

Research Note

The effects of dietary Selenium-yeast level on glutathione peroxidase activity, tissue Selenium content, growth performance, and carcass and meat quality of broilers

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ABSTRACT The present study was conducted to assess effects of selenium (Se)-yeast supplementation on glutathione peroxidase activity, Se levels in tissues, growth performance, carcass, and meat composition in broilers. A total of 275 one-d-old Cobb 500 broilers of both sexes were randomly allotted to 1 of 5 treatments during a 42-d period. The 5 treatments differed only in Se content: group 1 had no additional Se (background only); groups 2, 3, and 4 received 0.3 mg/kg of added Se from the beginning of the trial until d 21, whereas in the second half of the study (from d 22 to 42), these groups received 0.3, 0.6, and 0.9 mg/kg of added Se, respectively; and group 5 received 0.9 mg/kg of Se for the entire experimental period. At the end of the study, the control group showed significantly lower ($P < 0.01$) glutathione peroxidase activity in blood plasma compared to Se-supplemented

groups. Regarding Se concentration in various tissues, the groups receiving Se yeast showed higher plasma, feces, and meat Se contents than the control group ($P < 0.01$). Supplementation of Se improved broilers' body weight, weight gain and feed conversion ratio ($P < 0.01$). Dressing percentage was lower in the control group and the group with 0.3 mg/kg of added Se compared to other experimental groups (0.6 and 0.9 mg/kg of dietary Se). The proportion of less valuable carcass parts (wings and legs) was higher ($P < 0.01$) in the group fed the basal diet compared to groups supplemented with 0.9 mg/kg of Se. Initial and ultimate pH values differed among experimental groups ($P < 0.05$). Supplementation of Se improved the broiler's antioxidative resistance, growth performance, carcass quality, and chemical composition of meat.

Key words: selenium, broiler, glutathione peroxidase, growth performance, carcass quality

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INTRODUCTION

Selenium (Se) has a number of important biological roles, including regulation of glutathione peroxidase (GSH-Px) activity, immune function, health, and productivity (Surai, 2002; Surai and Fisinin, 2014). The nutritional Se requirement for broiler chickens throughout the growth period is 0.15 mg Se/kg in the diet (National Research Council, 1994). Se concentrations in feed ingredients vary greatly depending on the plant species and in particular, the Se status of the soil. Some regions, including the Balkans, are Se-deficient areas (Jovanović et al., 1998; Oldfield, 2002; Pešut et al., 2004) and addition of recommended quantities of Se to

feed is necessary to maintain good health and intensive production (Surai, 2002). The efficacy of Se in inducing Se-containing enzymes in vivo and in vitro depends on its chemical form (Ortuno et al., 1996). Nowadays, a few differing sources of Se are used as feed supplements, including inorganic and organic forms of Se, as well as nano Se. However, organic forms of Se and nano Se proved to be more active, less toxic, and accumulate at higher levels in all tissues than inorganic salts (Payne and Southern, 2005; Tiwary et al., 2006; Wang et al., 2007; Zhang et al., 2008; Mohapatra et al., 2014; Suchý et al., 2014).

As Se is a part of the enzyme GSH-Px, involved in antioxidative defense (Arthur, 2000), many authors observed that dietary Se raised the activity of GSH-Px in serum and tissues of broilers (Yoon et al., 2007; Wang and Xu, 2008; Jiang et al., 2009; Heindl et al., 2010; Wang et al., 2011; Zhou and Wang, 2011; Cai et al.,

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2012; Chen et al., 2013; Boostani et al., 2015). On the contrary, other authors did not observe any effect of dietary Se on GSH-Px activity in broilers (Choct et al., 2004; Leeson et al., 2008; Heindl et al., 2010; Cichoski et al., 2012). Furthermore, adding Se to broiler diets improved the Se content in plasma and tissues and led to production of Se-enriched meat (Choct et al., 2004; Zhou and Wang, 2011; Chen et al., 2013; Briens et al., 2014; Baltić et al., 2015).

Moreover, there are numerous contradictory reports regarding effects of Se on growth performance and carcass quality in poultry (Choct et al., 2004; Payne and Southern, 2005; Ryu et al., 2005; Ševčíková et al., 2006; Dlouha et al., 2008; Upton et al., 2008; Perić et al., 2009; Zhou and Wang, 2011; Cai et al., 2012; Hada et al., 2013; Oliveira et al., 2014; Boostani et al., 2015). Although the maximum allowed level of Se in feed is 0.5 mg/kg (European Commission, 2014), in this study we wanted to investigate how higher concentrations of organic Se than that recommended and allowed could influence the antioxidative status of broilers, animal growth, carcass, and meat quality. Therefore, the aim of the present study was to assess the effects of Se-yeast supplementation on GSH-Px activity, Se level in tissues, growth performance, and carcass composition in broilers.

MATERIALS AND METHODS

Animals, Housing, and Trial Duration

The experimental protocol was approved by the Veterinary Directorate of the Serbian Ministry of Agriculture, Forestry and Water Management and the Ethics Committee of the Faculty of Veterinary Medicine, University of Belgrade. A total of 275 one-d-old broilers of both sexes and the same origin (Cobb 500) were used in this study during a 42-d period. Birds were randomly

assigned to 1 of 5 experimental groups. Each pen was bedded with straw and contained 1 experimental group ($n = 55$ animals, stocking density = $0.17 \text{ m}^2/\text{head}$). Water and feed were supplied ad libitum throughout the study. On the first day of the trial, all chickens were marked by individually numbered leg rings and weighed.

Experimental Diets

During the whole experimental period, each group of animals was fed with 1 of 5 experimental diets that differed only in Se content. Basal diets were formulated to meet or exceed the nutritional requirements for broilers according to recommendations (National Research Council, 1994), including starter (d 1 to 21), grower (d 22 to 35), and finisher (d 36 to 42) (Table 1). The experimental diets were prepared by adding specific amounts of organic Se in the form of Se yeast *Saccharomyces cerevisiae* (ALKOSEL® R397, Lallemand Inc., Canada) to the basal diets. Se was added at different levels: broiler group 1 had no additional Se (background only); groups 2, 3, and 4 received 0.3 mg/kg of added Se from the beginning of the trial until d 21, whereas in the second half of the trial (from d 22 to 42), these groups received 0.3, 0.6, and 0.9 mg/kg of added Se, respectively; and group 5 received 0.9 mg/kg of Se for the entire experimental period (Table 2).

Measurements and Analyses

Feedstuffs Composition and Se Content The ingredients and chemical composition (calculated analyses) of the basal diets are listed in Table 1. Moreover, the total Se content was determined for each experimental diet by hydride generation atomic absorption spectrophotometry—HGAAS (THERMO SOLAAR S4

Table 1. Ingredients and chemical composition of diets.

Ingredient (g/kg)	Starter (1 to 21 d)	Grower (22 to 35 d)	Finisher (36 to 42 d)
Corn	478.9	586.5	660.0
Soybean meal 44%	210.0	127.0	140.0
Soy grits 35%	250.0	247.0	160.0
Soybean oil	18.0	–	–
Limestone	15.5	14.0	15.0
Mono-calcium phosphate	12.5	11.0	10.0
Vitamin-mineral premix 0.5% ¹	10.0	10.0	10.0
Sodium chloride	3.5	3.5	3.5
^{DL} -Methionine	1.6	1.0	1.5
Calculated analyses (g/kg)			
ME (MJ/kg)	13.05	13.09	13.07
Crude protein	222.8	192.1	177.4
Lysine	12.7	10.5	9.0
Methionine + cysteine	8.5	6.6	6.6
Ca	9.0	8.0	7.6
P	7.7	6.1	5.4

¹Vitamin-mineral premix provided per kg of diet: Vitamin A 12 999 IJ, Vitamin D3 4,950 IJ, Vitamin E 75 mg, Vitamin K3 3 mg, Vitamin B1 3 mg, Vitamin B2 7.95 mg, Vitamin B6 4.05 mg, Vitamin B12 0.0195 mg, Vitamin C 19.95 mg, Biotin 0.15 mg, Niacin 60 mg, Calcium pantothenate 15 mg, Folic acid 1.95 mg, Iodine 1.0005 mg, Selenium 0.3 mg, Choline chloride 399.9 mg, Iron 39.99 mg, Copper 15 mg, Manganese 99.9 mg, Zinc 99.9 mg, Methionine 2,100 mg, Lysine 1,200 mg.

Table 2. Selenium content of the experimental broiler diets supplemented with different levels of selenium.

Group	d 1 to 21		d 22 to 42	
	Se expected (mg/kg)	Total estimated (mg/kg)	Se expected (mg/kg)	Total estimated Se (mg/kg)
1	Background only	0.11	Background only	0.11
2	0.3	0.41	0.30	0.41
3			0.60	0.71
4			0.90	1.01
5	0.9	1.01	0.90	1.01

VP90 system, Thermo Fisher Scientific, Waltham, MA, USA) and presented in Table 2.

Blood Sampling Blood samples were obtained by puncture of the wing vein on d 1, 21, and 42 of the study. On the first day of the trial, blood was randomly collected from 10 animals, and on d 21 and 42, blood was taken from 6 birds in each group to determine GSH-Px activity and Se concentration in plasma. Blood was taken into heparinized vacutainers and centrifuged at $2,500 \times g$ for 20 min at 20°C. Thereafter, plasma samples were collected into microtubes and stored at -18°C for later processing.

Glutathione Peroxidase Activity (GSH-Px) GSH-Px activity in plasma was measured by the coupled test (Günzler et al., 1974) using a Cecil CE 2021 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK) at 37°C, $\lambda = 366$ nm. The results were expressed in microkatal per liter ($\mu\text{kat/L}$), where 1 katal is the SI unit equivalent to the amount of enzyme required to convert 1 mole of substrate per second.

Se analysis in plasma, feces, breast, and thigh muscle and calculation of Se assimilation efficiency (AE) Blood samples were collected at the beginning of the study and on d 21 and 42 to determine Se level in plasma. For analysis of Se content in feces, 6 samples from each experimental group were taken on d 21 and 42 of the study. At the end of the study, (d 42) 6 breast (*pectoralis major* muscle) and thigh meat samples were collected from each experimental group to determine Se content in meat. Samples were subsequently mineralized using microwave-assisted mineralization with nitric acid and hydrogen peroxide, followed by quantification by hydride generation atomic absorption spectrophotometry.

In addition, 6 birds from each experimental group were placed in individual cages on d 21 and 42 for measuring daily feed intake and feces weight. Se assimilation efficiency (**AE**) was calculated by the mass balance method (Wang and Fisher, 1999), as follows:

$$\text{AE}(\%) = (\text{Ingested Se} - \text{Excreted Se}) / \text{Ingested Se}$$

Ingested Se = daily feed intake

× analytical feed Se concentration

Excreted Se = daily feces weight × feces Se concentration.

Growth Performance All birds were weighed on d 1, 21, and 42 to obtain average body weight and weight

gain for each experimental group. Feed consumption per pen was recorded during the periods 1 to 21 d, 22 to 42 d and 1 to 42 d, and these values were used to calculate feed conversion ratios (FCR), which were calculated as the ratio between feed intake and bird weight gain.

Carcass and Meat Quality At the end of the study, animals were slaughtered and hot carcass weight was recorded for 30 carcasses per broiler group. After 24 h of storage at 2°C, cold carcass weight was measured to calculate the dressing percentage on cold carcass weight and associated carcass losses during chilling. Furthermore, 30 carcasses from each group were separated into breast, drumsticks with thighs, wings, neck, back with pelvis, and abdominal fat. The different parts were weighed and their percentage of total cold carcass weight was calculated.

Measurement of pH of 30 carcasses from each experimental group was carried out approximately 45 min ($\text{pH}_{45\text{min}}$) and 24 h ($\text{pH}_{24\text{h}}$) after slaughter on *pectoralis superficialis* muscle using a pH-meter Testo 205 (Germany). At 24 h postmortem, 6 thigh and breast meat samples from each experimental group were packed in polyethylene bags and kept at -18°C until analysis of moisture (ISO, 1442:1997), lipid (ISO, 1443:1973), protein (ISO, 937:1978), and ash (ISO, 936:1998) contents.

Statistical Analysis

Statistical analysis of the results was elaborated using software GraphPad Prism version 5.00 for Windows (GraphPadSoftware, San Diego, CA, USA, www.graphpad.com). All parameters were described by means and pooled standard error of means (**SEM**). One-way ANOVA with Tukey's post test was performed to assess the significance of differences among experimental groups. Pearson's correlation was used to determine relationships among GSH-Px activity in plasma between 21-d-old and 42-d-old birds, and Se concentrations in various tissues. Values of $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Table 3 shows the results of GSH-Px activity in plasma, Se concentration in various tissues, and Se AE in relation to the Se level in the broiler diets. At 21

Table 3. Effects of different levels of selenium in diet on plasma GSH-Px activity, selenium concentration in plasma, feces, and meat of broilers and selenium assimilation efficiency.

Parameter	n	d 1 to 21 22 to 42	Group with added Se (mg/kg)					SEM	P value
			1 0	2 0.3	3 0.3	4 0.3	5 0.9		
Plasma GSH-Px (μ kat/L)	10	1			32.16 ^{X,Z} \pm 2.30			–	–
	6	21	28.40 ^{B,Y}		35.97 ^{B,Y}		47.88 ^{A,X,Y}	7.42	<0.0001
	6	42	5.54 ^{A,X,Y}	14.26 ^{B,a,X,Y}	17.24 ^{C,X,Y}	18.10 ^{C,b,X,Y}	19.51 ^{D,Z,Y}	2.33	<0.0001
Plasma Se (mg/kg)	10	1			0.16 ^{X,Y} \pm 0.02			–	–
	6	21	0.089 ^{A,X}		0.182 ^{B,Z}		0.222 ^{C,X}	0.018	<0.0001
	6	42	0.077 ^{A,Y}	0.198 ^B	0.211 ^{B,X}	0.221 ^{B,X,Z}	0.240 ^{B,Y}	0.025	<0.0001
Feces Se (mg/kg)	6	21	0.076 ^A		0.297 ^B		0.747 ^C	0.041	<0.0001
	6	42	0.058 ^A	0.134 ^A	0.274 ^B	0.276 ^B	0.438 ^C	0.047	<0.0001
Breast Se (mg/kg)	6	42	0.113 ^A	0.320 ^B	0.470 ^C	0.616 ^D	0.859 ^E	0.021	<0.0001
Thigh Se (mg/kg)	6	42	0.112 ^A	0.286 ^B	0.353 ^C	0.554 ^D	0.707 ^E	0.026	<0.0001
AE (%)	6	21	63.00 ^A	65.83 ^A	68.34 ^B	68.43 ^B	76.06 ^C	2.018	<0.0001
	6	42	70.95 ^{A,b}	83.44 ^{B,b}	79.14 ^B	86.30 ^C	77.26 ^{a,D}	2.804	<0.0001

AE: assimilation efficiency; SEM: pooled standard error of means; within a row, means with the different letter significantly differ, A, B, C, D— $P < 0.01$; a, b— $P < 0.05$; within a column, means with the same letter significantly differ, X, Y, Z— $P < 0.01$.

Data are given as means and SEM.

d, GSH-Px activity in plasma was higher ($P < 0.01$) in the group receiving 0.9 mg/kg of Se from the beginning of the trial compared to other groups with low dietary Se content (0 and 0.3 mg of added Se/kg). At the end of the study, the control group showed significantly lower ($P < 0.01$) GSH-Px activity compared to Se-supplemented groups. In addition, the group with 0.3 mg/kg of added Se had lower GSH-Px activity compared to groups 4 and 5 (0.9 mg/kg of added Se). According to age, all 42-d-old broilers showed significantly lower ($P < 0.01$) GSH-Px activity compared to 1-d-old and 21-d-old broilers.

Regarding Se concentration in various tissues, the groups receiving Se yeast showed higher plasma, feces, and meat Se contents than the control group ($P < 0.01$). At the end of trail, higher levels of Se in feces were found in groups with 0.6 and 0.9mg/kg of dietary Se (groups 3, 4, and 5) compared to groups with 0 and 0.3 mg of added Se/kg. Furthermore, Se contents in breast and thigh meat increased significantly ($P < 0.01$) with the level of Se supplementation; thus, the highest value was observed in broilers fed diets supplemented with 0.9 mg/kg of Se yeast.

In addition, AE of Se was lower ($P < 0.01$) in the control group at d 21 (63.00%) compared to groups with 0.6 and 0.9 mg/kg of added Se, as well as in the control group (70.95%) at d 42 compared to other broiler groups ($P < 0.05$).

The relationship between plasma GSH-Px activity at 21 and 42 d of age and Se content in various tissues is presented in Table 4. Significant correlations ($P < 0.01$) were found among activity of selenoenzyme and Se concentrations in plasma, feces, and meat, except for GSH-Px activity and Se plasma content in 21-d-old broilers. Higher Pearson correlation coefficients were determined for GSH-Px activity measured on d 42 compared to those on d 21 of the trial. As Se is an essential component of the enzyme GSH-Px involved in

the antioxidative defense (Arthur 2000), in this study, the activity of the enzyme was greatly improved by dietary Se supplementation. Those results are in agreement with previous reports (Yoon et al., 2007; Wang and Xu, 2008; Jiang et al., 2009; Wang et al., 2011; Zhou and Wang, 2011; Hu et al., 2012; Chen et al., 2013; Boostani et al., 2015; Chadio et al., 2015), confirming that dietary Se elevates blood GSH-Px activity in Se-supplemented broilers. The linear correlation between Se concentration and GSH-Px activities of the blood and various tissues has been well documented (Pavlata et al., 2001). In accordance with this, in our study, high positive correlations were determined between GSH-Px activities and Se levels in tissue. Furthermore, in the present study, higher GSH-Px activity in 42-d-old birds was observed when 0.9 mg/kg of dietary Se was added to the diet compared to groups with lower Se levels (0 and 0.3 mg/kg of added Se). However, no significant differences in GSH-Px activity were determined between birds supplemented with 0.6 and 0.9 mg/kg of Se yeast. This indicates that the elevation of the plasma GSH-Px activity reached a plateau, which was also observed by other authors (Fischer et al., 2008; Zhou and Wang, 2011; Cai et al., 2012; Hu et al., 2012; Chen et al., 2013; Baltić et al., 2015; Taylor and Sunde, 2016). Regardless of dietary Se level, all groups of broilers showed a decline in GSH-Px activity at 42-d-old, which is in agreement with other studies (Pappas et al., 2005; Farahat et al., 2008; Baltić et al., 2015; Chadio et al., 2015). It seems that older birds have less need for synthesis of GSH-Px, because ingested Se could be used for producing several selenoproteins besides GSH-Px (Cichoski et al., 2012), and which would likely depend on avian metabolic needs (Daun and Akesson, 2004).

The present study revealed that broilers that received Se-supplemented diets exhibited a significant increase in plasma, feces, and meat Se levels compared with the control group, which is in accordance with previous

Table 4. Correlations among plasma GSH-Px activity on d 21 and 42 and Se concentration in plasma, feces, and meat of broilers (n = 30).

Parameters	Se plasma (21 d)	Se plasma (42 d)	Se feces (21 d)	Se feces (42 d)	Se breast	Se thigh
GSH-Px (21 d)	0.32 ^{ns}	0.47 ^{**}	0.67 ^{***}	0.60 ^{***}	0.59 ^{***}	0.56 ^{**}
GSH-Px (42 d)	0.74 ^{***}	0.80 ^{***}	0.61 ^{***}	0.74 ^{***}	0.77 ^{***}	0.74 ^{***}

ns—no significant difference; ** $P < 0.01$; *** $P < 0.001$.

reports (Choct et al., 2004; Yoon et al., 2007; Wang and Xu, 2008; Heindl et al., 2010; Zoidis et al., 2010; Wang et al., 2011; Zhou and Wang, 2011; Cai et al., 2012; Hu et al., 2012; Chen et al., 2013; Briens et al., 2014; Baltić et al., 2015). In addition, the AE of Se increased with dietary Se level and was higher in groups fed diets with the highest Se content (0.9 mg/kg) compared to the control group. Similar observations were reported by Baltić et al. (2015) in 49-d-old ducks, whereas Kirchgessner et al. (1997), Choct et al. (2004), and Yoon et al. (2007) found that Se was more efficiently retained when the concentration of Se in the diet was low. As in our study an organic form of Se was used, opposite to the studies of Kirchgessner et al. (1997) and Choct et al. (2004) (which utilized inorganic Se), the higher AE in our study could be a consequence of better absorption of organic Se by active transport (Wolfram et al., 1989) and higher deposition in muscle proteins (Schrauzer, 2000; Kim and Mahan, 2003) compared to inorganic Se.

The high Se level and GSH-Px activity in plasma of 1-d-old broilers observed in this study could be a consequence of dietary Se supplementation of hens, which was also observed by other authors (Surai, 2000; Paton et al., 2002; Pappas et al., 2005; Payne et al., 2005). Previous work has shown that maternal Se supplementation increases the activity of GSH-Px as well as Se concentration in tissues of the offspring at hatching, and those effects are sustained for several weeks after hatching (Surai, 2000; Pappas et al., 2005). Our results showed that the control group, plasma Se concentration decreased in 21-d-old broilers, whereas GSH-Px activity decreased in 42-d-old broilers, as was similarly found by Pappas et al. (2005) and Chadio et al. (2015). This later decrease of GSH-Px activity is probably due to the delay of the regeneration time of the erythrocytic pool (Bounous and Stedman, 2000).

Growth Performance of Broilers

Table 5 indicates that supplementation of Se improved body weight, weight gain, and feed conversion ratio in the broilers. After 21 d, the control group had the lowest body weight, weight gain, and the highest feed conversion ratio ($P < 0.01$), whereas the group receiving 0.9 mg/kg of Se (group 5) achieved the best growth performance results ($P < 0.01$). At the end of the study, groups with 0.9 mg/kg of dietary Se (groups 4 and 5) had higher ($P < 0.01$) body weight and

weight gain compared to other groups with lower Se contents in their diets. During the second half of the trial (from d 22 to 42), the highest weight gain ($P < 0.01$) was observed in broilers supplemented with 0.9 mg/kg Se yeast. Moreover, the FCR for the overall period of the study was higher ($P < 0.01$) in the control group compared to broilers in groups 2, 4, and 5 (0.3 and 0.9 mg of added Se/kg). Although it is recommended that 0.15 mg/kg of Se be added to feed in order to achieve good health and optimal growth of poultry (National Research Council, 1994), in this study, much higher levels of organic Se were used (0.11 to 1.01 mg/kg of analyzed Se in diet). Organic Se proved less toxic than inorganic Se, although very high levels of organic Se in diet (3 and 10 mg/kg) were determined to impair animal growth (Zoidis et al., 2010; Briens et al., 2014, respectively). Our results showed that supplementation of Se improved body weight, weight gain, and feed conversion ratio, even in groups supplemented with 0.9 mg/kg of Se. These results are consistent with reports of other authors (Choct et al., 2004; Ševčíková et al., 2006; Upton et al., 2008; Zhou and Wang, 2011; Marković et al., 2014; Baltić et al., 2016). On the contrary, it was also observed that Se supplementation in broilers did not have any effect on growth performance (Payne and Southern, 2005; Ryu et al., 2005; Perić et al., 2009; Cai et al., 2012; Chen et al., 2013; Briens et al., 2014; Boostani et al., 2015; Chadio et al., 2015). Although the control group received 0.11 mg/kg of dietary Se, for these fast-growing broilers, this amount was not sufficient to achieve optimal growth; consequently, we measured lower growth performance (lower body weight, weight gain, and higher FCR) in these animals. Se is important for animal growth, as it is a part of the enzyme group of iodothyroninedeiodinases, involved in the metabolism of thyroid hormones that are necessary for normal growth and development (Arthur, 1991). Better activation of thyroid hormones by increased Se content may explain the improved growth performance we measured (Choct et al., 2004).

Carcass Quality

The effects of dietary Se supplementation on carcass characteristics and yield of carcass cuts in broilers are shown in Table 6. Higher hot and cold carcass weights ($P < 0.05$) were determined in groups with 0.9 mg of

Table 5. Growth performance of broilers receiving diets with different levels of selenium. Data are given as means and SEM (n = 55 per group).

Parameter	d	Group with added Se (mg/kg)					SEM	P value
		1	2	3	4	5		
		1 to 21 22 to 42	0 0	0.3 0.3	0.3 0.6	0.3 0.9		
Body weight (g)	1	47.09	45.83	46.33	45.09	46.58	1.16	0.0563
	21	499.14 ^A	564.66 ^B	598.82 ^B	599.18 ^B	753.45 ^C	22.67	<0.0001
	42	1,820.47 ^A	1,995.33 ^B	1,995.33 ^B	2,180.33 ^C	2,181.33 ^C	57.98	<0.0001
Weight gain (g)	1 to 21	452.14 ^A	518.57 ^B	552.50 ^B	553.68 ^B	706.86 ^C	22.73	<0.0001
	22 to 42	1,307.60 ^B	1,420.43 ^B	1,384.67 ^B	1,599.87 ^A	1,418.87 ^B	61.66	<0.0001
	1 to 42	1,773.00 ^A	1,949.00 ^B	1,949.60 ^B	2,133.67 ^B	2,135.13 ^C	57.92	<0.0001
Feed intake (g)	1 to 21	981.13		981.82		1,045.46	–	–
	22 to 42	2,902.33	2,872.73	3,008.09	3,209.77	2,978.64	–	–
	1 to 42	3,883.46	3,854.55	3,989.91	4,191.59	4,024.09	–	–
Feed conversion ratio	1 to 21	2.22 ^A	1.96 ^B	1.80 ^B	1.82 ^B	1.48 ^C	0.09	<0.0001
	22 to 42	2.26	2.09	2.23	2.04	2.14	0.10	0.0658
	1 to 42	2.21 ^A	2.00 ^B	2.07 ^a	1.98 ^B	1.90 ^{B,b}	0.06	<0.0001

SEM: pooled standard error of means; within a row, means with the different letter significantly differ, A, B, C— $P < 0.01$; a— $P < 0.05$.

Table 6. Carcass characteristics and yield of carcass cuts (weight and proportion) of broilers fed with diets containing different levels of selenium.

Parameter	d	Group with added Se (mg/kg)					SEM	P value
		1	2	3	4	5		
		1 to 21 22 to 42	0 0	0.3 0.3	0.3 0.6	0.3 0.9		
HCW (g)		1,210.50 ^{a,A}	1,324.90 ^{b,B}	1,364.43 ^{A,b}	1,478.50 ^{C,a}	1,487.20 ^D	57.76	<0.0001
CCW (g)		1,179.20 ^{a,A}	1,290.90 ^{b,B}	1,329.77 ^{A,b}	1,441.00 ^{C,a}	1,450.07 ^D	56.38	<0.0001
CL (%)		2.66	2.65	2.61	2.61	2.57	0.13	0.2785
DP (%)		64.80 ^{A,a}	64.11 ^A	66.54 ^B	66.14 ^{b,B}	66.46 ^B	0.91	<0.0001
Breast	(g)	398.00 ^A	438.87 ^B	465.40 ^{A,a}	511.87 ^{C,b}	508.00 ^D	24.43	<0.0001
	(%)	33.75 ^b	33.89 ^b	34.95	35.47 ^a	35.05	0.84	0.0037
Drumsticks with thighs	(g)	340.20 ^{a,A}	372.87 ^{b,B}	378.67 ^{B,b}	404.33 ^{B,b}	411.13 ^{B,b}	17.09	<0.0001
	(%)	28.87	28.86	28.55	28.12	28.31	0.57	0.1574
Wings	(g)	127.47 ^A	134.73 ^{a,B}	137.80 ^{B,b}	144.40 ^{B,a}	147.67 ^{C,b}	4.85	<0.0001
	(%)	10.83 ^A	10.49 ^a	10.43	10.04 ^{B,b}	10.20 ^B	0.24	<0.0001
Neck, back with pelvis	(g)	294.27 ^{a,A}	325.67 ^{b,B}	332.07 ^{A,b}	358.60 ^{C,a}	369.07 ^D	15.03	<0.0001
	(%)	24.94	25.31	25.08	24.89	25.46	0.72	0.6854
Legs	(g)	72.67	73.93	73.40	75.67	78.80	4.43	0.198
	(%)	5.83 ^A	5.41 ^B	5.26 ^B	5.00 ^B	5.13 ^B	0.25	<0.0001
Abdominal fat	(g)	11.60 ^{B,b}	12.07 ^{B,b}	13.33 ^B	19.73 ^{A,b}	15.80 ^a	2.11	<0.0001
	(%)	0.97 ^B	0.93 ^B	1.00 ^B	1.36 ^{A,a}	1.10 ^b	0.15	<0.0001

HCW: hot carcass weight; CCW: cold carcass weight; CL: chilling losses of carcass weight; DP: dressing percentage of cold carcass weight; SEM: pooled standard error of means; within a row, means with the different letter significantly differ, A, B, C, D— $P < 0.01$; a, b— $P < 0.05$.

Data are given as means and SEM (n = 30 per group).

added Se/kg (groups 4 and 5) compared to the groups with lower levels of dietary Se. Chilling losses did not significantly differ among the broiler groups. Dressing percentage was lower in the control group and the group with 0.3 mg/kg of added Se compared to other experimental groups (0.6 and 0.9 mg/kg of dietary Se).

Basic cuts of broiler carcasses (breast, drumsticks with thighs, wings, neck, back with pelvis, legs, and abdominal fat), calculated as weight and percentage of carcass, significantly differed among the broiler groups. Supplementation of Se significantly improved the yield of carcass cuts, where the control group and the group with 0.3 mg/kg of added Se had lower ($P < 0.05$) weight of breast, drumsticks with thighs, wings, neck, back with pelvis, and abdominal fat compared to groups with 0.9 mg of added Se/kg. Moreover, the proportion of less

valuable carcass parts (wings and legs) was higher ($P < 0.01$) in the group fed with basal diet compared to groups supplemented with 0.9 mg/kg of Se. In addition, the group 4 broilers (0.9 mg/kg of Se) had a higher ($P < 0.05$) proportion of breast meat compared to groups with lower dietary Se content (0 and 0.3 mg/kg of added Se), and the highest proportion of abdominal fat (1.36%). Our results showed that Se supplementation, along with its positive effects on body weight, also increased hot and cold carcass weights. Similarly, organic forms of Se increased eviscerated weight in 1 study (Choct et al., 2004), whereas other authors did not find any effect of Se supplementation on carcass weight or dressing percentage (Choct and Naylor, 2004; Payne and Southern, 2005; Ševčíková et al., 2006; Heindl et al., 2010; Chen et al., 2013). It is possible that

organic Se may have enhanced the efficiency of protein deposition or water retention in tissues (Choct et al., 2004). That could be an explanation for higher dressing percentage determined in our study in high-Se-treated groups (0.6 and 0.9 mg/kg) compared to low-Se-treated groups (0 and 0.3 mg/kg). Furthermore, in our study, Se supplementation did not significantly influence carcass chilling losses, whereas others observed that dietary Se reduced chilling loss and drip loss in poultry (Choct and Naylor, 2004; Choct et al., 2004; Jiang et al., 2009; Perić et al., 2009; Zhou and Wang, 2011; Cai et al., 2012). As Se is involved in antioxidant defense of the body (Surai and Dvorska, 2002), improved antioxidant status may promote the maintenance of cell membrane integrity (Cheah et al., 1995), which could ultimately result in reduced drip loss and chilling loss.

With regard to yields of carcass parts, groups with higher dietary Se levels had higher weights of carcass parts compared to groups with lower dietary Se content. Some other studies previously reported this (Choct et al., 2004; Upton et al., 2008; Marković et al., 2014), whereas others did not observe any effect of dietary Se on carcass cut yields (Choct and Naylor, 2004; Payne and Southern, 2005; Ševčíková et al., 2006; Heindl et al., 2010; Hada et al., 2013; Oliveira et al., 2014; Baltić et al., 2016). Furthermore, in our study, the proportion of breast in the carcasses was lower, whereas the proportion of less valuable carcass parts (wings and legs) was higher in low-Se-treated groups compared to high-Se-treated groups. This increase in the proportion of breast in groups receiving the higher Se content likely reflected greater protein assimilation and improved growth by the Se-enhanced metabolism of thyroid hormones that regulated animal growth (Arthur, 1991; Jianhua et al., 2000). In addition, our results showed that abdominal fat content increased as dietary Se level increased. It is possible that dietary Se influences metabolism of fat, because high-Se diets containing 0.4 to 3.0 mg of Se/kg of diet for extended periods of time induced hyperinsulinemia, hyperglycemia, insulin resistance, glucose

intolerance, hyperlipidemia, and obesity (Zhou et al., 2013).

Meat Quality

The control group had higher $\text{pH}_{45\text{min}}$ ($P < 0.05$) compared to the group receiving 0.9 mg/kg of Se for the entire experimental period (group 5) (Table 7). At 24 h postmortem, the pH of broilers supplemented with 0.3 mg/kg Se yeast was higher compared to controls and group 5. The lipid content of breast meat significantly ($P < 0.05$) differed between the control (0.92%) and group 3 broilers (0.54%). Lower protein content of breast meat was found in group 2 broilers (22.66%) compared to the groups with 0.9 mg/kg added Se (23.80%, 23.64%). Higher protein content of thigh meat was determined in group 4 (20.74%) compared to groups with lower dietary Se level (0.3 and 0.6 mg of added Se/kg). Groups with lower levels of dietary Se (0 and 0.3 mg/kg of added Se) had higher ash content in breast meat compared to groups with 0.9 mg/kg of added Se. One of the most important meat quality parameters is pH value. Adding Se to diet had no effects on pH values of poultry meat (Perić et al., 2009; Boiago et al., 2014; Göçmen et al., 2016). On the contrary, in our study, the initial pH value ($\text{pH}_{45\text{min}}$) was lower in the group with the highest dietary Se level (group 5 broilers) compared to the control group, which was observed previously (Baltić et al., 2015). Those differences could be explained by the fact that initial pH values were measured starting from group 1 and moving through to group 5, and because pH value has an intensive decline immediately after slaughter (Smith et al., 1992), the highest values were observed in the group 1 birds. At 24 h after slaughter, pH values were lower in the group 1 (control) and group 5 broilers compared to the group with 0.3 mg/kg of added Se. Zhan et al. (2007) found an increasing trend of the pH value of loin muscle in Se-treated pigs. The higher pH value of Se-treated meat is probably due to higher depletion of hydrogen

Table 7. Meat characteristics (breast and thigh) of broilers fed with diets containing different levels of selenium.

d	Parameter	Muscle	n	Group with added Se (mg/kg)					SEM	P value
				1	2	3	4	5		
1 to 21				0	0.3	0.3	0.3	0.9		
22 to 42				0	0.3	0.6	0.9	0.9		
	$\text{pH}_{45\text{min}}$	Breast	30	6.37 ^a	6.28	6.26	6.30	6.16 ^b	0.08	0.0032
	$\text{pH}_{24\text{h}}$	Breast	30	5.84 ^a	6.06 ^{b,A}	5.95	5.96	5.80 ^B	0.10	0.0005
	Moisture (%)	Breast	6	72.32	72.52	72.86	72.57	73.20	0.79	0.4621
		Thigh	6	74.64	74.36	74.79	74.56	74.68	1.51	0.9936
	Lipid (%)	Breast	6	0.92 ^a	0.86	0.54 ^b	0.64	0.64	0.18	0.0133
		Thigh	6	3.36	3.38	2.82	3.08	3.06	0.75	0.7455
	Protein (%)	Breast	6	22.97	22.66 ^{A,a}	23.41	23.80 ^B	23.64 ^b	0.46	0.0034
		Thigh	6	19.64	19.52 ^a	19.24 ^A	20.74 ^{b,B}	19.70	0.60	0.0064
	Ash (%)	Breast	6	1.12 ^{B,a}	1.24 ^A	0.99 ^B	0.92 ^B	0.94 ^{b,B}	0.09	<0.0001
		Thigh	6	1.05 ^b	1.05 ^b	1.16 ^a	1.11	1.12	0.05	0.0054

SEM: pooled standard error of means; within a row, means with the different letter significantly differ, A, B,— $P < 0.01$; a, b— $P < 0.05$. Data are given as means and SEM.

peroxide (H_2O_2) that is catalyzed by the enzyme GSH-Px (Boiago et al., 2014). Previous studies in broilers showed that oxidative stress increases thigh meat lightness (color), speeds up pH drop after slaughter, and increases drip loss in breast meat (Zhang et al., 2011; Chen et al., 2017; Wang et al., 2017). Thus, the decline of meat quality in the breast muscle of broilers might be related to oxidative damage caused by H_2O_2 (Chen et al., 2017). On the other hand, low ultimate pH value (pH_{24}) of the high-Se-treated group (0.9 mg/kg) could be a consequence of higher glycogen content at slaughter, as muscle glycogen level at death largely determines the ultimate pH (Le Bihan-Duval et al., 2008). Se has been shown to mediate a number of insulin-like actions including the stimulation of glucose uptake and regulation of glycolysis, gluconeogenesis, and fatty acid (FA) synthesis (Zeng and Combs, 2008), and thus, high glycogen content in muscles could be a consequence of high dietary intake of Se.

Regarding chemical composition of meat, we found an increasing trend of protein content in breast and thigh meat and decreasing trend of lipid content in breast meat along with increasing dietary levels of Se. Similarly, Ibrahim et al. (2011) found that broilers fed with Se had higher crude protein content in muscles compared to unsupplemented-Se groups. This increase in protein content might be due to the aforementioned greater protein assimilation by Se-enhanced metabolism of thyroid hormones (Arthur, 1991; Jianhua et al., 2000). Opposite to our results, other authors observed that intramuscular fat content of meat increased as the supplemented level of Se increased (Ševčíková et al., 2006; Pappas et al., 2012; Baltić et al., 2015). As there are contradictory reports regarding Se impact on metabolism of fat (Hawkes and Keim, 2003; Hawkes et al., 2008; Rayman et al., 2008), further investigations are necessary to clarify the effects of Se on metabolism of fat in growing broilers.

CONCLUSION

The results of the current study showed that Se supplementation significantly increased GSH-Px activity broiler plasma and Se contents in plasma, feces, and muscles of broilers. Growth performance of chickens was improved after adding Se to diet, even in groups supplemented with 0.9 mg/kg of Se. Furthermore, better carcass quality was determined after enriching broiler diets with Se, as Se increased the dressing percentage and the proportion of breast meat in the carcasses. Moreover, chemical analysis of meat revealed that Se supplementation increased the protein content and decreased the lipid content of meat. As the addition of supranutritional Se doses to chicken diets, at levels well below those causing toxicity, improved the broiler's antioxidant resistance, growth performance, carcass quality, chemical composition of meat and enriched their meat with Se, in intensive broiler production, the rec-

ommended quantity of 0.15 mg/kg of dietary Se should be thoroughly revised.

SUPPLEMENTARY DATA

Supplementary data are available at *Poultry Science* online.

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