Polish Journal of Veterinary Sciences Vol. 28, No. 1 (2025), 83–91

DOI 10.24425/pjvs.2025.154016

Original article

Short-term influence of oral supplementation with selenitetriglycerides on hematological and biochemical measurements in sheep of Kamieniecka breed

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Abstract

The aim of this study was to determine the effect of oral administration of selenitetriglycerides on the selenium level and selected hematological and biochemical parameters in Kamieniecka sheep. Sheep in the experimental group received a preparation in the form of selenitetriglycerides orally at a dose of 2 ml per animal for the next 7 days (1 mg Se/kg body weight). Blood was collected from each mother sheep: on day 0 before Se administration, and then on days 1, 7, 14, 21, and 30 of the experiment. Serum selenium concentration in the control group was similar on all sampling days and ranged from 75.38 µg/l to 88.34 µg/l. In the experimental group, the selenium level was the lowest before supplementation and increased significantly during supplementation ($p \leq 0.01$), starting from the 1st day of the experiment, to reach the peak value on the 7th day (791.72 µg/l). The selenium concentration was significantly higher ($p \leq 0.01$) compared to the baseline values even on the 30th day of the experiment (214.78 µg/l) 23 days after the end of selenitetriglyceride supplementation. The research showed that the dose of selenitetriglycerides was safe and effectively improved the Se level in the tested Kamieniecka sheep. Monitored hematological and biochemical parameters showed that supplementation does not negatively affect any vital functions of the body. It was found that oral administration of selenitetriglycerides is an effective and safe form of selenium supplementation in sheep mothers before reproduction.

Keywords: biochemical and hematological parameters, Kamieniecka sheep, selenium level, selenitetriglycerides, supplementation

Introduction

Selenitroglycerides are a completely new form of organic selenium that is characterized by low toxicity. Selenium has various biological roles, from participation in antioxidant and detoxification processes to antiviral, antibacterial and anticancer properties (El-Bayoumy 2001, Pavlata et al. 2012). Selenium status is one of the most commonly used indicators of selenium concentration in organs and tissues. It is determined based on the concentration of selenium in whole blood, plasma and serum. Another indicator is the measurement of glutathione peroxidase (GSH-Px) activity in red blood cells (Pehrson et al. 1999, Enjalbert et al. 2006).

Selenium is a trace element that performs a number of functions in living organisms, including: it is a component of many enzymes controlling metabolic pathways, it is also involved in the metabolism of thyroid hormones, controls reproductive functions and has neuroprotective effects (Barceloux 1999, Maiorino et al. 1999, Gorini et al. 2021). This microelement also has anti-proliferative and anti-inflammatory properties and stimulates the immune system. In fulfilling its functions, it cooperates with vitamin E and sulfur-containing amino acids. Selenium deficiency can lead to reduced health status in livestock and economic losses on farms (Mehdi and Dufrasne 2016). Selenium has various biological roles, from participation in antioxidant and detoxification processes, through stimulation of B cell proliferation and production of IgM and IgG and antiviral, antibacterial and anticancer properties (El-Bayoumy 2001, Pavlata et al. 2012).

Nutritional Muscular Dystrophy (NMD), also known as white muscle disease, is the most common clinical disorder caused by selenium deficiency in ruminants. Young animals with hyposelenosis are also more susceptible to respiratory and gastro-intestinal infections. In adult individuals, selenium deficiency impairs fertility, contributes to the formation of ovarian cysts and increases embryonic mortality in the first 3-4 weeks after insemination (Ishii et al. 2002, Hemingway 2003, Palmieri and Szarek 2011). Placental retention is one of the most frequently encountered fertility disorders that accompany selenium deficiency. Selenium and vitamin E facilitate neutrophil migration to the mammary gland and enhance the bactericidal effects of neutrophils, thus shortening and alleviating the symptoms of clinical mastitis (Moeini et al. 2009).

Selenitetriglycerides are a completely new form of organic selenium in the +4 - oxidation state as a result of the modification of selenic acid and sunflower oil. These compounds are synthesized by esterification of pre-oxidized triglycerides into hydroxyl derivatives with selenic acid (Stańczyk et al. 2010). Selenitetriglycerides are lipophilic and are easily distributed in the body. Studies on rats have shown that oral administration of 2% and 5% solutions of selenitetriglycerides results in the highest selenium levels in the kidneys and the liver, with much lower levels found in the brain, spleen, lungs, intestines and heart. Metabolism of this form of selenium in the rat takes place mainly in the liver, and the element is excreted mainly by kidneys – selenium was completely eliminated from the body within 24 hours of the supplementation (Jastrzębski et al. 1997).

The low toxicity of selenium released from selenitetriglycerides is a considerable advantage. According to the literature data, the lethal dose of this microlement after supplementation in an inorganic form ranges from 1 to 5 mg kg^{-1} BW (Koller and Exon 1986). Studies on rats (Jastrzębski et al.1995) have shown that the average lethal dose (Se LD50) in supplementation with 2% selenitetriglycerides was 100 mg kg^{-1} BW, and 68 mg kg^{-1} BW with a 10% solution. Such high tolerance to selenitetriglycerides is extremely beneficial and indicates that they are nearly 30 times less toxic (at a concentration of 2%) in rats than preparations containing sodium selenate.

There are few publications on the use of selenitetriglycerides in farm animals. Due to the fact that so far there are so few publications regarding the influence of selenitetriglycerides on the organism of sheep, the aim of the research was to determine the effect of oral administration of selenitetriglycerides on the level of selenium and selected haematological and biochemical parameters in these animals.

Materials and Methods

Experimental design

The research was carried out with the consent of the Local Ethics Commission for Animals (approval no. 34/2021, 19 May 2021). The research was conducted on 30 Kamieniecka breed female sheep kept at the Komalwy breeding farm in the Warmia-Mazury Voivodeship, Poland. Sheep for the experiment were selected using the analogue method from a base herd of 350 ewes used for breeding. All sheep were three years old average body weight 57 kg (between 55 to 60 kg), and three months after lambing. Before the selection all animals were examined by USG (ForVet Dramiński) for the possible presence of pregnancy. All of them were not pregnant. The animals were kept in conditions that met the requirements for individual farm animal welfare and were kept throughout the year in a free-stall, on deep straw bedding. All sheep were fed the

same diet in the TMR (total mixed ration) using account the ad libitum feed method. The TMR consisted of the following components: grass silage (45%), corn silage (30%), hay (20%); concentrate mix (4.5%) and mineral and Milafos L vitamin mix (0.5%). The concentrate mixture included oat (50%), wheat (30%), corn (10%) and soy (10%) meals. All feeds were balanced in accordance with the norms and mothers' needs in specific physiological periods. On average, one sheep per day consumed: 2.8 kg of grass and corn silage; 0.6 kg of meadow hay; 0.6 kg of concentrate mixture.

The sheep were divided into 2 groups: control (C-Se) and experimental (E-Se), 15 animals in each group. Only the experimental group received a preparation in the form of selenitetriglycerides. The control group did not receive any supplement.

Sheep in the experimental group received a preparation in the form of selenitetriglycerides orally at a dose of 2 ml per animal for 7 days (1 mg Se/kg body weight) (from day 0). This dose was selected based on previous experimental studies on rats (Jastrzębski et al. 1997) and sheep (Zagrodzki et al. 2000). The liquid preparation was administered in the morning via a calibrated oral drip.

Blood sample collection and analysis

Blood was collected from the external jugular vein of each sheep six times into tubes containing a clot activator (9 ml, Vacuette, Greiner Bio-One, France) for serum analyses of selenium and biochemical parameters, into vacutainers containing K2 EDTA (4 ml, Vacuette, Greiner Bio-One, France) for hematological analysis and into tubes containing lithium heparin (6 ml, Vacuette, Greiner Bio-One, France) for determination of glutathione peroxidase. Samples taken for selenium estimation were stored at -20°C for further determination, the other analyses were conduced within 3 h after sampling. The first sample was collected on day 0 before Se administration and subsequent samples were collected on the 1st, 7th, 14th, 21st, and 30th day of the experiment.

Hematology

The following hematological parameters were determined in whole blood samples: red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), white blood cell count (WBC), percentage of neutrophils (% NEUT), percentage of lymphocytes (% LYMPH), percentage of monocytes (% MONO), percentage of eosinophils (% EOS) and percentage of basophils (%BASO). The determination of these parameters was performed using the flow cytometry based on laser light scatter (ADVIA 2120, Siemens Healthcare Diagnostics, Tarrytown, USA).

Biochemistry

The following biochemical parameters were determined: concentrations of glucose, total protein, albumin, triglycerides (TRIG), cholesterol (CHOL), non-esterified fatty acids (NEFA), activity of aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and gamma-glutamyl transpeptidase (GGT) using a Cormay ACCENT 200 Automatic Biochemical Analyzer and Cormay diagnostic kits (Łomianki, Poland). Serum Se concentration was determined by hydride generation-flame atomic absorption spectrometry (Unicam 939 Solar Spectrophotometer). The activity of glutathione peroxidase (GSH-Px) was measured in whole blood by the kinetic method using cumene hydroxide and phosphate buffer in an Epoll 20 analyzer using a Ransel diagnostic kit (Randox Laboratories, Crumlin, UK).

Statistical analyses

Statistical analysis was performed in TIBCO Statistica 13.1 (TIBCO Software Inc., Palo Alto, CA). All indicators were summarized using the arithmetic mean ($x \pm SD$). Shapiro-Wilk tests were used in order to check compliance with normal distribution. The analysis of parametric variables was conducted using The ANOVA test. To ascertain the difference between variables the post-hoc Tukey-test was used. The differences were considered as statistically significant if a p-value of a statistical test was below 0.05.

Results

There were no any significant differences in any hematological parameters between the groups of sheep and between the days of sampling (Table 1). All hematological parameters remained within reference intervals. The same situation was observed in relation to biochemical parameters. There were no significant differences in both groups of the sheep and no changes over time in all parameters (Table 2). The serum selenium level in the control group was similar in all days of sampling and remained between 75.38 µg/l and 88.34 µg/l (Fig. 1, Table 3). In the experimental group the level of selenium was the lowest before supplementation and it then increased significantly ($p \leq 0.01$), starting on day 1 of the experiment, to reach

Table 1. Hematological parameters of examined sheep (Mean + SD).

Parameter	Group	Day of experiment					
		0	1	7	14	21	30
RBC (10^6/µl)	C-Se	9.21±0,36	9.51±0.21	9.74±0.48	9.53±0.46	9.19±0.54	9.01±0.67
	E-Se	10.15±0.65	10.79±0.71	10.36±0.58	10.32±0.66	10.19±0.77	10.41±0.76
HGB (g/dl)	C-Se	10.78±0.81	11.11±0.96	11.38±0.91	11.05±0.72	10.65±0.61	10.25±0.93
	E-Se	11.98±1.11	12.72±1.14	12.01±0.85	12.11±1.01	11.65±1.17	11.78±1.14
HCT (%)	C-Se	33.48±1.76	34.45±2.19	35.55±2.25	34.65±2.04	33.53±1.39	31.77±2.67
	E-Se	36.60±3.13	39.18±3.07	37.73±2.62	37.67±2.99	37.25±3.51	37.13±3.49
MCV (Fl)	C-Se	36.43±2.04	36.21±1.89	36.56±2.14	36.68±1.97	36.57±2.02	35.28±2.18
	E-Se	36.03±0.95	36.28±0.86	36.68±0.95	36.31±1.15	36.63±0.98	35.68±1.11
MCH (pg)	C-Se	11.92±1.02	11.65±0.95	11,68±0.82	11.61±0.86	11.68±0.86	11.35±0.91
	E-Se	11.81±0.35	11.87±0.35	11.56±0.38	11.63±0.33	11.65±0.34	11.51±0.32
MCHC (g/Dl)	C-Se	32.27±0.84	32.15±1.06	31.93±0.57	31.63±0.92	31.71±0.71	32.12±0.75
	E-Se	32.72±0.43	32.40±0.57	31.81±0.51	32.03±0.45	31.53±0,62	32.22±0.49
PLT (10^3/µl)	C-Se	412.83±116.34	435.12±127.64	398.518±127.94	348.52±137.61	379.01±108.21	407.83±161.15
	E-Se	443.67±205.94	444.51±209.89	406.53±182.38	400.17±199.29	394.67±133.77	433.67±144.31
WBC (10^3/µl)	C-Se	5.99±1.42	6.61±1.57	6.68±2.05	5.88±1.82	6.12±1.62	5.66±1.63
	E-Se	6.41±1.92	5.94±2.63	6.64±1.92	6.36±1.96	6.35±1.51	5.91±1.56
NEUT (%)	C-Se	25.83±7.89	28.78±9.01	31.48±8.67	23.22±6.57	26.11±8.29	25.21±4.89
	E-Se	24.02±6.25	25.27±8.31	28.86±12.31	21.68±6.39	27.97±7.46	26.15±8.66
LYMPH (%)	C-Se	56.79±8.56	53.63±8.11	53.55±9.61	59.17±8.87	60.82±9.75	60.65±10.42
	E-Se	57.98±5.78	55.14±8.07	54.17±8.01	58.62±6.94	57.61±3.92	59.11±12.33
MONO (%)	C-Se	4.21±1.76	4.47±1.75	4.23±1.81	4.88±0.97	3.13±1.31	2.11±0.96
	E-Se	4.13±1.91	5.45±2.18	4.93±1.09	5.35±1.64	3.68±1.62	1.78±0.45
EOS (%)	C-Se	11.42±5.21	10.72±4.06	9.15±3.56	11.19±5.39	8.21±4.24	9.77±3.58
	E-Se	12.23±3.74	13.15±4.54	10.31±3.84	12.78±3.54	9.22±3.94	11.41±2.17
BASO (%)	C-Se	0.54±0.25	0.72±0.31	0.56±0.21	0.53±0.31	0.48±0.14	0.61±0.17
	E-Se	0.56±0.19	0.67±0.18	0.55±0.22	0.52±0.12	0.31±0.16	0.33±0.21

RBC – red blood cell count, HGB – hemoglobin concentration, HCT – hematocrit, MCV – mean corpuscular volume, MCH – mean corpuscular hemoglobin, MCHC – mean corpuscular hemoglobin concentration, PLT – platelet count, WBC – white blood cell count, % NEUT – percentage of neutrophils, % LYMPH – percentage of lymphocytes, % MONO – percentage of monocytes, % EOS – percentage of eosinophils, %BASO - percentage of basophils.

the peak value on day 7 of supplementation (791.72 µg/l) and to decrease gradually with consecutive blood sample collections. It was significantly higher ($p \leq 0.01$) than at baseline even on day 30 of the experiment (214.78 µg/l) – 23 days after selenitetriglycerides supplementation ended (Fig. 1, Table 3). Serum selenium level the experiment group was significantly higher ($p \leq 0.01$) during the entire experiment (except day 0) compared with the control animals.

Activity of GSH-Px was similar in the control animals through the entire experiment (Fig. 1, Table 3). In the supplemented animals activity of this enzyme increased significantly ($p \leq 0.01$) in the second sampling, reached the peak value (564.08 IU/gHB) on the 14th day of the experiment and started to decrease gradually to the last day of the experiment where it reached 430.36 IU/gHB (Table 3). On all sampling days (except day 0) activity of GSH-Px in the experimental group was significantly ($p \leq 0.01$) higher compared with the control group.

Discussion

The administration of selenitetriglycerides did not significantly influence the number of red cells, HGB concentration, HCT, red cells indices and number of platelets in the sheep. Similar results were obtained in calves (Żarczyńska et al. 2021), where a single supplementation with selenitetriglycerides did not significantly affect the number of erythrocytes and platelets. This fact is confirmed by previous research, which indicated that supplementation with inorganic selenium does not stimulate erythropoiesis processes taking place

Table 2. Biochemical parameters of examined sheep (Mean + SD).

Parameter	Group	Day of experiment					
		0	1	7	14	21	30
Glucose (mmol/l)	C-Se	3.06±0.23	3.12±0.31	2.81±0.34	2.88±0.24	3.16±0.21	3.33±0.51
	E-Se	2.97±0.37	3.17±0.43	2.77±0.45	2.92±0.31	2.94±0.23	3.16±0.41
Total protein (g/l)	C-Se	74.35±7.61	73.91±4.04	74.01±7.66	73.78±5.16	77.06±7.61	73.31±5.62
	E-Se	75.33±4.61	75.06±4.95	76.22±5.65	75.08±5.22	74.91±4.61	73.36±6.69
Albumine (g/l)	C-Se	39.01±1.19	39.41±1.19	38.11±1.41	38.01±1.04	38.55±1.97	39.31±1.33
	E-Se	40.91±2.11	40.15±2.34	39.91±2.02	40,33±1.95	38.41±1.56	40.28±1.98
TRIG (mmol/l)	C-Se	0.21±0.06	0.28±0.09	0.23±0.11	0.26±0.03	0.23±0.12	0.20±0.11
	E-Se	0.24±0.05	0.28±0.05	0.25±0.04	0.24±0.12	0.21±0.07	0.19±0.09
CHOL (mmol/l)	C-Se	1.61±0.28	1.65±0.26	1.59±0.25	1.68±0.25	1.55±0.23	1.71±0.24
	E-Se	1.56±0.55	1.75±0.16	1.71±0.19	1.74±0.18	1.71±0.32	1.93±0.14
NEFA (mmol/l)	C-Se	0,14±0.04	0.12±0.02	0.15±0.06	0.14±0.04	0.11±0.03	0.15±0.02
	E-Se	0.16±0.03	0.11±0.03	0.12±0.03	0.13±0.03	0.12±0.02	0.13±0.02
AST (U/l)	C-Se	85.83±15.13	90.17±13.64	87.16±11.73	87.51±15.25	82.12±9.33	101.33±31.45
	E-Se	92.11±23.24	99.33±16.09	97.51±14.23	95.51±24.88	84.33±12.12	103.15±25.08
LDH (U/l)	C-Se	1027.22±43.29	1137.15±131.37	1061.55±67.46	1070.83±57.15	1143.12±95.29	1115.66±125.91
	E-Se	1155.12±158.41	1222.51±84.33	1168.33±100.47	1208.02±159.34	1326.16±121.41	1237.54±123.66
GGT (U/l)	C-Se	72.51±15.26	71.67±15.35	69.33±14.91	64.51±17.37	63.16±11.66	68.66±31.45
	E-Se	71.16±13.76	68.52±12.07	63.52±9.24	64.13±10.12	68.83±12.04	67.16±10.98

TRIG – triglycerides, CHOL – cholesterol, NEFA – non-esterified fatty acids, AST – aspartate aminotransferase, LDH – lactate dehydrogenase, GGT – gamma-glutamyl transpeptidase.

in the bone marrow (Snarska et al. 2018). On the other hand, other studies conducted on kids and lambs (Soliman 2015, Barwary et al. 2016, Mohamed et al. 2017) showed that supplementation with inorganic forms of selenium had a significant impact on the morphological parameters of blood. This may be related to age, physiological condition, Se level in the diet of the studied animals or differences in the selenium sources used in the above-mentioned studies. Supplementation with selenitetriglycerides also had no effect on the white blood cell count. This is consistent with the results obtained by Żarczyńska et al. (2021), where no such impact was also observed. However, the results obtained by Soliman (2015) indicate a positive effect of supplementation with an inorganic form of selenium on the increase in the total number of leukocytes and the percentage of lymphocytes in lambs. It should be emphasized, however, that these studies were performed on young animals, where the effect of selenium supplementation on the white blood cell count may be more visible.

No significant changes in serum glucose levels were observed during the experiment in both groups of animals. Similar results were observed by other authors (Abbas 2002, Domínguez-Vara et al. 2009). In turn, research conducted on rats showed that selenium can influence glycemic processes in the body, i.e. insulin reaction, glycolysis and pyruvate metabolism (Jabłońska et al. 2016). Similarly, Ebrahimi et al. (2009) observed a significant decrease in glucose levels in calves drinking milk with the addition of an organic form of selenium. However, the above-mentioned experiments did not refer to the study of the effect of selenium supplementation in the form of selenitetriglycerides. During the experiment, the lack of effect of supplementation on the level of albumin and total protein was also noticed. Similar results in the case of selenitetriglyceride supplementation were observed in a group of tested camels and calves (Żarczyńska et al. 2020, Żarczyńska et al. 2021). On the other hand, studies on the effect of selenium on the level of total protein and albumin in the serum of goats have shown that selenium can stimulate protein biosynthesis, and its supplementation causes an increase in the level of total protein in the blood (Reczyńska et al. 2019), but such effects were observed during much longer studies – after 160 days of oral supplementation of selenium. Studies conducted on sheep by other authors (El-Shahat and Abdel Monem 2011, Soliman et al. 2012) showed an increase in the level of total protein in the serum of animals supplemented with selenium. Other studies (Fatma et al. 2019) showed that differences in total protein concentration depend not only on the form of Se, but also on the breed of sheep.

Results of the present study found no effect of Se supplementation on serum total cholesterol concentra-

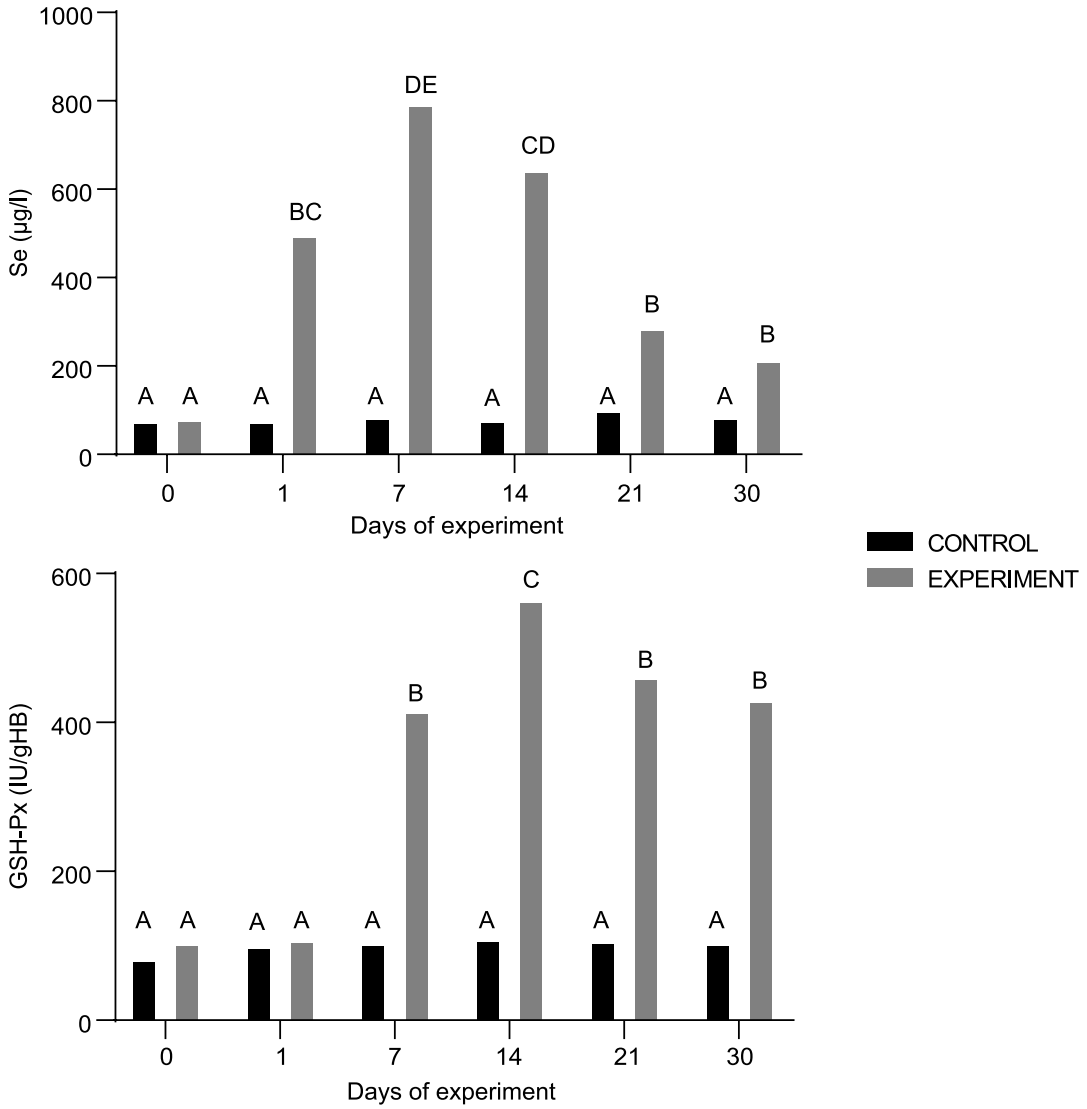

Fig. 1. Serum selenium concentration (Se) and activity of glutathione peroxidase (GSH-Px) in examined sheep. Bars with different letters are significantly different at $p<0.05$.

Table 3. Level of selenium and activity of GSH-Px in examined sheep (Mean + SD).

Parameter	Group	Day of experiment					
		0	1	7	14	21	30
Se (µg/l)	C-Se	$75.38^{A}\pm8,13$	$76.95^{A}\pm10.14$	$84.38^{A}\pm16.15$	$77.36^{A}\pm12.33$	$88,34^{A}\pm14,33$	84.33 ± 15.13
	E-Se	$76,61^{A}\pm12,48$	$^{xx}497,48^{BC}\pm79.65$	$^{xx}791.72^{DE}\pm233.45$	$^{xx}644.38^{CD}\pm193.14$	$^{xx}287.08^{B}\pm44.33$	$^{xx}214.78^{B}\pm32.61$
GSH-Px (IU/gHB)	C-Se	$101.35^{a}\pm22.61$	$99.91^{a}\pm15.04$	$103.01^{a}\pm14.66$	$108.78^{a}\pm20.16$	$106.06^{a}\pm17.61$	$104.31^{a}\pm16.62$
	E-Se	$104.33^{a}\pm20.15$	$108.06^{a}\pm24.95$	$^{xx}415.22^{b}\pm28.65$	$^{xx}564.08^{c}\pm39.22$	$^{xx}460.91^{b}\pm35.61$	$^{xx}430.36^{b}\pm32.69$

Se – serum selenium concentration, GSH-Px – activity of glutathione peroxidase.
$^{A,B/a,b}$ – statistically significant differences at $p\leq0.01$ marked on the right side in rows.
^{xx} – statistically significant differences at $p\leq0.01$ marked on the left in the columns.

tion. These results are similar to those obtained by Błażejak-Grabowska et al. (2022) where no changes in cholesterol levels were observed in sheep supplemented with the inorganic form of selenium. On the other hand Shinde et al. (2009) found that Se administration increased the concentration of total cholesterol and its HDL (High-density lipoprotein) fraction in calves. The authors explained this observation by the positive effect of increased blood selenium concentration on pancreatic function, which facilitated the absorption and digestion of dietary fat. Similarly to cholesterol, there was no effect of supplementation on serum triglyceride levels, which is consistent with the results obtained by Żarczyńska et al. (2021) in calves.

Our own research also showed no effect of the use of selenitetriglycerides on the serum NEFA level. The results of studies conducted on heifers during transport indicate the impact of selenium on reducing the NEFA level in the case of severe lipolysis associated with transport stress (Jung et al. 2023). In our own research, the animals were not exposed to any negative factors and the possible impact of supplementation with this microelement on this indicator was not observed.

An increase in the activity of liver enzymes such as AST, LDH or GGT (especially AST) may be an indicator of selenium poisoning in ruminants (Reczyńska et al. 2019). The results obtained for the activity of these enzymes in all tested animals do not indicate any such risk and do not show any impairment of liver function. Similar results using selenitetriglycerides were obtained in studies conducted on calves and camels (Żarczyńska et al. 2020, Żarczyńska et al. 2021). Supplementation with selenitetriglycerides, at the dose used, had no significant effect on the biochemical indices monitored in this study. There was no effect of supplementation on glucose concentrations, indicators of protein, fat metabolism and liver enzymes.

The concentration of selenium in the supplemented sheep increased significantly on the first day after the administration of selenitetriglycerides and reached its maximum level on the 7th day of the experiment, when the serum concentration of selenium reached 791.72 µg/l, which was ten times higher than the initial value. Significantly higher selenium concentrations in sheep from this group compared to control animals persisted until the end of the experiment. Though comparable studies with this form of Se are not available in sheep, previous studies using on intramuscular injection of sodium selenite increased serum Se concentration two days after supplementation, while oral provision of selenized yeast increased lamb's blood Se within six days (Qin et al. 2007). The level of this element obtained in our research on the seventh day of supplementation was very high and exceeded the reference values for sheep (Błażejak-Grabowska et al. 2022). Despite such a high concentration of this microelelement, no clinical symptoms of possible selenium poisoning were observed, which indicates that selenitetriglycerides are a safe source of this micronutrient for sheep. In a study performed on lambs (Tiwary et al. 2006), in which a single ruminal bolus containing sodium selenate (3 or 4 mg Se kg^{-1} BW) or selenomethionine (4, 6 or 8 mg Se kg^{-1} BW) was administered the authors observed symptoms of poising on such tachypnea, respiratory distress, multifocal myocardial necrosis or pulmonary alveolar vasculitis with pulmonary edema and hemorrhage. It should be emphasized that the dose of selenitetriglycerides administered to sheep was selected on the basis of previous studies (Sochacka et al. 2014, Żarczyńska et al. 2020, Żarczyńska et al. 2021). Therefore, it seems appropriate to conduct further tests to determine the toxicity of the tested dose of selenium preparation and its possible modification. In the control group the level of selenium was stable during the entire experiment and remained within the reference values for sheep.

Supplementation with selenitetriglycerides significantly increased the activity of glutathione peroxidase on the seventh day of the experiment, the peak of this activity was reached on day 14, while significantly increased GSH-Px activity was observed until the end of the experiment. Philipo et al. (1987) explained that the time which elapses from selenium supplementation to the increase in GSHPx activity results from the fact that selenium is first used to replenish tissue reserves and only then to synthesize peroxidase. On the other hand, Arthur et al. (2000) additionally explained that this period is influenced by the mechanisms of selenium incorporation into erythrocytes during erythropoiesis and the time necessary for the biosynthesis of the enzyme itself. The increase in GSH-Px activity found here is similar to findings from studies conducted in mice (Sochacka et al. 2014) and humans (Książek et al 2013), but conflicts with results from previous studies in sheep (Zagrodzki et al. 2000). There was no increase in plasma glutathione peroxidase, cytosolic glutathione peroxidase, type I and type II iodothyronine deiodinases and thioredoxin reductase in the brain, adrenal glands, kidneys, liver and thyroid of sheep supplemented orally with 60 mg (cir. 1.2 mg/kg^{-1} BW) of selenitetriglycerides per animal per day for a month (Zagrodzki et al. 2000). The activity of plasma GSHPx described in this experiment increased insignificantly up to the 10th day of the research (from 67.0 U/l to 115.6 U/l), but by the 14th day of the experiment, it had significantly decreased (to 54.1 U/l) and continued to decline until the end of the experiment. Similarly, no significant increase in GSH-Px activity in whole blood was observed during 28 days of oral selenitetriglyceride supplementation (Zagrodzki et al. 2000). Differences in GSH-Px activity observed between our result and results obtained by these authors may be related to the fact that the described studies were carried out on another breed of sheep with another type of feeding. On the other hand, in studies performed on lambs (Sobiech and Kuleta 2002) the authors observed on increase of GSH activity until the second week of the experiment after a single injection of inorganic preparation of selenium, which is similar to the results obtained in our own research.

In summary we can conclude that oral administration of selenitetriglycerides is an effective and safe form

of selenium supplementation in sheep. The obtained results showed that the dose of selenitetriglycerides used is safe and effective in improving Se status and the monitored hematological and biochemical parameters indicate that the supplementation did not have a negative effect on the functioning of the organism. Since the research was performed on a limited group of animals administered only one dose of selenitetriglycerides, it would be advisable to continue the research on a much larger number of sheep with different doses of the preparation in order to determine the optimal dose and the optimal duration of supplementation.

Funded by the Minister of Science under „the Regional Initiative of Excellence Program"

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