

Comparison of the effectiveness of selenium and vitamin E supplementation on the health of the mammary gland of sheep

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Article Details: Received: 2020-10-14 | Accepted: 2020-11-27 | Available online: 2021-01-31

<https://doi.org/10.15414/afz.2021.24.mi-prap.75-79>



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In this study we observed the effect of peroral and parenteral supplementation of Selenium (Se) and vitamin E (VTE) on the selected antioxidant parameters in blood and on the incidence of mastitis in ewes after the first lambing. Of the 350 sheep of Improved Walachian and Lacaune breeds, situated in eastern Slovakia under standard zootechnical and animal hygiene conditions, we selected thirty one-year-old ewes before their first lambing. The ewes were divided into three groups according to the method of supplementation of Se and VTE. The blood samples from *vena jugularis* were collected on day 30 before lambing (control sampling) and on days 3 and 30 after lambing. Ewes were checked for mastitis on days 10 and 30 after lambing. On the day of the control sampling the ewes from the B1 group were perorally supplemented with 0.3 mg Se/kg/DM in the form of Na₂SeO₃ and 50 IU/vitamin E/kg/DM, while ewes from the B2 group were injected subcutaneously Se with VTE at a dose of 6.6 mg/Se and 1,000 IU/VTE. Changes in the concentration of Se and VTE in the blood plasma of ewes were determined only in the perorally supplemented group (B1) on day 3 after lambing. At 30 days after lambing the the incidence of mastitis in this group was half as lower (10%) as in the control group (20%).

Keywords: sheep, mastitis, supplementation, glutathione peroxidase, selenium, bacteria

1 Introduction

Most diseases in ewes occur at or just after lambing, which is a period associated with immune suppression, resulting in an increased susceptibility to infections. The increasing immune suppression before lambing is multifactorial but is associated with endocrine changes and decreased intake of critical nutrients. Among the most important nutrients, but often deficient in compound feeding stuffs, involved in the biological functions and antioxidative activity are vitamin E (VTE), and selenium (Se) compounds (Person et al., 2007; Kafizadeh et al., 2014).

Selenium is one of the essential trace elements that together with VTE protect the body against the oxidative damage. The presence of Se in soil on the territory of EU is very variable, from an average of 0.05–0.1 ppm. In recent years, it is confirmed that the Central Europe is area with very low concentrations of selenium in the soil (Grešáková et al., 2013).

Deficiencies of Se and VTE are frequently detected also on farms in the central and northern Europe, which indicates necessity of supplementation of products containing selenium and vitamin E. Application of synthetic injectable forms of Se and α -tocopherol seems to be the most effective solution to fulfilling the requirements of the organism on both antioxidants, especially before lambing, when the peroral supplementation fails to increase the reduced concentration of these components in the blood plasma of sheep (Pavlata et al., 2002; Spears and Weiss, 2008).

The aim of the present study was to compare the effect of parenteral and peroral supplementation of Selenium (Se) and vitamin E (VTE) on their levels and activity of glutathione peroxidase (GPx) in blood as well as their effect on the incidence of mastitis in ewes during the first month after lambing.

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2 Material and methods

2.1 Sheep housing

The practical part of the study was carried out in the herd with 350 sheep of Improved Walachian and Lacaune breeds, located in eastern Slovakia and kept under standard zootechnical and animal hygiene conditions. In the months of April to October, ewes are thrown into pastures. In the winter, sheep are housed in sheep-stall on deep litter. For most ewes, lambing takes place from late January to mid-February. During the lambing period, sheep were fed with daily ration of hay (2.8 kg), clover silage (0.9 kg), triticale grain (0.3 kg) and wheat bran (0.15 kg) with a Se content of up to 0.1 mg/kg dry matter and VTE 48 IU/kg dry matter (DM). All ewes had *ad libitum* access to water.

2.2 Milking sheep

Sheep are milked twice a day until the end of summer season in the early and late afternoon after coming from the pastures. With approaching decreasing milk production and approaching drying time, the milking frequency is reduced to once a day. On the farm there is a 2 × 14 machine double-row milking parlor (Alfa Laval Agri), where are the aisles, a staff room, a milk cooling and storage room and a waiting room. One sheep milking interval is about 2 to 3 minutes at 40.5 kPa. The milking process is completed by manual milking, thereby increasing fattiness in the milk and better emptying the mammary gland. The milking process is finished by soaking the dips in the disinfectant solution and storing the milk in a cooling tank.

2.3 Selection of experimental animals

Selection of groups and the method of administration of Se and VTE. After ultrasound examination, approximately on day 120 of pregnancy, thirty one-year-old ewes before the first lambing (yearling ewes), of average weight of 37 ± 0.8 kg, were selected for this study. The ewes were randomly allocated to three groups, 10 animals in each. The first group of ewes (B1) was orally supplemented with sodium selenite at a dose of 0.3 mg/kg DM together with VTE at a dose of 50 mg/kg DM one month before the expected lambing. At this time the second group of ewes (B2) was parenterally supplemented with Selevit inj. a. u. v. (Biotika a.s., SR) in content with Tocoferoli acetat (25 mg/ml) and Natrii selenis (2.2 mg/ml) together with Erevit 300 sol. inj. in content with Tocoferoli acetat (300 mg/ml). The total single dose per sheep was 3 ml of Selevit inj. a. u. v. together with Erevit 300 sol. at a dose of 3 ml/pc (Biotika a.s., SR), i.e. 6.6 mg/Se and 1,000 IU/α-tocopherol acetate per head. The control group of ewes (C) was not supplemented with Se or VTE. The feed contained 90.4 µg Se per kg/DM and 48 IU vitamin E per kg/DM.

2.4 Collection of samples

One month before the onset of parenteral administration of Se and VTE, on days 3 and 30 after lambing, 10 ml samples of blood were collected from the jugular vein of all selected ewes into 2 tubes one containing an anticoagulant (100 µl lithium heparin) and one without anticoagulant. The samples were then transported for further processing in a cooling box at a temperature of +4 °C. In a laboratory, 2 ml of blood was withdrawn from each heparinized sample for determination of GPx activity, and blood plasma and serum were obtained from the residual content by centrifugation at 3000 rpm. for 15 min. All samples were kept frozen at -54 °C until analysis.

2.5 Clinical examination

Each ewe was clinically examined on days 10 and 30 after lambing when 10 ml of milk was aseptically collected for laboratory examination and the California mastitis test (CMT). During the clinical examination, ewes were observed for clinical signs of mastitis, such as swelling, fever, dehydration, pain, inappetence, etc., and mammary glands were examined by palpation. The health of the udder and individual forms of mastitis (subclinical and clinical) based on clinical signs, abnormal udder secretions, CMT scores, bacteriological examination with positive culture result, were classified according to Fthenakis (1995) and Vasil et al. (2018).

Bacteriological examination was performed according to the commonly accepted rules (Vasil' et al., 2018). Suspect colonies (*Staphylococcus* spp., *Streptococcus* spp. and *Enterobacteriaceae* spp.) were isolated on blood agar, cultivated at 37 °C for 24 h and subjected to detailed biochemical identification using the STAPHY-test, STREPTO-test, or ENTERO-test and employing a software TNW Pro 7.0 (Erba-Lachema, CZ).

2.6 Analysis of samples

Selenium concentration in the blood and feed samples was determined after wet digestion in a LS 1200 module microwave oven (Milestone, USA) using a Zeman 4100 atomic absorption spectrometer (Perkin Elmer, USA) according to Pavlata et al. (2012). The GPx activity in the blood was determined using a Glutathione peroxidase assay kit (Randox-Ransel, UK) according to Paglia and Valentine (1967). Determination of VTE in the blood plasma was performed by the HPLC method according to Hess et al. (1991).

The determination of VTE in the collected and homogenized sample of the mixed feed was performed after its saponification and extraction by the HPLC method according to Smith et al. (1997).

2.7 Statistical analysis

One-way Anova with the Dunnett's post test were used to compared the levels of Se, α -Toc and GPx activity in the blood of ewes from the B1 and B2 groups compared to the control group (C). The values of $P < 0.05$ were considered significant. Results are presented as means (M) \pm standard deviation (SD).

3 Results and discussion

Table 1 shows the plasma levels of VTE, Se and GPx activity in the blood of examined ewes. On day 3 after lambing increased plasma levels of Se and VTE were observed in the orally supplemented group (B1) compared to the control group.

Similar findings increasing of concentrations of Se in the blood and colostrum have been made in sheep after peroral supplementation with addition 0.3 mg Se of kg/DM in form of Na_2SeO_3 (Pavlata et al., 2012).

Apart to the increased Se and VTE, no significant changes in GPx activity were detected in this group. In the parenterally supplemented group of sheep (B2) was not detected changes of Se and VTE levels as well as in GPx activity throughout the monitored period.

Misurova et al. (2009) noted that blood Se values above 100 $\mu\text{g/l}$ corresponded to GPx activity of 700 $\mu\text{kat/l}$. In our study, Se values above 120 $\mu\text{g/l}$ were recorded in the all monitored groups, but neither oral nor parenteral supplementation affected the activity of GPx the values of which persisted below the level of 700 $\mu\text{kat/l}$ throughout the study (Table 1).

Table 1 Evaluation of antioxidant and oxidative parameters in the blood of ewes

Period	Parameter	Unit	C	B1	B2
			M \pm SD	M \pm SD	M \pm SD
30 days before lambing	VTE	mg/l	2.35 \pm 0.22	2.40 \pm 0.27	2.30 \pm 0.32
	Se	$\mu\text{g/l}$	131.6 \pm 11.5	134.8 \pm 9.8	128.1 \pm 8.4
	GPx	$\mu\text{kat/l}$	648.7 \pm 19.3	655.3 \pm 45.9	642.4 \pm 52.7
3 days after lambing	VTE	mg/l	1.75 \pm 0.20 ^a	3.60 \pm 0.50 ^b	2.05 \pm 0.38
	Se	$\mu\text{g/l}$	126.9 \pm 16.0 ^a	202.4 \pm 17.3 ^b	140.2 \pm 21.1
	GPx	$\mu\text{kat/l}$	613.2 \pm 38.9	627.8 \pm 37.5	609.6 \pm 32.3
30 days after lambing	VTE	mg/l	2.50 \pm 0.37	3.4 \pm 0.38	2.35 \pm 0.27
	Se	$\mu\text{g/l}$	128.2.6 \pm 12.0	153.5 \pm 25.4	130.7 \pm 13.6
	GPx	$\mu\text{kat/l}$	678.6 \pm 39.0	661.1 \pm 73.5	669.1 \pm 57.5

C – control group of ewes, B1 – orally supplemented group of ewes one month before lambing, B2 – parenterally supplemented group of ewes one month before lambing; a, b – values in the row with different superscript letters differ significantly at $P < 0.05$

Biological function of selenium through GPx activity is complemented by vitamin E, which also shows the effects of a cellular antioxidant. Antioxidants are necessary to prevent some disorders in female reproduction such are reducing morbidity, incidence of mastitis during lactation, retained placenta, infertility, increased incidence of endometritis and ovarian cysts.

According to Cohen et al. (1991) serum a-tocopherol concentrations above 4.0 mg/ml have been reported to be adequate in ruminants. Despite the increased levels of VTE in the peroral supplemented group, on the 3rd day after lambing, its low concentrations (<4.0 mg/ml) was observed during the observed period in all groups (Table 1).

Table 2 shows the incidence of mastitis in the monitored groups of sheep. On day 10 after lambing, clinical examination and laboratory diagnosis of bacterial agents of intramammary infections (IMI) showed the lowest incidence of positive udder-halves in the orally supplemented group. The positive effect of oral supplementation of VTE and Se was also manifested in B1 on day 30 after lambing when the incidence of IMI was 10% from all examined udders which was half as less as compared to the control and B2 groups.

Table 2 Occurrence of mastitis in ewes after the first lambing on days 10 and 30

Group	10 days after lambing			30 days after lambing		
	number of examined halves	positive halves		number of examined halves	positive halves	
	<i>n</i>	<i>n</i>	%	<i>n</i>	<i>n</i>	%
C	20	5	25.0	20	4	20.0
B1	20	1	5.0	20	2	20.0
B2	20	2	10.0	20	4	10.0

C – control group of ewes, B1 – orally supplemented group of ewes one month before lambing, B2 – parenterally supplemented group of ewes one month before lambing

4 Conclusions

We confirmed that the peroral supplementation of the products containing selenium and vitamin E to pregnant sheep showed a positive effect on the increase in selenium and VTE levels in the blood plasma on the 3rd day after lambing. This supplementation reduced also the intramammary infection in a group of ewes supplemented orally with Se and VTE 30 days after lambing.

Acknowledgements

This study was supported by the Slovak grants APVV No. SK-PL-18-0088, KEGA No. 006UVLF-4-2020, and VEGA No. 1-0529-19: The effect of environmental agents of mastitis in dairy cows and ewes on the production and degree of oxidative stress.

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