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Blood glutathione peroxidase activity and some immunological markers in sheep: the effect of vitamin E and selenium

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ABSTRACT :This research set out to identify which cellular immunity parameters—metabolic activity, phagocytic activi-ty, and lymphocyte blastogenic response—were affected by the administration of selenium and vitamin E. Two groups were formed from nine pregnant sheep ranging in weight from 42 to 66 kg. Five sheep in one group received 5 milligrams of vitamin E and 0.4 milligrams of selenium per kilogram of body weight subcutaneously before lambing, while four in the other group received no therapy and acted as a control. All of the sheep had blood samples taken before the therapy, at 14 and 30 days post-lambing. In the samples obtained 14 days and 30 days after lambing, respectively, the control group and the sheep treated with vitamin E and selenium had substantially lower whole blood GSH-Px activity ($P < 0.01$, and $P < 0.001$, respectively). On days 14 and 30, after lambing, the examination of immunological measures revealed a decrease in these markers. The phagocytic activity index of leukocytes and neutrophils were significantly affected by the provided preparation ($P < 0.001$ and $P < 0.05$, respectively).

Keywords: sheep; cellular immunity; selenium; vitamin E; glutathione preoxidase

INTRODUCTION

Vitamin E and selenium are antioxidants that are re- lated to immune function in domestic animals (Finchand Turner, 1996). Vitamin E is a powerful antioxidant that prevents the formation of lipid hydroperoxides fromunsaturated phospholipids present in subcellular mem- branes (McDowell, 1989). Selenium as an essential com- ponent of glutathione peroxidase reduces potentially harmful oxygen radicals such as hydrogen peroxides and lipid hydroperoxides (Rotruck et al., 1973). A bio-chemical role was recently established for selenium as acomponent of an enzyme, GSH-Px, which functions along with vitamin E in the cells to control peroxidation (Van Vleet, 1980).

An increase in reactive oxygen molecules (ROM) arises when oxidative metabolic reactions are increasedas in aerobic exercise, pregnancy, stress, tissue injury, and infection (Nockels, 1996). In stress, many hormonessuch as glucocorticoids and epinephrine are produced. In addition, it was shown in calves (Reddy et al., 1987) and mice (Lim et al., 1981) that blood cortisol and corticosteron levels decreased after vitamin E dietary supplementation.

A number of investigators have demonstrated that circulating neutrophils, peritoneal macrophages, andpulmonary alveolar macrophages from selenium-vitamin E deficient animals have low amounts of glu- tathione peroxidase activity and decreased microbicidal ability (Serfass and Ganther, 1975; Bayne and Arthur,1979).

A great attention has recently been focused on the role of vitamin E and selenium in protection leukocytes and macrophages during phagocytosis, the mechanism whereby mammals immunologically kill invading bacteria.Both vitamin E and GSH-Px are antioxidants that protect phagocytic cells and surrounding tissues from oxidative attack by free radicals produced by the respiratoryburst of neutrophils and macrophages during phagocy- tosis (Baboir, 1984; Baker and Cohen, 1983). The protec-tion of cell membranes

and other cellular components of immune cells against lipid peroxidation is probably the most important mechanism of vitamin E in the immune response (Bendich, 1990).

Cellular defences appear to be particularly vulnerable to a deficiency. Phagocytes from selenium-deficientcattle fail to kill ingested microbes (Boyne and Arthur, 1979). In addition, the performance of phagocytes can be improved by selenium/vitamin E injections (Gyang etal., 1984).

The present investigation aimed to determine the ef-fect of vitamin E and selenium administration on blood glutathione peroxidase activity as well as on some immmunological functions in sheep.

Starting two weeks prior to anticipated lambing, ninepregnant Merino sheep weighing 42 to 66 kg, aged threeto four years, were available for the present experiment. During the experiment, the sheep were housed and fed daily concentrates of 0.5 kg BAK (BAK is a concentratefor sheep), its composition as depicted in Table 1, meadow hay and water were available ad libitum. The same feeding program continued throughout the experi-ment duration, and the sheep were under a constant surveillance during the experiment.

The sheep were di-vided into two groups. The first group (n = 5) was ad-ministered a single subcutaneous injection of 5 mg tocopheryl acetate and 0.4 mg of selenium as sodium se-lenite per kg body weight (Selevit inj.: 25 mg tocopherylacetate and 2.2 mg sodium selenite in 1 ml, Biotika) andthe second group was not treated (n = 4) it served ascontrol.

Table 1. Composition of the concentrate (BAK) Ingredient Amount

Protein 126.0 g/kg	
Digestible protein	97.0 g/kg
Dry matter	860 g/kg
Crude fibre	80 g/kg
Calcium	6 g/kg
Phosphorous	6 g/kg
Sodium	5 g/kg
Vitamin A	25 000 iu
Vitamin E	2 500 iu
Vitamin D	10 mg/kg

Blood samples were obtained from the sheep at threestages. At the beginning of the experiment (two weeksbefore lambing), prior to vitamin E and selenium adminis-tration, 14 days after lambing and 30 days after lambing. The glutathione peroxidase activity (GSH-Px) in whole blood was determined spectrophotometrically ac-cording to the modification of the technique of Pagliaand Valentine (1967) using a commercial kit (Randox, Ire-land). Enzymatic activity was expressed as U/g haemo-globin (Hb).

Metabolic activity of phagocytes was tested by de-termination of their tetrazolium reduction activity (Mareček and Procházková, 1986) and the results indi-cated as metabolic activity index (MA-I). Phagocytic ac-tivity of phagocytes was tested according to Větvička etal. (1982) by using microspheric hydrophilic metacrylateparticles (MSHP) method and the results given as phago-cytic activity index. Lymphocyte blastogenesis was tested by the fluorescence assay with ethidium bromide(Nagahata et al., 1986) and results are expressed as stimulation index.The values are expressed as mean ± standard devia-tion and analysed by a two-way analysis of variance (one repeated factor: time, one grouping factor: treat- ment) and Dunnett's test was performed in order to check each group differences at each time of sampling using a computer program. Student's t-test was used to evaluate the treatment effect between groups.

RESULTS

The mean initial GSH-Px activities of the group treated with vitamin E and selenium preparation were similar to the control group before vitamin E and sele- nium treatment (Figure 1). 14 days and 30 days afterlambing, the GSH-Px activity was significantly higher in the treated group ($P < 0.01$; $P < 0.001$, respectively) than in the control group. The same variations were ob- served in mean GSH-Px activities of treated group on days 14 and 30 after lambing in comparison with the ini-tial level, and these changes were highly significant ($P < 0.01$). At the end of the experiment (30 days after lambing), GSH-Px activity in control group was lowerthan the initial level (49.05 ± 17.78 vs. 49.68 ± 22.97), but this decrease was not significant ($P > 0.05$). ANOVA revealed that both phagocytic activity in- dex of neutrophils and phagocytic activity index of leukocytes showed significant ($P < 0.05$; $P < 0.01$, re-spectively) effects as a result of supplementation. 14 days after lambing, the phagocytic activity index of

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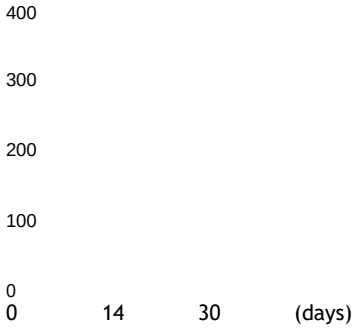
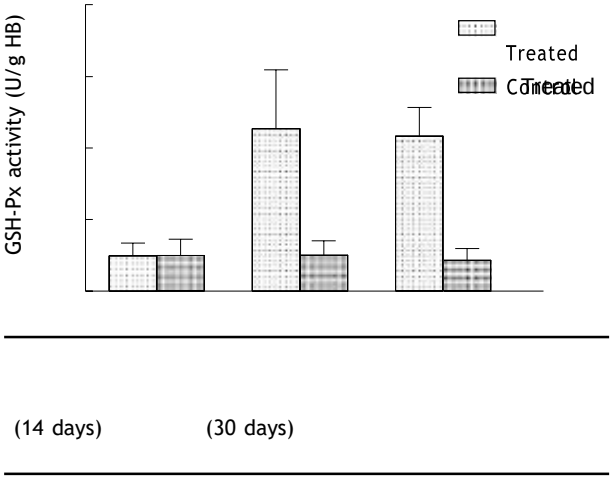


GSH-Px activity (U/g HB)

Groups	Before treatment	After parturition	After parturition (O day)
Treated	49.05 ± 17.78	226.6 ± 82.57**A	216.6 ± 40.09***A
Control	49.68 ± 22.97	50.22 ± 20.14	42.70 ± 16.73
Time effect P < 0.0001		Group effect P < 0.0001	

AP < 0.01 vs 0; **P< 0.01 vs control; ***P < 0.001 vs control

Figure 1. Whole blood glutathione peroxidase activity in sheeptreated with vitamin E and selenium and in non-treated sheep



leukocytes decreased significantly (P < 0.01) in control group in comparison with treated group (5.07 ± 1.39 vs. 7.66 ± 0.45), whereas 30 days after lambing, there was a tendency in treated group for the phagocytic ac- tivity index of leukocytes to be higher than in controlgroup (8.42 ± 1.97 vs. 5.14 ± 2.94) but these differences were not significant (Figure 2). The phagocytic activity index of neutrophils was lower in control group than in treated group (5.62 ± 1.72 vs. 8.48 ± 1.93), but this de-crease was not significant (Figure 3). There were no dif-ferences in the phagocytic activity index of neutrophils between the sampling periods in both groups.

Evaluation of the metabolic activity index showed in-significant differences (P > 0.05) between and withintreated sheep and control sheep during the experiment as illustrated in Figure 4. The effect of vitamin E and se-lenium administration on the blastogenic response of lymphocytes was examined and the results are ex- pressed as stimulation index (Figure 5). As indicated,there were no differences in stimulation index betweencontrol and vitamin E and selenium injected sheep. Al- though there were no significant differences in stimula-tion index between both groups, there was a tendency in treated sheep for the stimulation index to be higher than in control sheep 14 days after lambing which was based on the high initial value of stimulation index intreated sheep.

DISCUSSION

GSH-Px assays offer a rapid and simple alternative towhole blood selenium estimation for the diagnosis ofselenium deficiency, avoiding the matter of selenium concentration. The enzyme is very stable in erythro- cytes (Wilson and Judson, 1976) and there is a high cor-relation between erythrocyte GSH-Px activity and whole blood GSH-Px activity (Kováč and Sankari, 1988) and they are therefore suitable for routine diagnostic pur- poses. In the treated group, the GSH-Px increase ap-pears to respond to selenium and vitamin E injection.

The decline in selenium concentration during latepregnancy and lactation has already been reported forselenium-deficient sheep (Lacetera et al., 1999). Thepresent study demonstrated that in sheep of controlgroup, lactation was probably responsible for worsen-ing the GSH-Px activity status. GSH-Px activity in bloodwas independent of dietary vitamin E (Siddonns andMills, 1981). Therefore, our study demonstrated that theinjection of 0.4 mg/kg body weight before lambing wasresponsible for a lasting increase in the GSH-Px activity.In cattle, major stresses that increase blood cortisol

concentration are castration, weaning, handling, de-horning, parturition, water source, forced exercise, neo-natal diarrhea, shipping, and certain conditions that may cause pain (Roth and Kaeberle, 1982). Thus, thesheep of this experiment were possibly under stress. Supplementation of selenium more than required hasalready been shown to enhance the immune response incattle and several non-ruminant species (Stowe et al., 1988; McDowell, 1992). The results of Finch and Turner (1989) suggest that whole blood GSH-Px activity is a poor indicator of immunological responses. The results presented here indicate that the changes of immuno- logical responses and GSH-Px activity were not the same after vitamin E and selenium administration.

It was reported that vitamin E supplementation en- hanced phagocytosis (Hogan et al., 1990). A sufficientvitamin E concentration in phagocytic cells seems toplay an important role in optimal development of chemi-cal processes during phagocytosis (Boxer, 1990). On day 0, the significant difference between the groups in phagocytic activity index of leucocytes was probablydue to different concentrations of serum constituents which have an effect on immune response. Vitamin Eand selenium used in our study led to significant differ-ences in phagocytic functions as shown by phagocytic activity indexes of leukocytes and neutrophils. Politis et al. (1995) indicated that functions of blood macrophages and neutrophils are depressed during the early postpartum period in cows. During our experiment,at the beginning, on 14 and 30 day after lambing, sheepin both groups showed no significant differences in the values of stimulation index and metabolic activity index.It has been reported that vitamin E supplementation in cattle enhances lymphocyte blastogenesis (Reddy et al., 1986; Eicher-Pruiett et al., 1992) Although the means did not differ significantly between groups in thepresent study, blastogenesis of lymphocytes (stimula- tion index) in treated group tended to have higher val- ues than the control group.

There were declines in the immunological parametersinvestigated that seemingly due to the effect on parturi- tion and lactation are considered as stress factors.

In conclusion, a single vitamin E and selenium injec- tion led to a significant rise in whole blood GSH-Px ac- tivity. The increase was similar after lambing (14 and 30days). An evaluation of vitamin E and selenium injec-tion effects on immunological parameters showed dis- cernible effects on phagocytic function, as measured by phagocytic indexes of leukocytes and neutrophils, while they had no effect on either blastogenesis as mea-sured by stimulation index or metabolic activity as mea-sured by metabolic activity index.

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