Effect of vitamin E and selenium on blood glutathione peroxidase activity and some immunological parameters in sheep

K. MILAD[1], O. RÁCZ[2], A. ŠIPULOVÁ[2], V. BAJOVÁ[1], G. KOVÁČ[1]

[1]*University of Veterinary Medicine, Košice, Slovak Republic*
[2]*Faculty of Medicine, P. J. Šafarik University, Košice, Slovak Republic*

ABSTRACT: The objective of this study was to determine the effect of vitamin E and selenium administration on glutathione peroxidase (GSH-Px) activity and selected parameters of cellular immunity (metabolic activity, phagocytic activity, lymphocyte blastogenic response). Nine pregnant sheep with body weight of 42 to 66 kg were divided into two groups. Before lambing, the first group (n = 5) was given subcutaneously 5 mg vitamin E and 0.4 mg selenium per kg body weight while the second group (n = 4) was given no treatment and served as control. Blood samples from all sheep were collected before the treatment, 14 days after lambing and 30 days after lambing. Whole blood GSH-Px activities were significantly higher in the vitamin E and selenium treated sheep than in the control in the samples taken 14 days after lambing and 30 days after lambing ($P < 0.01$; $P < 0.001$, respectively). The evaluation of immunological parameters showed declines in immunological parameters measured on days 14 and 30 after lambing. The administered preparation led to significant effects ($P < 0.001$; $P < 0.05$) on phagocytic activity index of leukocytes and phagocytic activity index of neutrophils, respectively.

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

INTRODUCTION

Vitamin E and selenium are antioxidants that are related to immune function in domestic animals (Finch and Turner, 1996). Vitamin E is a powerful antioxidant that prevents the formation of lipid hydroperoxides from unsaturated phospholipids present in subcellular membranes (McDowell, 1989). Selenium as an essential component of glutathione peroxidase reduces potentially harmful oxygen radicals such as hydrogen peroxides and lipid hydroperoxides (Rotruck *et al*., 1973). A biochemical role was recently established for selenium as a component 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 increased as in aerobic exercise, pregnancy, stress, tissue injury, and infection (Nockels, 1996). In stress, many hormones such 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, and pulmonary alveolar macrophages from selenium-vitamin E deficient animals have low amounts of glutathione 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 respiratory burst of neutrophils and macrophages during phagocytosis (Baboir, 1984; Baker and Cohen, 1983). The protection 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-deficient cattle fail to kill ingested microbes (Boyne and Arthur, 1979). In addition, the performance of phagocytes can be improved by selenium/vitamin E injections (Gyang *et al*., 1984).

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

MATERIAL AND METHODS

Starting two weeks prior to anticipated lambing, nine pregnant Merino sheep weighing 42 to 66 kg, aged three to 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 concentrate for sheep), its composition as depicted in Table 1, meadow hay and water were available *ad libitum*. The same feeding program continued throughout the experiment duration, and the sheep were under a constant surveillance during the experiment. The sheep were divided into two groups. The first group (n = 5) was administered a single subcutaneous injection of 5 mg tocopheryl acetate and 0.4 mg of selenium as sodium selenite per kg body weight (Selevit inj.: 25 mg tocopheryl acetate and 2.2 mg sodium selenite in 1 ml, Biotika) and the second group was not treated (n = 4) it served as control.

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 three stages. At the beginning of the experiment (two weeks before lambing), prior to vitamin E and selenium administration, 14 days after lambing and 30 days after lambing.

The glutathione peroxidase activity (GSH-Px) in whole blood was determined spectrophotometrically according to the modification of the technique of Paglia and Valentine (1967) using a commercial kit (Randox, Ireland). Enzymatic activity was expressed as U/g haemoglobin (Hb).

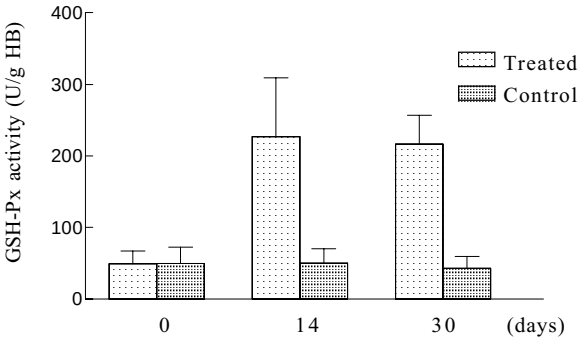
Metabolic activity of phagocytes was tested by determination of their tetrazolium reduction activity (Mareček and Procházková, 1986) and the results indicated as metabolic activity index (MA-I). Phagocytic activity of phagocytes was tested according to Větvička *et al*. (1982) by using microspheric hydrophilic metacrylate particles (MSHP) method and the results given as phagocytic 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 deviation and analysed by a two-way analysis of variance (one repeated factor: time, one grouping factor: treatment) 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 selenium treatment (Figure 1). 14 days and 30 days after lambing, 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 observed in mean GSH-Px activities of treated group on days 14 and 30 after lambing in comparison with the initial 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 lower than 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 index of neutrophils and phagocytic activity index of leukocytes showed significant ($P < 0.05$; $P < 0.01$, respectively) effects as a result of supplementation. 14 days after lambing, the phagocytic activity index of



Groups	GSH-Px activity (U/g HB)		
	Before treatment (O day)	After parturition (14 days)	After parturition (30 days)
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 $P < 0.0001$

$^{A}P < 0.01$ vs 0; $^{**}P < 0.01$ vs control; $^{***}P < 0.001$ vs control

Figure 1. Whole blood glutathione peroxidase activity in sheep treated 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 activity index of leukocytes to be higher than in control group (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 decrease was not significant (Figure 3). There were no differences in the phagocytic activity index of neutrophils between the sampling periods in both groups.

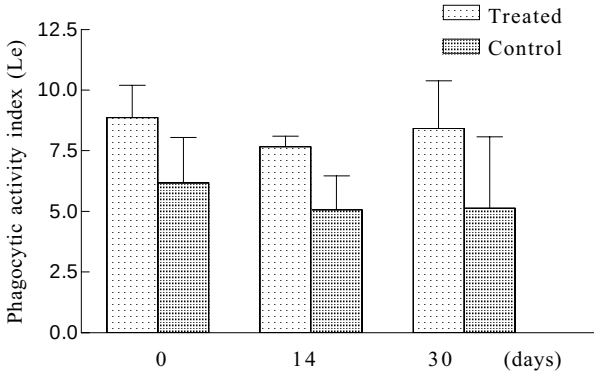
Evaluation of the metabolic activity index showed insignificant differences ($P > 0.05$) between and within treated sheep and control sheep during the experiment as illustrated in Figure 4. The effect of vitamin E and selenium administration on the blastogenic response of lymphocytes was examined and the results are expressed as stimulation index (Figure 5). As indicated, there were no differences in stimulation index between control and vitamin E and selenium injected sheep. Although there were no significant differences in stimulation 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 in treated sheep.

DISCUSSION

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

The decline in selenium concentration during late pregnancy and lactation has already been reported for selenium-deficient sheep (Lacetera *et al.*, 1999). The present study demonstrated that in sheep of control group, lactation was probably responsible for worsening the GSH-Px activity status. GSH-Px activity in blood was independent of dietary vitamin E (Siddonns and Mills, 1981). Therefore, our study demonstrated that the injection of 0.4 mg/kg body weight before lambing was responsible for a lasting increase in the GSH-Px activity.

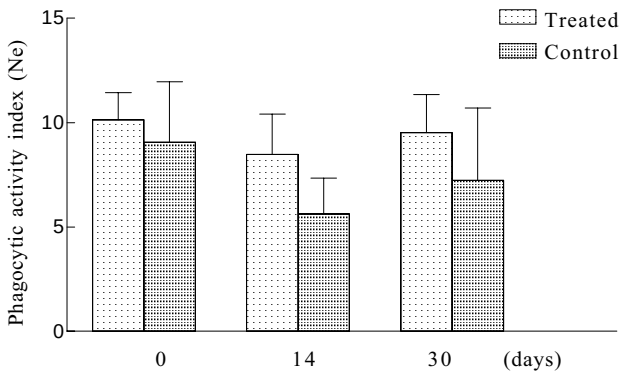
In cattle, major stresses that increase blood cortisol concentration are castration, weaning, handling, dehorning, parturition, water source, forced exercise, neonatal diarrhea, shipping, and certain conditions that



Groups	Phagocytic activity index of leukocytes		
	Before treatment (O day)	After parturition (14 days)	After parturition (30 days)
Treated	8.87 ± 1.34*	7.66 ± 0.45**	8.42 ± 1.97
Control	6.18 ± 1.87	5.07 ± 1.39	5.14 ± 2.94
Time effect = n.s.		Group effect $P < 0.001$	

** $P < 0.01$ vs control; * $P < 0.05$ vs control; n.s. = not significant

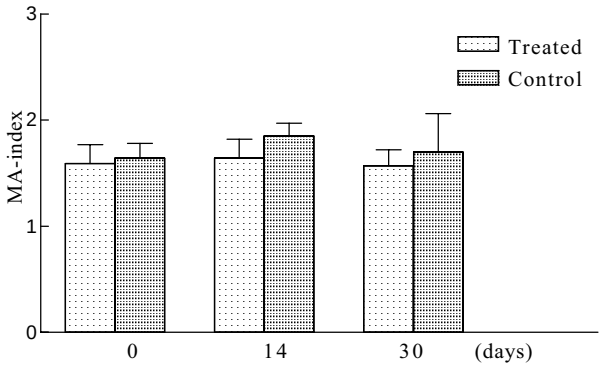
Figure 2. Phagocytic index of leukocytes isolated from sheep treated with vitamin E and selenium and from non-treated sheep



Groups	Phagocytic activity index of neutrophils		
	Before treatment (O day)	After parturition (14 days)	After parturition (30 days)
Treated	10.13 ± 1.30	8.48 ± 1.93	9.52 ± 1.83
Control	9.06 ± 2.90	5.62 ± 1.72	7.24 ± 3.45
Time effect = n.s.		Group effect $P < 0.05$	

n.s. = not significant

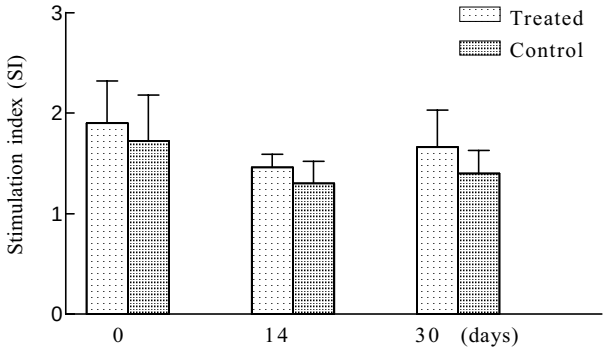
Figure 3. Phagocytic index of neurophils isolated from sheep treated with vitamin E and selenium and from non-treated sheep



Groups	Metabolic activity index (MA-I)		
	Before treatment (O day)	After parturition (14 days)	After parturition (30 days)
Treated	1.59 ± 0.18	1.64 ± 0.18	1.57 ± 0.15
Control	1.64 ± 0.14	1.85 ± 0.11	1.70 ± 0.36
Time effect = n.s.		Group effect = n.s.	

n.s. = not significant

Figure 4. Metabolic activity index of sheep treated with vitamin E and selenium and in non-treated sheep



Groups	Stimulation index		
	Before treatment (O day)	After parturition (14 days)	After parturition (30 days)
Treated	1.90 ± 0.42	1.46 ± 0.13	1.66 ± 0.37
Control	1.72 ± 0.46	1.30 ± 0.22	1.40 ± 0.23
Time effect = n.s.		Group effect = n.s.	

n.s. = not significant

Figure 5. Stimulation index (lymphocyte blastogenic response to Con A) in sheep treated with vitamin E and selenium and in non-treated sheep

may cause pain (Roth and Kaeberle, 1982). Thus, the sheep of this experiment were possibly under stress.

Supplementation of selenium more than required has already been shown to enhance the immune response in cattle 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 immunological responses and GSH-Px activity were not the same after vitamin E and selenium administration.

It was reported that vitamin E supplementation enhanced phagocytosis (Hogan *et al*., 1990). A sufficient vitamin E concentration in phagocytic cells seems to play an important role in optimal development of chemical processes during phagocytosis (Boxer, 1990). On day 0, the significant difference between the groups in phagocytic activity index of leucocytes was probably due to different concentrations of serum constituents which have an effect on immune response. Vitamin E and selenium used in our study led to significant differences 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, sheep in 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 the present study, blastogenesis of lymphocytes (stimulation index) in treated group tended to have higher values than the control group.

There were declines in the immunological parameters investigated that seemingly due to the effect on parturition and lactation are considered as stress factors.

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

REFERENCES

Baboir B.M. (1984): The respiratory burst of phagocytes. J. Clin. Invest., *73*, 599.

Baker S.S., Cohen H.J. (1983): Altered oxidative metabolism in selenium-deficient rat granulocytes. J. Immunol., *130*, 2856.

Bayne R., Arthur J.F. (1979): Alterations of neutrophil function in selenium-deficient cattle. J. Comp. Pathol., *89*, 151–158.

Bendich A. (1990): Antioxidant vitamins and their functions in immune responses. Adv. Exp. Med. Biol., *262*, 35–55.

Boxer L.A. (1990): The role of antioxidants in modulating neutrophil functional response. Adv. Exp. Med. Biol., *262*, 19–33.

Boyne R., Arthur J.R. (1979): Alterations of neutrophil function in selenium deficient cattle. J. Comp. Pathol., *89*, 151–158.

Eicher-Pruiett S.D., Morrill J.L., Blecha F., Higgins, J.J., Anderson N.V., Reddy P.G. (1992): Neutrophil and lymphocyte response to supplementation with vitamins C and E in young calves. J. Dairy Sci., *75*, 1635–1642.

Finch J. M., Turner R.J. (1989): Enhancement of bovine lymphocyte responses: a comparison of selenium and vitamin E supplementation. Vet. Immunol. Immunopath., *23*, 245–256.

Finch J.M., Turner R J. (1996): Effects of selenium and vitamin E on immune responses of domestic animals. Res. Vet. Sci., *60*, 97–106.

Gyang E.O., Stevens J.B., Olson, W.G., Tsitsamis S.D., Usenik E.A. (1984): Effects of selenium – vitamin E injection on bovine polymorphonucleated leucocytes phagocytosis and killing of *Staphylococcus aureus*. Am. J. Vet. Res., *45*, 175–177.

Hogan J.S., Smith K.L., Weiss W.P., Todhunter D.A., Schockey W.L. (1990): Relationships among vitamin E, selenium, and bovine blood neutrophils. J. Dairy Sci., *73*, 2372–2378.

Kováč G., Sankari S. (1988): Glutathione peroxidase activity, selenium concentration in the blood and the masticatory muscle of cattle. Folia Veter., *32*, 79–93.

Lacetera N., Bernabucci U., Ronchi B., Nardone A. (1999): The effect of injectable sodium selenite on immune function and milk production in Sardinian sheep receiving adequate dietary selenium. Vet. Res., *30*, 363–370.

Lim T.S., Putt N., Safranski D., Chung C., Watson R.R. (1981): Effect of vitamin E on cell-mediated immune response and serum corticosteron in young and maturing mice. Immunology, *44*, 289–295.

Mareček D., Procházková J. (1986): Micro-NBT test. In: Procházková J., John C. (eds.): Selected Diagnostic Methods of Immunology (In Czech). Avicenum, Praha. 219–222.

McDowell L. R. (1989): Vitamins in Animal Nutrition. Comparative Aspects to Human Nutrition. Academic Press. 93–131.

McDowell L.R. (1992): Minerals in Animal and Human Nutrition, Academic Press, Ine., San Diego. 295–351.

Nagahata H., Noda H., Abe T. (1986): Evaluation of bovine lymphocyte blastogenic response by fluorometric DNA synthesis assay. Jpn. J. Vet. Sci., *48*, 23–28.

Nockels C.F. (1996): Antioxidants improved cattle immunity following stress. Anim. Feed Sci. Technol., *62*, 59–68.

Paglia D.E., Valentine W.N. (1967): Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med., *70*, 158–169.

Politis I., Hidiroglou M., Batra T.R., Gilmore J.A., Gorewit R.C., Scherf H. (1995): Effects of vitamin E on immune function of dairy cows. Am. J. Vet. Res., *56*, 179–184.

Reddy P.G., Morrill J.L., Minocha H.C., Morrill M.B., Dayton A.D., Frey R.A. (1986): Effect of supplemental vitamin E on the immune system of calves. J. Dairy Sci., *69*, 164–171.

Reddy P.G., Morrill J.L., Minocha H.C. (1987): Vitamin E is immunostimulatory in calves. J. Dairy Sci., *70*, 993–999.

Roth J.A., Kaeberle M.L. (1982): Effect of glucocorticoids on the bovine immune system. J. Am. Vet. Med. Assoc., *180*, 894–901.

Rotruck J.T., Pope A.L., Ganther H.E., Swanson D.G., Hafeman. D.G., Hoexstra W.G. (1973): Selenium: biochemical role as a component of glutathione peroxidase. Science, *179*, 588–590.

Serfass R.E., Ganther H.E. (1975): Defective microbicidal activity in glutathione Peroxidase-deficient neurophils of selenium-deficient rats. Nature, *255*, 640–641.

Siddonns R.C., Mills C.F. (1981): Glutathione peroxidase activity and erythrocyte stability in calves differing in selenium and vitamin E status. Brit. J. Nutr., *46*, 345–356.

Stowe H.D., Thomas J.W., Jonhnson T., Marteniuk J.V., Morrow D.A., Ullrey D.E. (1988): Responses of dairy cattle to long-term and short-term supplementation with oral selenium and vitamin E. J. Dairy Sci., *71*, 1830–1839.

Van Vleet J.F. (1980): Current knowledge of selenium – vitamin E deficiency in domestic animals. J. Amer. Vet. Med. Assoc., *176*, 321–325.

Větvička V., Fornusek I., Kopeček J., Kaminková J., Kašpárek I., Vranova M. (1982): Phagocytosis of human leukocytes: A simple micromethod. Immunol. Lett., *5*, 97–100.

Wilson P.S., Judson G.J. (1976): Glutathione peroxidase activity in bovine and ovine erythrocytes in relation to blood selenium concentration. Brit. Vet. J., *132*, 428–434.

Received: 00–01–28
Accepted after corrections: 00–12–05

Corresponding Author:

Prof. MVDr. Gabriel Kováč, DrSc., University of Veterinary Medicine, Komenského 73, 041 81 Košice, Slovak Republic
Tel. +421 95 633 21 11–15, fax +421 95 632 36 66, e-mail: kovac@uvm.sk