Dietary organic selenium supplementations affect oxidative stability of chilled and frozen chicken meat

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Summary

The aim of the study was to determine the effects of supplementary dietary organic selenium on oxidative stability and quality of cooled and deep-frozen chicken breast and thigh muscles. The research was undertaken on broilers Cobb 500 subdivided to three randomly assigned groups: inorg$Se_{0.3}$ (sodium selenite added to feed at 0.3 mg·kg^{-1}), org$Se_{0.3}$ (organic selenium added to feed at 0.3 mg·kg^{-1}) and org$Se_{0.5}$ group (organic selenium added to feed at 0.5 mg·kg^{-1}). Samples were probed while stored at 4 °C (immediately after cooling, and after 1 day, 3 days and 7 days) and at –20 °C (after 30 days and 90 days) after slaughter. Organic selenium feed supplementation resulted in selenium muscle enrichment. Increased deposition of selenium in breast muscles was found in all groups, but only in org$Se_{0.5}$ group in thigh muscles. Higher glutathione peroxidase activities on the day of slaughter in both muscles, and glutathione as well as catalase were determined in tight muscles. Addition of organic selenium at 0.3 mg·kg^{-1} and 0.5 mg·kg^{-1} of feed improved oxidative stability during the first few days of storage, with more pronounced effects in the breast muscle.

Keywords

chicken; muscle; oxidative stability; organic selenium

Oxidative changes are the main non-microbiological factor affecting meat quality [1–3]. Main causes affecting meat quality in periods of cool storage in refrigerators and deep freezers are lipid peroxidation and colour changes [4–7]. Chicken meat is especially sensitive to oxidation due to its high polyunsaturated fatty acids content and, therefore, it is believed that the degree of discoloration represents the intensity of the oxidative processes.

Muscle cells in both live animals and meat have various mechanisms of antioxidative protection. They include enzymatic mechanisms (glutathione peroxidase, GSH-Px; superoxide dismutase, SOD; catalase, CAT) and non-enzymatic mechanisms (glutathione, albumin, uric acid, vitamins E, A and C) [8–11]. Thereby, not all antioxidative compounds are equally important. For instance, GSH-Px has a greater role in cell oxidative protection than SOD or CAT [12]. CAT and SOD are bound enzymes, wherein SOD catalyses transformation of superoxide anion to H_2O_2, which is then degraded by catalase, while GSH-Px can degrade both H_2O_2 and lipid peroxides originating from the process of lipid peroxidation [8]. Intracellular GSH-Px comprises two proteins, cytosolic GSH-Px and phospholipid hydroperoxide GSH-Px, which are present in the nucleus, mitochondria and cytosol [13].

Besides being an important source of proteins, feedstuffs of animal origin are an important source of macroelements and microelements, in-

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cluding selenium [14–18]. Selenium is an essential nutritional supplement in chicken feed, important for the improvement of both health and production traits of chicken. It increases the quality of meat for human consumption [6, 19–24]. Selenium can be added to feed in inorganic form (selenates, selenites) or in organic form (selenomethionine, selenocysteine) [16]. Selenomethionine exerts its antioxidative activity by slowing down oxidation processes and by incorporation into selenoproteins [25]. Selenium deposition in tissues elevates GSH-Px activity [26–29].

Most of the research regarding dietary micromineral supplements is aimed at their health effects or deposition in tissues [9, 30], but there are also reports on the use of selenium supplementation to investigate the effect on meat quality, on parameters such as water holding capacity, pH or colour [31].

Technological processes, such as cooling, freezing, thawing and storing can change meat appearance, its sensory and processing characteristics. Therefore, the objective of this research was monitoring the effects of organic selenium supplementation in feed and its tissue deposition in connection to oxidative stability of chilled and frozen chicken breast and thigh muscle. This was accomplished by measuring the activity of GSH-Px, SOD and CAT, and concentrations of glutathione and lipid peroxide as indicators of oxidative stress.

MATERIALS AND METHODS

The study protocol was approved by the Ethics Committee of Faculty of Veterinary Medicine (251-61-01/139-10-39). The research was undertaken on 252 broilers Cobb 500 of both sexes. They were reared on the floor, with food and water available *ad libitum*. Balanced groups were formed according to the amount and type of selenium in the feed (N = 14 for each group). First group ($orgSe_{0.3}$) was given organic selenium in a content of 0.3 mg·kg^{-1} of feed (commercial formulation Sel-Plex; Alltech, Lexington, Kentucky, USA). The second group ($orgSe_{0.5}$) was given organic selenium in a content of 0.5 mg·kg^{-1} of feed (commercial formulation Sel-Plex). Control group ($inorgSe_{0.3}$) was given a standard starter and grower feed containing sodium selenite in a content of 0.3 mg·kg^{-1} feed. Up until day 21, all groups were fed broiler starter feed and, in the period of 22-42nd day, grower feed (Tab. 1). Feed consumption and growth were balanced in all of the groups.

Fattening was finished on the 42nd day when the chicken were transported to slaughter house. After the slaughter, the carcasses were chilled to +2 °C during 1.5–2 h in a tunnel system.

After cooling, breast (*m. pectoralis superficialis)* and thigh muscle (*m. gastrocnemius*) were stored in a refrigerator at a temperature + 4–8 °C and a part of them in a freezing chamber at –20 °C. Samples for determination of selenium concentration were withdrawn immediately after chilling and kept frozen at –20 °C until the analyses were made. Antioxidative enzymes activity and peroxidation intensity were determined immediately after chilling, after 1, 3 and 7 days of storage in a refrigerator, as well as after 30 and 90 days of storage at –20 °C.

Samples were homogenized in 0.14 mol·l^{-1} KCl at 46.66 Hz with cooling for 90 s using homogeniser Schütt Homgen^{plus} (Schütt Labortechnik, Göttingen, Germany) and centrifuged at 20 000 ×*g* for 30 min at 4 °C (3K15; Sigma, Osterode am Harz, Germany). Then, activity of glutathione peroxidase (GSH-Px, EC 1.11.1.9), superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC

Tab. 1. Ingredients and nutrient content of diets.

Ingredients and nutrient content [g·kg^{-1}]	Starter	Grower
Ingredient		
Maize	509	566
Soybean meal (44% of crude protein)	396	362
Protein Gold	20	0
Mineral and vitamin mixture Kuškovit	50	50
Vegetable oil	25	22
Nutrient composition (calculated)		
Crude protein	221	201
Crude fat	52.4	50.5
Crude fibre	38.7	37.6
Calcium	10.4	9.6
Lysine	13.3	11.7
Methionine	6.4	5.8
Metabolizable energy [MJ·kg^{-1}]	14.76	14.86

Protein Gold (Kušić promet, Sveti Ivan Zelina, Croatia) contains crude protein min. 50 %, moisture max. 13 %, crude fibre max. 4.5 %, methionine + cystine min. 2.5 %; lysine min. 4 %, threonine min. 1.5 % and metabolizable energy min. 13.6 MJ·kg^{-1}.

Mineral and vitamin mixture Kuškovit (Kušić promet) comprising: vitamin A 270 000 IU·kg^{-1}, vitamin D_3 40 000 IU·kg^{-1}, vitamin E 600 mg·kg^{-1}, vitamin K_3 50 mg·kg^{-1}, biotin 2 mg·kg^{-1}, thiamine 20 mg·kg^{-1}, riboflavin 95 mg·kg^{-1}, pantothenic acid 245 mg·kg^{-1}, pyridoxine 40 mg·kg^{-1}, niacin 800 mg·kg^{-1}, vitamin B_{12} 300 mg·kg^{-1}, vitamin C 300 mg·kg^{-1}, choline 10 000 mg·kg^{-1}, folic acid 10 mg·kg^{-1}. Mineral content in Kruškovit is manganese 1 500 mg·kg^{-1}, iron 725 mg·kg^{-1}, zinc 1 000 mg·kg^{-1}, copper 80 mg·kg^{-1} and iodine 15 mg·kg^{-1}.

1.11.1.6) and content of glutathione (GSH) and lipid peroxides (LPO) were determined.

GSH-Px and SOD activities were assayed on a SABA 18 (AMS, Roma, Italy) automatic analyser using the commercial Ransel and Ransod reagent kits (both Randox, Crumlin, United Kingdom), respectively. CAT activity, GSH and LPO content were determined using spectrophotometer Thermospectronic Helios delta (Unicam, Cambridge, United Kingdom) by previously published methods [32–34]. Selenium content in chicken muscles was determined by inductively coupled plasma-mass spectrometry technique (ICP-MS) in Agilent 7500cx (Agilent Technologies, Santa Clara, California, USA). Enzyme activities and lipid peroxides content were calculated per gram of protein. Selenium content was expressed per gram of muscle tissue.

Results (N = 14 for each group for enzymes activities and content of selenium, glutathione and lipid peroxide; N = 90 for each group for body weight, weight gain, feed consumption and feed conversion ratio) were statistically processed using Statistica 9 software (StatSoft, Palo Alto, California, USA) and expressed as mean ± standard deviation. The significance of differences was tested by variance analysis and post hoc Tukey's test if the distribution of data was normal, or by Kruskal-Wallis variance and Wilcoxon T-test analysis if the null hypothesis was rejected. The level of statistical significance was set at $p \leq 0.05$.

RESULTS

The body weight, weight gain, feed consumption and feed conversion ratio in the control group fed diets supplemented with inorganic and in groups fed diets supplemented with organic selenium did not differ significantly (Tab. 2).

Values of the SOD activity are presented in Tab. 3. On the day of the slaughter, SOD activ-

Tab. 2. Body weight, weight gain, feed consumption and feed conversion ratio of chicken fed feed with sodium selenite or organic selenium.

Group	Body weight [g]			Weight gain [g]		Feed consumption [g]		Feed conversion ratio	
	1st day	21nd day	42nd day	1–21 days	22–42 days	0–21 days	22–42 days	0–21 days	22–42 days
inorg$Se_{0.3}$	42.4 ± 0.3^a	801.5 ± 10.2^a	2668.2 ± 44.8^a	759.0 ± 10.2^a	1865.0 ± 45.0^a	1173.3	3681.3	1.55	1.97
org$Se_{0.3}$	42.0 ± 0.3^a	825.2 ± 9.4^a	2682.1 ± 48.1^a	783.2 ± 9.4^a	1822.9 ± 47.3^a	1264.5	3716.1	1.61	2.10
org$Se_{0.5}$	41.5 ± 0.3^a	795.2 ± 7.8^a	2660.3 ± 37.1^a	751.9 ± 7.9^a	1867.2 ± 37.1^a	1249.3	3538.5	1.66	1.94

Results are expressed as mean ± standard error for body weight and gain, and mean for feed consumption (N = 90 for each group). Different letters in superscripts refer to a significant difference between control and organic selenium groups at $p \leq 0.05$. inorg$Se_{0.3}$ – sodium selenite 0.3 mg·kg^{-1} of feed, org$Se_{0.3}$ – organic selenium 0.3 mg·kg^{-1} of feed, org$Se_{0.5}$ – organic selenium 0.5 mg·kg^{-1} of feed.

Tab. 3. Superoxide dismutase activity in chicken breast and thigh muscles during storage.

	Superoxide dismutase activity [U·g^{-1}]					
Storage temperature	+4 °C				–20 °C	
Sampling time	0th day	1st day	3rd day	7th day	30th day	90th day
m. pectoralis superficialis						
inorg$Se_{0.3}$	1397 ± 85^a	1280 ± 58^a	1500 ± 135^a	1199 ± 71^a	943 ± 51^a	1291 ± 77^a
org$Se_{0.3}$	2019 ± 114^b	1390 ± 68^a	1192 ± 45^b	1106 ± 57^a	833 ± 27^a	1599 ± 87^a
org$Se_{0.5}$	1821 ± 89^b	1371 ± 47^a	1213 ± 42^a	1182 ± 49^a	878 ± 36^a	1555 ± 72^a
m. gastrocnemius						
inorg$Se_{0.3}$	2244 ± 136^a	1115 ± 96^a	1340 ± 75^a	2151 ± 105^a	2759 ± 133^a	1749 ± 126^a
org$Se_{0.3}$	2374 ± 132^a	1725 ± 120^b	1303 ± 54^a	2404 ± 79^a	3009 ± 173^a	2574 ± 136^b
org$Se_{0.5}$	2506 ± 208^a	1736 ± 162^b	1262 ± 74^a	2096 ± 87^a	2979 ± 202^a	2533 ± 96^b

Results are calculated per gram of protein and expressed as mean ± standard error (N = 14 for each group). Different letters in superscript refer to a significant difference between control and organic selenium groups at $p \leq 0.05$.
inorg$Se_{0.3}$ – sodium selenite 0.3 mg·kg^{-1} of feed, org$Se_{0.3}$ – organic selenium 0.3 mg·kg^{-1} of feed, org$Se_{0.5}$ – organic selenium 0.5 mg·kg^{-1} of feed.

Tab. 4. Glutathione peroxidase activity in chicken breast and thigh muscles during storage.

	Glutathione peroxidase activity [U·g^{-1}]					
Storage temperature	+4 °C				–20 °C	
Sampling time	0th day	1st day	3rd day	7th day	30th day	90th day
m. pectoralis superficialis						
inorgSe$_{0.3}$	43.3 ± 2.7^{a}	44.2 ± 1.3^{a}	55.8 ± 6.1^{a}	42.3 ± 3.0^{a}	49.1 ± 4.1^{a}	42.1 ± 3.1^{a}
orgSe$_{0.3}$	49.9 ± 2.6^{a*}	43.3 ± 2.4^{a}	43.8 ± 3.5^{a}	42.6 ± 2.9^{a}	43.9 ± 3.2^{a}	52.79 ± 4.3^{a}
orgSe$_{0.5}$	60.9 ± 4.1^{b}	49.9 ± 3.9^{a}	46.3 ± 2.8^{a}	47.7 ± 2.3^{a}	49.3 ± 4.9^{a}	52.7 ± 3.2^{a}
m. gastrocnemius						
inorgSe$_{0.3}$	90.9 ± 5.3^{a}	43.1 ± 4.3^{a}	78.1 ± 4.5^{a}	108.1 ± 7.8^{a}	127.0 ± 6.2^{a}	93.7 ± 4.9^{a}
orgSe$_{0.3}$	90.3 ± 3.6^{a*}	61.3 ± 5.9^{a}	67.5 ± 3.2^{a}	104.0 ± 5.8^{a}	110.0 ± 7.2^{a}	101.6 ± 5.3^{a}
orgSe$_{0.5}$	115.2 ± 9.3^{b}	68.0 ± 6.3^{b}	61.2 ± 4.1^{a}	92.5 ± 6.9^{a}	122.8 ± 9.5^{a}	100.7 ± 7.6^{a}

Results are calculated per gram of protein and expressed as mean±standard error (N = 14 for each group). Different letters in superscript refer to a significant difference between control and organic selenium groups at $p \leq 0.05$, * – significant difference between two organic selenium groups at $p \leq 0.05$.
inorgSe$_{0.3}$ – sodium selenite 0.3 mg·kg^{-1} of feed, orgSe$_{0.3}$ – organic selenium 0.3 mg·kg^{-1} of feed, orgSe$_{0.5}$ – organic selenium 0.5 mg·kg^{-1} of feed.

Tab. 5. Glutathione content in chicken breast and thigh muscles during storage.

	Glutathione [μmol·g^{-1}]					
Storage temperature	+4 °C				–20 °C	
Sampling time	0th day	1st day	3rd day	7th day	30th day	90th day
m. pectoralis superficialis						
inorgSe$_{0.3}$	27.6 ± 2.7^{a}	26.4 ± 1.5^{a}	46.7 ± 5.2^{a}	36.8 ± 2.6^{a}	33.5 ± 2.8^{a}	5.0 ± 0.9^{a}
orgSe$_{0.3}$	25.3 ± 1.3^{a}	22.7 ± 1.3^{a}	29.6 ± 1.5^{b}	32.3 ± 3.2^{a}	26.2 ± 2.4^{a}	6.8 ± 1.2^{a}
orgSe$_{0.5}$	26.1 ± 1.4^{a}	29.1 ± 2.5^{a}	29.0 ± 2.0^{b}	29.3 ± 1.6^{a}	35.98 ± 3.3^{a}	6.7 ± 1.1^{a}
m. gastrocnemius						
inorgSe$_{0.3}$	39.0 ± 2.6^{a}	21.7 ± 1.3^{a}	43.3 ± 2.3^{a}	37.3 ± 2.2^{a}	54.7 ± 3.7^{a}	8.7 ± 1.1^{a}
orgSe$_{0.3}$	34.9 ± 2.8^{a*}	25.5 ± 1.7^{a*}	36.3 ± 2.3^{a}	35.8 ± 3.4^{a}	54.6 ± 3.4^{a}	12.7 ± 1.6^{a}
orgSe$_{0.5}$	47.3 ± 3.5^{b}	35.9 ± 2.7^{b}	37.1 ± 2.3^{a}	45.5 ± 2.6^{a}	49.8 ± 3.7^{a}	13.4 ± 1.4^{a}

Results are calculated per gram of protein and expressed as mean ± standard error (N = 14 for each group). Different letters in superscript refer to a significant difference between control and organic selenium groups at $p \leq 0.05$, * – significant difference between two organic selenium groups at $p \leq 0.05$.
inorgSe$_{0.3}$ – sodium selenite 0.3 mg·kg^{-1} of feed, orgSe$_{0.3}$ – organic selenium 0.3 mg·kg^{-1} of feed, orgSe$_{0.5}$ – organic selenium 0.5 mg·kg^{-1} of feed.

ity in breast meat of orgSe$_{0.3}$ and orgSe$_{0.5}$ was significantly higher than in control group (p = 0.001 both). Dietary organic selenium supplementation led to a significantly higher SOD activity in thigh muscles of both experimental groups; on the first day of storage (p = 0.007 for orgSe$_{0.3}$, p = 0.006 for orgSe$_{0.5}$). Dietary organic selenium supplementation in contents of 0.3 mg and 0.5 mg per kilogram of feed resulted in significantly higher SOD activity on 90th day of storage (p = 0.001 both).

Tab. 4 shows GSH-Px activity. Activity of this enzyme in *m. pectoralis superficialis* was significantly higher in orgSe$_{0.5}$ (p = 0.032) on the day of slaughter than in the control and orgSe$_{0.3}$ groups (p = 0.050 both). Thigh muscle GSH-Px activity in orgSe$_{0.5}$ was significantly higher than in the control and orgSe$_{0.3}$ groups immediately after slaughter and chilling (p = 0.034 and p = 0.028, respectively), and on the first day of storage for orgSe$_{0.5}$ group (p = 0.001).

Tissue content of glutathione is presented in Tab. 5. Content of GSH in *m. pectoralis superficialis* on third day of storage in control group was significantly higher compared to orgSe$_{0.3}$ and orgSe$_{0.5}$ (p = 0.001 both). Higher GSH content in both muscles in orgSe$_{0.5}$ than in orgSe$_{0.3}$ was found on the first day of storage (p = 0.020 both). Content of GSH in thigh muscle was significantly higher in orgSe$_{0.5}$ on the day of slaughter and

Tab. 6. Catalase activity in chicken breast and thigh muscles during storage.

	Catalase [U·g^{-1}]					
Storage temperature	+4 °C				–20 °C	
Sampling time	0th day	1st day	3rd day	7th day	30th day	90th day
m. pectoralis superficialis						
inorgSe$_{0.3}$	5.5 ± 1.0^{a}	8.0 ± 1.0^{a}	10.9 ± 7.2^{a}	6.3 ± 0.9^{a}	10.5 ± 1.1^{a}	8.2 ± 1.0^{a}
orgSe$_{0.3}$	5.1 ± 0.9^{a}	5.5 ± 0.9^{a}	6.6 ± 1.0^{a}	4.6 ± 0.8^{a}	8.2 ± 1.3^{a}	10.4 ± 1.2^{a}
orgSe$_{0.5}$	6.1 ± 0.9^{a}	6.5 ± 1.1^{a}	8.5 ± 1.3^{a}	6.5 ± 0.7^{a}	8.5 ± 0.9^{a}	8.7 ± 0.7^{a}
m. gastrocnemius						
inorgSe$_{0.3}$	26.7 ± 3.9^{a}	8.7 ± 1.4^{a}	19.3 ± 2.6^{a}	12.7 ± 1.7^{a}	24.6 ± 2.8^{a}	4.8 ± 1.1^{a}
orgSe$_{0.3}$	30.2 ± 3.6^{a*}	16.6 ± 3.4^{a}	12.1 ± 1.5^{a}	13.9 ± 1.4^{a*}	24.3 ± 3.1^{a}	9.3 ± 1.5^{b*}
orgSe$_{0.5}$	53.71 ± 7.9^{b}	35.9 ± 2.7^{a}	13.8 ± 1.5^{a}	7.8 ± 1.4^{a}	17.1 ± 2.1^{a}	5.1 ± 0.5^{a}

Results are calculated per gram of protein and expressed as mean ± standard error (N = 14 for each group). Different letters in superscript refer to a significant difference between control and organic selenium groups at $p \leq 0.05$, * – significant difference between two organic selenium groups at $p \leq 0.05$.
inorgSe$_{0.3}$ – sodium selenite 0.3 mg·kg^{-1} of feed, orgSe$_{0.3}$ – organic selenium 0.3 mg·kg^{-1} of feed, orgSe$_{0.5}$ – organic selenium 0.5 mg·kg^{-1} of feed.

Tab. 7. Lipid peroxide content in chicken breast and thigh muscles during storage.

	Lipid peroxide [nmol·g^{-1}]					
Storage temperature	+4 °C				–20 °C	
Sampling time	0th day	1st day	3rd day	7th day	30th day	90th day
m. pectoralis superficialis						
inorgSe$_{0.3}$	99.0 ± 10.8^{a}	123.6 ± 6.8^{a}	166.5 ± 17.8^{a}	79.7 ± 4.2^{a}	153.7 ± 27.6^{a}	145.6 ± 9.5^{a}
orgSe$_{0.3}$	110.1 ± 9.5^{a*}	110.7 ± 6.3^{a}	90.8 ± 2.8^{b}	68.8 ± 3.1^{b}	199.9 ± 9.1^{a}	152.7 ± 11.4^{a}
orgSe$_{0.5}$	150.3 ± 8.6^{b}	114.5 ± 5.4^{a}	102.4 ± 5.3^{b}	74.8 ± 3.9^{ab}	126.9 ± 11.2^{a}	143.7 ± 11.1^{a}
m. gastrocnemius						
inorgSe$_{0.3}$	93.1 ± 6.5^{a}	131.2 ± 15.2^{a}	103.5 ± 5.8^{a}	191.5 ± 12.9^{a}	163.9 ± 30.7^{a}	201.2 ± 17.3^{a}
orgSe$_{0.3}$	101.7 ± 13.1^{a}	117.7 ± 7.6^{a}	107.7 ± 11.2^{a}	233.1 ± 34.8^{a}	189.9 ± 29.4^{a}	253.2 ± 13.9^{b}
orgSe$_{0.5}$	116.8 ± 8.9^{a}	115.1 ± 9.0^{a}	127.6 ± 12.8^{a}	293.9 ± 23.6^{a}	173.7 ± 11.2^{a}	219.6 ± 10.0^{a}

Results are calculated per gram of protein and expressed as mean ± standard error (N = 14 for each group). Different letters in superscript refer to a significant difference between control and organic selenium groups at $p \leq 0.05$, * - significant difference between two organic selenium groups at $p \leq 0.05$.
inorgSe$_{0.3}$ – sodium selenite 0.3 mg·kg^{-1} of feed, orgSe$_{0.3}$ – organic selenium 0.3 mg·kg^{-1} of feed, orgSe$_{0.5}$ – organic selenium 0.5 mg·kg^{-1} of feed.

on first day of storage compared to control group (p = 0.050 and p = 0.001, respectively). We found a pronounced decrease in GSH in both muscles in all groups after 90 days of freezing.

Tab. 6 shows CAT activity in chicken tissue. CAT activity in breast muscle was not significantly changed during this experiment. Activity of CAT was higher in thigh muscles of orgSe$_{0.5}$ group immediately after slaughter compared to orgSe$_{0.3}$ and control groups (p = 0.025 and p = 0.004, respectively). Higher activity of CAT was determined in orgSe$_{0.5}$ than in orgSe$_{0.3}$ on the 7th day of storage (p = 0.025). CAT activity determined after 90 days of storage was significantly higher in orgSe$_{0.3}$ than in control and orgSe$_{0.5}$ groups (p = 0.046 and p = 0.036, respectively).

Lipid peroxidation intensity according to different selenium forms and contents in the diet is presented in Tab. 7. Lipid peroxidation after slaughter was significantly higher in breast muscle of orgSe$_{0.5}$ group compared to control and orgSe$_{0.3}$ groups (p = 0.003 and p = 0.025, respectively). On the third and 7th day of storage, the LPO content in breast muscle of control group was significantly higher than in organic selenium supplemented groups (p = 0.002 both). Lipid peroxidation processes in meat during storage intensified in particular in the thigh muscle, being significantly higher in orgSe$_{0.3}$ group than in the control (p = 0.006).

Data on selenium content are presented in Fig. 1. The content of selenium in breast muscles on the day of slaughter was significantly higher in groups fed organic selenium compared to inorgSe$_{0.3}$ group (p = 0.001 both). Selenium content in thigh muscles was significantly higher only in orgSe$_{0.5}$ group (p = 0.001).

DISCUSSION

Oxidative stress and the changes it incurs on fatty acids and proteins can change the smell, taste, texture and consistency of meat, which diminishes its nutritional quality [35, 36]. Therefore, proper chilling and storage procedures are of crucial importance for maintaining meat quality and it processing characteristics. Meat antioxidative systems, both enzymatic and non-enzymatic, have protective effect during chilling and freezing [37].

In this study, the results of the final weight and consumption of food in general coincide with the results found in literature [15, 16, 20] that show no difference in weight gain or feed consumption of chicken fed feed with different content of selenium as sodium selenite or selenomethionine. Furthermore, present results confirm results of Bostani et al. [38] and Skřivan et al. [39] who proved that different forms of selenium, oxidative stress and their interactions had no effect on the feed intake, daily body gain and feed conversion ratio. Contrary to our results, Upton et al. [10] showed that the final weight of chicken and the feed intake and gain were greater in the group receiving selenized yeasts compared to the group treated with inorganic selenium. Also, Yang et al. [40] found that organic selenium increased daily intake and food conversion.

SOD activity in breast and thigh muscles was higher in both groups that were fed feed supplemented with organic selenium. In breast muscles, SOD activity was higher on the day of slaughter and after 1 day of storage, whereas in thigh meat, SOD activity was higher on 1st, 7th and 30th day of refrigerated storage, which is in accordance with results of Jiang et al. [41] and Wang et al. [42] for SOD activity in breast muscles. Also, SOD activity in breast muscles in the present study continuously decreased during refrigerated storage, while SOD activity was higher in frozen meat, in particular in thigh muscles (Tab. 3). This could be ascribed to different types of metabolism, as SOD activity is associated with the numbers of mitochondria [43].

A higher GSH-Px activity was found in this study in orgSe$_{0.5}$ group on the day of slaughter in breast muscles and in thigh muscles immediately after chilling as well as on the first day of storage. These findings are in agreement with the results of Rajashree et al. [6] who showed that 0.5 mg·kg^{-1} of organic selenium could be an excellent source of selenium as it improved the meat quality through enhanced selenium retention, higher GSH-Px activity and decreased lipid peroxidation rate. Different from the above results, Dlouhá et al. [4] found the highest activity of GSH-Px in breast muscle of broilers fed feed supplemented with sodium selenite.

Selenium is essential for catalytic functions of GSH-Px, while research done by Kuricová et al. [14], Choct et al. [15] and Payne and Southern [16] showed that organic selenium is more effectively deposited in muscles than inorganic selenium, which is in accordance with higher selenium content (Fig. 1) and GSH-Px activity we found in muscles of orgSe$_{0.5}$ group in this study (Tab. 4). In this way, oxidative stability of chicken muscle is enhanced [44, 45] because selenium can elevate and maintain GSH-Px muscle activity, while it also preserves cell membrane integrity. The results of the present study showed that GSH-Px activity was higher in thigh than *m. pectoralis superficialis*, i.e. higher in oxidative than glycolytic muscles. DeVore et al. [46] and Daun and Akesson [17] found GSH-Px activity to be higher in oxidative muscles, due to different metabolism and, therefore, different susceptibility towards oxidative damage [17] during life and *post mortem* [37]. Tissues containing higher amounts of antioxidative enzymes would be expected to be more stable towards lipid peroxidation but, because they contain

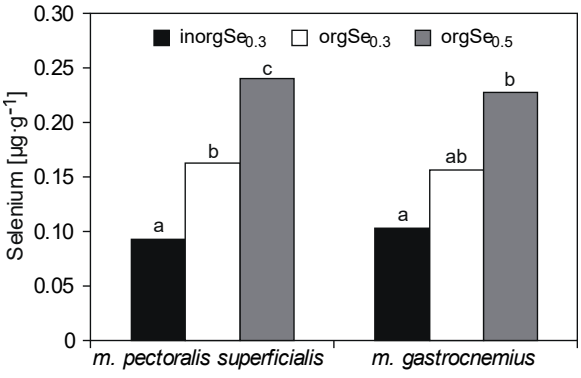

Fig. 1. Selenium content in chicken breast and thigh muscles.

Results are calculated per gram of protein and expressed as mean ± standard error (N = 14 for every group). Different letters above columns refer to a significant difference between control and organic selenium groups at $p \leq 0.05$. inorgSe$_{0.3}$ – sodium selenite 0.3 mg·kg^{-1} of feed, orgSe$_{0.3}$ – organic selenium 0.3 mg·kg^{-1} of feed, orgSe$_{0.5}$ – organic selenium 0.5 mg·kg^{-1} of feed.

more fats and iron that could be pro-oxidative, they are more susceptible to peroxidation [17]. Therefore, supplementing feed with selenium can delay the process of lipid peroxidation [46].

Selenium deposition in breast muscle in this research was proportional to the amount of organic selenium added to feed and significantly higher in thigh muscle only when 0.5 mg·kg^{-1} organic selenium was added. Enhanced selenium deposition in muscles was found by Payne and Southern [16], Leeson et al. [47] in chicken and hens, Mikulski et al. [48] and Cantor et al. [49] in turkeys with the amount of deposition in pectoral muscles being correlated to the amount added in feed according to Krstić et al. [50] and Baltic et al. [51] in breast and thigh muscle of ducks. Furthermore, Zia et al. [52] showed that selenium content in chest and thigh in Se-yeast fed birds were significantly increased compared to sodium selenite group.

Catalase activity in *m. pectoralis superficialis* in this study was independent of both the form and the amount of selenium added to the feed. This finding is contrary to Jiang et al. [41] who found elevated activity of this enzyme due to the added dietary selenomethionine in broiler feed. In thigh muscles in this study, catalase activity was higher immediately after slaughter and on first day of storage when 0.5 mg·kg^{-1} organic selenium was added, which is similar to results of Hala et al. [22]. The enzyme was stable in pectoral muscles during the storage, while the activity in thigh muscles decreased. This might have been be due to aforementioned metabolic differences between muscle types [37, 43, 53–55]. In nandu (*Rhea americana*) meat [56] as well as in chicken, beef and pork meat [37], catalase was stable during 2–3 months at –20 °C. Its role in meat oxidative stability seems, therefore, quite important.

The content of GSH was affected in both muscles of chicken in org$Se_{0.5}$ group on the first day of storage, when its content was elevated. After 90 days of storage at –20 °C, GSH contents decreased 4–5 times in all of the groups. This is in accordance with Nair and Latha [57]. The decline is known to be negatively correlated to temperature and duration of the storage [58]. Decrease in GSH content was suggested to cause decrease in GSH-Px activity and decrease in oxidative stability of meat. GSH functions directly in neutralization of free radicals and serves in the maintenance of the reduced forms of the antioxidant vitamin C and vitamin E, which are important for meat quality [31].

The changes that take place after the slaughter affect the balance between pro- and antioxidative molecules, due to changes in their contents. In this study, no protective effect of organic selenium supplementation was determined on oxidative stability in thigh and breast muscles during storage. After the slaughter, lipid peroxidation in both muscles was more intensive in groups fed feed supplemented with organic selenium (Tab. 7). Similar results were reported by Dokupilová et al. [59] in thigh muscles of rabbits. Positive effect of organic selenium in this research was observed in breast muscles on the 3rd and 7th day of storage. Moreover, increased oxidative stability of meat with organic selenium supplementation was determined by Skřivan et al. [39], Dlouhá et al. [4], Dhumal et al. [5] and Boiago et al. [7] in chicken as well as by Mikulski et al. [48] in turkeys.

In conclusion, feed supplementation with organic selenium resulted in selenium enrichment in muscles. Addition of organic selenium at 0.3 mg·kg^{-1} and 0.5 mg·kg^{-1} of feed had protective effect on the oxidative stability noticeable during the first few days of storage, with more pronounced effects in the breast muscle.

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