# Productive traits, selenium status and blood metabolic status in fattening lambs affected by selenium biofortified corn

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## Abstract

The widespread selenium (Se) deficiency in soil and in feedstuffs from such soil, needs to be prevented by various methods. Recently, biofortification of Se has been carried out by soil fertilization and foliar application of crops. The aim of this study was evaluate the productive traits, Se status and blood metabolic profile in fattening lambs affected by Se biofortified corn. The research was conducted with 20 Merinolandschaf lambs of the average age of 70 days, during 30 days of fattening. The feed mixture of the control group contained corn (0.014 mg Se/kg dry matter, DM); in the experimental group (Se-BC), corn was biofortified (0.278 mg Se/kg DM). The feed mixture, hay and water were offered to lambs ad libitum. Blood was collected from the jugular vein on days 0 and 30 of experiment. Haematological indices were determined in whole blood, whereas concentrations of biochemical indices, enzyme activities, and concentrations of metabolic hormones were determined in serum. After the slaughter, samples of musculus semimembranosus, liver, kidney, lungs, spleen, peritoneum and heart tissues were taken. No significant differences were obtained in productive traits when feeding lambs with Se-BC. Higher concentrations of Se in lungs and liver (control: 0.26 and 0.75 mg/kg, respectively; Se-BC: 0.33 and 0.92 mg/kg, respectively) was determined in Se-BC compared to control. Higher activity of glutathione peroxidase enzyme in serum, higher erythrocyte count, higher content of haemoglobin and haematocrit in whole blood of Se-BC lambs were determined. The results of the study indicate the posibility of using Se-BC in lambs' diets.

### Biofortification, lambs, blood parameters, production, selenium

Selenium (Se) is an essential trace element for animals and humans, and has multiple biological activities. Thus, low dietary intake of Se can cause health disorders in humans and animals (Gianidis et al. 2016). In livestock, Se deficiency is also responsible for the white muscle disease, with clinical signs that include lesions in skeletal and/or hearth muscle (Malagoli et al. 2015). It is well known that Se is a major component of a number of functional selenoproteins such as glutathione peroxidase (GPx), iodothyronine deiodinase and thioredoxin reductase, which protect the cell membrane from lipid peroxidation.

The lack of Se in food can be eliminated by its dietary supplementation, by enriching food with Se (fortification) and increasing Se concentrations in feedstuffs (biofortification) (Stein 2010; Novoselec et al. 2018). Agronomic biofortification of food crops, especially staples such as cereals, which are consumed widely, may be an effective component of a food system strategy to reduce Se malnutrition (Lyons 2018). In Se deficient areas, several means of selective supplementation are available, e.g. organic Se-yeast can be added to the feed, or inorganic sodium selenite can be added to mineral mixes for free choice consumption (Hall et al. 2017). Biofortification refers to the application of mineral fertilizers to desired crops, combined with grains with increased ability to adopt microelements as an approach not only to increase the concentration of microelements in edible crops but also to

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improve the yield on soils where microelements or Se are exhausted or unavailable (Borg et al. 2009). Plants assimilate Se as selenate, selenite or organic Se compounds such as selenocysteine (SeCys) and selenomethionine (SeMet) but cannot assimilate elemental Se or metal selenides (White et al. 2004). The selenates are transported through the membrane of the roots by high-affinity sulphate transporter and Se by phosphate transporters (Li et al. 2008). Selenite is soon converted to organic Se compounds, while Se is transferred to xylenes and assimilated into organic Se compounds and distributed within the plant. In recent years, research has been carried out on biofortification, or enrichment of forage with Se (pasture and hay) and their effect on animal productivity and animal health. Besides. Hall et al. (2009) investigated the Se status in sheep grazing the pastures fertilised with Se in areas with normally low forage Se concentrations which resulted in significantly higher concentrations of Se in whole blood of sheep. Hall et al. (2013) reported a significant linear increase in the concentration of blood Se and body weight of weaned calves fed with bio-fortified alfalfa hay during 7 weeks. Feeding cows in lactation with Se-enriched silage showed greater bioavailability of Se in milk and blood as well as similar production traits compared to cows fed with silage containing addition of Se-yeast as well as to cows fed with addition of inorganic Se-silage (Séboussi et al. 2016). Mehdi et al. (2015) found a significant increase in the concentration of Se in the muscles, lungs, kidneys, liver and plasma of young beef bulls fed cereals enriched with Se (barley and spelt). Monitoring of the metabolic profile by determining the concentration of biochemical and haematological indices in the blood of small ruminants gives us a clearer picture of their nutritional and health status even before the changes are visible in the animal (Antunović et al. 2017). Considering that Southeastern Europe is a deficient in Se concentrations of soil (Pešut et al. 2004; Antunović et al. 2010a; Valčić et al. 2013; Ademi et al. 2015), biofortification could be a good solution to overcome Se deficiency in the animal-human chain. Thus, the aim of this study was to research whether the addition of Se biofortified corn in diets influences the production traits. Se status and metabolic profile of fattening lambs.

## **Materials and Methods**

Bioethics Committee for Research on Animals of the Faculty of Agrobiotechnical Sciences Osijek established that the research was carried out under the legal provisions of Animal Protection Act (NN 133/06, NN 37/13, and NN 125/13).

### Animals and feeding

The research was conducted on 20 Merinolandschaf lambs after weaning, of the average age of 70 days, during 30 days of fattening. The lambs which were in good health and evenly represented in terms of sex (50%  $\Im$ : 50%  $\Im$ ) were divided in two groups. The feed mixture of the control group contained 23% corn (0.014 mg Se/kg dry matter, DM), while 23% biofortified corn (0.278 mg Se/kg DM) was used in the experimental group (Se-BC). Feed mixture, hay (0.0306 mg/kg Se) and water were offered to lambs *ad libitum*. Foliar application of Se (Na-selenate, 10 g Se/ha) occurred on corn hybrids Bergxxon (RWA, FAO group 400). Ingredients of feed mixtures and chemical composition of feed mixtures are presented in Table 1.

## Feed analyses

Feed composition was determined with standard methods (AOAC 2006). The concentrations of minerals in solutions of digested feed samples were determined by inductively coupled plasma (ICP; Optima 2100 DV; PerkinElmer, Massachusetts, USA). All feed mixture samples were dried and ground into a fine powder using a heavy metal-free ultracentrifugal mill (ZM 200, Retsch GmbH, Haan, Germany) or knife mill (GM 200). All samples were digested with 10 ml of a 5:1 mixture of HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> at 180 °C for 60 min in a microwave oven (Mars 6; CEM, North Carolina, USA). Each batch of samples run on the ICP was analysed with an internal pooled plasma control and with the reference material prepared in the same way as other plant samples. All samples were analysed in duplicate.

## Blood analyses

Blood was collected from the jugular vein (10 ml) on days 0 and 30 of experiment into serum vacutainer tubes and vacutainer tubes containing ethylenediamine tetra-acetic acid (EDTA) after morning feeding. Determination

Table 1. Composition of feed mixture.

Ingredients	Feed mixture (%)
Corn	23
Barley	38
Wheat bran	20
Soybean meal	4
Extruded soybean	14
Mineral-vitamin premix*	1
Se content in corn (control group)	0.014 mg/kg
Se content in BC (experimental group)	0.278 mg/kg
Chemical composition (%):	
Dry matter	90.21
Crude proteins	15.62
Ether extract	5.01
Crude fibres	5.86
Ash	6.15
NEL, MJ ME/kg	7.7
Total Se content, mg/kg of feed mixture (control group)	0.162
Total Se content in BC, mg/kg of feed mixture (experimental group)	0.221

BC - biofortified corn; NEL - net energy for lactation

\*1 kg of mineral-vitamin premix contained: 1 000 000 I.U. vitamin A; 150 000 I.U. vitamin D<sub>3</sub>; 1 500 mg vitamin E; 50 mg vitamin B<sub>4</sub>; 100 mg vitamin B<sub>1</sub>; 200 mg vitamin B<sub>2</sub>; 200 mg vitamin B<sub>6</sub>; 1 mg vitamin B<sub>12</sub>; 500 mg Ca panthotenate; 1000 mg niacin; 20 000 mg choline chloride; 4000 mg FeSO<sub>4</sub>; 800 mg CuSO<sub>4</sub>; 3 500 mg Mn oxide; 5 000 Zn sulphate; 20 mg cobalt chloride; 10 000 mg Mg sulphate; 80 mg potassium iodide.

of haematological indices including the number of leukocytes (WBC), erythrocytes (RBC) and thrombocytes (PLT), as well as the content of haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), the mean haemoglobin content in erythrocytes (MCH) and the mean haemoglobin concentration in erythrocytes (MCHC) in whole blood of lambs was carried out on an automatic 3-part differential haematology analyzer Sysmex PocH-100Iv (Sysmex Europe GmbH, Hamburg, Germany).

Blood serum was separated by centrifugation (10 min) at 1,600 g. Following indicators were determined in serum: mineral concentrations (Ca, inorganic P, Mg, Fe), biochemical indices (urea, glucose, cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, total protein, albumin, globulin,  $\beta$ -hydroxybutyrate [BHBA]) and enzyme activity (alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP], creatine kinase [CK] and  $\gamma$ -glutamyl transferase [GGT]). The concentration of BHBA was measured with the colorimetric method (RANBUT, Randox Laboratories Ltd., Crumlin, UK). All these indices were analyzed with Olympus AU400 (Olympus, Tokyo, Japan). Concentration of very low density lipoproteins (VLDL) was calculated with triglycerides/5.

The activity of glutathione peroxidase (GPx) in serum was determined by Ransel<sup>®</sup> kits (Randox Laboratories Ltd., Crumlin, UK) and the activity of total superoxide dismutase (SOD) with RANSOD<sup>®</sup> kits (Randox Laboratories Ltd., Crumlin, UK) on an automatic analyzer Olympus AU 400 (Olympus, Tokyo, Japan). In serum, concentrations of total triiodothyronine (T<sub>4</sub>), thyroxine (T<sub>4</sub>), and leptin concentrations were determined using commercial kit enzyme immunoassay (DE4568, DE4569, and DEE007, respectively), whereas the concentration of IGF-I was determined using commercial kit enzyme immunoassay for the quantitive determination of the human insulin-like growth factor (IGF-I) (DEE007) according to the ELISA procedure.

#### Carcass traits and tissue analyses

After slaughtering and exsanguination of 6 lambs per group, samples of the semimembranosus muscle (MS), liver, kidney, lungs, spleen, peritoneum and heart tissues were taken. Values of carcass colour were determined in MS 45 min after slaughtering. Values of pH1 were taken 45 min *post mortem* and pH2 24 h after slaughter and cooling. The pH was measured with a handheld contact pH meter (Mettler Toledo, Greifensee, Switzerland) with a piercing type electrode. Values of colour were measured according to the CIE L\* a\* b\* system (CIE 1976). Afterwords, the dressing percentage was calculated (live body weight - weight of carcass/100). The weighing and noting the body condition score of lambs was carried out according to Russel (1991) at every sampling. After that, daily gains of lambs were calculated. The concentrations of Se in solutions of digested samples of fresh animal tissues were determined with ICP.

## Statistical analyses

The mean values were obtained by the MEANS procedure of SAS  $9.4^{\text{®}}$ . Analysis of variance was performed with the ANOVA procedure, and the differences between groups were processed with the Tukey test and declared at the level P < 0.05 using the model:

 $Y_{ii} = \mu + D_i + e_{ii}$ 

where:  $Y_{ii}$  = dependent observation;  $\mu$  = overall mean;  $D_i$  = effect of diet treatment;  $e_{ii}$  = residual.

# Results

Feeding lambs with mixtures containing biofortified corn did not influence the production traits such as body weight, daily gain, or body condition score (Table 2) compared to the control. Similarly, the carcass traits of lambs, pH values and colour parameters of MS were not influenced by Se-BC (Table 3).

Table 2. Productive traits of lambs fed with feed mixtures containing biofortified corn.

Index	Day of measuring	g Group		SEM	Р
		Control	Se-BC		
Body weight (kg)	0	22.83	23.23	0.40	0.64
	30	29.80	30.98	0.79	0.47
Daily gain (g)	0-30	232.40	258.33	29.58	0.67
Body condition score	0	3.53	3.58	0.03	0.44
	30	3.58	3.76	0.06	0.17

SEM - standard error means; Se-BC - feed mixture containing Se biofortified corn.

Table 3. Carcass traits, colour indices, and pH values of musculus semimebranosus of lambs fed with mixtures containing biofortified corn.

Carcass traits	Group		SEM	Р
	Control	Se-BC		
Slaughter weight (kg)	30.05	29.58	1.12	0.85
Hot carcass weight (kg)	14.70	14.29	0.46	0.69
Dressing (%)	48.90	48.53	0.65	0.80
Forestomach and intestines (kg)	8.15	8.16	0.46	0.99
Organ weights* (kg)	1.30	1.33	0.04	0.76
Skin and lower legs (kg)	3.85	3.44	0.17	0.27
Colour indices and pH values				
L	36.88	36.97	0.15	0.79
a	15.48	15.27	0.14	0.48
b	1.14	1.11	0.18	0.66
pH <sub>1</sub>	6.79	6.81	0.03	0.79
pH <sub>2</sub>	5.63	5.62	0.02	0.60

SEM - standard error means; Se-BC - feed mixture containing Se biofortified corn;pH1 - 45 min *post mortem*, pH2 - 24 h *post mortem*; \*weight of lungs, heart, liver and spleen.

Higher concentrations of Se in lungs and liver by 26.92% and 25.12%, respectively, were determined in Se-BC lambs compared to control (Table 4). A tendency of increase (P = 0.05) in Se concentration was determined in spleen, with 18% of change, compared to control. While, concentrations of Se in MS, kidney, heart and peritoneum of lambs fed with

Tissue	Group		Change (%)	SEM	Р
	Control	Se-BC			
MS	0.13	0.13	-	0.02	0.99
Kidney	1.07	1.19	+11.21	0.06	0.15
Liver	0.75	0.92	+25.12	0.03	0.04
Heart	0.23	0.24	+4.35	0.01	0.59
Spleen	0.33	0.39	+18.18	0.01	0.05
Peritoneum	0.03	0.04	+33.33	0.01	0.55
Lungs	0.26	0.33	+26.92	0.01	< 0.001

Table 4. Concentrations of Se (mg/kg fresh matter) in different tissues of lambs fed with mixtures containing biofortified corn.

MS - musculus semimembranosus; SEM - standard error of mean; Se-BC - feed mixture containing Se biofortified corn

Se-BC did not differ compared to the control. By analyzing the haematological indices (Table 5), higher RBC, HGB, and HCT contents were determined in whole blood of Se-BC lambs. The activity of GPx in the serum of the experimental group at the end of the study was higher by 36.47% compared to the control (Fig. 1).

Indices	Time of measuring	Group		SEM	P
		Control	Se-BC		
WBC (×10 <sup>9</sup> /l)	0	10.94	11.11	0.62	0.89
	30	7.59	7.45	0.30	0.82
RBC (×10 <sup>12</sup> /l)	0	10.46	9.86	0.49	0.55
	30	8.15	9.57	0.34	0.04
HGB (g/l)	0	121.50	109.90	5.17	0.27
	30	92.60	105.90	3.21	0.03
HCT (g/l)	0	0.39	0.38	0.02	0.84
	30	0.34	0.38	0.01	0.03
MCV (fl)	0	38.43	40.91	0.86	0.15
	30	41.95	39.89	0.91	0.27
MCH (pg)	0	11.75	11.27	0.30	0.44
	30	11.46	11.11	0.19	0.36
MCHC (g/l)	0	308.70	274.70	11.47	0.14
	30	274.70	280.20	5.85	0.65
PLT (×10 <sup>9</sup> /l)	0	557.40	535.80	41.65	0.80
	30	657.20	637.50	53.88	0.86

Table 5. Haematological indices of lambs fed with mixtures containing biofortified corn.

SEM - standard error of mean; Se-BC - feed mixture containing Se biofortified corn; WBC - number of leukocytes; RBC - erythrocytes; HGB - contents of haemoglobin; HCT - haematocrit; MCV - mean corpuscular volume; MCH - average haemoglobin content in erythrocytes; MCHC - mean haemoglobin concentration in erythrocytes; PLT - platelet count

Activity of SOD was not affected when lambs were fed with Se-BC, compared to control (Fig. 2). Feeding lambs with Se-BC did not influence concentrations of Ca, P-inorganic, Mg, Fe and Se in serum, while the rest of biochemical indices in serum did not differ in Se-BC lambs (Table 6). Concentrations of leptin, IGF-I,  $T_3$  and  $T_4$  in lambs' serum were not influenced by Se-BC (Table 7).

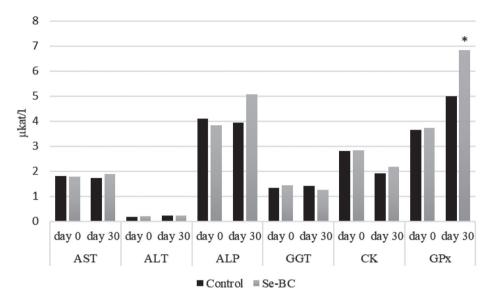


Fig. 1. Enzyme activities in serum of lambs fed with mixtures containing biofortified corn (Se-BC)

\*P < 0.05; AST - aspartate aminotransferase; ALT - alanine aminotransferase; ALP - alkaline phosphatase; GGT -  $\gamma$ -glutamyl transferase; CK - creatine kinase; GPx - glutathione peroxidase

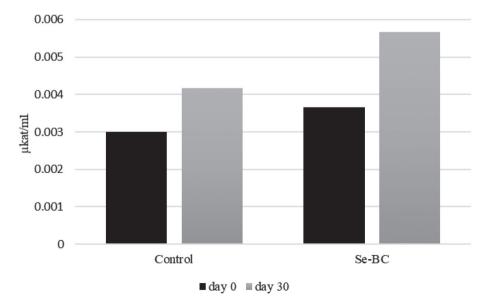


Fig. 2. Superoxide dismutase activity in serum of lambs fed with mixtures containing biofortified corn (Se-BC)

Indices T	Time of measuring	Group		SEM	Р
	-	Control	Se-BC		
Ca (mmol/l)	0	2.61	2.83	0.07	0.06
	30	2.58	2.65	0.04	0.40
P-anorganic (mmol/l)	0	2.78	3.10	0.12	0.17
	30	2.72	2.87	0.05	0.47
Mg (mmol/l)	0	1.05	1.00	0.03	0.46
	30	1.25	1.19	0.06	0.63
Fe (µmol/l)	0	32.20	37.20	2.36	0.30
	30	27.38	33.57	1.88	0.10
Se (mg/l)	0	0.06	0.05	0.004	0.49
	30	0.12	0.13	0.003	0.52
Glucose (mmol/l)	0	3.81	3.94	0.10	0.54
	30	4.92	4.57	0.12	0.15
Urea (mmol/l)	0	3.47	5.03	0.45	0.08
	30	6.26	6.69	0.27	0.44
Cholesterol (mmol/l)	0	2.38	2.35	0.19	0.94
	30	1.37	1.40	0.08	0.90
HDL- cholesterol (mmol/	l) 0	1.19	1.15	0.07	0.76
	30	0.69	0.79	0.04	0.22
LDL-cholesterol (mmol/l)	0	1.04	1.09	0.12	0.85
	30	0.57	0.50	0.05	0.53
VLDL (mmol/l)	0	0.06	0.05	0.01	0.91
	30	0.05	0.05	0.01	0.48
Triglycerides (mmol/l)	0	0.32	0.24	0.03	0.14
	30	0.25	0.23	0.02	0.49
Total proteins (g/l)	0	61.51	66.38	1.47	0.06
	30	68.85	65.35	1.09	0.11
Albumins (g/l)	0	30.30	32.68	0.69	0.06
	30	30.24	31.27	0.42	0.23
BHB (mmol/l)	0	0.31	0.33	0.03	0.79
	30	0.32	0.36	0.02	0.33

Table 6. Biochemical indices in serum of lambs fed with mixtures containing biofortified corn.

SEM - standard error of the mean; Se-BC - feed mixture containing Se biofortified corn; HDL - high-density lipoprotein; LDL - low-density lipoprotein; VLDL - very low-density lipoprotein; BHB - beta-hydroxybutyrate

Table 7. Concentrations of metabolic hormones in serum of lambs fed with mixtures containing biofortified corn.

Tissue	Gr	SEM	Р	
	Control	Se-BC		
Leptin (ng/ml)	2.85	1.51	0.64	0.22
IGF-I (ng/ml)	341.33	265.65	54.43	0.52
T3 (ng/ml)	1.39	1.57	0.10	0.38
T4 (nmol/l)	80.47	81.20	5.95	0.96

SEM - standard error of mean; Se-BC - feed mixture containing Se biofortified corn; IGF-I - insulin-like growth factor;  $T_3$  - triiodothyronin;  $T_4$  - thyroxin

# Discussion

Feeding lambs with Se biofortified corn did not lead to any change in production traits, likely due to feeding with feed mixtures which were isoproteic, isolipidic and isoenergetic. Many studies determined an increased Se concentration in the liver and muscle with supplementation of Se in diets of lambs reared in a Se-deficient area (Qin et al. 2007; Antunović et al. 2009). Qin et al. (2007) indicated that Se concentrations were the highest in the kidneys, intermediate in the liver, and the lowest in the muscle of lambs. Similar range was determined in the present study, with the highest concentrations in the liver, and the lowest in MS of lambs, with increased deposition in liver and lungs in Se-BC lambs. Ranches et al. (2017) determined higher Se concentrations in the liver of calves fed biofortified hay (7.7 mg/kg DM of Se) compared to control (0.1 mg Se/kg DM), during 21 and 42 days. Also, in their study liver Se was much higher in lambs fed Se-enriched hay compared to lambs receiving sodium selenite in grain-based supplement.

Hall et al. (2013) concluded that in areas with low Se in forage, feeding beef calves with Se-enriched alfalfa hay during the weaning, improved growth and survival in the feedlot. Mehdi et al. (2015) determined increase of Se concentration in muscles, lungs, kidneys, liver and blood plasma of weaned calves fed diets containing 173  $\mu$ g/kg DM of Se in Se-enriched spelt and barley compared to control diet (58  $\mu$ g Se/kg DM). Ranches et al. (2017) determined higher Se concentrations in plasma of weaned calves fed Se-biofortified hay, compared to control (49.6 vs. 15.7 ng/ml). In the present study, the reason for the absence of significant increase of Se in muscle and serum of lambs was probably due to the fact that it was a short term study, and Se was determined in serum, not in whole blood. Weaned lambs in the first days consume less feed which can also be a limiting factor in Se storage in meat and blood. The above-mentioned suggests that lambs at the beginning of the study were Se-deficient because adequate levels of Se in blood of sheep are 0.12–0.15 mg/l as determined by Aitken (2001). One of the reasons could be that foliar application was usually found to be more efficient than soil application for Se (Gupta and Gupta 2017).

Significantly higher RBC, HGB and HCT content in whole blood of Se-BC lambs could be related to the protective effect of Se on cell membrane and organelles against oxidative damage and increase their life span (Shi et al. 2017). Positive influence of Se on haematological indices in Se-supplemented lambs was also determined by Faixova et al. (2007) in goat kids. The activity of the enzyme in the serum of the experimental group at the end of the study was significantly different only in GPx activity. Se-BC lambs had higher GPx activity in serum relative to control group by 36.47%. Increase of GPx activity in serum of lambs fed with supplementation of organic Se in the Se deficient area was determined by numerous authors (Qin et al. 2007; Antunović et al. 2009; Antunović et al. 2013).

Concentrations of metabolic hormones in serum of lambs did not differ depending on the dietary treatments (Table 7). There was a noticeably higher but not significant concentration of T<sub>3</sub> hormones in serum of Se-BC lambs compared to the control. Novoselec et al. (2017) found a significant increase in T<sub>3</sub> hormone activity during supplementation with Se in lactating sheep. Selenium supply is prioritised to the thyroid under conditions of Se restriction (Lyons 2018). Changes of blood thyroid hormone concentrations are an indirect measure changes in thyroid gland activity and circulating thyroid hormones, which can be considered as indicators of the metabolic and nutritional status of the animals. Concentration of leptin in the lambs' serum is influenced by the energy supply through diet (Antunović et al. 2010b). Serum leptin sensitivity to energy balance is reduced during periods of negative energy balance in sheep (Tokuda et al. 2001). The IGF-I plays a pivotal role in regulating the proliferation, differentiation and specific functions of many

cell types (Baxter 1986). As determined in the present study, metabolic horomones such as  $T_3$ ,  $T_4$ , leptin and IGF-I were maintained when feeding lambs with Se-BC, which indicates preserved nutritional status due to energy balance.

The Se-BC in the lambs' diets maintained production traits, Se status and metabolic profile of lambs. Besides, it improved Se concentrations in lungs and liver, but not in the serum and MS indicating the posibility of using Se-BC in lambs' diets. Therefore, it is necessary to include biofortified corn containing different concentrations of Se in diets and longer duration of experiment in further research as well as use of biofortified corn, not only by foliar application but also in combination with soil. This will contribute to more comprehensive conclusions when using Se-BC in fattening lambs.

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