



## RESEARCH ARTICLE

### Influence of Dietary Supplementation with Selenium on Blood Metabolic Profile and Thyroid Hormones Activities in Fattening Lambs

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#### ABSTRACT

The aim of the investigation was to determine influence of dietary supplementation with selenium on blood metabolic profile and thyroid hormones activities in fattening lambs. The study included 36 Merinolandschaf lambs during fattening period (50 days) divided into three groups (control, Exp-I and Exp-II). The control group diet was not supplemented with Se, whereas the diet of Exp-I was supplemented with 0.3 mg/kg inorganic selenium (sodium selenite) and Exp-II with 0.3 mg/kg organic selenium (Sel-Plex). Concentration of minerals (Ca, P-inorganic, K, Na, Fe and Cl), biochemical indicators (urea, glucose, cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, creatinine, total protein, albumin), enzyme activity (ALT, AST, GGT, LDH and GSH-Px) and thyroid hormone activities ( $T_3$ ,  $T_4$ ) were determined. Significantly higher ( $P < 0.01$ ) selenium content and GSH-Px activity as well as the lower content of Ca was determined in the blood of Exp-II and Exp-I groups compared to control group ( $93.89$  and  $67.69$ :  $34.11 \mu\text{g L}^{-1}$ ;  $607.98$  and  $556.92$ :  $247.52 \mu\text{kat L}^{-1}$ ;  $2.38$  and  $2.39$ :  $2.76 \text{ mmol L}^{-1}$ ). Significantly higher ( $P < 0.01$ ) selenium content in the in blood of lambs from Exp-II compared to Exp-I group was determined. Significantly higher ( $P < 0.05$ ) concentrations of HDL-cholesterol and  $T_3$  hormone activity were found in Exp-II group compared to the Exp-I and control groups. The results showed lower blood selenium content in control group and indicate the justification of adding selenium, especially organic selenium in lamb's diets, raising the concentration of Se, GSH-Px enzyme, HDL-cholesterol and  $T_3$  hormones improving the health status, especially in areas deficient in selenium.

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#### INTRODUCTION

Selenium is an essential trace element for promoting human and animal health (Saleh *et al.*, 2013; Khan *et al.*, 2013; Łukaszewicz *et al.*, 2013; Mashkoor *et al.*, 2013) whose dietary intake is not sufficient in many parts of the world. Forages in many areas of the world do not provide adequate dietary selenium for livestock. Selenium, a component of enzyme glutathione peroxidase, in combination with vitamin E serves as a biological antioxidant to maintain cellular integrity (Qureshi *et al.*, 2010). The concentration of selenium in animal organism

depends on its intake of food (Surai, 2006) therefore in some countries it is necessary to add a selenium supplement in the diet. In recent years, various sources of selenium that are used as supplements in the diets of sheep and lambs have been investigated (Antunović *et al.*, 2009; Pavlata *et al.*, 2011; Hall *et al.*, 2012). These are primarily sodium selenite and sodium selenate as inorganic sources of selenium, which are usually provided in mineral premixes or are injected. Organic selenium sources are selenomethionine and selenocysteine which are found in selenium yeast or in feeds grown on selenium rich soils (Stewart *et al.*, 2012). Previous research has

shown better bioavailability and utilization of organic selenium in the body (Pavlata *et al.*, 2011; Hall *et al.*, 2012). Very few studies were carried out with sheep and lambs which indicate the metabolic profile and thyroid hormone activity when feeding with different selenium supplementation diets. Previous studies have usually included the determination of only a small number of blood parameters involved in determining the effects of addition of selenium in the diets of sheep and lambs (Faixova *et al.*, 2007; Steen *et al.*, 2008). However, the addition of selenium in the diets of animals may cause certain metabolic changes that may affect their productivity and health status (Salman *et al.*, 2013). Therefore, more blood parameters in blood have to be incorporated in research with aim to get overall information about metabolic path and influence of selenium supplement in the lamb's diet. The aim of the investigation was to determine influence of dietary supplementation with selenium on blood metabolic profile and thyroid hormones activities in fattening lambs.

## MATERIALS AND METHODS

The study included 36 Merinolandschaf lambs during fattening period (50 days). Lambs were divided into three groups depending on dietary treatments with 12 lambs. Lambs were an average age of 60 days, healthy and in good condition. Lambs were fed with feed mixture (16% CP; 11.25 MJ NEL kg<sup>-1</sup>, 0.069 mg kg<sup>-1</sup> Se) and meadow hay (10% CP, 4.3 MJ NEL kg<sup>-1</sup>, 0.0041 mg kg<sup>-1</sup> Se) *ad libitum*. The control group diet was not supplemented with Se, whereas the diet of the experimental group I (Exp-I) was supplement with 0.3 mg kg<sup>-1</sup> of inorganic selenium (sodium selenite), and experimental group II (Exp-II) with 0.3 mg kg<sup>-1</sup> of organic selenium (Sel-Plex™, Alltech Inc.). In the beginning and the end of the experiment there were no significant differences in body weights of lambs depending on dietary treatments (control group: 18.51 and 33.66 kg; Exp-I group: 18.56 and 34.22 kg; Exp-II group: 18.60 and 34.43 kg).

The blood was collected on the 50<sup>th</sup> day of fattening from the jugular vein (10mL) into the vacuum tubes Venoject® (Venoject®, Sterile Terumo Europe, Leuven, Belgium) and with EDTA (ethylenediamine tetra-acetic acid) which were kept in a water bath at 37°C. Afterwards serum was isolated by a 10-minute centrifugation (3000 revolutions/min) and frozen at -20°C until assayed. Concentrations of minerals (Ca, P, K, Na, Fe and Cl), biochemical parameters (urea, glucose, cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, creatinine, total protein and albumin) and enzyme activity (ALT, AST, GGT and LDH) were determined with Olympus System Reagents (OSR), manufactured and distributed by Olympus Diagnostic GmbH (Irish Branch), Lismeehan, Ireland, manufactured for Olympus Diagnostic GmbH, Hamburg, using Olympus AU 600 apparatus. Serum for determination of selenium was diluted (1+4) with 0.2% solution of Triton X-100 (Merck). The concentration of total selenium in serum of lambs was determined by atomic absorption spectrometry, graphite technique (GFAAS) on the apparatus Analyst 800/Zeeman (Perkin-Elmer) with the addition of Pd and Mg (NO<sub>3</sub>)<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 (glutathione peroxidase) was determined in whole blood of lambs with commercial "Ransel" kit (Randox Laboratories Ltd, London, UK). Method for determination of GSH-Px is based on catalytic oxidation of glutathione by cumen hydroxide peroxide, and for reading is used spectrophotometer UV/VIS JENWAY 6305. Concentrations of total T<sub>3</sub> and T<sub>4</sub> in blood serum were determined by means of duplicate determinations using commercial kits for clinical use in humans (Abbott Laboratories, Abbot Park 60064 IL, USA) by Imx-Abbott immunoanalyzer (AIA; IMX System ser. No. 21482, 04660103). Methods for determination of T<sub>3</sub> and T<sub>4</sub> were Microparticle Enzyme Immunoassay (MEIA) and Fluorescence Polarization Immunoassay (FPAI). Sensitivity of the assay was less than 0.4 nmol L<sup>-1</sup> (T<sub>3</sub>) and 12.8 nmol L<sup>-1</sup> (T<sub>4</sub>). Mean recovery rates were 98.6%.

The results between groups were statistically evaluated by using ANOVA procedure (SAS 9.3). Differences were considered as significant at the level of 0.05 or less. The means and their standard deviation and standard errors were also calculated.

## RESULTS

At the end of the experiment there were non-significant differences in body weights of fattening lambs depending on dietary treatments with selenium. Daily gains of lambs were 303, 313 and 317g in control, Exp-I and Exp-II group, respectively.

Influence of dietary supplementation with selenium on blood content of minerals and biochemical parameters in lambs (Table 1 and 2) showed no significant variation compared to physiological values, expect higher concentration of P-inorganic, urea and triglycerides as well as slightly lower Ca and cholesterol concentration in experimental groups. In the lamb blood of the experimental groups (Exp-I and Exp-II) fed with feed mixture supplementation with selenium (inorganic and organic sources) significantly higher (P<0.01) Se content and lower Ca content was found compared to control group. Results from Exp-II group presented significantly (P<0.01) higher content of Se compared to Exp-I group.

Exp-II group of lambs showed significantly higher concentrations of HDL-cholesterol compared to control and Exp-I groups. A similar trend, but no significant difference (P>0.05) was found for concentrations of triglycerides and total cholesterol. The higher concentration of urea and triglycerides and lower cholesterol concentration in blood of lambs showed moderate effect of dietary supplementation with selenium on the metabolism of fats. The Exp-II group of lambs fed with the addition of organic selenium showed significantly higher concentration of HDL-cholesterol compared to lambs of control and Exp-I groups in blood serum of lambs. A similar trend, but no significant difference was found for concentrations of triglycerides and total cholesterol.

Enzyme activity in the blood of lambs did not significantly differ depending on the dietary supplementation with selenium, except for GSH-Px that

**Table 1:** Serum values (mean±SD) of mineral content in lamb

Minerals	Groups			Reference values [Lepherd et al., 2009]
	Control	Exp-I	Exp-II	
Ca (mmol L <sup>-1</sup> )	2.76±0.54 <sup>A</sup>	2.39±0.66 <sup>B</sup>	2.38±0.68 <sup>B</sup>	2.42-2.92
P (mmol L <sup>-1</sup> )	4.04±0.25	3.77±0.69	4.12±0.40	1.88-3.34
K (mmol L <sup>-1</sup> )	4.54±1.43	4.49±0.91	4.90±1.25	4.40-5.90
Na (mmol L <sup>-1</sup> )	133.61±37.45	135.81±28.94	142.37±33.05	142.00-152.00
Cl (mmol L <sup>-1</sup> )	103.00±17.26	100.56±17.30	103.74±21.37	95.00-103.00
Fe (µmol L <sup>-1</sup> )	35.19±17.91	29.92±17.78	28.82±14.44	29.70-39.70 <sup>1</sup>
Se (µg L <sup>-1</sup> )	34.11±16.34 <sup>A</sup>	67.69±20.23 <sup>B</sup>	93.89±15.07 <sup>C</sup>	> 30 <sup>2</sup>

A, B, C-(P<0.01), <sup>1</sup>Kaneko et al. (2008), <sup>2</sup>Puls (1994)-critical levels. In Exp-I and Exp-II diet supplemented with inorganic and organic selenium, respectively.

**Table 2:** Serum (mean±SD) of biochemical indicators of lambs

Indicators	Groups			Reference values <sup>*</sup>
	Control	Exp-I	Exp-II	
Glucose (mmol L <sup>-1</sup> )	4.81±0.63	4.42±0.99	4.58±0.85	2.70-4.80
Urea (mmol L <sup>-1</sup> )	9.56±1.71	8.81±1.79	9.42±1.45	5.00-9.10
Kreatinin (mmol L <sup>-1</sup> )	62.25±6.98	60.80±7.26	64.00±7.52	35.00-64.00
Total proteins (g L <sup>-1</sup> )	59.73±14.03	53.97±14.96	53.43±16.59	51.00-64.00
Albumine (g L <sup>-1</sup> )	32.60±1.22	31.96±0.76	33.46±1.59	30.00-37.00
Triglyceride (mmol L <sup>-1</sup> )	0.28±0.08	0.26±0.15	0.30±0.16	0.0-0.2 <sup>1</sup>
Cholesterol (mmol L <sup>-1</sup> )	1.28±0.03	1.21±0.40	1.30±0.48	1.35-1.97 <sup>1</sup>
HDL-cholesterol (mmol L <sup>-1</sup> )	0.65±0.16 <sup>a</sup>	0.67±0.13 <sup>a</sup>	0.75±0.13 <sup>b</sup>	-
LDL-cholesterol (mmol L <sup>-1</sup> )	0.48±0.25	0.45±0.55	0.48±0.25	-

<sup>\*</sup>Lepherd et al. (2009); a, b-(P<0.05), <sup>1</sup>Kaneko et al. (2008). In Exp-I and Exp-II diet supplemented with inorganic and organic selenium, respectively.

ranged according to reference values (Table 3). In control group lambs had the highest activity of the most of examined enzymes, which was on the upper limit of physiological values. The opposite trend was observed only for the GSH-Px, that showed significantly higher activity (P<0.01) in the lambs blood from experimental groups (Exp-I and Exp-II) compared to the control group. Influence of different dietary supplementation with selenium on blood activities of thyroid hormones in lambs (Table 4) showed no significant variation compared to physiological values. Activity of thyroid hormones was different in the blood of lambs from experimental groups fed different sources of selenium. In fact, there was a significantly higher (P<0.05) activity of T<sub>3</sub> in the blood of Exp-II group fed with addition of organic compared to Exp-II and control groups of lambs.

## DISCUSSION

At the end of the experiment there were non-significant differences in body weights of fattening lambs depending on dietary treatments with selenium. The reason for that could be due to fact that lambs in control group had only lack of Se in blood, and not deficient of selenium, resulting in similar body weight. Similar results were obtained by Johansson *et al.* (1990) and Dominguez-Vara *et al.* (2009).

Dietary supplementation with selenium showed no significant variation in blood content of minerals and biochemical indicators in lambs compared to physiological values (Kaneko *et al.*, 2008; Lepherd *et al.*, 2009). Higher concentration of P-inorganic, urea and triglycerides in all groups as well as slightly lower Ca and cholesterol concentrations in experimental groups were determined. Higher P concentration in lambs' blood serum may be associated with age. It is known that lambs have a higher P concentration in the blood in comparison with adult sheep, because it decreases as age of lambs progress (Antunović *et al.*, 2005). The determined higher levels of selenium in the lambs' blood of experimental

groups fed with selenium supplementation indicate the need for addition of selenium in the diets because in control group it was on the lower limit according to reference values. Above mentioned indicates that the level of selenium in the body depends on its content in the diets observed by Cristaldi *et al.* (2005). According to previous studies (Pavlata *et al.*, 2011; Hall *et al.*, 2012) it is more efficient to utilize organic sources of selenium compared to inorganic, as demonstrated in the present study as well. In generally, organic selenium is absorbed and retained more readily by ruminants than inorganic selenium (Qin *et al.*, 2007). Similar results carried out in lambs found Faixova *et al.* (2007). The concentration of Ca in the trial with selenite was determined with selenite injected to sheep intramuscular by Malecki *et al.* (2002) that agrees with results from the present study. They pointed out that this concentration is probably a consequence of increased pH in the rumen of sheep due to increased activity of protozoa and cellulolytic bacteria aimed to increasing the intensity of fiber and protein degradation in the rumen, which can lead to an increase of pH value.

In the blood of Exp-II group of lambs fed with the addition of organic selenium, significantly higher concentration of HDL-cholesterol was observed compared to lambs of control and Exp-I group. Kumar *et al.* (2008) did not find significant differences in total cholesterol, total protein, albumin and globulin when adding sodium selenite in the lambs' diet compared to group fed without the addition of selenium. In growing lambs fed with supplementation of Se, Zn and vitamin E caused increasing of HDL fraction in blood plasma (Gabryszuk *et al.*, 2007). Shinde *et al.* (2009) also found significantly higher content of HDL-cholesterol in buffalo calves fed with the addition of selenium (sodium selenite) compared to those without added selenium. Authors reported that this is probably the reason of Se essentiality maintaining the integrity of the pancreas, therefore effective digestion and absorption of fats.

Lambs of control group had the highest activity of the most examined enzyme, which was on the upper limit of

**Table 3:** Serum enzymes (mean±SD) activities in lambs

Enzymes	Groups			Reference values*
	Control	Exp-I	Exp-II	
AST (U L <sup>-1</sup> )	143.77±58.60	105.83±36.65	138.08±55.87	83.00-140.00
ALT (U L <sup>-1</sup> )	20.69±9.23	15.58±6.72	12.58±7.82	6.00-20.00 <sup>1</sup>
GGT (U L <sup>-1</sup> )	71.90±19.55	73.33±17.46	73.61±26.87	56.00-110.00
ALP (U L <sup>-1</sup> )	487.40±122.76	432.33±125.94	465.25±198.97	184-627 <sup>2</sup>
LDH (U L <sup>-1</sup> )	435.25±110.11	363.80±176.59	415.80±113.18	238.00-440.00 <sup>1</sup>
GSH-Px (μkat L <sup>-1</sup> )	247.52±34.10 <sup>A</sup>	556.92±70.68 <sup>B</sup>	607.98±107.24 <sup>B</sup>	> 600 <sup>3</sup>

\*<sup>1</sup>Lepherd et al. (2009); A, B-(P<0.01), <sup>1</sup>Kaneko et al. (2008), <sup>2</sup>Borjesson et al. (2000), <sup>3</sup>Pavlata et al. (2012). In Exp-I and Exp-II diet supplemented with inorganic and organic selenium, respectively.

**Table 4:** Thyroid hormones (mean±SD) activities in blood of lambs

Hormones	Groups			Reference values*
	Control	Exp-I	Exp-II	
T <sub>3</sub> (nmol L <sup>-1</sup> )	1.48±0.16 <sup>a</sup>	1.33±0.19 <sup>a</sup>	1.68±0.33 <sup>b</sup>	1.25-2.00
T <sub>4</sub> (nmol L <sup>-1</sup> )	102.3±16.0	93.8±10.3	101.3±11.2	63.95-103.1

a,b-(P<0.05); \*Antunović et al. (2010). In Exp-I and Exp-II diet supplemented with inorganic and organic selenium, respectively.

physiological values. The opposite trend was observed for GSH-Px. Significantly higher GSH-Px activity (P<0.01) was observed in lambs from experimental groups (Exp-I and Exp-II) compared to the control group. Above mentioned indicates the lack of selenium in diet of lambs from control group. According to White and Rewell (2007) upper limit of physiological values for AST, ALT, LDH is 150 U/L, 20 U/L, and 440 U/L, respectively (Kaneko *et al.*, 2008). Reference values for GSH-Px activity in sheep blood was greater than 600 μkat L<sup>-1</sup> of whole blood (Pavlata *et al.*, 2012). Determined lower GSH-Px activity, in relation to physiological values (Pavlata *et al.*, 2012.), may be due to influence of age considering that the reference values were observed in adult sheep. Antunović *et al.* (2009) carried out trial with lambs fed different dietary supplementation of selenium (inorganic and organic sources) resulting in significantly higher activity of the blood GSH-Px enzyme, and lower activities of ALT and LDH compared to the control group without addition of selenium. In these researches a similar trend of mentioned enzymes activity was found but differences among groups were not significant. The present study approved significantly higher activity of GSH-Px in lambs from experimental groups in comparison with control group that is in agreement with results observed by Quin *et al.* (2007), Faixova *et al.* (2007), Vignola *et al.* (2007), Juniper *et al.* (2008), and in sheep by Panev *et al.* (2013).

Influence of dietary supplementation with selenium on the blood activity of thyroid hormones in lambs of the present study showed no significant variation compared to physiological values observed by Antunović *et al.* (2010). Activity of thyroid hormones was different in lambs' blood from experimental groups fed with different sources of selenium. There was a significantly higher activity of T<sub>3</sub> in the blood Exp-II group of lambs compared to Exp-I and control group of lambs. Similar changes in thyroid hormone activity with addition of inorganic and organic sources of selenium in diets of lambs from Dubrovnik sheep was determined by Antunović *et al.* (2009), although differences were not significant. Likewise, the study by Shinde *et al.* (2009) showed significantly higher activity of T<sub>3</sub> hormone in the blood of buffalo calves that were fed with the addition of selenium (sodium selenite), compared to those without added selenium. Explanation

may be due to fact that type I iodothyronine-5'-deiodinase is a Se dependent enzyme, which is responsible for the deiodination of T<sub>4</sub> to T<sub>3</sub>. Similar observations of thyroid hormone activity in lambs fed with the addition of selenium observed Bik *et al.* (1998).

**Conclusion:** Supplementation of Se in lambs' diets could be an efficient solution for raising the concentration of Se in lambs' blood consequently raising the GSH-Px, HDL-cholesterol and T<sub>3</sub> hormones, with small decline of Ca. The results indicate a lack of selenium in the blood of lambs by control group and the justification of adding selenium, especially organic selenium, in lambs' diets in areas deficient in selenium.

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