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**ORIGINAL PAPER**

# Effect of selenium and vitamin E supplementation on erythropoiesis in young goats

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**Abstract**

Selenium is one of the most commonly supplemented microelements in ruminants because deficiency of this trace element is a global problem that can lead to several diseases, especially in young animals during the neonatal period. The aim of this study was to assess the activity of the hematopoietic process based on cytological evaluation of caprine bone marrow smears in goats supplemented with selenium and vitamin E during the neonatal period. Twelve clinically healthy goats at the age of 2 days participated in the study. They were divided into two equal groups ($n$=6). The goats from the experimental group received a single dose of Se and vitamin E on the second day of life. Bone marrow was collected 2 times, stained with the MGG method, and subjected to cytological evaluation. Peripheral blood was sampled 3 times and peripheral blood smears were analyzed. The bone marrow cells in the group receiving the supplement absorbed dyes faster and more intensively than the same cells in the control animals. The number of erythroblasts was higher in goats from the experimental group than in the control group, which indicated the intensification of erythropoiesis in animals supplemented with selenium and vitamin E. The results of morphological tests clearly indicated an increase in the number of erythrocytes and a higher hemoglobin concentration in the group receiving selenium and vitamin E. Selenium and vitamin E supplementation has a positive impact on the erythropoiesis in bone marrow and can prevent neonatal anemia in goats.

**Keywords:** bone marrow, erythropoiesis, selenium, vitamin E, young goats

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# INTRODUCTION

Iron (Fe), copper (Cu), manganium (Mn), zinc (Zn), and selenium (Se) are the most commonly supplemented microelements in ruminants (Moeini et al. 2011). Se is an antioxidant element that plays a key role in immune processes and anti-cancer protection. It is a trace element, very modestly distributed in nature as it constitutes a small percentage of the earth's crust. Selenium deficiency in ruminants primarily occurs in areas where acidic soils are poor in this element (Pavlata et al. 2003). Clinical signs include incorrect posture, lying down, and development of inflammation (Arthur et al. 2003, Ghany--Hefnawy et al. 2010). Nutritional muscular dystrophy can be also observed during Se deficiency (Pavlata et al. 2000, 2001, 2005*a*, Deger et al. 2008, Katz et al. 2009, Kozat 2009, Żarczyńska et al. 2017). Literature data also confirm the toxic effect of this element on the animals' health; however, such an effect is observed much less frequently (Tiwary et al. 2006). The intensity of symptoms associated with Se deficiency increases after wet and cool summer months, and after the use of superphosphate and sulfur based fertilizers (Ghany-Hefnawy et al. 2010).

Selenium plays an important role in the process of erythropoiesis by regulating the redox reactions. These reactions lead to the formation of the reactive oxygen species (ROS) that exhibit a destructive effect on the erythrocytes lifespan, and the breakdown of hemoglobin molecules (Nagababu et al. 2003). Due to the fact that iron, heme and globin chains can cause high levels of oxidative stress mediated by free radicals, selenium plays a regulatory role in erythropoiesis outside the bone marrow through being incorporated into selenocysteine. This process, although initially effective, is detrimental to the erythroid development and, as a result, may lead to anemia (Weiss, dos Santos 2009)

In healthy humans and animals (especially young ones), the process of erythropoiesis in bone marrow is very effective, being able to ensure full development of all stages and forms of erythropoietic cells. The entire, very complex process of erythropoiesis is regulated by erythropoietin (EPO), which is a glycoprotein peptide hormone. Depending on the body's needs, numerous erythrocytes may be released into the peripheral blood, transporting oxygen to cells and tissues. In cases of increased erythropoiesis, juvenile forms of erythrocytes: basophilic, polychrome and eosinophilic erythroblasts, are also released into the peripheral blood. Reticulocytes are the final stage in erythrocyte maturation. The presence of these cells indicates that the process of erythropoiesis in bone marrow is proceeding properly (Ghaffari 2008).

Laboratory tests performed during Se deficiency show normocytic or microcytic anemia, often in association with iron deficiency states. Studies conducted on rodents prove that Se deficiency contribute to the development of anemia, especially in cases of cell aging and inflammation (Yang et al. 2004).

Se in animals is mainly found in muscles and kidneys. It is not stored in organs and tissues, but is incorporated into proteins, through which it performs its function. These proteins are referred to as selenoproteins. One of the most important selenoproteins is glutathione peroxidase (GSH-Px) – the first enzyme proven to play a role in the process of erythropoiesis (Kaushal et al. 2011). A decrease in the activity of this enzyme causes defragmentation of cell membranes, an increase in the intracellular $Ca^{2+}$ concentration, and damage to mitochondria causing apoptosis (Abutarbush, Radostits 2003). A decrease in GSH-Px activity can also lead to erythropoiesis disorders, resulting in the development of thalassemia and inflammation, which lead to the formation of oxidative stress in redox reactions (Beatty et al. 2000). Glutathione peroxidase is the only enzyme that can prevent harmful lipid peroxidation by reducing lipid peroxides to alcohols. Studies in mice indicate that the quantification of erythropoiesis indices in the blood, bone marrow and spleen showed the presence of anemia, with an increase in the number of erythroid precursor cells and reticulocytes (Kawatani et al. 2011).

Vitamin E is an important fat-soluble antioxidant that protects against lipid peroxidation initiated by free radicals (Azzi, Stocker 2000, Azzi 2007). Its effect on the course of hematopoiesis has not been clearly clarified; however, when its concentration is reduced, the incorporation of selenium into selenoproteins is limited, leading to the aggravation of anemia. (Pavlata et al. 2005*b*, Altamura et al. 2020).

The aim of this study was to assess the activity of the hematopoietic process based on the cytological evaluation of caprine bone marrow smears in goats supplemented with selenium and vitamin E during the neonatal period.

## MATERIAL AND METHODS

All experimental procedures received approval of the Local Ethics Committee for animal experiments in Olsztyn (Resolution No. 28/2021).

The study was performed on one herd of Polish White Improved goats. 12 young (2 days old) clinically healthy and properly fed goats in good physical condition were chosen to participate in the experiment. They were randomly divided into three equal groups ($n$=6) of 3 females and 3 males – experimental (EXP) – animals that on the 2$^{nd}$ day of life received a single intramuscular injection (2 ml per animal) of vitamin E and Se – tocopherol acetate – 50 mg, sodium selenite – 0.5 mg (Eurovet Animal Health BV; control (CONT) – animals that did not receive any additional Se and vitamin E supplementation.

The animals were housed in a free-stall barn, meeting the welfare requirements for farm animals in the EU law. All young goats were kept with their dams and fed their milk throughout the duration of the study. Dams' diet is showed in Table 1. Moreover, animals had unlimited access to Multi-

Table 1

Chemical composition of dams' diet (% of fresh matter)

| Specification | CJ® concentrate | Cereal straw | Meadow hay | Dried beet pulp |
|---|---|---|---|---|
| Dry matter (%) | 88.99 | 90.02 | 85.15 | 92.32 |
| Crude ash (%) | 5.59 | 8.00 | 8.99 | 4.92 |
| Crude protein (%) | 19.32 | 4.25 | 7.43 | 4.98 |
| Crude fat (%) | 3.42 | 1.51 | 0.91 | 0.76 |
| Crude fiber (%) | 6.92 | 42.1 | 27.39 | 14.63 |
| Selenium (mg $kg^{-1}$) | 0.15 | 0.025 | 0.023 | 0.047 |
| Gross energy MJ $kg^{-1}$ | 16.14 | 17.01 | 16.38 | 15.93 |

Lisal salt licks (NaCl – 94%, water – insoluble substances – max. 4%, Mg 2000 mg $kg^{-1}$, Co – 18 mg $kg^{-1}$, Zn – 810 mg $kg^{-1}$, Mn – 830 mg $kg^{-1}$, I – 100 mg $kg^{-1}$, Se – 10 mg $kg^{-1}$. Because the feeding plan in this herd included constant access to licks for all animals, the dams' supply of selenium from the licks was assumed to be the same for both groups.

The activity of the hematopoietic process was assessed based on the cytological evaluation of bone marrow smears and morphology of peripheral blood. The effectiveness of used supplementation was assessed based on the GSH-Px activity and Se and vitamin E concentrations in peripheral blood.

Bone marrow samples were collected under local anesthesia 2 times – on $2^{nd}$ day of life and after 25 days, and were used to prepare smears for bone marrow cytological evaluation.

Blood for hematology and biochemistry (GSH-Px activity, Se and vitamin E concentrations) was collected 3 times – on $2^{nd}$ day of life, and then 15 and 25 days after the supplementation.

All laboratory tests and cytological evaluation of bone marrow smears were performed at the Clinical Laboratory for Large Animals, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences in Brno.

Bone marrow (approximately 1 ml) was collected with a 63 mm long 18 G bone marrow biopsy needle, from $3^{rd}$-$4^{th}$ rib in the sternum area into a 1 ml tube without anticoagulant. Xylazine at a dose of 0.05 mg $kg^{-1}$ of body weight (Sedazin, Biovet Puławy, Poland) was used for premedication. The area around the insertion of the biopsy needle was anesthetized with 3 ml of lignocaine solution. The sampling site was prepared according to standard surgical procedures.

Immediately after collection, due to the rapid processes of clot formation, smears were made on previously prepared and labeled slides (Marienfeld, Germany) and stained with the May-Grünwald-Giemsa method (MGG). Bone marrow staining time was 80 seconds with May-Grünwald stain and 5 min with Giemsa stain. During cytological evaluation of the bone marrow smears, 1000 cells of all developmental lines were counted with a hematology counter SH-96/24D by Alchem.

Blood was collected from the jugular vein using a Vacumed blood collection system into a 1 ml collection tube with a clot activator and a 1 ml collection tube with $K_2$EDTA (Vacumed®, Italy).

Peripheral blood morphology included: hemoglobin concentration (Hb), number of red blood cells (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), reticulocyte percentage (RETIC%) and absolute reticulocyte count (RETIC $\cdot 10^9$) and was determined using a Siemens diagnostic hematology analyzer ADVIA 2120i.

Peripheral blood smears were stained with the May- Grünwald -Giemsa method (MGG) – staining time for May-Grünwald stain – 3 min and for Giemsa stain – 5 min, and then evaluated under light microscope (Zeiss).

Se concentration was determined in the serum. Samples were mineralized in a mixture of nitric and perchloric acids in a ratio of 3:1. The mineralization was carried out in an electric aluminum heating block with temperature programming, raising the temp. from 120 to 200°C in 2-3 hours. After cooling down, concentrated hydrochloric acid was added to the colorless product of mineralization, and heated at 80°C for 20 min in order to reduce $Se^{6+}$ to $Se^{4+}$. Simultaneously with the test samples, reagent samples and samples with the addition of Se (0.050 and 0.100 μg $g^{-1}$ serum) were prepared. In addition, the method for Se determination was verified by analyzing the certified reference material: BCR No 184 – Lyophilized Bovine Muscle; certified value – 0.183 μg $g^{-1}$, marked value – 0.171 μg $g^{-1}$. Selenium content after mineralization was determined by flame atomic absorption spectrophotometry (flame acetylene – air) using the hydride generation method. The analysis was performed with a Unicam 939 Solar atomic absorption spectrophotometer, equipped with an Optimus data station, background correction (deuterium lamp), and a Unicam VP 90 hydride generation adapter.

Activity of glutathione peroxidase (GSH-Px) was measured from the whole blood in an Accent-200 biochemical analyzer (Cormay Group, Poland), using a Ransel reagent kit (Randox, UK).

The serum vitamin E concentration was determined by high-performance liquid chromatography (HPLC) using a HP-1050 chromatograph by Hewlett Packard and ClinRep kits (Recipe Chemical). The flow rate was 1.5 ml $min^{-1}$ at a wavelength of 325 nm.

Statistical analysis was performed using the ANOVA test (blood samples) and the post-hoc Kruskal-Wallis test, and *T*-student test (bone marrow) with Statistica 10 software (StatSoft Tibco, USA). The differences were considered statistically significant at $p \leq 0.05$.

# RESULTS

Table 2. shows the percentage distribution of cells of the erythroblastic cell line in bone marrow of goats from both groups. The first morphologically

Table 2

Percentage distribution of the erythroblastic cell line of the bone marrow sampled from both groups

| Specification | | | PROERBL (%) | BASO ERBL (%) | POLY ERBL (%) | ORTHO ERBL (%) |
|---|---|---|---|---|---|---|
| Day 0 | control | mean | 3.89 | 6.65 | 13.1 | 9.5 |
| | | SD | 0.98 | 0.99 | 2.33 | 1.94 |
| | experimental | mean | 4.22 | 6.89 | 14.59 | 9.31 |
| | | SD | 1.52 | 2.61 | 1.21 | 1.23 |
| Day 25 | control | mean | 4.01 | 7.06 | 15.09 | 9.43 |
| | | SD | 2.02 | 1.57 | 0.76 | 1.55 |
| | experimental | mean | $7.11^{*A}$ | 11.76 | $18.88^{*}$ | $14.47^{*A}$ |
| | | SD | 2.33 | 0.97 | 0.96 | 1.76 |

Explanations: PROERBL – proerythroblast, BASO ERBL – basophilic erythroblast, POLY ERBL – polychromatic erythroblast, ORTHO ERBL – orthochromatic erythroblast, $^{A}$ – statistically significant difference at $p \leq 0.05$ between day 0 and 25$^{th}$, * – statistically significant difference at $p \leq 0.05$ between groups

distinguishable cell in this cell line is the proerythroblast with a large, well-stained navy blue nucleus surrounded by blue cytoplasm slightly brighter around the nucleus. The largest group are polychromatic erythroblasts – 18.88%. It seems interesting that the presence of eosinophilic erythroblasts with a lobed nucleus was observed in the bone marrow smears of EXP group. This may be related to the intensity of the erythropoiesis process. The percentage of these cells was high – 14.47%, which was a statistically significant difference between the groups.

In order to properly interpret the hematological tests, peripheral blood morphology and cytological analysis of peripheral blood smears were performed. The results of the tests are presented in Table 3. The results of these tests clearly indicate significant activation of the hematopoietic processes through a significant increase in RBC with an increase in the concentration of hemoglobin (Hgb). Table 4 shows GSH-Px activity, selenium and vitamin E concentrations. Glutathione peroxidase activity increased significantly on day 25 of the experiment in the EXP group. In this group, an increase in the selenium content was also noted at the same time of the experiment, which was a statistically significant difference between the groups.

Table 3

Haematological parameters of peripheral blood from both groups

| Specification | | | RBC ($x10^{12}$ $l^{-1}$) | HGB (g $l^{-1}$) | MCH (fmol) | MCV (fl) | MCHC (g $l^{-1}$) | RDW (%) | RETIC (%) | RETIC ($x10^9$) |
|---|---|---|---|---|---|---|---|---|---|---|
| Day 0 | control | mean | 9.22 | 85.44 | 0.53 | 28 | 300 | 17.7 | 0.86 | 29.2 |
| | | SD | 0.74 | 6.33 | 0.02 | 1.06 | 10.9 | 0.75 | 0,25 | 1.18 |
| | experimental | mean | 9.56 | 86.51 | 0.53 | 32 | 300 | 18.9 | 0.98 | 30.1 |
| | | SD | 0.76 | 6.41 | 0.03 | 1.07 | 11.1 | 0.77 | 0.22 | 1.21 |
| Day 15 | control | mean | 9.66 | 88.43 | 0.54 | 27 | 290 | 18.8 | 0.81 | 30.8 |
| | | SD | 0.77 | 6.52 | 0.02 | 1.07 | 16.9 | 0.81 | 0.21 | 1.23 |
| | experimental | mean | 10.33 | 88.54 | 0.53 | 30 | 300 | $19.1^{A}$ | 0.84 | 31.3 |
| | | SD | 1.34 | 6.53 | 0.03 | 1.22 | 15.8 | 0.93 | $0.23^{A}$ | 1.24 |
| Day 25 | control | mean | 9.88 | 91.33 | 0.56 | 30 | 300 | 20.1 | 0.83 | 32.2 |
| | | SD | 1.23 | 8.04 | 0.04 | 1.30 | 16.5 | 1.1 | 0.22 | 1.39 |
| | experimental | mean | 10.89 | $93.22^{A}$ | 0.56 | 30 | 310 | 23.7 | $0.96^{*}$ | $38.6^{*}$ |
| | | SD | 1.55 | 8.34 | 0.04 | 1.33 | 20.3 | 1.34 | 0.26 | $1.54^{*}$ |

Explanations: hemoglobin concentration (Hb), number of red blood cells (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), reticulocyte percentage (RETIC%), absolute reticulocyte count (RETIC $\cdot 10^9$), $^{A}$ – statistically significant difference at $p \leq 0.05$ between day 0 and $25^{th}$, * – statistically significant difference at $p \leq 0.05$ between groups

Table 4

Se, vitamin E concentrations and GSH-Px activity in both groups

| Specification | | Se (µmol $l^{-1}$) | | Vit. E (µg $ml^{-1}$) | | GSH-Px (U $g^{-1}Hb^{-1}$) | |
|---|---|---|---|---|---|---|---|
| | | experimental | control | experimental | control | experimental | control |
| Day 0 | mean | 40.15 | 42.28 | 2.76 | 2.89 | 43.4 | 39.5 |
| | SD | 5.12 | 6.54 | 1.16 | 1.77 | 3.32 | 3.03 |
| Day 15 | mean | $64.33^{*A}$ | 39.98 | $3.67^{*A}$ | 2.67 | $126.3^{*A}$ | 42.8 |
| | SD | 7.15 | 5,59 | 1.78 | 1.36 | 13,51 | 4.97 |
| Day 25 | mean | $61.22^{*A}$ | 43.29 | $3.36^{*A}$ | 2.56 | $175.9^{*A}$ | 45,3 |
| | SD | 6.34 | 5.98 | 1.67 | 1.21 | 21.51 | 5.98 |

$^{A}$ – statistically significant difference at $p \leq 0.05$ between day 0 and $25^{th}$, * – statistically significant difference at $p \leq 0.05$ between groups

## DISCUSSION

Due to the limited amount of research on the intensification of caprine erythropoiesis after vitamin E and Se supplementation, we decided to undertake this difficult research topic. Postnatal deficiency anemia is observed in many newborn ruminants, regardless of the species. The decrease in the number of erythrocytes and in the hemoglobin concentration in young animals is explained by the transformation of fetal hemoglobin (HbF) into adult hemoglobin (Hb) – Al-Habsi et al. (2007), Brun-Hansen et al. (2006). In goats, this process is preceded by the HbF conversion into juvenile hemoglobin detected from 40 to 120 days postpartum (Johnson et al. 2002).

Deficiencies of microelements, to a large extent including Se, may affect the erythropoietic activity of bone marrow, contributing to the development of anemia, especially in neonatal period. This is related to the earlier lysis of the erythrocytic membranes and, consequently, to a decreased number of these cells in the peripheral blood. The result is a gradual deterioration of the oxygen supply to cells, tissues and organs, and a slow decline in the animal's health.

Hoffman (2007) and Kamada et al. (2007) proved that the addition of Se increases the activity of the hematopoiesis, as well as activating the functions of cells from individual developmental lines e.g. erythropoietic, granulopoietic etc. Snarska et al. (2018), in the research conducted on fallow deer kept in captivity, proved the effect of Se and vitamin E on the processes of hematopoiesis in this ruminant species. They showed a statistically significant increase in the percentage of proerythroblasts in fallow deer 25 days after receiving additional Se and vitamin E supplement. An increase in the number of erythrocytes and hemoglobin concentration was also observed at that time. Our results are consistent with the aforementioned studies.

A study by An et al. (2011) showed the influence of micronutrients on the erythropoiesis occurring in the erythroblastic islands in the bone marrow and the differentiation of erythroid cells. Research by Kawatani et al. (2011) conducted on mice indicates the superior role of Se and selenoproteins in the regulation of erythropoiesis in bone marrow by controlling redox reactions leading to erythroid damage. The result of this complex process was a decrease in the hematocrit value, a decrease in the concentration of hemoglobin and an increase in the volume of erythrocytes. These results suggested that in mice the redox reaction results in the formation of ineffective erythropoiesis. These studies clearly emphasized the protective effect of selenium and selenoproteins on the proper course of erythropoiesis and elimination of the effects of erythroid damage.

The standard measure of erythropoiesis disorders due to oxidative stress is a decrease in hemoglobin concentration in erythrocytes. This was proven in the study of Liao et al. (2018) on mice in cases of acute anemia as a result

of irradiation and strong oxidative stress resulting in damage to erythrocyte membranes, reducing the cells lifespan.

The relationship between Se concentration and GSH-Px activity has been described by e.g. Pavlata et al. (2011). Similar research results were obtained by Harapin et al. (2000), indicating a correlation between the activity of glutathione peroxidase, and the content of selenium in peripheral blood. We came to similar conclusions, demonstrating a clear upward trend in the GSH-Px activity in EXP group 15 days after Se and vitamin E supplementation. At that time, increased activity of erythropoiesis was also observed among polychromatic and eosinophilic erythroblasts. Literature data indicates that the blood Se concentration in newborn goats is lower by about 40% compared to their mothers (Misurova et al. 2009). The study by Illek et al. (2002) indicated a significant effect of selenium supplementation of pregnant heifers on the Se concentration and the activity of glutathione peroxidase in newborn calves, thus emphasizing the importance of this microelement on animal health. The study of Ramirez et al. (2004) conducted on sheep with selenium deficiency demonstrated the undeniably increased mortality in animals due to deficiency of this element.

Selenium deficiency shortens the lifespan of erythrocytes by damaging their cell membrane, and has a significant impact on the decrease in the activity of glutathione peroxidase, which protects erythrocytes against oxidation and degeneration of cell structures by hydrogen peroxide (Pavlata et al. 2005*a*, Faixova et al. 2007, Qin et al. 2007, 2011). In the study of Pavlata et al. (2003), the authors clearly emphasize the importance of selenium transfer from mothers to newborn calves in terms of their health, and further development, which depends on the concentration of this element in mothers.

During our research, we observed that proerythroblasts have a different number of nucleoli (from 2 to 4). Research by Snarska et al. (2018) conducted on fallow deer, demonstrated that Se and vitamin E supplementation has a stimulating effect on erythropoiesis, increasing the percentage of immature cells of the erythroblastic cell line in the bone marrow. Cytological evaluation of bone marrow in fallow deer showed a similar number of nucleoli in the group receiving supplementation. Our results presented in this publication show that the process of erythropoiesis in bone marrow in EXP group was definitely more active compared to the CONT group.

The serum concentration of vitamin E in young ruminants varies and depends on its low ability to be transported throughout the placenta. Therefore, the concentration of this compound during neonatal period depends on its content in colostrum and dam's milk. The studies by Pavlata et al. (2005), Schneider (2005), Kojouri, Shirazi (2007) indicate that colostrum and milk of dams with vitamin E deficiency did not protect their offspring against vitamin E deficiency. Another factor affecting the concentration of vitamin E is its transport in the body by lipoprotein particles (Bourne et al. 2008).

Research by Pavlata et al. (2005b) conducted on calves showed a positive effect of selenium and vitamin E supplementation in the course of advanced states of deficiency of this vitamin. Our research showed that the increased concentration of vitamin E was correlated with the increased Se concentration and the activity of GSH-Px in the EXP group 25 days after supplementation. Moreover, an increase in hemoglobin (Hb) and the number of RBCs was observed at that time. Higher percentages of polychromatophilic and eosinophilic erythroblasts were observed during cytological evaluation of bone marrow smears. Increased reticulocyte count and percentage were observed in the peripheral blood on the 25$^{th}$ day of the experiment, in EXP group, which is a measurable indicator of effective erythropoiesis due to the ability to carry oxygen.

## CONCLUSIONS

The experiment and its results allow us to assume that vitamin E and Se supplementation in 2-day-old goats effectively improves the process of erythropoiesis in bone marrow, protecting against the loss of hemoglobin in the early developmental period, and contributes to protecting those animals against the effects of anemia that may develop during their neonatal period.

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