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Research paper

The effect of sodium selenate on biochemical and morphological parameters of blood and reproductive indicators of sheep of selected breeds used in the Czech Republic

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Abstract

Introduction: In the Czech Republic, there is a deficiency of selenium in sheep due to its low level of occurrence in soil, thus in fodder. Selenium deficiency in these animals can manifest in a number of diseases. Therefore, sheep should be supplemented with preparations containing selenium with vitamin E, because Se is closely related to vitamin E, which plays an important role in the immune system.

Aim: The aim of the study was to assess the selenium status of selected sheep breeds in the Czech Republic and to determine the effect of selenium and vitamin E supplementation on selected hematological, biochemical and enzymatic blood indicators and on reproductive indicators.

Material and methods: The research material consisted of sheep, used in the Czech Republic, of three breeds: Šumavka (16), Valaszka (16) and Zwartbles (16). At the 3rd month of gestation, the sheep were injected with sodium selenate with vitamin E. In the blood of sheep, hematological, biochemical and enzymatic parameters were analized, and reproductive indicators of sheep were determined.

Results and discussion: The concentration of Se in sheep was quite low at the beginning of the experiment (from 0.145 (AVR group) to 0.219 μmol/l (BZR group) and the highest concentration (1.322 μmol/l I BZR group) on the test III. It was shown in experimental groups that with increasing selenium concentration, GSH-Px activity increased from the lowest level of 53.77 (ASR group) to the highes: 222.88 U/gHb (BZR group). In sheep from experimental groups (ASR, AVR, BZR groups) better fertility was obtained, amounting to 96.3%, 95.5%,89.4%, respectively.

Conclusions: In all groups of sheep there was a selenium deficiency. Supplementation turned out to be an effective method of compensating for its deficiency. The proper selenium status ensured better reproductive indicators for sheep.

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1. INTRODUCTION

The sheep population in 2019 in the Czech Republic amounted to about 180 000 heads.[1] For example, in the world in 2018 year, there were about 1.2 billion sheep (43% in Asia), and in the European Union countries in 2019 there were 74.6 million sheep and goats, including: Spain (24%), Romania (16%), Greece (16%), France (11%), Italy (11%). In Poland, according to the data of the Central Statistical Office, the sheep population in 2019 was 267 729 heads,[2] while according to the Polish Sheep Association in 2019 there were 77 647 ewes in 963 herds with an average number of 80.6 in a herd.[3] In the Czech Republic, 18 390 ewes were inspected for breeding performance in 2019 year. Sheep production in the Czech Republic accounts for 0.4% of the total livestock production and 0.2% of the total production of the agricultural sector.[4]

Sheep as the so-called small ruminants have lower nutritional and maintenance requirements in comparison to large ruminants, e.g. beef cattle.[5] Used for milk or meat, they can be kept both in small and large farms. The number of herds and the intensity of rearing is related to the production of the basic raw materials obtained from these animals, which are currently: meat (mutton, lamb) and milk (processed into cheese, kefir and yoghurt) and their consumption. Many consumers are looking for food rich in health-promoting ingredients, e.g. lactoferrin,[6] fatty acids,[7] which come from various animal species and may improve their health status.[8,9] The production of such food is pro-ecological and closely related to the environment.[1]

Sheep are mostly kept on pastures, or in the winter season they use hay produced from pastures. Due to the deficiency of some elements in the soil, there may also be a deficiency of these elements in their organisms, e.g. selenium (Se). Se deficiency occurs not only in Czech Republic and the Poland but also in many countries around the world.[10] Se deficiency in sheep can manifest itself in a number of diseases, such as nutritional muscular dystrophy, food degeneration of the liver, disturbances in the proper functioning of the ovaries or mulberry heart disease.[11]

In order to avoid Se deficiencies, sheep should be supplemented with preparations containing mentioned element. This way the body of sheep is enriched with this element, not violating the health status of sheep, thus not reducing the health-promoting quality of lamb or mutton. The addition of Se to the feed in the form of a supplement increases the level of antibodies, the phagocytic activity of neutrophils and macrophages and when stimulated with mitogens, it contributes to an increase in the number of T lymphocytes.[12] The most easily assimilated are selenates (SeO_4) and amine compounds of Se.[13] The main form of supplying the animal organism with Se ions are selenomethionine and selenocysteine. The absorption of Se from the feed in monogastric animals reaches 80%, while in ruminants (polygastric) it reaches 51%.[14] This element is used significantly more by young ruminants (calves, lambs, kids) than adults, because they have undeveloped rumen functions as the rumen microorganisms of adult animals can incorporate Se into their structures.[15]

One of the diseases in sheep herds, that results from Se and vitamin E deficiency is the white muscle disease (WMD), which results in changes in the ultrastructure of the muscles. These changes lead to degeneration and extensive muscle damage or necrosis as well as proliferation of connective tissue. Se shows a close relationship with vitamin E and sulfur amino acids. The effect of Se and vitamin E deficiency are changes in the ultrastructure of muscles, which are manifested by a more pronounced accumulation of chromatin around the edges of the cell nucleus, a decrease in glycogen concentration below the detection limit, greater swelling of the mitochondria and fragmentation of mitochondrial cristae. The sarcomeres are also often broken. These changes lead to degeneration and extensive muscle damage or necrosis and growth of connective tissue.[16]

2. AIM

The aim of the study was to assess the Se status of selected sheep herds in the Czech Republic and to determine the impact of the applied supplementation with this element on selected hematological, biochemical and enzymatic indicators of sheep blood as well as on reproductive indicators.

3. MATERIAL AND METHODS

The research was conducted in 2021. The research material consisted of 48 ewes of three local breeds of sheep used in 3 herds in the Czech Republic. Farm A – 16 pieces Šumavka (AS) and 16 pieces Valašska (AV); Farm B – 16 pieces Zwartbles (BZ) (Table 1). Ewes/mothers were divided into groups according to age (over 3 years) and race with body weight (breeds: AS 60–70 kg; AV 50–60 kg and BZ 60–70 kg). All selected sheep were after mating and pregnant. Sheep, using the analogue method, were divided into subgroups with appropriate nomenclature for particular groups and subgroups. From farm A, the Šumavka breed was selected: ASC (control group) and ASR (research group), and the Valašska breed: AVC (control group) and AVR (research group). On the other hand, from farm B the Zwartbles breed was selected: BZC (control group) and BZR (research group).

Sheep in the year-round system were kept on pastures in a free system. In winter, they sheltered in wooden buildings with hay racks, drinkers and places for licks. The feeding in winter was dominated by hay of a very good quality (ad libidum) and minerals in the form of various licks with molasses. In summer, they took full advantage of the very good quality pastures and mineral licks. Silage and concentrated fodder were not used in the feeding. The mating started in November, when sheep were in very good condition after the summer feeding season.

The study began in February 2021, when the sheep were in the 3 months pregnant. Pregnancy was confirmed by an ultrasound examination performed with the Mindray DP50 (Mindray Medical, Poland) scanner equipped with an abdominal probe in the 3.5–5.0 MHz frequency range. The

first blood sampling was performed on days 90–95 of pregnancy defined as 'time zero' (test I). On the same day, after blood sampling, sheep from the ASR, AVR and BZR groups were injected with sodium selenate with vitamin E (Se + E) from EuroVet Health BV, composed of: 50 mg of tocopherol acetate, 0.5 mg sodium selenate and 1 mL solvens ad. The preparation was administered intramuscularly in an amount depending on their body weight, i.e. 0.10 mL per 1 kg of body weight. So sheep of the breeds: Šumava and Zwartbles were given 6 mL per animal, while Valaszka sheep were administered with 5 mL per animal of preparation. Subsequent blood collections took place: on the 7th (test II) and 14th (test III) day from the starting date. Blood was donated from the zygomatic vein. For hematologic tests, whole blood samples were used in test tubes containing the sprayed ethylene diamine tetra acetic acid (EDTA-K2) solution. Hematological parameters: red blood cell (RBC) counts, white blood cell (WBC) counts, hemoglobin (HBG), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were all determined in full blood, using the Vet Animal Blood Counter 18 (Horiba ABX SAS, France).

Blood samples for biochemical tests were collected into polyethylene tubes containing clot activating beads. Samples for biochemical analyses were centrifuged immediately after collection (10 min, 3000 rpm). The obtained serum was stored in individual test tubes at –18°C until further analysis. Biochemical analyses of the serum were performed within 4 h after collection. The following biochemical parameters were determined: glucose (GLU), the activity of aspartate transaminase (AST), lactate dehydrogenase (LDH), concentrations of cholesterol (CHO), triglycerides (TRI), and Se. The following analytical methods were used: GLU concentration was determined with the use of glucose oxidase,[17] CHO by the colorimetric method with CHO esterase and CHO oxidase,[18] TRI by the enzymatic method with glycerophosphate oxidase,[19] AST activity by the kinetic method[20] and LDH activity by the kinetic method with Cormay reagents.[21] All biochemical examinations were performed using an ACCENT 200 automated chemistry analyzer (PZ Cormay, Poland) and commercial Cormay diagnostic kits. Serum Se levels were determined in triplicate by graphite furnace atomic absorption spectrometry in LaboKlin Laboratory for Clinical Diagnostics KG (Bad Kissingen, Germany). The activity of glutathione peroxidase (GSH-Px) was determined in whole blood by the method described for the RANSEL glutathione peroxidase enzyme activity kit (Randox-Ransel, Randox Laboratories, Crumlin, Antrim, UK).

Analyzing the breeding index of the ewes' herd, the following were given:

$$\text{fertility} = \frac{N \text{ ewes}}{N \text{ ewes intended for breeding}} \times 100\%,$$

$$\text{prolificacy} = \frac{N \text{ lambs born}}{N \text{ lambs}} \times 100\%,$$

$$\text{reared lambs} = \frac{N \text{ lambs weaned}}{N \text{ lambs born live}} \times 100\%,$$

and breeding performance =

$$= \frac{N \text{ weaned lambs}}{N \text{ ewes intended for breeding}} \times 100\%.$$

Obtained data was statistically analyzed in the Statistica v. 13.1. One-way ANOVA was performed to calculate the arithmetic mean and standard error of mean (SEM). The significance of differences between the studied groups was carried out at the confidence level of $P \leq 0.01$ and $P \leq 0.05$ using the Newman–Keulus test.[22]

4. RESULTS AND DISCUSSION

Analyzing the results of the hematological parameters of sheep from the control and experimental groups, no statistically significant differences were found between them (Table 1). In studies II and III there was a slight increase in the number of WBC and RBC when compared to study I. However, there was a decrease in HBG concentration and a decrease in HCT in subsequent studies. Red cell indices, i.e. MCV, MCH and MCHC, remained at a similar level in all animals throughout the experiment and were within reference values.[23]

GLU concentration in sheep of both groups was the highest in the study I and ranged from 6.61 mmol/L (group ASC) to 6.67 mmol/L (group AVC) and it showed a downward trend in subsequent studies (Table 2). Literature data indicate a possible effect of Se supplementation on GLU metabolism in the body. Studies carried out on diabetic rats proved that after administration of Se, there was a significant reduction in the GLU content (from 50% to 80%) in the serum of these animals.[24] Analyzing the CHO concentration, it was shown that all studies showed a significant increase in its content as sheep were saturated with Se and vitamin E (Table 2).

In authors' research, a gradual decrease in serum TG concentration was observed (Table 2). The highest level of 1.11 mmol/L (AVR group) was demonstrated in study I (term 0), and the lowest in study III (14 days after administration) of 0.51 mmol/L (group AVC). Such a state is confirmed by the reports of Brzezińska et al.[25]

The concentration of Se (Table 2) in sheep determined in our own research was quite low at the beginning of the experiment, i.e. in study I (term 0) and ranged from 0.145 μmol/L (AVR) to 0.219 μmol/l (BZR). The increase in the concentration of this element in sheep was influenced by the administration of Se preparation, because already in study II the values were higher in sheep from experimental groups and ranged from 0.398 μmol/L (ASR) to 0.445 μmol/L (AVR). However, these values, were still less than the values recognized as physiological for the species. Pavlata et al. claim that the concentration of Se in the serum of sheep within the limits of 0.823 μmol/L is completely sufficient.[26] According to Caox (1992), the sufficient content is approximately 0.866–1.14 μmol/L of serum.[27] The highest concentration of Se was found in the serum of sheep from experimental groups in test

Table 1. Hematological parameters in blood ewes.

Parameters	Farm A				Farm B		SEM	*P* value
	AS		AV		BZ			
	ASC	ASR	AVC	AVR	BZC	BZR		
	Test I – term 0							
WBC, 10^9/L	7.25	7.29	7.14	7.18	7.29	7.53	1.062	0.794
RBC, 10^{12}/L	11.42	11.45	11.40	11.44	11.37	11.46	0.563	0.711
HGB, g/dL	13.67	13.95	13.46	13.98	14.64	14.98	0.421	0.990
HCT, %	35.12	35.49	35.31	35.72	36.11	36.33	3.633	0.821
MCV, fl	32.04	31.12	33.12	31.09	32.26	31.12	0.346	0.943
MCH, pg	10.21	10.34	10.22	10.41	10.28	10.33	0.131	0.87
MCHC, g/dL	34.10	34.12	34.08	34.11	34.17	34.24	0.523	0.952
	Test II – 7 days from term 0							
WBC, 10^9/L	7.31	7.64	7.22	7.58	7.37	7.82	1.042	0.784
RBC, 10^{12}/L	12.49	13.37	13.78	14.08	12.87	13.68	0.486	0.638
HGB, g/dL	12.74	12.93	12.99	13.82	12.73	12.98	0.384	0.831
HCT, %	34.63	34.93	34.47	34.92	35.04	35.22	3.531	0.821
MCV, fl	30.13	31.23	29.32	32.88	28.31	30.55	0.249	0.833
MCH, pg	9.23	9.48	9.30	9.79	9.28	9.52	0.125	0.753
MCHC, g/dL	34.00	34.18	33.98	34.22	34.23	34.85	0.577	0.982
	Test III – 14 days from term 0							
WBC, 10^9/L	8.49	8.92	8.45	8.37	8.83	8.47	1.132	0.813
RBC, 10^{12}/L	13.50	13.94	14.08	14.47	15.32	15.84	0.671	0.922
HGB, g/dL	11.75	11.83	11.69	11.78	11.79	11.94	0.325	0.962
HCT, %	33.75	34.03	33.55	33.89	33.25	33.77	3.840	0.934
MCV, fl	31.76	32.33	27.19	30.02	29.15	33.72	0.406	0.728
MCH, pg	9.04	10.33	10.18	9.21	10.85	10.34	0.175	0.694
MCHC, g/dL	33.98	34.32	34.02	34.59	33.78	34,82	0.441	0.897

Table 2. Biochemical parameters in blood ewes.

Parameters	Farm A				Farm B		SEM	*P* value
	AS		AV		BZ			
	ASC	ASR	AVC	AVR	BZC	BZR		
	Test I – term 0							
Se, μmol/L	0.189^{a}	0.193^{A}	0.197a	0.145^{A}	0.182	0.219^{A}	0.002	0.542
GLU, mmol/L	6.61	6.65	6.67	6.63	6.62	6.66	0.123	0.375
CHO, mmol/L	2.12	2.14^{a}	2.04	2.13^{a}	2.12	2.13^{a}	0.049	0.863
TRI, mmol/L	0.94^{a}	1.07^{a}	0.87	1.11^{a}	1.00^{a}	1.01^{a}	0.003	0.482
	Test II – 7 days from term 0							
Se, μmol/L	0.167a	0.398B	0.188	0.445^{B}	0.169	0.419^{B}	0.003	0.531
GLU, mmol/L	6.40	6.21	6.48	6.63	6.51	6.27	0.111	0.344
CHO, mmol/L	2.31	2.29	2.12	2.23	2.21	2.37	0.032	0.745
TRI, mmol/L	0.76	0.99	0.82	0.99	0.68b	0.94	0.004	0.631
	Test III – 14 days from term 0							
Se, μmol/L	0.217^{b}	0.995^{C}	0.222^{b}	1.015^{C}	0.194	1.322^{C}	0.004	0.589
GLU, mmol/L	6.49	5.94	5.61	5.84	6.37	6.27	0.168	0.415
CHO, mmol/L	2.42	2.63^{b}	2.21	2.60^{b}	2.29	2.57^{b}	0.052	0.922
TRI, mmol/L	0.55^{b}	0.59^{b}	0.51	0.57^{b}	0.61^{b}	0.63^{b}	0.003	0.549

III, when 14 days passed from the administration of the Se preparation. These values, ranging from 0.995 μmol/L (ASR) to 1.322 μmol/L (BZR), were statistically significantly higher than the values recorded at term 0 (Table 2). The applied Se supplementation in the form of an intramuscular injection of sodium selenate increased its concentration in the sheep serum. The dynamics of changes in serum Se concentration found in our own research do not differ from that determined by other authors. In study on lambs Qin et al. recorded the highest concentration of Se within 2 weeks of its administration – similar to authors' own study.[13]

An available method for diagnosing Se deficiency is the determination of glutathione peroxidase (GSH-Px) activity in RBC derived from the heparinized blood. The average activity of this enzyme in sheep is approx. 110 U/gHb.[28] When the serum concentration of Se drops below 0.38 μmol/L, and the peroxidase activity drops below 25 U/gHb, then muscle dystrophy, the so-called white muscle disease or nutritional muscle dystrophy (WMD), may occur in small ruminants. Other important enzymes that can be used in this evaluation may be AST and creatine kinase (CK). In animals suspected of the subclinical course of the WMD disease, this activity may be approx. 1000 U/L.[29] In own research, the activity of GSH-Px in the so-called term 0 (test I) was significantly below the physiological norm for small ruminants (Table 3). In study II, i.e. within 7 days of administration of the preparation, the GSH-Px activity reached the physiological norm, but only in the experimental groups, ie ASR, AVR and BZR. Much higher values of GSH-Px activity occurred on day 14 from the preparation administration (test III) and ranged from 222.88 U/gHb (BZR) to 195.82 U/gHb (ASR).

Hudman et al. reports that symptoms of WMD occur below 25 U/gHb.[30] According to Pavlata et al. the activity of GSH-Px is a very good reflection of the Se status of the organism, as there is a proven high correlation between the activity of GSH-Px and the concentration of Se.[31] The enzyme most frequently detected in various types of hepatopathies and myopathies is AST. In own research, the activity of AST increased slightly (without statistically significant differences) in the control groups, while it decreased in the experimental groups. In test III, it reached the lowest value from 128.06 U/L (ASR) to 142.31 U/L (AVC). The results of these studies did not differ from those obtained by Sobiech et al., which may indicate the absence of pathological changes in the muscle tissue of the studied sheep.[32]

The enzyme specific for muscle tissue is LDH. In own research, LDH activity showed a tendency similar to AST (Table 3). Increases in LDH were observed in test II and test III over time after administration. The highest increase in LDH activity was recorded in test III in sheep from control groups for which the average value of this indicator was 629.66 U/L. The studies by Salplacht and Necas indicate that the activity of LDH in ruminant tissues may be approximately 100–150 times higher than in serum.[32] The enzyme with the greatest affinity for muscle tissue is CK. A permanent increase in CK activity indicates an ongoing disease process and is correlated with the degree of pathological changes. In our study, the increase in CK activity was significant at $P \leq 0.05$ in the control groups of sheep and occurred between the mean of tests III and II and test I. In test III, it reached the highest value of 144.74 U/L in the control group (ASC) (Table 3). This increase may indicate small degenerative changes in the muscles but without clinical symptoms. It should also be noted that there are quite large individual differences between the activity of CK in the animals of the control and experimental groups. Although the CK activity found in test III is the highest, it is lower than that reported by other authors who relate their values to other species.[33]

Table 4 shows the breeding performance indicators of sheep in the experiment. Analyzing the racial aspect, the low-

Table 3. Activity of selected enzymes.

Parameters	Farm A				Farm B		SEM	*P* value
	AS		AV		BZ			
	ASC	ASR	AVC	AVR	BZC	BZR		
	Test I – term 0							
GSH-Px, U/gHb)	49,03	53.77[a]	47.09	55.46[a]	42.83	57.48[a]	0.002	0.542
AST, U/L	124.72[a]	121.31	126.58[a]	122.43a	124.33[a]	119.22[a]	21.23	0.164
LDH, U/L	528.49[a]	533.06	536.43[a]	522.44[a]	533.20[a]	544.87[a]	4342	0.474
CK, U/L	110.95[a]	102.84	112.04[a]	109.52[a]	112.71[a]	110.11	18.41	0.511
	Test II – 7 days from term 0							
GSH-Px, U/gHb	47.78	119.21[b]	44.32	145.87[b]	49.44	119.59[b]	0.003	0.531
AST, U/L	128.34	122.01	133.27	128.04	131.20[b]	127.17[b]	19.07	0.132
LDH, U/L	615.03[b]	524.05	613.33[b]	532.35[a]	544.11[a]	543.82[a]	7105	0.652
CK, U/L	123.49[a]	104.34	121.08	115.57	120.51	112.37	15.72	0.674
	Test III – 14 days from term 0							
GSH-Px, U/gHb	47.46	195.82[c]	42.38	215.41[c]	44.74	222.88[b]	0.004	0.589
AST, U/L	139.71[b]	128.06	142.31[b]	131.13[b]	140.20[b]	134.21[b]	17.20	0.133
LDH, U/L	635.21[b]	534.07	633.48	585.09[b]	620.28[b]	562.25[b]	8033	0.752
CK, U/L	144.74[b]	111.69	136.17[b]	124.94[b]	135.74[b]	118.10	16.20	0.495

Table 4. Reproductive indicators of sheep from the research herds (%).

Parameters	Farm A				Farm B		SEM	*P* value
	AS		AV		BZ			
	ASC	ASR	AVC	AVR	BZC	BZR		
Fertility	91.2[a]	96.3[b]	90.1[a]	95,5[b]	83,2[c]	89.4[a]	0.294	0.041
Prolificacy	134.2[a]	139.9[b]	101.4[c]	118.7[d]	178.3[e]	188.4[f]	22.45	0.033
Survived (reared) lambs	113.5[a]	125.1[b]	92.3[c]	98.9[d]	152.3[e]	167.6[f]	18.34	0.049
Reproductive performance	98.4[a]	112.6[b]	86.3[c]	91.5[a]	134.8[d]	145.9[e]	17.67	0.005

Comments: [a, b, c] differ significantly for $P \leq 0.05$.

est fertility was demonstrated in the Zwartbles breed and the highest in the Šumava breed. On the other hand, the highest fertility concerned the Zwartbles breed and the lowest – the Valashka breed. This was confirmed by statistically significant differences at $P \leq 0.05$. Moreover, also statistically significantly, the lowest fertility, prolificacy and rearing of lambs were demonstrated in sheep from the control groups in relation to the research groups. In the case of reproductive performance, there were also differences between the breeds and research groups. The best reproductive performance indicator was found in the research groups (ASR, AVR and BZR). However, when analyzing the racial factor it was the Zwartbles race. These results were similar to those reported by SChOK.[4]

5. CONCLUSIONS

(1) The obtained results show that in all groups of sheep there was a selenium deficiency.
(2) The applied supplementation of this element turned out to be an effective way to compensate for its deficiency.
(3) In groups of sheep in which Se supplementation was not used, there was a slight increase in the activity of AST, LDH and CK.
(4) A positive correlation was demonstrated between the increase in Se concentration and GSH-Px activity.
(5) Proper Se status ensured better reproductive indicators of sheep in research groups (ASR, AVR and BZR).

Conflict of interest

Authors have no conflict of interest.

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