Kids were randomly divided into 4 equal groups and fed a basal diet consisted of concentrate mixture and oat straw to meet their nutrient requirement. Group 1 served as control (without any supplementation), whereas animals in groups 2, 3 and 4 were supplemented either 0.3 mg selenium (Se) as Se-yeast, 100 mg of DL-α-tocopheryl acetate or both. This feeding practice lasted for 180 days, during which fortnightly body weights of kids were recorded. Blood was collected at day 0, 60, 120 and 180 of experimental feeding and analysed for α-tocopherol, Se and activity of antioxidant enzymes i.e. catalase (CAT), super oxide dismutase (SOD), glutathione peroxidase (GSH-Px) and malonaldialdehyde (MDA) concentration as a measure of lipid per-oxidation (LPO). Results revealed that the activity of erythrocyte GSH-Px and SOD and MDA concentration as a measure LPO and serum Se were significantly higher in all the 3 experimental groups as compared to control. Similarly, the concentration of α-tocopherol in serum was significantly higher in group 2, 3 and 4 as compared to group 1. However, growth rate of the kids and concentration of CAT did not differ among the different treatments. It was concluded that vitamin E and Se-yeast supplementation had no effect on body weight gain, but improved the antioxidant status of the kids in terms of erythrocyte GSH-Px, SOD activity and LPO.

Key words: Antioxidant enzymes, Growth, Kids, Selenium yeast, Vitamin E

Vitamin E, a non-enzyme scavenger of free radicals (Mac Pherson 1994), acts as a lipid soluble antioxidant in cell membranes (Noguchi *et al*. 1973) similarly selenium, a component of glutathione peroxidase enzyme (Rotruck *et al*. 1973), destroys free radicals in the cytoplasm. Normally oxidation and production of free radicals is an integral part of cell metabolism in animals and oxygen is the ultimate electron receptor in an electron flow system. However, when the electron flow becomes uncoupled, it leads to production of free radicals (Papas 1996), which are destroyed by glutathione peroxidase, a selenium containing enzyme, whereas vitamin E reacts with peroxide radicals to yield stable hydro peroxide. NRC (2007) recommended a dietary level of 0.3 mg selenium and 100 IU of vitamin E /kg DM for growing goats. However, Samanta *et al*. (2006) and Shinde *et al*. (2007) suggested that relatively higher levels of selenium and vitamin E supplementation may improve animal performance, which may be due to enhanced immunity. Little information is available on the effect of supplementation of organic selenium and vitamin E on growth and antioxidant status of goats (*Capra hircus)*. Therefore, the present research work was conducted in male goat kids to study the effect of selenium yeast and vitamin E supplementation on their growth and antioxidant status.

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MATERIALS AND METHODS

Animals, feeding and management: Male kids (24), procured from the Institute, were adapted on the experimental diet comprising concentrate mixture and oat straw for 1 month during which they were treated against ecto- and endo-parasites and subsequently at regular intervals. All the kids were vaccinated against foot-and-mouth disease and *peste des petits of ruminants* (PPR). These animals (average body weight 6.8±0.20 kg) were distributed in 4 different groups of 6 animals in each on the basis of their body weights following randomized block design, and were kept in a well ventilated shed with individual feeding and watering arrangements. Kids in all the 4 groups were fed on concentrate mixture and oat straw to meet their nutrient requirements for 50 g daily weight gain (NRC 2007). The concentrate mixture consisted (%) of crushed maize grain 30, soybean meal 37, wheat bran 30, mineral mixture 2 and common salt 1. Treatments were: group 1 (control), without any supplementation, group 2

supplemented with 0.3 mg Se from selenium yeast, group 3 supplemented with 100 mg DL-α-tocopheryl acetate and group 4 supplemented with both 0.3 mg organic Se and 100 mg DL-α-tocopheryl acetate/ kg DM, Oat straw was provided to the animals after total consumption of concentrate mixture. All the kids were offered about 100 g of the available green [(oat (*Avena sativa*)/ berseem (*Trifolium alexandrium*)/maize (*Zea mays*)] fodder once a week to meet their vitamin A requirements. Clean and fresh drinking water was provided twice a day to all the animals. This feeding practice lasted for 180 days, during which fortnightly body weights were recorded to assess their growth rate. Blood was collected at zero day and subsequently at an interval of 60 days up to 180 days of experimental feeding to estimate antioxidant enzymes activity, lipid per oxidation, alpha tocopherol and selenium concentration.

Processing of blood samples and preparation of erythrocyte pellet: Whole blood (5 ml) 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 1 litre in distilled water) as anticoagulant. The blood samples were centrifuged at 3,000 rpm for 10 min at 4ºC and plasma and buffy coat were separated. 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 1litre) (Yagi *et al*. 1989). RBC diluted to 1:1 in PBS was used for the estimation of haemoglobin. For the estimation of catalase, super oxide dismutase (SOD), lipid peroxide (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 RBC haemolyzate of 1: 20 dilution.

Estimation of antioxidant enzymes: Lipid per oxidation in RBC hemolyzate was determined by Placer *et al*. (1966); wherein, the concentration of malonaldialdehyde (MDA) in nmol of MDA/mg haemoglobin was calculated using the extinction coefficient of 1.56×108/M/cm (Utley *et al.* 1967). Catalase (CAT) was assayed in erythrocytes by the method of 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 Marklund and Marklund (1974) with certain modifications as suggested by Minami and Yoshikawa (1979). Glutathione peroxidase (GSH-Px) activity was determined as per Paglia and Valentine (1967).

Estimation of vitamins and selenium in feeds and serum: The concentration of α-tocopherol in concentrates mixture and oat straw offered to the experimental goats was estimated by HPLC (McMurray *et al*. 1980). The α-tocopherol concentration in serum was determined (Milne and Botnen 1986) using high performance liquid chromatography (HPLC). The α-tocopherol standard was procured commercially and diluted using ethanol. 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 serum samples was estimated in the double acid (HNO_3 and $HClO_4$; 4:1) mixture digested sample by atomic absorbance spectrophotometer 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.

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

RESULTS AND DISCUSSION

The chemical composition of concentrate mixture and oat straw is presented in Table 1.

Table 1. Chemical composition of feeds (% DM basis)

Attributes	Concentrate mixture	Oat straw
Organic matter	91.9	93.6
Crude protein	20.4	4.3
Ether extract	2.3	1.2
Neutral detergent fibre	34.5	78.3
Acid detergent fibre	11.6	57.1
Hemicelluloses	22.9	21.2
Cellulose	9.5	43.9
Calcium (Ca)	1.57	0.85
Phosphorus (P)	0.86	0.14
Selenium (ppm)	0.12	0.11
α-tocopherol (ppm)	13.75	1.90

The crude protein content of the concentrate mixture and oat straw was 20.4 and 4.3%, respectively, whereas the α-tocopherol and selenium concentration in concentrate mixture and oat straw were 13.75, 1.90 and 0.12 and 0.11 mg kg^{-1}, respectively.

Data on growth performance and average daily gain (ADG) are presented in Table 2.

Table 2. Growth performance of kids in different groups

Attributes	Group				SEM
	1	2	3	4	
Initial body weight (kg)	6.74	6.67	6.68	6.63	0.16
Final body weight (kg)	13.39	12.89	13.33	12.79	0.24
Weight gain (kg)	6.65	6.22	6.65	6.16	0.20
Average daily gain (g)	36.9	34.6	36.9	34.2	0.89

Supplementation of selenium yeast and vitamin E had no effect on the growth performance of kids as ADG was similar (P>0.05) in both the Se and vitamin E supplemented groups as compared to control group. Contrary to these findings, Kumar *et al.* (2009) in lambs observed increased growth rate in organic Se supplemented animals than control. Similarly, Nicholson *et al.* (1991) in calves and Wichtel *et al.* (1994) in yearling heifers observed increased growth rate in animals supplemented with Se. However,

similar to our results, Dominguez-Vara *et al*. (2009) observed no difference in growth rate of finishing lambs fed 0.3 mg Se-yeast/kg DM as compared to control. Mudgal *et al*. (2007) in male buffalo calves and McClure and Mahan (1988) in lambs also did not observe any effect of selenium supplementation on their growth rate. Similarly, supplementation of vitamin E in the diet of lambs (Turner *et al.* 2002) and calves (Cusack *et al.* 2005) had no effect on their average daily gain as compared to non-supplemented animals. Shinde *et al.* (2008) observed that supplementation of vitamin E (300 IU) and Se (0.3ppm) or both (300 IU vitamin E and 0.3ppm Se) in the diet of buffalo calves had no effect on their growth and ADG.

Lipid per-oxidation (LPO) and activity of different antioxidant enzymes, i.e. catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in the RBC of kids in different groups at different time intervals have been presented in Table 3.

Table 3. Antioxidant enzyme status of kids in different groups

Group							P value		
	0	60	120	180	Mean	SEM	G	P	G×P
GSH-PX (µmole NADPH oxidized/ g Hb/ min)									
1	17.2	17.4	16.4	17.8	17.2[a]	0.61	0.00	0.00	0.95
2	16.2	19.5	20.1	20.8	19.2[b]	0.78			
3	17.3	20.5	20.7	21.4	20.0[b]	0.55			
4	16.7	20.8	22.0	23.2	20.7[b]	0.47			
Catalase (U/ mg of Hb)									
1	1.6	1.6	1.6	1.6	1.6	0.04	0.53	0.55	0.90
2	1.6	1.7	1.7	1.8	1.7	0.09			
3	1.6	1.8	1.7	1.8	1.7	0.06			
4	1.6	1.8	1.9	2.2	1.9	0.11			
SOD (U/ mg of Hb)									
1	21.4	22.7	22.6	21.7	22.1[a]	0.52	0.04	0.00	0.93
2	21.6	24.1	25.5	26.8	24.5[b]	0.71			
3	21.8	25.4	28.3	30.0	26.4[b]	0.62			
4	21.7	28.9	30.0	30.6	27.8[c]	0.58			
LPO (µmole MDA formed/ mg of Hb)									
1	1.7	1.6	1.6	1.6	1.6 [a]	0.09	0.02	0.04	0.15
2	1.7	2.1	2.1	1.9	2.0 [b]	0.12			
3	1.7	2.0	1.9	2.3	2.0 [b]	0.14			
4	1.6	1.9	2.4	2.5	2.1 [b]	0.07			

[abc]Means bearing different superscripts in a column differ significantly (P<0.05).

The overall mean RBC GSH-Px activity was significantly (P<0.05) higher in group 2, 3 and 4 as compared to control group indicating that the supplementation of either organic Se or vitamin E or both significantly improved the RBC GSH-Px activity. Contrary to our findings, Cerri *et al*. (2009) reported that supplementation of 0.3 ppm organic and inorganic selenium in the ration of dairy cows from 25 days pre-partum to 70 days of lactation had no effect on serum glutathione peroxidise activity. However, similar to our findings, Arthington (2008) in beef steers, Kumar *et al*. (2009) in lambs and Shi *et al*. (2011) in goats observed an increase in RBC GSH-Px activity due to selenium yeast supplementation as compared to control group. Sandhu and Singha (2003) reported that buffalo calves injected with 250 mg vitamin E and 7.5 mg Se weekly for 1 month had a significant increase in their erythrocyte GSH-Px activity. Shinde *et al*. (2007) observed higher level of GSH-Px activity in buffalo calves supplemented with 0.3 ppm of Se and 300 IU of DL- α- tocopheryl acetate in male buffalo calves for 180 days. It was further observed that catalase (CAT) activity of erythrocytes did not differ (P>0.05) among the different treatment groups. Similar to our findings, Sandhu and Singha (2003) and Shinde *et al*. (2007) reported that supplementation of vitamin E and Se had no effect on their erythrocyte CAT activity. Similarly, Walsh *et al*. (1993) also did not find any effect of supplemental 0.438ppm Se on tissue CAT activity of crossbred calves. The mean SOD enzyme activity was significantly (P<0.05) different among different groups being 22.1, 24.5, 26.4 and 27.7 U/mg of Hb, in groups 1, 2, 3 and 4, respectively. Contrary to our findings, buffalo calves that were injected with 250 mg vitamin E and 7.5 mg Se weekly for 1 month had shown a decreased erythrocyte SOD activity (Sandhu and Singha 2003).

Malonaldialdehyde (MDA) values, indicator of lipid per-oxidation, were significantly (P<0.05) higher in all the 3 treatment groups as compared to control group. Contrary to our results, Sandhu and Singha (2003) observed decreased (P<0.05) concentration of MDA in male buffalo calves which were intramuscularly injected with vitamin E and Se (250 mg and 7.5 mg, respectively) at weekly intervals for 1 month. Similarly, Mudgal *et al.* (2012) also observed decreased MDA values in buffalo calves supplemented with 0.3 ppm Se. Contrary to these Brady *et al.* (1978) reported that female white tailed deer fed either a control diet (0.04 ppm Se) or Se supplemented diet (0.2 ppm) for 1 year had no effect on the MDA values. Shinde *et al.* (2007) also reported that supplementation of 0.3 ppm Se and 300 IU of DL- α- tocopheryl acetate in male buffalo calves for 180 days had no effect on erythrocyte lipid per-oxidation. However, similar to our results, crossbred calves

Table 4. Serum selenium and vitamin E concentration in different groups of kids

Group							P value		
	0	60	120	180	Mean	SEM	G	P	G×P
Selenium (ppb)									
1	190	182	207	216	199[a]	12.38	0.00	0.00	0.00
2	182	247	270	326	256[c]	15.22			
3	175	221	256	240	223[b]	17.10			
4	184	253	281	370	272[d]	16.59			
Alpha tocopherol (µg/ml)									
1	1.52	1.81	2.34	3.27	2.23[a]	0.12	0.00	0.01	0.04
2	1.56	2.21	2.98	3.58	2.58[a]	0.27			
3	1.51	3.64	5.17	5.42	3.93[b]	0.19			
4	1.58	3.98	5.76	6.17	4.37[c]	0.16			

[abcd] Means bearing different superscripts in the same column differ significantly (P<0.05).

supplemented with sodium selenite (0.25 mg/kg body weight/ day) for 6 weeks had higher activity of MDA in treatment group as compared to control (Kaur *et al*. 2003). Concentration of α-tocopherol and Se in blood serum of kids of different groups and at different period intervals are presented in Table 4.

The overall mean α-tocopherol concentration in serum was significantly (P<0.05) higher in groups 3 and 4 (supplemented with vitamin E and vitamin E+Se, respectively) when compared with groups 1 and 2. Similar to our findings, others (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 serum α-tocopherol concentrations. However, Se supplementation had no effect on the serum α-tocopherol concentration in Holstein calves (Weiss *et al*. 1990) and in male buffalo calves (Mudgal *et al*. 2012). The overall mean Se levels (ppb) in serum of kids were significantly (P<0.05) different in 4 groups being lowest in group 1, and highest in group 4. There was also a significant (P<0.05) increase in serum Se concentration at 60,120 and 180 days. Groups 2, 3 and group 4 had significantly (P<0.05) higher levels of Se as compared to group 1 indicating that the plasma Se level in the supplemented groups was increased. Similarly Pherson and Johnson (1985) in young cattle bulls, Weiss *et al.* (1990) in Holstein cows and Rowntree *et al.* (2004) in Hereford cows also reported that supplemental Se increased the blood levels of Se.

Our results suggested that supplementation of vitamin E and selenium yeast significantly improves the antioxidant status of kids in terms of erythrocyte GSH-Px, SOD activity and LPO accompanied with the enhanced level of these nutrients in the serum.

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