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Research Article

Antioxidant enzyme activities in anoestrus buffalo heifers supplemented with vitamin E and selenium

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ABSTRACT

The fifteen anoestrus buffalo heifers were divided into three groups viz. Group-I (control); Group-II-supplemented orally with 3500 IU vitamin E i.e. α-tocopherol acetate per week per animal and Group-III-supplemented orally with α-tocopherol acetate +14 mg selenium as sodium selenite per week per animal; for two months. Supplementation of vitamin E alone increased erythrocytic glutathione peroxidase activity but decreased the level of lipid peroxidation and the activities of superoxide dismutase and glucose-6-phosphate dehydrogenase. Supplementation of selenium along with vitamin E resulted in the similar changes but the effect was slightly higher on the level of lipid peroxidation and glutathione peroxidase activity. The data indicates that selenium synergises the action of vitamin E in improving the antioxidant status as well as reproductive performance.

Key words: Anoestrus buffalo, antioxidant enzymes, lipid peroxidation, vitamin E, selenium

Reactive oxygen metabolites (ROM) are unavoidable products of normal metabolic processes. Imbalance between the production of ROM and their safe disposal can initiate oxidative chain reactions and lipid peroxidation (Miller *et al.*, 1993). These reactions, if not controlled can cause extensive tissue damage, which may affect membrane permeability and enzyme function. Antioxidants such as vitamin E, vitamin C, β-carotene and the enzymes like superoxide dismutase and glutathione peroxidase are critical for body's defense against extensive production of ROM. Inadequate dietary antioxidants may lead to suboptimal reproductive performance by peroxidative damage to steroiodogenic enzymes (Staats *et al.*, 1988). Vitamin E is the main biological chain breaking antioxidant essential for growth, reproduction, prevention of various diseases and integrity of tissues (Quereshi *et al.*, 1997). The activity of glutathione peroxidase in the blood of dairy cattle was associated with the incidence of anoestrus or subestrus (Jukola *et al.*, 1996). Although selenium deficiency is not observed in Punjab, the role of dietary selenium supplementation along with vitamin E needs to be studied as selenium is known to increase the antioxidant action of vitamin E. In the present investigation, anoestrus buffalo heifers were supplemented with vitamin E and selenium and the levels of lipid peroxidation and antioxidant enzymes - superoxide dismutase, glutathione peroxidase and glucose-6-phosphate dehydrogenases were evaluated along with reproductive performance.

MATERIALS AND METHODS

The animals maintained at the diary farm of Punjab Agricultural University, Ludhiana under standard conditions of feeding and management were used for the study. Fifteen anoestrus buffalo heifers (2-4 years of age) with inactive and smooth ovaries as examined by per rectal palpation and showing sexual quiescence for at least three preceding reproductive cycles were divided into three groups.

Group I (n = 5)	Control (anoestrus heifers)
Group II (n = 5)	Anoestrus heifers supplemented orally with 3500 IU vitamin E(α-tocopherol acetate) per week per animal.

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Group III (n = 5) Anoestrus heifers supplemented orally with 3500 IU vitamin E(α-tocopherol acetate) + 14 mg selenium (sodium selenite) per week per animal.

Supplementation was continued for two months. The blood samples were collected once before supplementation in anoestrus heifers and at weekly intervals for a month after the withdrawal of supplementation.

The blood samples were collected aseptically from the jugular vein and immediately transferred to the laboratory in an ice box. For the preparation of hemolysate, blood was taken in a centrifuge tube up to the marked level and washed thrice with the normal saline. The hemolysate was prepared by adding distilled water upto the marked level with constant shaking. Lipid peroxidation was estimated in the hemolysate by the method of Placer *et al.* (1966). The activities of superoxide dismutase (SOD), glutathione peroxidase (GPX) and glucose-6-phosphate dehydrogenase (G6PD) were assayed in the hemolysate using the methods of Nishikimi *et al.* (1972), Hafeman *et al.* (1974) and Bergmeyer (1974), respectively. The results were subjected to analysis of variance on computer using randomized block design in CPCSI software developed by Dr. H.S. Cheema, Associate Professor of Statistics, Punjab Agricultural University, Ludhiana.

RESULTS AND DISCUSSION

Lipid peroxidation: The results for erythrocytic lipid peroxidation have been expressed in terms of malondialdehyde (MDA) content. The endogenous erythrocytic MDA levels and H_2O_2 induced erythrocytic MDA levels (with 0.5% and 1.5% H_2O_2 in the reaction medium) decreased significantly in groups II and III as compared to group I (Table 1). The levels of endogenous erythrocytic MDA were significantly lower in group III as compared to group II at Ist, 2nd and 3rd weeks after supplementation, whereas the levels of H_2O_2 (0.5%) induced erythrocytic MDA were significantly lower in group III than group II at 2nd and 3rd week after supplementation. However, H_2O_2 (1.5%) induced erythrocytic MDA level was significantly lower in group III at all the samplings after supplementation as compared to group II. Vitamin E and selenium deficiencies increase indices of lipid peroxidation in ruminant calves (Walsh *et*

Table 1. Erythrocytic lipid peroxidation (n mol MDA/mg Hb) in anoestrus buffalo heifers supplemented with vitamin E and selenium

Group	Before supplementation	Weeks after supplementation				CD5%		
		1	2	3	4	Mean	Replicates	Treatments
				Endogenous MDA				
I	368.9±4.16[a]	374.2±1.37[a]	369.2±1.32[a]	368.0±9.25[a]	366.6±1.44[a]	369.5 ±2.00	10.85	18.79
II	385.0±19.3[a]	294.3±6.09[b]	258.7±10.22[b]	239.3±12.0[b]	224.2±4.16[b]	254.1±18.53*		
III	374.2±3.94[a]	264.8±12.59[c]	239.1±10.2[c]	215.7±10.2[c]	210.3±10.20[b]	232.4±15.26*		
				0.5% H_2O_2 induced MDA				
I	569.7±3.11[a]	571.8±2.54[a]	571.4±2.95[a]	567.6±2.36[a]	564.4±2.53[a]	568.8 ±2.16	NS	20.39
II	565.7±1.82[a]	491.8±1.79[b]	454.28±8.53[b]	422.3±2.65[b]	414.6±1.32[b]	445.7±21.54*		
III	566.9±4.05[a]	477.5±12.82[b]	415.6±6.17[c]	397.9±1.79[c]	417.9±2.34[b]	427.3±21.25*		
				1.5% H_2O_2 induced MDA				
I	864.3±3.65[a]	864.9±1.48[a]	863.9±3.44[a]	862.9±3.06[a]	861.1±3.27[a]	863.2 ±0.99	10.57	18.31
II	852.0±2.63[a]	801.0±3.76[b]	758.7±13.10[b]	724.3±3.64[b]	713.9±1.97[b]	749.5±24.09*		
III	847.7±0.76[a]	779.1±13.45[c]	704.9±10.65[c]	688.5±7.20[c]	680.2±5.34[c]	713.2±27.64*		

The values with same superscripts within a column do not differ significantly.
Asterisk(*) indicates the mean values significantly different from the respective pretreatment values.

al., 1993). Vitamin E interacts directly with lipid peroxides in plasma lipoproteins and cell membranes to neutralize them (Pironi *et al.*, 1998). In the present results too, vitamin E supplementation has been found to decrease the lipid peroxidation level in anoestrus buffalo heifers. The sparing as well as synergistic actions are thought to result from the ability of both tocopherol and selenium dependent GPX to decrease the production of lipid peroxidation products (Machlin and Bendich, 1987). In our results, lipid peroxidation increased progressively with 0.5% and 1.5% H_2O_2 in the reaction medium but in all cases, it decreased with vitamin E and selenium.

Enzyme activities: The erythrocytic SOD activity (Table 2) decreased significantly in group II and group III as compared to group I anoestrus heifers at 4th week after supplementation and decreased significantly in group III as compared to group I at 3rd week after supplementation. However, the difference between the activity of SOD in group II and group III was non-significant at all the samplings after supplementation. Superoxide dismutase disproportionates superoxide to hydrogen peroxide, which is metabolized in the intracellular compartments by selenium dependent GPX. Superoxide anion can react with hydrogen peroxide to form hydrogen peroxide radical, which reacts with poly-unsaturated acids to generate lipid peroxides (Pironi *et al.*, 1998). The increased activity of erythrocytic SOD in anoestrus heifers could be attributed to physiological upregulation of this enzyme in an attempt to diminish the superoxide radical challenge. Supplementation of vitamin E and selenium might be responsible for relieving the load of oxidative stress in anoestrus heifers, thus lowering the erythrocytic SOD activity in anoestrus heifers supplemented with vitamin E and selenium.

The erythrocytic activity of G6PD (Table 2) decreased significantly in groups II and III as compared to group I heifers. However, the difference between the activity of G6PD in group II and group III was non-significant at all the samplings after supplementation. A number of species can upregulate the activity of G6PD in an attempt to mitigate the effects of peroxidative challenge (Walsh *et al.*, 1993). This may be the possible reason for the higher activity of G6PD in unsupplemented group I anoestrus heifers. Supplementation of vitamin E and selenium has lowered the erythrocytic G6PD activity along with the decrease in lipid peroxidation thus relieving the oxidative stress, which was observed earlier too in

Table 2. Erythrocytic antioxidant enzyme activities in anoestrus buffalo heifers supplemented with vitamin E and selenium

Group	Before supplementation	Weeks after supplementation				CD5%		
		1	2	3	4	Mean	Replicates	Treatment
Superoxide dismutase (U/mg Hb)								
I	10.30±0.049^a	10.31±0.06^a	10.29±0.06^a	10.29±0.06^a	10.38±0.09^a	10.32±0.026	0.109	0.189
II	10.17±0.026^a	9.16±0.063^a	8.72±0.050^a	8.55±0.064^{ac}	8.45±0.091^b	8.72±0.194*		
III	10.21±0.055^a	8.85±0.064^a	8.61±0.087^a	8.29±0.088^{bc}	8.07±0.144^b	8.45±0.208*		
Glucose-6-phosphate dehydrogenase (µ mol NADPH/min/g Hb)								
I	3.22±0.091^a	3.19±0.080^a	3.21±0.106^a	3.18±0.084^a	3.16±0.138^a	3.18±0.011	0.223	0.386
II	3.21±0.215^a	2.46±0.181^b	2.20±0.186^b	2.08±0.193^b	1.93±0.159^b	2.16±0.135*		
III	3.18±0.167^a	2.61±0.168^b	1.97±0.046^b	1.89±0.059^b	1.81±0.075^b	2.07±0.225*		
Glutathione peroxidase(U/mg Hb)								
I	13.12±0.112^a	13.12±0.10^a	13.09±0.12^a	13.12±0.11^a	13.16±0.10^a	13.12±0.01	0.364	0.631
II	13.37±0.264^a	14.28±0.41^b	14.51±0.37^b	14.89±0.33^b	15.02±0.34^b	14.68±0.20*		
III	13.20±0.223^a	14.73±0.34^b	15.99±0.14^c	17.19±0.12^c	17.40±0.16^c	16.33±0.75*		

The values with same superscripts within a column do not differ significantly.
Asterisk(*) indicates the mean values significantly different from the respective pretreatment values.

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postpartum anoestrus buffaloes supplemented with vitamin E and selenium(Anita *et al.*, 2004).

The activity of GPX in erythrocytes (Table 2) increased significantly in group II and group III as compared to group I. The GPX activity was higher in group III as compared to group II at 2nd , 3rd and 4th weeks after supplementation. Selenium and vitamin E used separately are able to combat the oxidative stress; used together, they are even more efficacious (Jukola *et al.*, 1996). Supplementation with selenium and/or vitamin E increased the blood GPX activity in cows and heifers (Osame *et al.*, 1992; Wichtel *et al.*, 1996). The increased GPX activity along with the ecrease in lipid peroxidation justifies the antioxidative action of vitamin E and selenium.

In group II, four animals exhibited estrus at 133 days after the treatment and two animals became pregnant at 153 days after the treatment. In group III, all the five animals exhibited estrus at 51 days after the treatment and two animals were pregnant at 61 days after the treatment. The animals in group I remained anoestrus through out the experimental period. Hence the results reveal that supplementation of selenium along with vitamin E improves the antioxidant status as well as the reproductive performance of anoestrus heifers.

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