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ORIGINAL PAPER

The effect of selenium and vitamin E supplementation on thrombopoiesis in young goats*

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Abstract

Selenium deficiency is still a global problem in livestock husbandry, and this micronutrient is commonly supplemented in ruminants around the world, especially during the neonatal period. The aim of this study was to assess the influence of selenium and vitamin E supplementation in young goats in the neonatal period on the activity of the process of thrombopoiesis based on cytological evaluation of bone marrow smears. Twelve clinically healthy goats at the age of 2 days divided into two equal groups (n=6) participated in the study. Only animals from the experimental group received a single dose of Se and vitamin E on the second day of life. Bone marrow was collected three times (2nd day of life and after 15 and 25 days), stained with the MGG method, and subjected to cytological evaluation. Peripheral blood smears were also analyzed three times. 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 numbers of megakaryoblasts and megakaryocytes were higher in goats from the experimental group than in the control group, which indicated the intensification of thrombopoiesis in animals supplemented with selenium and vitamin E. The results of the morphological tests clearly indicated an increase in the number of platelets and a higher platelet volume in experimental group. It can be concluded that selenium and vitamin E supplementation has a positive impact on the thrombopoiesis in caprine bone marrow.

Keywords: caprine bone marrow, selenium, thrombopoiesis, vitamin E, young goats

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INTRODUCTION

Selenium is an essential micronutrient and an integral part of antioxidant systems (Nève 1995). It is also crucial for the biosynthesis of selenoproteins containing selenocysteine. Low Se levels can cause muscle degeneration in young ruminants and pigs. On the other hand, selenium deficiency in adult ruminants causes a decrease in milk yield, endocrine disorders and impaired immunity (Herdt et al. 2011). In human medicine, low selenium levels are associated with increased incidence of cancer, vascular diseases, Alzheimer's disease and haematopoietic disorders (Rayman 2012). Se deficiency also contributes to the presence of oxidative stress due to the fact that this element constitutes important antioxidant enzymes, e.g. glutathione peroxidase, which is often used for indirect detection of oxidative stress in animals (Hejel et al. 2021) In turn, excessively high blood concentrations of this element adversely affect animal health, but such cases are recorded much less frequently (Tiwary et al. 2006).

Thrombocytes are the final stage of differentiation of megakaryocytes, and their formation occurs through the isolation of the precursor forms. The process of megakaryocyte formation is closely connected to the differentiation of the erythroblastic cell line (Guan et al. 2017). The bipotent precursor cell (megakaryocyte/erythrocyte progenitor cell – MEP) proliferates in order to form megakaryocytes. These cells have the ability to cleave fragments of their cytoplasm. Megakaryocytes are multilobed giant platelet--producing cells found on the outer surface of the vascular sinuses in the bone marrow. Cytoplasmic extensions of mature megakaryocytes protrude into the lumen of the vascular sinuses, where they transform into proplatelets, and then into individual platelets. Entire megakaryocytes rarely migrate into the vascular sinuses, and occasionally they may be seen in animal blood smears. Precursor cells belong to the common myeloid and granulocytic cell line (common myeloid progenitor – CMP) – Yang et al. (2023). However, direct differentiation of megakaryocytes, without the CMP precursor, is also described. The late phase of megakaryocyte differentiation is the maturation of their precursor - the promegakaryoblast, accompanied by endomitosis (Kaushansky 2015).

Thrombocytes are fully differentiated structures in which no further cell divisions take place. However, despite the absence of a nucleus, the synthesis and transcription of mitochondrial DNA, as well as protein synthesis from megakaryocytic mRNA, was confirmed in these cells. Thrombocytes are the smallest, enucleated cytoplasmic fragments that contain typical cellular organelles and exhibit active metabolism due to the presence of mitochondria. A highly specialized system of membrane receptors, secretory granules and the cytoskeleton determine their high reactivity. The fundamental role of platelets is to maintain proper haemostasis, but they are also actively involved in inflammation and immune processes, as well as in the progression of cancer.

The selenium concentration in platelets is extremely high and depends on its incorporation into immature cellular structures. This makes it possible to avoid Se deficiency even in cases of its reduced supply. The process of thrombopoiesis and the number of PLTs resulting from its activity actively responds to changes in the concentration of selenium in mammals (Dalir-Naghadeh et al. 2015).

The response of the human platelets to increased Se concentration is characterized by a significant increase in the platelet activity of glutathione peroxidase (GPx) that demonstrates greater sensitivity than GPx activity in serum or erythrocytes. It can be explained by a shorter lifespan of platelets compared to the one of erythrocytes. Moreover, platelet GPx activity has been found to be more sensitive in response to increased Se intake in humans than plasma or erythrocyte GPx. Unlike in plasma, whole blood or erythrocytes, haemoglobin does not affect Se markers in platelets. Platelets not only have the ability to actively synthesize proteins, but also to create new structures that are functionally and structurally similar to the platelets they derived from.

Hematopoiesis and thrombopoiesis are dependent on thrombopoietin, which is homologous to erythropoietin. This glycoprotein hormone activates signalling proteins and gradual proliferation of megakaryocytes, the synthesis of platelet proteins and the loss of proliferation capacity, leading to the formation of a polyploid nucleus due to subsequent endomitotic divisions.

An extremely important element of normal erythropoiesis and thrombopoiesis is GPx (Kaushal et al. 2011). A decrease in its activity causes defragmentation of cell membranes, apoptosis, and increased concentration of calcium ions (Abutarbush, Radostits 2003). It can also lead to disruption of erythropoiesis, resulting in the development of thalassemia and inflammation due to the formation of oxidative stress in redox reactions (Beatty et al. 2000) and the gradual slowing down of haematopoiesis. GPx is the only enzyme that can prevent harmful lipid peroxidation by reducing lipid peroxides to alcohols.

Research on mice indicate that the quantitative parameters of haematopoiesis in the blood, bone marrow and spleen showed anaemia with an increase in the number of erythroid precursor cells, and thus also cells of the thrombocytic cell line (Kawatani et al. 2011).

Vitamin E is a protector against lipid peroxidation initiated by free radicals (Azzi, Stocker 2000, Azzi 2007). Its influence on the course of haematopoiesis has not been unequivocally determined, however, during its decreased concentration, the incorporation of selenium into selenoproteins is limited, contributing to the severity of anaemia, and affecting the fragmentation of cytoplasm fragments from megakaryocytes (Pavlata et al. 2005, Altamura et al. 2020).

The aim of this study was to evaluate the effect of selenium and vitamin E supplementation on thrombopoiesis in young goats.

MATERIALS AND METHODS

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

Twelve young (2 days old), clinically healthy and properly fed goats in good physical condition were chosen from one herd of Polish White Improved goats to participate in the experiment. The animals were randomly divided into two equal groups (n=6): control – animals that did not receive any additional Se and vitamin E supplementation, and experimental – animals that on the 2nd day of life received a single intramuscular injection of vitamin E and Se (2 ml per animal) - tocopherol acetate - 50 mg (approx. 16.67 mg kg^{-1}), sodium selenite - 0.5 mg (approx. 0.17 mg kg^{-1}) (Eurovet Animal Health BV, Holand), which is a commonly used dosage of vitamin E and selenium for young goats.

The animals were housed in a free-stall barn, meeting the requirements for the farm animals' welfare in accordance with the EU law. All goats were kept with their dams and fed their milk throughout the entire experiment. Moreover, animals had unlimited access to Multi-Lisal salt licks (NaCl 94%, water-insoluble substances max. 4%, Mg 2000 mg kg^{-1}, Co 18 mg kg^{-1}, Zn (zinc) 810 mg kg^{-1}, Mn 830 mg kg^{-1}, I (Iodine) 100 mg kg^{-1}, Se (selenium) 10 mg kg^{-1}. Considering the fact that the feeding program in this herd included constant access to licks for all animals, the micronutrient supply of the dams was assumed to be the same for both groups.

The effectiveness of the administered supplement was assessed based on the GPx activity and Se and vitamin E concentrations in peripheral blood, and the activity of thrombogenesis based on the cytological evaluation of bone marrow smears and morphology of peripheral blood.

Bone marrow samples were collected under local anesthesia 3 times: 2nd day of life and after 15 and 25 days (on the 17th and 27th day of life), and were used to prepare smears for bone marrow cytological evaluation. 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 a biopsy needle was locally anesthetized with 3 ml of lignocaine solution. The sampling site was prepared according to standard surgical procedures. Approximately, 1 ml of bone marrow was collected with a 63 mm long 18 G bone marrow biopsy needle, from 3rd-4th rib in the sternum area, into a 1 ml tube without anticoagulant. Bone marrow smears were made immediately after collection on previously prepared and labeled slides (Marienfeld) and stained with May- Grünwald-Giemsa method (MGG). Bone marrow staining time was 80 seconds with May-Grünwald stain and 5 minutes with Giemsa stain. During cytological evaluation of the bone marrow smears, 1000 cells of all cells from thrombocytic cell line (megakaryoblasts, promegakariocytes, megakariocytes) were counted with a hematology counter SH-96/24D by Alchem.

Blood for hematological and biochemical tests was collected 3 times: 2nd day of life (day 0), after 15 and 25 days after supplementation. Blood was collected from the jugular vein using the VACUMED blood collection system into a 1 ml collection tube with clot activator and into a 1 ml collection tube with K2EDTA (VACUMED®, Italy).

Peripheral blood morphology included platelet count (PLT) and mean platelet volume (MPV), which were determined on a Siemens diagnostic hematology analyzer ADVIA 2120i. Peripheral blood smears were stained with May-Grünwald-Giemsa method (MGG), where the staining time for May-Grünwald stain was 3 minutes and for Giemsa stain was 5 minutes and then each sample was evaluated under light microscope (Zeiss).

The methods for determining selenium and vitamin E concentrations are described and presented in the following paper (Snarska et al. 2023). The use of the supplement resulted in significantly higher serum concentrations of selenium and vitamin E in the experimental group 15 days after the supplement administration.

The results were statistically analyzed using the ANOVA and Levene's tests with Statistica 10 software (StatSoft Tibco, USA). The differences were considered statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

The results of the blood morphology are presented in Table 1. These results clearly indicate a significant activation of hematopoietic processes in the experimental animals already on the 15th day of the study, demonstrated as a significant ($p<0.05$) increase in the platelet count (PLT), which continued to the end of the experiment. An increase in platelet volume was also observed during this period in those animals. Statistical analysis of MPV

Table 1

The platelet blood parameters (mean ± SD)

Specification	Control			Experimental		
Day	0	15	25	0	15	25
PLT	146.5±4,82*	145.5±12.74**	151.3±13.50***	189.5±22.82 *	195.8±19.73**	194.7±48.05***
MPV	3.53±0.26*	3.65±0.43**	3.73±0.45***	5.57±1.18$^{A, B, *}$	6.58±1.45$^{A, c, **}$	7.53±1.56$^{B, C, ***}$

Explanations: ^{A} – statistically significant difference ($p<0.05$) in experimental group between 0 and 15 days, ^{B} – statistically significant difference ($p<0.05$) in experimental group between 0 and 25 days, ^{C} – statistically significant difference ($p<0.05$) in experimental group between 15 and 25 days, * statistically significant difference ($p<0.05$) between groups in day 0, ** statistically significant difference ($p<0.05$) between groups in day 15, *** statistically significant difference ($p<0.05$) between groups in day 25

showed significant differences ($p<0.05$) between the control and experimental group on days 15 and 25. Cytological evaluation of peripheral blood smears showed that the cleaved cytoplasmic fragments were larger, and the platelets were richly granulated in animals that received additional selenium and vitamin E supplement. The number of platelet aggregates consisting of an uncountable number of platelets was also higher in the experimental animals (Figures 1, 2).

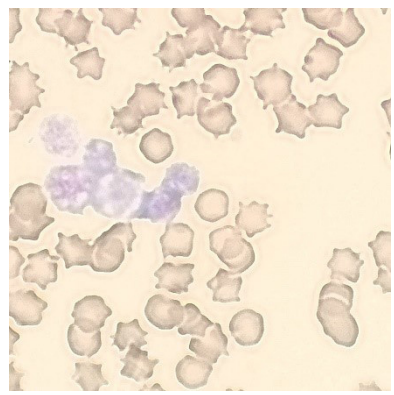

Fig. 1. Giant platelets, similar in size to erythrocytes in blood smear (experimental group on day 15, MGG staining method, magnification x1000)

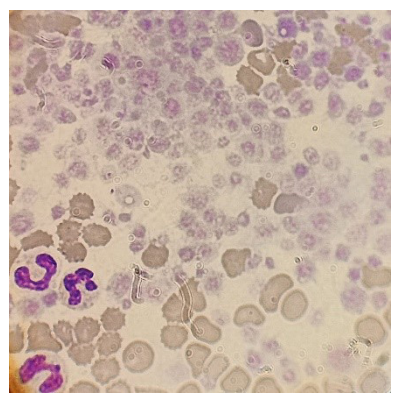

Fig. 2. Numerous large and giant platelets in blood smear (experimental group on day 25, MGG staining method, magnification x1000)

The results of the cytological evaluation of bone marrow smears are shown in Table 2. Cytological evaluation of the bone marrow indicate the activation of thrombopoiesis processes from the 15th day after selenium and vitamin E supplementation in the experimental animals. The numbers of megakaryoblasts and megakaryocytes were higher in the experimental animals throughout the entire experiment ($p<0.05$). Numbers of those cells also differed significantly ($p<0.05$) between day 15 and 25, and between the beginning and the end of the experiment in the experimental group. Such

Table 2

The average number of cells from the thrombocytic cell line in relation to 1,000 of all bone marrow cells (mean ±SD)

Specification	Control			Experimental		
Day	0	15	25	0	15	25
Megakaryoblasts	0.5±0.73*	0.83±0.69**	1.33±0.47***	1.5±0.5$^{B,\,*}$	1.5±0.49$^{C,\,**}$	2.5±0.5$^{C,\,***}$
Promegakaryocytes	0	0	0	0	0	0
Megakaryocytes	0.17±0.37*	0.17±0.76**	0.5±0.5***	1.5±0.76$^{B,\,*}$	1.5±0.58$^{C,\,**}$	2.17±069$^{C,\,***}$

Explanations: ^{A} – statistically significant difference (p<0.05) in experimental group between 0 and 15 days, ^{B} – statistically significant difference (p<0.05) in experimental group between 0 and 25 days, ^{C} – statistically significant difference (p<0.05) in experimental group between 15 and 25 days, * statistically significant difference (p<0.05) between groups in day 0, ** statistically significant difference (p<0.05) between groups in day 15, *** statistically significant difference (p<0.05) between groups in day 25

changes were not observed in the control animals. Moreover, the bone marrow cells in the group receiving the supplement absorbed dyes faster and more intensively than the same cells in the control animals (Figure 3).

The authors of this publication decided to address the subject of cytological evaluation of the bone marrow in ruminants because this topic is rarely discussed in veterinary research. Among the available scientific literature, there are no studies on the effect of selenium and vitamin E supplementation on thrombopoiesis in the caprine bone marrow.

One of the first research in this field (Kiem 1988) indicated that platelets could be an excellent detector of selenium deficiency. This research proved that selenium deficiency and a decreased platelet counts were the factors causing increased incidence of cardiac and vascular diseases in humans and more than half of the determined selenium derived from the progenitor cells of thrombocytic line.

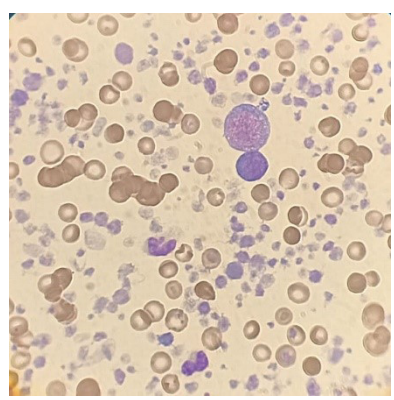

Fig. 3. Numerous large platelets and immature cells of the white blood cell line in bone marrow smear (experimental group on day 25, MGG staining method, magnification x1000)

The research showed that selenium supplementation increases GPx activity and actively stimulates hematopoiesis. Our results indicate a statistically significant increase in the number of platelets in peripheral blood in the group receiving selenium and vitamin E on the 15th day of the study. An and Mohandas (2011) came to similar conclusions, demonstrating the importance of proper mineral balance during hematopoiesis and subsequent cell differentiation. In the current experiment, the mean platelet volume in blood morphology began to increase already on the 15th day from the start of supplementation and significantly increased on the 25th day of the experiment in animals that received additional selenium.

The results of our research also showed a significant effect of selenium and vitamin E on the stimulation of thrombopoiesis in the bone marrow in terms of the number of immature forms of the cells. Research on sheep, showed that platelet parameters and platelet count in animals supplemented with selenium are a promising proxy for the currently used indicators of Se status in sheep, regardless of the source of Se (Dalir-Naghadeh et al. 2015). Selenium plays a key role in the hematopoiesis by regulating the redox reaction,. These reactions favor the formation of reactive oxygen species, which have a destructive effect on the erythrocytes and platelets lifespans (Nagababu et al. 2003).

Research by Hoffman (2007) and Kamada et al. (2007) showed that the addition of selenium has a stimulating effect on the hematopoietic processes of every cell line. A study conducted on fallow deer (Snarska et al. 2018) also confirmed the stimulating effect of selenium and vit. E on hematopoiesis demonstrated by increased activity of the erythroblastic line. Studies conducted on rodents indicate that Se deficiency inhibits hematopoiesis and contributes to the development of inflammation (Kaushal et al. 2011).

Our research showed that megakaryocytes in the group receiving selenium with vitamin E cleaved off larger fragments of a highly granulated cytoplasm, they absorbed pigments more intensively than control animals. The number of megakaryocytes was higher in goats from the experimental group than in the control group, which indicates the intensification of thrombopoiesis in animals supplemented with selenium and vitamin E. Similar conclusions were drawn by Snarska et al. (2018) in a study on fallow deer.

It is known that selenium supplementation induces quick activation of hematopoietic processes, especially in the erythroblastic and platelet cell lines. Often this response is also associated with increased activity of GPx (Shi et al. 2011). Dalir-Naghadeh et al. (2015) point to the shortening of platelet lifespan to 8-14 days compared to the extension of erythrocyte survival of about 150 days in sheep during selenium supplementation. These results clearly indicated that erythrocytes did not show the ability to incorporate Se into GPx. Based on the results obtained by the aforementioned authors, it was shown that the GPx activity of blood platelets and the concentration of selenium in platelets were a reliable parameter to assess the

selenium status in sheep. Therefore, a delay between Se supplementation and the highest Se concentration and GPx activity would be expected in whole blood as well as in erythrocytes compared to platelets.

Abutarbush and Radostits (2003) show that a decrease in GPx activity causes defragmentation of cell membranes and an increase in intracellular Ca^{2+} concentration and damage to cell nuclei, leading to the development of normocytic anemia. In the course of this condition, a clear change in the morphology of platelets can be observed, consisting in changing their shape from disc-shaped, small structures to clearly activated forms. Such conclusions were also reached by the authors of this publication in the course of their own research.

CONCLUSIONS

The results allow us to assume that vitamin E and Se supplementation in young goats effectively improves the process of thrombopoiesis in bone marrow, which translates into better platelet parameters in whole blood.

Author contributions

Conceptualization – A.S., D.G.; methodology – A.S., L.R.; laboratory analyses – A.S.; data curation – A.S., D.G.; statistical analysis – L.R.; original draft preparation – A.S., D.G.; review – A.S.; editing – D.G. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interests. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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