

*Original study*

## Status of selenium and correlation with blood GSH-Px in goats and their kids in organic breeding fed with different levels of organic selenium

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### Abstract

The aim of this research was to determine how the supplement of organic selenium in the diet at different concentrations reflects on the content of blood Se and GSH-Px activity and their correlation in goats and their kids in organic breeding. The study included 24 Alpine goat breeds during lactation and their kids. Goats were divided into three groups depending on dietary treatments. Goats were fed with the cereals mixture 1 kg/per day in control group (C). Organic selenium supplied as selenised yeast in the first experimental group (E-I) 0.015 % and in the second experimental group (E-II) 0.030 % in cereals mixture. The addition of organic selenium significantly ( $P<0.01$ ) increased blood Se of goats and kids in E-I and E-II groups in relation to C group and higher ( $P<0.05$ ) in E-II in comparison with the E-I group. Concentrations of Se followed by GSH-Px activity, and were higher in the E-I and E-II groups in comparison with C group. In the blood of suckling kids has determined higher ( $P<0.01$ ) GSH-Px activity in E-I and E-II groups in relation to C group. It was also determined higher GSH-Px in the blood of kids E-II group compared to the E-I group. Significant correlations was found between Se levels and GSH-Px activity in goats and kids, but were significant only in the goats of E-II group. The results obtained concentration of Se and GSH-Px in the blood goats and kids showed the validity of adding prganic selenium into food in organic breeding.

**Keywords:** goats, selenium, GSH-Px, organic selenium, blood, organic breeding

**Abbreviations:** C: control group, E: experiment group, GSH-Px: glutathione peroxidase

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## Introduction

Studying the concentration of selenium in foods of animal origin is very important for the supply of human population to these essential microelements. The concentration of selenium in the animal organism is dependent on its intake with food (Surai 2006). Selenium is necessary for growth and fertility in animals and for prevention of variety of disease conditions (Kolodziej & Jacyno 2005, Balicka-Ramisz *et al.* 2006, Gabryszuk *et al.* 2009, Marai *et al.* 2009). The bases of a meal in the feeding of ruminants, including goats are voluminous feed (95 %), while only 5 % are concentrated feed. Most of the voluminous feeds used in the feeding of small ruminants are produce on poorer quality soils, which are often high humidity and acidity, and that prevents plants to adopt quality of selenium from the soil, even if it is in the soil in sufficient quantities. In the world there are endemic areas where lack of selenium in the soil causes a lack of selenium in plants grown on these soils (Gupta & Gupta 2000, Antunović *et al.* 2010). Therefore, in many countries in the diet must add selenium. In recent years various studies are done with the addition of organic selenium in the food of small ruminants in different quantities (Kopić *et al.* 2007, Antunović *et al.* 2009b, Misurova *et al.* 2009a, Pavlata *et al.* 2011a, b). It was determined that organic selenium has better bioavailability and utilization in the body (Daniels 1996, Wolfram 1999, Pavlata *et al.* 2011a) and less excretion thus reducing environmental pollution. This is very important because in organic breeding are recommended the use of organic selenium in areas with selenium deficiency in the soil (Govasmark *et al.* 2005, Schlemmer *et al.* 2008, Steen *et al.* 2008). Selenium is an essential part of the enzyme glutathione peroxidase (GSH-Px) and plays an significance role in the intracellular a specific immune defence (Dercksen *et al.* 2007). Adequate methods for monitoring the supply of body selenium are related to the determination of its content in the blood and products supplemented animals and activity of GSH-Px in blood (Pavlata *et al.* 2011a, Antunović *et al.* 2009a, 2008, Lyons *et al.* 2007). Thomson *et al.* (2004) concluded that plasma or serum selenium reflects short-term status of selenium, erythrocyte selenium reflects longer-term status and hair or nail selenium reflect long-term selenium status in organism. Gerloff (1992) concluded that activity of GSH-Px can be indicator of long-term supplementation of organism with selenium, while determination blood selenium content indicator the short-term variation in the levels of this microelement. The aim of this investigation is to determine how the addition of organic selenium in the diet at different concentrations reflects on the content of selenium and GSH-Px in the blood of goats and their kids and their correlation to organic breeding.

## Material and methods

### *Animals, locations of investigation and diets*

The study included 24 Alpine goat breeds during lactation and their kids during the winter season. Goats were divided into three groups depending on dietary treatments and held by the »Regulation on organic production of animal products« (Ministry of Agriculture and Forestry of Croatia 2002). Goats were an average age of 4 years, healthy in third lactation, with one kid in the litter. Research began immediately after kidding and lasted 60 days. Goats were fed with the following cereals mixture: wheat, barley, corn and wheat bran (12 % crude

protein, 10 MJ NEL/kg) 1 kg/per day and meadow hay (11 % crude protein, 4.7 MJ NEL/kg) *ad libitum*. Organic selenium supplied as selenised yeast (Sel-Plex, Alltech Inc., Nicholasville, KE, USA). In the first experimental group (E-I) the Sel-Plex was added in cereals mixture (0.015 %) and in the second experimental group (E-II) the Sel-Plex (0.030 %). Beside milk, kids fed mentioned meal for goats at will. The content of selenium in feed of goats and kids is shown in Table 1.

Table 1

Selenium contents in goats' and kids' meals depending about nutrition treatments

Groups	Selenium content		
	Cereal mixture, mg/kg	Milk, µg/L	Meadow hay, mg/kg
Control (C)	0.060	7.39	0.0035
Experiment I (E-I)	0.075	10.57	0.0035
Experiment II (E-II)	0.090	22.18	0.0035

### Sampling and analyses

Blood was collected from the jugular vein (10 ml) into serum vacutainer tubes (Venoject, Sterile Terumo Europe, Leuven, Belgium) and ethylenediamine tetra-acetic acid (EDTA) after morning feeding on the 60th day of lactation. At the same time were taken blood samples from their kids. The EDTA tubes were inverted several times to ensure adequate mixing of the blood with anticoagulant. Blood serum was separated by centrifugation (10 min) at speeds of 3000 rpm. Concentrations of selenium in cereal mixture, meadow hay and goat milk were microwave assisted acid digested using concentrated nitric, perchloric and chloric acids (high pure) according to the method for Microwave Sample Preparation-MARS 5 (CEM, Matthews, NC, USA) in order to total the sample decomposition. Determination of selenium content was done by Continuous Hydride Generation Atomic Absorption method on PerkinElmer 2380 MHS-10 (PerkinElmer Inc., Waltham, MA, USA). Serum for determination of selenium was diluted (1+4) with 0.2 % solution of Triton X-100 (Merck KGaA, Darmstadt, Germany). The concentration of total selenium in serum of goats and kids was determined by atomic absorption spectrometry, graphite technique on the apparatus AAnalyst 800/Zeeman (PerkinElmer Inc., Waltham, MA, USA) with the addition of Pd and Mg ( $\text{NO}_3$ )<sub>2</sub> and matrix modifier (Pizent *et al.* 1999). The concentration was determined by standard addition method. Accuracy of the method was checked by determining the reference serum sample (Seronorm, Trace Elements in Serum, Sero AS, Billingstad, Norway). GSH-Px was determined in whole blood of goat and their kids with commercial »Ransel« kit (Randox Laboratories Ltd, London, UK). Method for determination of GSH-Px was based on catalytic oxidation of glutathione by cumen hidroxi peroxide, and for reading was used a Jenway 6305 UV/VIS spectrophotometer (Bibby Scientific Ltd, Staffordshire, UK).

### Statistical analysis

The results were statistically evaluated by using LSD test in Statistica 8 (StatSoft, Inc. Tulsa, OK, USA). Differences were considered as significant at the level of 0.05 or less. The means

and their standard deviation, min and max values as well as standard errors were also calculated. Pearson's correlation method and regression equation were used to assess the relation between blood concentration of selenium and GSH-Px activity in goat and their kids in Statistica 8.

## Results and discussion

Analysing the Table 2 it is visible that the addition of organic selenium significantly increased selenium content in the blood of goats and their kids who are consumed mixture with the addition of Se in comparison to the control group. Activity of GSH-Px was also followed selenium concentrations and was significantly higher in the E groups than in the C group. It was also determined significantly higher ( $P<0.05$ ) Se concentration and GSH-Px activity in the blood of goat E-II group compared to the E-I group. In the blood of suckling kids has determined significantly higher ( $P<0.01$ ) GSH-Px activity in both E groups compared to C group. It was also determined significantly higher ( $P<0.05$ ) GSH-Px in the blood of kids E-II group compared to the E-I group.

Table 2  
Blood selenium contents and activities of GSH-Px in goats and kids with different nutrition treatments

Values	Control		Groups		Experiment II	
	Goat	Kid	Experiment I Goat	Kid	Goat	Kid
Selenium, µg/L:						
Mean	36.18 <sup>A</sup>	19.17 <sup>A</sup>	72.30 <sup>Ba</sup>	35.87 <sup>Ba</sup>	82.15 <sup>Bb</sup>	44.20 <sup>Bb</sup>
Standard deviation	17.12	5.28	6.18	7.73	7.84	6.74
Max	70.00	28.56	80.94	44.00	93.82	54.30
Min	18.09	14.06	65.64	21.02	72.55	32.00
Standard error	6.05	1.87	2.18	2.73	2.77	2.38
GSH-Px, µkat/L:						
Mean	76.37 <sup>Aa</sup>	52.58 <sup>A</sup>	146.17 <sup>Ab</sup>	108.25 <sup>Ba</sup>	318.21 <sup>B</sup>	128.47 <sup>Bb</sup>
Standard deviation	38.53	13.06	39.48	15.57	66.41	19.10
Max	143.71	69.54	212.68	128.33	393.75	100.18
Min	20.12	34.15	97.72	90.00	218.43	158.56
Standard error	13.62	4.62	13.95	5.51	23.48	6.75

Mean with different superscript letters differ significantly: <sup>a,b</sup> $P<0.05$ , <sup>A,B</sup> $P<0.01$

Similar conclusions have obtained Pavlata *et al.* (2011a, b) and Misurova *et al.* (2009a), although the concentrations of selenium and GSH-Px activities in these research were higher. The higher concentration of selenium in blood after application of organically form selenium (selenomethionine) results from directly incorporated into tissue proteins instead of methionine (Shrauzer 2003). Increased selenium concentrations in goats milk, blood and plasma as well as GSH-Px activity in whole blood of goats' food with organic selenium determined Khaled & Illek (1999). Although placental transfer of selenium is more effective from the transport of selenium in milk (Pavlata *et al.* 2003), it is considered that the concentration of selenium and GSH-Px in the blood of goats after birth, is about 60 % of maternal concentrations (Misurova *et al.* 2009b). In our investigation selenium contents in

goat milk (from 7.39 in control group to 22.18 µg/L in E-II group; Table 1) were slightly lower in comparison by results (from 23.31 to 31.26 µg/L) in investigation by Petrera *et al.* (2009) and with results (from 9.2 to 31.2 µg/L) in investigation by Rodriguez *et al.* (2002) and similar with results Pechova *et al.* (2008). Petrera *et al.* (2009) with the addition of selenium yeast in food of goats are determine significantly increased selenium levels and increased GSH-Px, but without significance. The main problem in using GSH-Px activity as attest for selenium assessment is related to variability of results between different laboratories. This is associated with differences in methodologies, samples storage, transport of samples and other (Surai 2006). In the C group of goats and kids it is visible lack of selenium in the blood, which is connected with a reduced concentration in the diet (Table 1). Castillo *et al.* (2001) were determined influence of nutritional factors on the blood GSH-Px values in Assaf ovine breed. The limit value of deficiency in whole blood is 80 µg/L (Bickardt *et al.* 1999) and in serum 30 µg/L (Puls 1994). In Table 3 and Graphs 1, 2, 3, 4, 5 and 6 are shown the correlation and regression equation and curve between the concentration of blood selenium and GSH-Px of goats and their kids depending on the dietary treatments.

Table 3

Correlation and regression equation between blood selenium and GSH-Px activity in blood of goats and their kids

Correlation	Groups	Control		Selenium Experiment I		Experiment II	
		Goats	Kids	Goats	Kids	Goats	Kids
GSH-Px	C	0.65 ( $P>0.05$ )	0.59 ( $P>0.05$ )	-	-	-	-
	E-I	-	-	0.57 ( $P>0.05$ )	0.63 ( $P>0.05$ )	-	-
	E-II	-	-	-	-	0.77 ( $P<0.05$ )	0.66 ( $P>0.05$ )
Regression equation		Goats			Kids		
CI		GSH-Px=23.239+1.4688×Se			GSH-Px=24.573+1.4609×Se		
E-I		GSH-Px=-115.5+3.6185×Se			GSH-Px=63.067+1.2594×Se		
E-II		GSH-Px=-213.9+6.4770×Se			GSH-Px=45.350+1.8807×Se		

Mean with different superscript letters differ significantly: <sup>a,b</sup> $P<0.05$ ,  $P>0.05$ -ns

It is visible that high correlations were determined between these two parameters in goats, but were significant only in the E-II group. In the kids were also determined a high correlation between selenium and GSH-Px in the blood, but with no significant difference ( $P>0.05$ ). Mentioned points to relationship of these two indicators, although insignificant. The above shows the calculated regression equations and regression curves. In the research Pavlata *et al.* (2011b) were determined significant correlations ( $r=0.72$ ;  $P<0.01$ ) between the concentration of selenium in whole blood and GSH-Px in the blood of goats. In the blood of kids were determined significantly high correlation ( $r=0.93$ ;  $P<0.01$ ) between serum selenium and GSH-Px in whole blood of growing kids (Pavlata *et al.* 2005), as well as the results Misurova *et al.* (2009b) which have in kids found a strong significant correlation ( $r=0.64$ ;  $P<0.01$ ) between selenium concentration and GSH-Px.

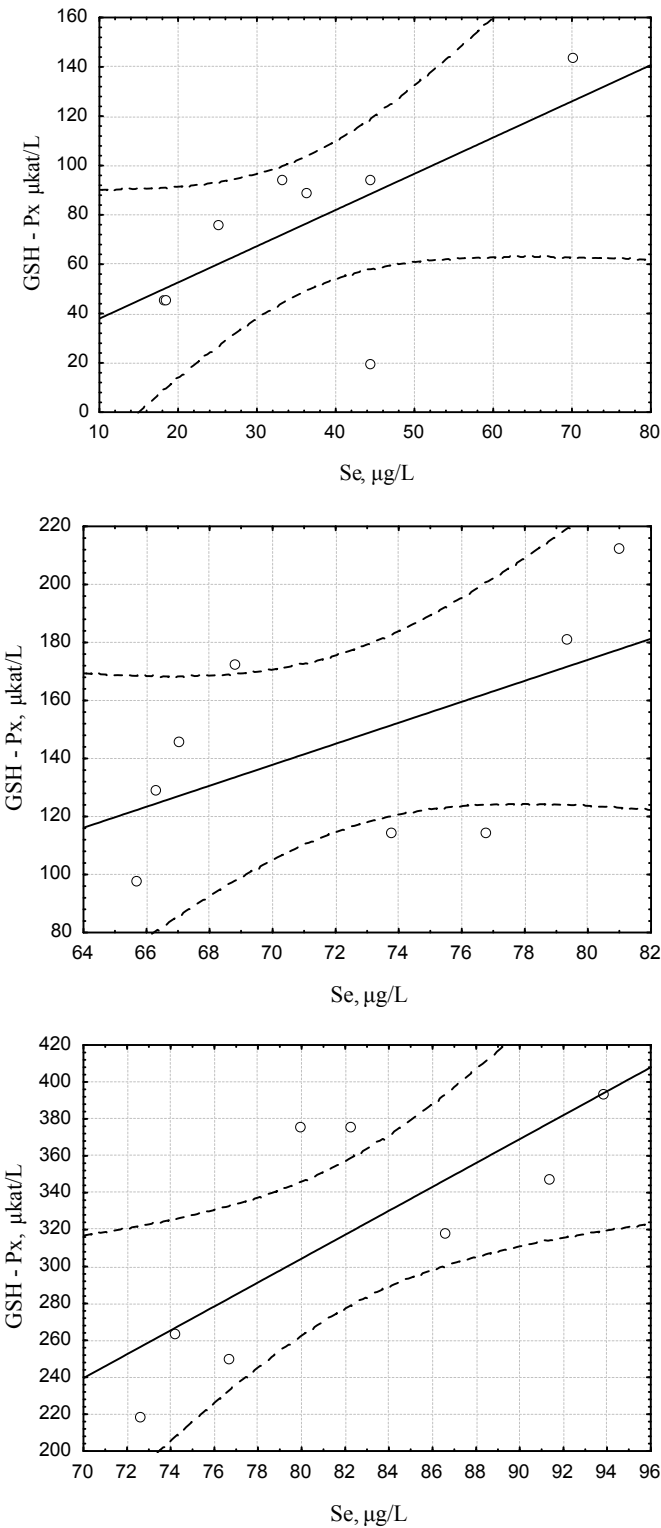


Figure 1, 2 and 3  
Regression curve for blood  
concentration of selenium  
and GSH-Px in goats from  
C group, E-I group and E-II  
group

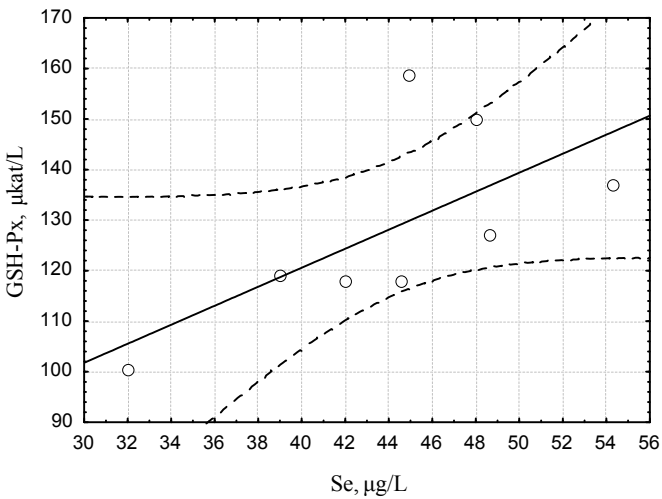
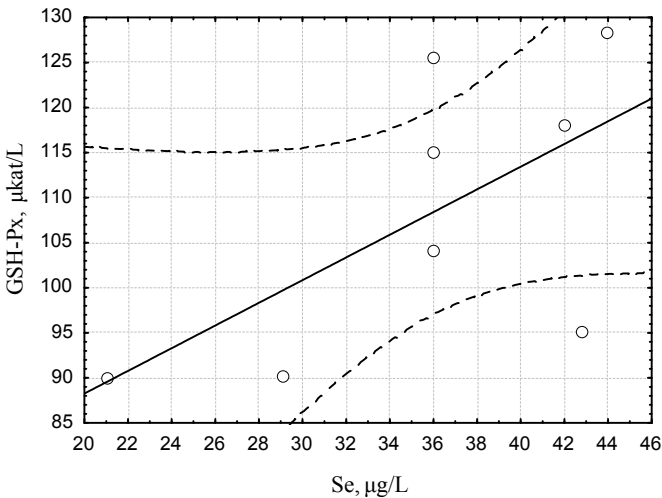
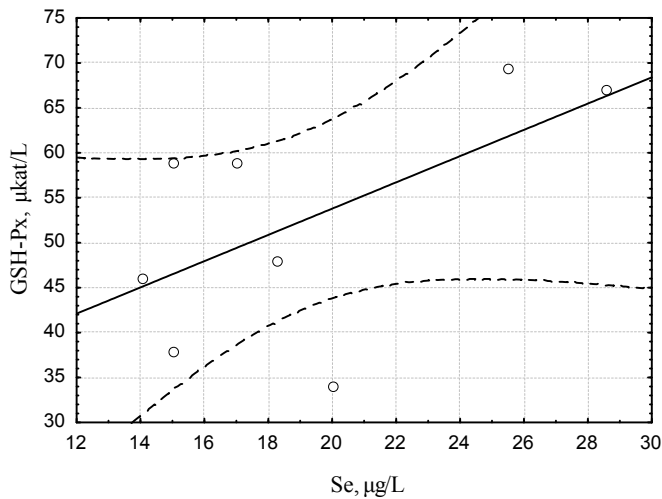


Figure 4, 5 and 6  
Regression curve for blood  
concentration of selenium  
and GSH-Px in kids from C  
group, E-I group and E-II  
group

In conclusion, the results obtained concentration of selenium and GSH-Px in the blood goats and their kids showed the validity of adding organic selenium into food in organic breeding.

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