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Research article

IMPROVEMENT OF ANTIOXIDATIVE ACTIVITY OF BROILER MUSCLES AFTER DIETARY MODULATION WITH SELENIUM AND METHIONINE

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The objective of the study was to compare the antioxidative capacity of broiler chicken breast and leg muscles after dietary modulation with selenium (Se) and methionine (Met). Free radical scavenging (ABTS, DPPH) and iron reduction (FRAP) activities were determined as the total antioxidative potential (TEAC), as well the enzyme activity of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx), in relation to concentrations of lipid peroxidation end products (TBARS). Analyses were performed on breast m. *pectoralis superficialis* and *profundus* individually and together. The studied leg muscles included *biceps femoris, gastrocnemicus, iliotiobialis, peroneus longus, sartorius, semimembranosus, semitendinosus* and all leg muscles together. Flex broiler chickens were fed diets supplemented with 6.7, 8.2, 9.7 and 11.2 g DL-methionine/kg feed and Se as sodium selenite and selenized yeast at 0.26, 0.38 and 0.50 mg Se/kg. Greater TEAC and enzyme activities were observed in leg than in breast muscles. Selenium did not change TEAC in muscles sets, but improved antiradical capacity in the *pectoralis major* and *minor, sartorius* and *biceps femoris*. The highest level of methionine increased TEAC in individual leg muscles. Selenium and methionine at the highest concentrations increased SOD activity in the entire group and individual muscles, while Se raised GPx activity. In conclusion, the diet supplementation with selenium and high concentrations of methionine had a greater impact on the antioxidative potential of individual than the whole set of chicken breast and leg muscles. The positive effect of the studied diet modulation could raise the quality and extend the shelf-life of fresh chicken meat.

Keywords: antioxidative enzymes, chicken individual muscles, methionine, selenium, total antioxidative potential

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INTRODUCTION

The total antioxidative potential (TEAC) of muscles, created by enzymatic (catalase (CAT), superoxide dismutase (SOD) and selenium dependent glutathione peroxidase [GPx]) and non-enzymatic (vitamin E, ubiquinols, cellular thiols) systems can be modified by animal feed [1–5]. Despite selenium (Se) being one of the most intensively studied components of animal diets, there are still inconsistent data on the effectiveness of Se forms (organic vs inorganic) on the activity of GPx and radicals scavenging capacity (ABTS, DPPH) and iron reduction (FRAP) [6–8]. Kuricova et al. (2003) did not find any differences between Se forms, whereas Mahmoud et al. (2003) reported that organic Se was more effective in reducing oxidative stress [9,10]. Methionine, a limiting amino acid in the diets of growing chickens due to its role in glutathione synthesis can also affect the antioxidative potential [11,12]. Most research conducted on TEAC has been focused on whole muscle groups i.e. breast muscles with anaerobic metabolism, containing more protein but lower fat and ferric compounds, and leg muscles with aerobic metabolism. The greatest morphological and physiological differences have been observed between individual muscles in the whole group, indicating varied susceptibility to oxidation [13]. Thus, it would be crucial to recognize the antioxidative potential of separate muscles collected from chicken breast and leg. The poultry meat industry is currently the key sector in Polish, as well as world, economy. Both, production and consumption of poultry meat is continuously growing and it is the most perspective for the future due to high economical efficacy and no religious and diet objections. The objective of the study was to compare the individual and whole groups of breast and leg muscles of broiler chickens in terms of antioxidative capacity after dietary modulation with selenium (Se) and methionine (Met). The study could be used to simplify the prediction of the quality and shelf-life of fresh meat.

MATERIAL AND METHODS

Animals and diets

All procedures carried out on the animals were approved by the Local Ethics Committee for animal experimentation.

A total of 165 one day old male Flex broiler chickens (Hubbard, Poland) (40.0 g ± 2.0 g) were reared in floor pens with sawdust litter. The environmental temperature during the experiment was gradually reduced from 32 to 21°C and the lighting program consisted of 24 h light for the first 10 days, then 6 h darkness daily. Relative humidity varied between 64 and 70%. Birds had unlimited access to drinking water and feed composed of a mashed form of maize, wheat and soya beans.

After two weeks the chickens were randomly allocated into 11 groups for different feed treatments: (I) fed with a control (non-supplemented) feed mixture (negative

Table 1. Composition of experimental diets

			Grower									
			Methionine				Organic Se			Inorganic Se		
	Starter	Control	Met 6.7 g	Met 8.2 g	Met 9.7 g	Met 11.2 g	0.26 mg	0.38 mg	0.50 mg	0.26 mg	0.38 mg	0.50 mg
Mashed maize	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Mashed wheat	39.7	43.4	43.1	42.8	42.5	42.1	43.2	43.2	43.1	43.3	43.3	43.3
Soy oil	3.30	4.70	4.80	4.90	5.00	5.10	4.80	4.80	4.80	4.80	4.80	4.80
Soy bean meal	32.4	27.3	27.3	27.4	27.5	27.6	27.3	27.3	27.3	27.3	27.3	27.3
Selenium yeast	/	/	/	/	/	/	0.037	0.075	0.113	/	/	/
Sodium selenite	/	/	/	/	/	/	/	/	/	0.001	0.002	0.003
Chalk	0.37	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Premix dka-s/g	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
DL-methionine 98%	0.232	0.228	0.381	0.534	0.687	0.840	0.228	0.228	0.228	0.228	0.228	0.228
EM (MJ)*	12.5	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0
Crude protein (g)	227	205	199	200	196	199	202	206	200	206	203	197
Crude fibre (g)	28.7	27.8	27.8	27.7	27.6	27.6	27.8	27.8	27.8	27.8	27.8	27.8
Crude fat (g)	52.1	66.7	66.8	69.9	70.4	71.7	67.9	67.1	68.7	68.7	68.1	68.8
Lysine (g)	12.6	11.4	11.6	11.5	11.7	11.7	11.4	11.5	11.8	11.7	11.5	11.4
Methionine (g)	5.42	5.17	6.73	8.18	9.87	11.10	5.29	5.30	5.26	5.14	5.25	5.14
Ca (g)	9.30	9.19	9.31	9.11	9.31	9.24	9.04	9.32	9.33	9.26	9.14	9.28
P (g)	4.80	4.64	4.68	4.69	4.66	4.78	4.74	4.65	4.63	4.73	4.63	4.64
Na (g)	1.72	1.68	1.70	1.73	1.67	1.67	1.67	1.72	1.69	1.71	1.73	1.70
Se (mg)	0.154	0.143	0.139	0.141	0.140	0.144	0.257	0.370	0.503	0.265	0.387	0.497

*1 kg of premix provided: vitamin A (retinyl acetate) 10,000 IU; vitamin D_3 (cholecalciferol) 2,000 IU; vitamin. E (DL-α-tocopheryl acetate) 20 mg; vitamin K (hetrazeen vit. K_3 free from menadione) 3 mg; vitamin B_1 (thiamine mononitrate) 2.5 mg; vitamin B_6 (pyridoxine HCl) 0.4 mg; vitamin B_{12} (cyanocobalamin) 0.015 mg; choline (choline chloride) 450 mg; folic acid 1.2 mg; nicotic acid 25 mg; panthothenic acid 8 mg; DL-methionine 1.0 mg, Fe (as $FeSO_4 \cdot H_2O$) 30 mg; Mn (as MnO_2) 74 mg; Cu (as $CuSO_4 \cdot 5\ H_2O$) 4 mg; Zn (as ZnO) 45 mg; I (as $Ca(IO_3)_2$) 0.3 mg; Co (as $CoCO_3$) 0.4 mg.

control); (II) fed with a basal diet with added 6.7 g DL-methionine per kg, (III) one kg of the diet containing 8.2 g DL-methionine, (IV) fed with 9.7 g DL-methionine per kg, (V) provided with 11.2 g DL-methionine in kg feed, (VI) supplied with a basal diet enriched with 0.26 mg/kg organic selenium (selenium yeast), (VII) diet containing 0.38 mg organic selenium per kg, (VIII) supplemented with 0.50 mg/kg organic selenium, (IX) with diet containing non-organic selenium (sodium selenite, Sigma-Aldrich) at the same levels as the organic form i.e. 0.26 mg/kg, (X) containing 0.38 mg inorganic selenium in one kg of the diet, and (XI) fed with 0.5 mg inorganic selenium per kg feed. The feed additives, selenium and methionine, concentrations used in the study were selected according to literature data and good husbandry practice with a special respect to animal welfare. It was also assumed that the highest concentration of selenium (0.5 mg/kg) and methionine (11.6 g/kg) did not reach toxic levels for chickens. All diets contained a selenium free vitamin premix (Table 1).

From day 1 to day 14 the chicks were supplied with a starter diet, then from day 22 to day 42 with grower feed mixtures. The crude protein content in the starter diet was about 220 g/kg, whilst in the grower feed about 200 g/kg. The energy value was calculated at the mean level of 12.2 and 12.7 MJ/kg, respectively. The feed mixtures were chemically analyzed with all the components listed in Table 1.

Muscle collection and preparation

After 42 days of the study all birds were slaughtered by spine dislocation. Breast and leg components were harvested manually, immediately chilled on ice and stored at -80ºC. Directly before analyses muscles were defrosted to -2ºC. Muscles from the left side of chicken carcass were analyzed as a whole group, whereas from the right half the following individual muscles were removed: *pectoralis superficialis* and *profundus* from the breasts and *biceps femoris, gastrocnemicus, iliotiobialis, peroneus longus, sartorius, semimembranosus* and *semitendinosus* from legs. All collected muscles were weighed and analyzed for protein content (Kjeltec TM2300 *FOSS,* Denmark). Hydrophilic fractions of the samples were prepared by homogenization of muscles in redistilled water (Mixer B-400 Buchi, Switzerland) and centrifuging, then used in total antioxidative capacity assays (ABTS, DPPH, FRAP, TBARS) [14].

Total antioxidative capacity (TEAC)

ABTS$^+$ assay

The total antioxidant capacity of the hydrophilic fraction of chicken breast and leg muscles was analyzed by Trolox-equivalent antioxidant capacity (TEAC) assay [15]. The formation of ABTS$^+$ radical cations was initiated by reacting 14 mM ABTS [2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)] (Sigma–Aldrich, Poland) with an equal volume of 4.9 mM potassium persulfate, followed by incubation in the dark at room temperature for 12–16 h. Before analysis the absorbance of the ABTS$^+$ solution was measured at 734 nm and adjusted to 0.700 (±0.02) by using 5.5 mM

PBS (pH 7.4, temp. 30°C). An aliquot of 10 µl meat homogenate or Trolox standard (0–1.2 mM in PBS) (Fluka Chemie Gmbh., Switzerland) was added to 1.0 ml of the $ABTS^+$ solution, mixed thoroughly and after 60 sec absorbance was measured at 734 nm, followed by a second measurement taken after 6 min of incubation at 30°C. The percentage inhibition of the blank absorbance was calculated for each Trolox standard reference and meat sample, respectively.

DPPH+ assay

Scavenging activity of the muscle samples was analyzed towards 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) (Sigma–Aldrich, Poland) [16,17]. The supernatant was collected after homogenization of the meat samples for 2 min at 3,000 rpm (B-400 Buchi, Germany) in distilled water and centrifuging at 5,000 G for 15 min. The supernatant was mixed with ethanol and DPPH radical solution and incubated at room temperature in the dark for 10 min before taking the absorbance measurement at 517 nm. The ability to scavenge the DPPH radical was expressed as µM Trolox per g wet muscle tissue.

FRAP assay

Ferric reducing antioxidant power (FRAP) assay was carried out on meat samples homogenized for 2 min at 3,000 rpm (B-400 Buchi, Germany) in potassium phosphate buffer (pH 7.2), centrifuged at 5,000 G for 15 min, and the supernatant collected [18]. Next, 1 ml aliquots were added to 3 ml FRAP buffer containing 10 mM TPTZ (2,4,6-Tris(2-pyridyl)-*s*-triazine) (Sigma–Aldrich, Poland) in 40 mM HCl and 20 mM Fe_2Cl_3 (Sigma–Aldrich, Poland) and added to 300 mM acetate buffer. Immediately after mixing the absorbance was measured at 593 nm. The antioxidant power of the samples was expressed as µM of Fe^{2+} per g wet muscle tissue.

TBARS assay

The extent of lipid oxidation was analyzed by thiobarbituric acid reactive substance (TBARS) assay [19]. Briefly, a 1.0 g of selected muscle tissue was homogenized with 10 ml of distilled water, then trichloroacetic acid (10 ml; 10%, w/v) was added to precipitate proteins. 4 ml of filtered (Whatman N1 filter paper) samples was mixed with 1 ml of 0.06 M thiobarbituric acid. Samples were incubated at 80 °C for 60 min and the absorbance was measured at 532 nm. The assay was calibrated using standard solutions of 1,1,3,3,-tetra-ethoxypropane in trichloroacetic acid. Results are expressed as mg of malondialdehyde (MDA) per kg wet muscle tissue.

Activity of antioxidant enzymes

Catalase (CAT, EC1.11.1.6) activity was measured per Aebi (1983) based on the hydrolysis of H_2O_2 by the enzyme and monitored for changes in absorbance at 240

nm during the initial 30 s. [20]. Catalase activity in muscles was determined after homogenization (0.05 M phosphate buffer, pH 7.0) and centrifugation at 7,000 G [21]. One unit (U) of catalase activity was defined as the amount of wet muscle tissue needed to decompose 1 mM of H_2O_2 in 1 min.

Superoxide dismutase (SOD, EC1.15.1.1) activity was measured using a Cayman Superoxide Dismutase Assay Kit No. 7060002, based on the utilization a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase. The muscles were homogenized with 20 mM cold HEPES containing 1 mM EGTA, 210 mM mannitol and 70 mM sucrose (pH 7.2), and centrifuged at 1,500 G. Absorbance of the collected supernatants was monitored for 20 min at 450 nm. One unit (U) of SOD activity was defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical per g of wet muscle tissue.

Glutathione peroxidase (GPx, EC1.11.1.9) activity was determined by the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of reduced glutathione and hydrogen peroxide (Cayman Chemicals, Kit No 703102). The supernatant of muscle tissues was prepared by homogenization with 50 mM cold Tris-HCl with 5 mM EDTA, 1mM DTT (pH 7.5) buffer and centrifuging at 10,000 G. The reaction was monitored at 340 nm for 5 min. One unit (U) of GPx activity was defined as the amount of enzyme needed to oxidize 1.0 mM of NADPH to $NADP^+$ per min at 25°C per g wet muscle tissue.

Statistical Methods

Collected data were statistically evaluated by one-factorial ANOVA using StatSoft Statistica® Software (2009). Tukey's test at $P < 0.05$ significance level was used to determine the differences between groups, standard error and standard deviation. The data are presented as an average value and accompanied by SEM (standard error).

RESULTS

Antioxidant characteristics of broiler breast muscles

Breast muscles collected from chickens fed the control diet, analyzed as a whole group, were characterized by the ability to scavenge DPPH free radicals at an average level of 6.1 mM Trolox/g tissue. Antioxidant activity was not dependent on the type or amount of feed additive used in the study. Breast muscles from the chickens fed a diet supplemented with 0.5 mg/kg organic selenium showed the highest antiradical activity against ABTS+ radicals (11.5 mM Trolox/g), and the lowest value (9.1 mM Trolox/g) was observed in the birds whose diets were supplemented with 0.5 mg/kg sodium selenite. There was no apparent effect of the applied supplementation to the poultry feed on the intensity of lipid oxidation (TBARS), nor on the capacity of the whole group of breast muscles to reduce iron (FRAP). Thus, the value of FRAP in breast muscles with predominant fast twitch fibers was about two times lower than in leg

muscles where red fibers are dominant. The ability of the chicken myofibrillar proteins extracted from the leg and back muscles to reduce the Fe3+ to Fe2+ was almost seven times higher than that analyzed for the wings and breast muscles.

Antiradical activity DPPH of m. *pectoralis major* collected from the chickens fed a diet containing inorganic selenium (0.38 mg/kg selenium) was highest at 14.9 mM Trolox/g, and the m. *pectoralis minor* at 12.3 mM Trolox/g. Significantly greater DPPH quenching activity was also observed for the muscles obtained from chickens fed a diet supplemented with 0.5 mg/kg of selenium yeast (app. 13.6 uM Trolox/g). The ability of individual breast muscles to scavenge ABTS free radicals did not differ significantly ($p < 0.05$) to the values for the whole muscle tissue group, with the exception of a lower antioxidative activity for muscles collected from birds fed the diet supplemented with selenium at 0.38 mg/kg.

The m. *pectoralis superficialis* of chickens fed a diet supplemented with 8.2 g/kg and 9.7 g/kg methionine expressed a significantly greater ability to scavenge a synthetic ABTS radical (11.7 mM Trolox/g and 14.0 uM Trolox/g, respectively). The application of selenium to the chicken diet increases the ability of the muscles to scavenge the synthetic free radical ABTS significantly between 2.0 and 8.0 times for the wings and outer breast (sodium selenite) muscles.

The ability to reduce Fe^{3+} to Fe^{2+} was similar for both the individual breast muscles i.e. *pectoralis major* and *minor*, and ranged from 0.69 μM Fe/g for muscles obtained from chickens fed a diet supplemented with 0.38 mg/kg of selenium yeast to 0.93 μM Fe/g for the control group. No effect of the addition of selenium to animal feed on an increase of FRAP and ABTS values were observed for individual breast muscles.

Antioxidant characteristics of broiler leg muscles

Total antioxidant activity in the whole group of chicken leg muscles (thigh and drumstick) was significantly greater ($p < 0.05$) than the chicken breast muscles. Leg muscle groups of Flex chickens had a greater superoxide dismutase activity (3.60 U/g) compared to breast muscle groups (2.04 U/g) (Table 2). The ability of the leg muscles to scavenge free DPPH radical ranged from 12.4 mM Trolox/g (chickens fed a diet supplemented with 0.38 mg/kg sodium selenite) to 17.5 mM Trolox/g (chickens fed a diet supplemented with 0.26 mg/kg selenium yeast Yarrowia lipolytica). No significant ($p < 0.05$) increase in free radical ABTS scavenging was observed in the chicken leg muscles by feed supplemented with selenium and methionine, with the exception of the group fed the diet supplemented with 6.7 g/kg methionine.

The capacity of the leg muscles to reduce Fe3+ was almost two times greater than the breast muscles with an average value of 1.13-1.36 mM Trolox/g. However, there were no significant differences in the capacity to reduce Fe3+ between groups, a clear trend of increased activity of the leg muscles after feed supplementation with organic selenium (0.5 mg/kg) and methionine (6.7 g/kg). In general, the capacity of the thigh myofibrillar protein to reduce Fe3+ to Fe2+ was higher than in breast myofibrillar

Table 2. Effect of the dietary treatment on the individual breast muscles antioxidant status parameters.

	Dietary treatment						SEM	p-value
	Control	Selenium yeast (mg/kg)			Sodium selenite (mg/kg)			
		0.26	0.38	0.5	0.26	0.38	0.5	
Pectoralis major								
GPx [1]	1.22±0.07 ab	1.18±0.28 ab	1.55±0.10 cd	2.65±0.17 f	1.03±0.31 a	1.42±0.04 bc	1.89±0.38 e	0.106	< 0.05
SOD [2]	2.04±0.21 a	1.87±0.08 a	1.95±0.12 a	2.63±0.11 bc	2.12±0.46 ab	2.71±0.09 c	3.04±0.13 cde	0.088	< 0.05
CAT [3]	273±15.3 cd	259±7.20 c	231±2.40 bc	389±5.00 ef	204±7.70 ab	183±6.80 a	260±4.00 c	1.192	< 0.05
DPPH [4]	6.09±0.58 a	6.29±0.33 a	5.70±0.65 a	6.47±0.26 a	6.21±0.31 a	6.13±0.19 a	6.54±0.43 a	0.086	< 0.05
ABTS [5]	10.2±0.59 bcd	9.34±1.00 bc	10.3±0.25 cd	11.5±1.78 d	10.2±0.23 bcd	9.52±1.05 bc	9.12±0.56 bc	0.210	< 0.05
FRAP [6]	0.681±0.04 a	0.740±0.07 a	0.746±0.08 a	0.686±0.03 a	0.687±0.08 a	0.658±0.05 a	0.682±0.08 a	0.012	0.10
TBARS [7]	3.22±0.11 a	3.09±0.17 a	3.26±0.26 a	3.06±0.24 a	2.89±0.13 a	3.00±0.16 a	3.08±0.15 a	0.038	< 0.05
Pectoralis minor									
GPx[1]	1.21±0.23 ab	1.48±0.12 bc	1.21±0.21 ab	1.74±0.22 cde	1.57±0.08 cde	1.82±0.03 de	1.73±0.19 cde	0.053	< 0.05
SOD [2]	3.60±0.12 e	3.39±0.55 de	3.47±0.45 e	4.18±0.30 f	2.87±0.36 cd	3.05±0.86 cde	4.99±0.29 g	0.151	< 0.05
CAT [3]	562±5.00 h	441±98.5 g	309±41.4 d	368±11.2 e	355±5.30 e	421±5.60 fg	441±3.60 g	1.605	< 0.05
DPPH.[4]	14.67±1.63 cd	17.49±2.80 e	13.78±1.14 bc	14.91±1.09 cd	15.69±0.74 d	12.36±0.67 b	13.01±0.51 b	0.389	< 0.05
ABTS [5]	8.78±0.41 bc	9.27±1.27 bc	7.09±1.54 a	9.77±0.62 bc	8.60±0.63 b	6.58±0.89 a	9.52±1.20 bc	0.276	< 0.05
FRAP [6]	1.29±0.06 cd	1.24±0.11 bcd	1.14±0.07 b	1.36±0.04 d	1.30±0.10 cd	1.17±0.13 b	1.20±0.08 bc	0.020	< 0.05
TBARS [7]	5.55±0.27 bc	5.61±1.09 bc	6.09±0.37 cd	4.77±0.54 b	7.39±2.14 e	6.34±0.09 cde	7.16±0.88 de	0.235	< 0.05

[1] Glutathione peroxidase expressed in U/g of wet tissue; [2] Superoxide dismutase, expressed in U/g of wet tissue; [3] Catalase, expressed in U/g of wet tissue; [4] Total antioxidant activity measured by DPPH assays. DPPH are expressed in µmol equivalents of Trolox. [5] Total antioxidant activity measured by $ABTS^{+}$ assays. $ABTS^{+}$ units are expressed in µmol equivalents of Trolox. [6] Total antioxidant activity measured by Ferric reducing antioxidant power (FRAP); expressed as µmol of Fe^{2+} equivalents/g of tissue.

protein. However, leg muscles were characterized by significantly ($p < 0.05$) greater TBARS value than breast muscles, mainly due to the greater fat concentration.

In the control group the highest ability to scavenge DPPH radical was observed for m. *sartorius* (14.3 mM Trolox/g), which made up 26% of the total leg muscle protein content with an average of 18.7 g/100 g. This muscle exhibited a relatively high ability to reduce iron (0.96 μM Fe/g), but also the lowest ability to scavenge ABTS radicals of all the analyzed leg muscles. The supplementation of chicken diets with selenium and methionine significantly ($p < 0.05$) increased the antioxidant activity of m. *sartorius* (up to 7.1 mM Trolox/g for 11.2 g of added methionine per kg in feed), and as well enhancing the capacity of iron reduction (FRAP).

Chicken m. *biceps femoris* with an average weight three times less than m. *sartorius*, was generally characterized by a greater ability to scavenge ABTS radicals and to reduce Fe III to Fe II, particularly in the case of muscles obtained from birds fed a diet enriched with selenium and methionine. Moreover, the amount of oxidized products (TBARS) analyzed in *biceps femoris* was significantly lower ($p < 0.05$) when the birds were supplied with diet containing 0.5 mg/kg of either organic or inorganic selenium. The results of the antioxidant status of individual leg muscles i.e. *biceps femoris, gastrocnemius, iliotiobialis, peroneus longus, sartorius, semimembranosus* and *semitendinosus*, are presented in Table 3.

Application of selenium compounds to broiler feed resulted in a significantly ($p < 0.05$) greater ability of *iliotiobialis* muscle to reduce iron (FRAP), but it had no positive effect on antiradical activity. Within the chicken thigh muscles, *semimembranosus* (only about 5.0% share of the whole leg muscle) and *semitendinosus* (approximately 10% of all leg muscle) were characterized by similar values of DPPH free radical scavenging ability (12.2 mM Trolox/g). A significant ($p < 0.05$) increase in antiradical activity was recorded only for bird feed supplemented with more than 8.2 g/kg methionine in m. *semitendinosus* and with more than 9.7 g/kg methionine and 0.38 mg/kg sodium selenite in m. *semimembranosus*. The use of greater amounts of methionine in the diet had a positive effect on the ability of the analyzed muscles to scavenge synthetic ABTS radicals, whereas selenium compounds increased iron reducing activity (FRAP). Moreover, such diet modifications also contributed to a significant ($p < 0.05$) inhibition of oxidative changes in m. *semitendinosus* and m. *semimembranosus* (TBARS). The antioxidant activity of m. *gastrocnemius* and m. *peroneus longus* towards ABTS and DPPH radicals was comparable to the scavenging ability of whole leg muscle groups, but the ability of m. *gastrocnemius* to reduce Fe^{3+} was significantly lower ($p < 0.05$), at an average of 1.1 mM Trolox/g. Application of selenium compounds and methionine to chicken diets resulted in a significantly ($p < 0.05$) lower content of malondialdehyde compared to the control animals.

Superoxide (SOD) activity

The breast muscles collected from the studied Flex chickens fed a standard diet were characterized by an average of 2.04 U/g SOD activity. Supplementation with selenium yeast up to 0.38 mg per kg in broiler feed did not change SOD activity. Significantly

($p < 0.05$) greater SOD activities (2.63-3.41 U/g) were detected for the group of breast muscles in birds fed the diets containing greater concentrations of methionine (11.2 g/kg) and Se (0.5 mg/kg), both as selenium yeast and sodium selenite. Similar correlations were obtained when analyzing the activity of superoxide dismutase in the individual m. *pectoralis major* (Table 2). In the case of the *pectoralis minor*, greater SOD activity in relation to the control group was observed when birds were supplied with a diet enriched with organic selenium at 0.5 mg/kg (2.70 U/g). Leg muscle groups of Flex chickens had a greater superoxide dismutase activity (3.60 U/g) compared to breast muscle groups (2.04 U/g) (Table 2).

Renerre et al. (1999) have shown that SOD has a higher activity in oxidative than in glycolytic muscle [22]. Like the breast muscle groups, a significant ($p < 0.05$) increase in SOD activity was observed in leg muscle groups after dietary supplementation with a high proportion of methionine and inorganic Se. Moreover, a clear tendency of increased SOD activity was observed in leg muscle groups of birds fed a diet containing 0.5 mg/kg Se in the form of selenized yeast (4.18 U/g). Within all the analyzed individual leg muscles of the control group of chickens, the lowest SOD activity was noted in m. *semitendinosus* (0.75 U/g), while SOD activity in other muscles ranged from 1.27 U/g for m. *sartorius* to 1.70 U/g for m. *peroneus longus* (Table 3). Application of selenium compounds (0.5 mg/kg) and methionine (11.2 g/kg) to the chicken diet significantly ($p < 0.05$) increased the activity of SOD in *semitendinosus, sartorius* and *biceps femoris* muscles. In the case of *semimembranosus, iliotiobialis, gastrocnemius* and *peroneus longus* muscles, no significant ($p < 0.05$) effect of diet supplementation on antioxidant enzyme activity was observed. The obtained results are in accordance with the findings of Pušić and colleagues (2018), who showed that the addition of organic selenium in the amount of 0.5 mg/kg feed did not lead to a significant increase in SOD activity in m. *gastrocnemius* [23]. Additionally, these authors found that supplementation with organic selenium in amounts of 0.3 and 0.5 mg/kg feed increased SOD activity in m. *pectoralis superficialis*.

Glutathione peroxidase (GPx) activity

An average GPx activity of breast and leg muscles of Flex chicken fed the control diet amounted to about 1.21 U/g. No apparent effect of dietary methionine on GPx activity in breast and leg muscles was noted, while the addition of selenium compounds did result in greater enzyme activity in the breast and leg muscles. Analysis of glutathione peroxidase in individual chicken breast muscles (m. *pectoralis major* and m. *pectoralis minor*) confirmed a significant ($p < 0.05$) positive effect of dietary selenium on GPx enzyme activity. In addition, m. *pectoralis minor* expressed greater GPx activity (1.43 U/g) also at a lower concentration of dietary organic selenium (0.26 mg/kg). The highest glutathione peroxidase activities within the control group were analyzed for m. *semimembranosus* (1.60 U/g), m. *biceps femoris* (1.41 U/g) and m. *semitendinosus* (1.31 U/g), whereas the lowest activities were observed for m. *gastrocnemius* (0.81 U/g) and m. *iliotiobialis* (0.90 U/g) (Table 3). Similar to SOD activity, the lowest GPx activity was observed in m. *gastrocnemius* and m. *iliotibialis*

Table 3. Effect of the dietary treatment on the individual leg muscles antioxidant status parameters.

	Dietary treatment												
	Control	DL-methionine (g/kg)				Selenium yeast (mg/kg)			Sodium selenite (mg/kg)			SEM	p-value
		6.70	8.20	9.70	11.2	0.26	0.38	0.50	0.26	0.38	0.50		
Sartorius													
GPx [1]	1.08	1.21	1.16	1.21	1.07	1.62	0.87	1.40	1.53	1.77	1.69	0.070	0.09
SOD [2]	3.40 ab	3.44 ab	3.19 ab	3.09 ab	5.71 d	3.77 bc	2.39 a	3.10 ab	2.82 ab	3.01 ab	4.94 cd	0.210	< 0.05
CAT [3]	864 g	602 cd	537 ab	488 a	637 cde	520 a	648 de	707 f	585 bc	652 de	671 ef	21.6	< 0.05
DPPH [4]	14.3 f	11.5 bcd	12.8 e	11.8 cde	11.7 cde	10.3 b	7.78 a	12.3 de	7.39 a	11.1 bc	10.3 b	0.432	< 0.05
ABTS [5]	4.02 a	6.18 cdef	6.56 def	5.72 cde	7.09 f	6.71 ef	6.01 cdef	4.40 ab	4.55 ab	5.40 bc	5.53 bcd	0.223	< 0.05
FRAP [6]	0.096 a	0.097 a	0.148 f	0.124 e	0.111 bcd	0.107 bc	0.103 ab	0.117 de	0.103 ab	0.111 bcd	0.113 cd	0.003	< 0.05
TBARS [7]	5.77 b	7.09 e	6.77 d	7.18 e	6.36 c	6.55 cd	5.77 b	5.23 a	5.59 b	6.36 c	6.41 c	0.132	< 0.05
Iliotiobialis													
GPx[1]	0.940 bcd	0.989 bcd	0.941 bcd	0.991 bcd	0.849 bc	0.916 bc	0.170 a	0.701 ab	1.23 bcd	1.47 d	1.39 cd	0.076	< 0.05
SOD [2]	3.81 bcd	2.91 abc	2.67 ab	2.56 ab	5.18 d	2.79 ab	1.25 a	2.12 ab	2.51 ab	2.70 ab	4.63 cd	0.243	< 0.05
CAT [3]	321 e	221 c	156 b	107 a	257 d	487 g	615 h	674 i	398 f	464 g	484 g	38.3	< 0.05
DPPH [4]	13.1 de	12.8 cde	10.1 a	17.3 f	13.8 e	12.7 cde	10.7 ab	11.4 abc	12.1 bcd	10.8 ab	11.6 abc	0.423	< 0.05
ABTS [5]	8.72 d	8.79 d	5.38 a	9.36 d	11.31 e	7.35 c	5.34 a	5.66 ab	5.91 ab	5.82 ab	6.18 b	0.503	< 0.05
FRAP [6]	0.095 a	0.090 a	0.155 e	0.095 a	0.109 cd	0.100 abc	0.099 abc	0.120 d	0.097 ab	0.109 cd	0.107 bc	0.004	< 0.05
TBARS [7]	6.59 e	5.45 ab	5.23 a	5.59 abcd	6.14 cde	5.32 a	5.00 a	6.18 de	5.95 bcd	5.55 abc	5.32 a	0.108	< 0.05
Biceps femoris													
GPx[1]	1.45 bc	1.02 ab	0.976 ab	1.03 ab	0.884 a	1.63 c	0.887 a	1.42 bc	1.45 bc	1.69 c	1.61 c	0.069	< 0.05
SOD [2]	3.56 a	3.33 a	3.08 a	2.98 a	5.60 b	3.74 a	2.35 a	3.06 a	3.50 a	3.69 a	5.62 b	0.220	< 0.05
CAT [3]	512 fg	556 h	491 ef	442 d	591 i	345 b	473 e	532 g	308 a	374 c	394 c	19.1	< 0.05
DPPH [4]	12.5 a	11.7 a	11.9 a	17.5 c	11.5 a	11.1 a	11.4 a	11.1 a	15.5 b	11.1 a	11.0 a	0.459	< 0.05
ABTS [5]	5.19 a	8.02 de	6.08 ab	8.98 e	6.94 bcd	7.26 cd	7.09 bcd	7.01 bcd	6.39 bc	5.14 a	7.50 cd	0.443	< 0.05
FRAP [6]	0.088 a	0.095 b	0.150 g	0.102 cd	0.110 e	0.103 cd	0.096 b	0.143 f	0.104 d	0.114 e	0.100 c	0.004	< 0.05
TBARS [7]	7.18 def	7.05 def	7.68 ef	6.55 cde	8.91 g	6.59 cde	5.64 bc	4.91 ab	8.18 fg	6.32 cd	4.27 a	0.296	< 0.05

cont. Table 3.

	Dietary treatment											
	Control	DL-methionine (g/kg)				Selenium yeast (mg/kg)			Sodium selenite (mg/kg)			SEM	p-value
		6.70	8.20	9.70	11.2	0.26	0.38	0.50	0.26	0.38	0.50		
Semimembranosus													
GPx[1]	1.64 bcde	1.53 abcd	1.48 abc	1.53 abcd	1.39 ab	1.92 def	1.17 a	1.70 bcdef	1.87 cdef	2.12 f	2.03 ef	0.062	< 0.05
SOD [2]	3.79 d	2.91 bc	2.66 bc	2.56 bc	5.45 e	3.12 bcd	1.74 a	2.45 ab	3.03 bc	3.22 cd	5.15 e	0.240	< 0.05
CAT [3]	921 g	496 bc	431 ab	382 a	531 c	689 e	819 f	876 fg	573 cd	655 de	674 e	37.2	< 0.05
DPPH [4]	12.4 b	11.8 b	11.8 b	16.1 d	12.1 b	12.5 b	11.0 b	11.1 b	9.49 a	14.2 c	11.3 b	0.379	< 0.05
ABTS [5]	7.62 de	7.92 ef	8.55 fg	9.21 g	5.40 ab	5.09 a	7.75 ef	5.67 ab	7.52 de	6.41 bc	6.94 cd	0.265	< 0.05
FRAP [6]	0.105 a	0.106 ab	0.151 c	0.108 ab	0.140 c	0.105 a	0.121 b	0.105 a	0.117 ab	0.115 ab	0.138 c	0.004	< 0.05
TBARS [7]	9.77 f	8.95 e	5.36 a	6.68 b	10.2 f	7.00 bc	7.55 cd	8.91 e	7.18 bc	9.86 f	7.95 d	0.323	< 0.05
Semitendinosus													
GPx[1]	1.35 ab	1.17 ab	1.12 ab	1.17 ab	1.03 ab	1.23 ab	0.486 a	1.02 ab	1.63 b	1.88 b	1.80 b	0.092	< 0.05
SOD [2]	2.88 cde	2.52 bcd	2.27 abc	2.17 ab	4.79 f	3.06 de	1.67 a	2.38 bc	3.18 e	3.37 e	5.30 f	0.231	< 0.05
CAT [3]	466 d	510 e	445 d	396 c	545 f	397 c	525 ef	584 g	116 a	183 b	202 b	33.3	< 0.05
DPPH [4]	12.0 cd	12.0 cd	13.3 d	15.3 e	15.7 e	10.8 abc	9.98 ab	10.9 abc	9.65 a	11.8 c	11.3 bc	0.423	< 0.05
ABTS [5]	8.03 b	9.08 c	7.30 b	9.23 c	7.62 b	5.64 a	8.03 b	8.00 b	6.97 b	7.47 b	5.69 a	0.215	< 0.05
FRAP [6]	0.108 c	0.096 a	0.147 h	0.101 abc	0.136 g	0.116 d	0.100 ab	0.124 ef	0.104 bc	0.130 f	0.123 e	0.003	< 0.05
TBARS [7]	11.3 f	7.50 e	7.68 e	6.32 bcd	5.55 b	4.45 a	6.09 bc	6.73 cde	7.27 de	7.23 de	7.14 cde	0.362	< 0.05
Gastrocnemicus													
GPx[1]	0.855 a	1.19 abc	1.14 ab	1.19 abc	1.05 a	1.63 de	0.887 a	1.42 bcd	1.54 cde	1.79 e	1.69 de	0.069	< 0.05
SOD [2]	3.83 d	2.74 bc	2.49 abc	2.33 ab	5.00 e	3.18 cd	1.80 a	2.51 abc	2.59 bc	2.78 bc	4.71 e	0.213	< 0.05
CAT [3]	388 e	133 abc	86.5 ab	70.0 a	65.0 a	161 bc	295 d	348 de	147 abc	177 c	159 bc	23.3	< 0.05
DPPH [4]	12.4 b	11.7 b	12.3 b	17.9 e	17.4 e	12.8 bc	12.0 b	12.0 b	14.7 d	14.1 cd	9.43 a	0.531	< 0.05
ABTS [5]	7.64 bc	9.40 ef	6.95 ab	9.00 de	6.67 ab	8.20 cd	6.37 a	6.73 ab	6.63 ab	6.59 ab	6.54 ab	0.234	< 0.05
FRAP [6]	0.101 bc	0.093 a	0.149 f	0.100 b	0.105 d	0.104 cd	0.095 a	0.109 e	0.103 bcd	0.105 d	0.100 b	0.003	< 0.05
TBARS [7]	7.09 g	6.09 e	6.23 e	5.36 d	5.55 d	5.36 d	3.77 a	4.64 b	6.68 f	5.64 d	5.00 c	0.196	< 0.05

cont. Table 3.

	Dietary treatment											
	Control	DL-methionine (g/kg)				Selenium yeast (mg/kg)			Sodium selenite (mg/kg)			SEM	p-value
		6.70	8.20	9.70	11.2	0.26	0.38	0.50	0.26	0.38	0.50		
Peroneus longus													
GPx[1]	1.19 ab	1.15 ab	1.10 ab	1.15 ab	1.01 ab	1.43 bc	0.679 a	1.21 ab	1.77 cd	2.01 d	1.93 cd	0.088	< 0.05
SOD [2]	3.92 cd	2.44 ab	2.19 a	2.09 a	4.70 f	4.11 de	2.75 b	3.40 c	2.43 ab	2.62 ab	4.55 ef	0.203	< 0.05
CAT [3]	461 ef	398 c	333 b	284 a	433 de	491 fg	618 h	678 i	427 cd	494 fg	513 g	23.7	< 0.05
DPPH [4]	12.5 ab	11.9 ab	12.9 b	16.1 d	19.1 e	11.0 a	13.3 bc	10.9 a	14.8 cd	13.3 bc	12.0 ab	0.519	< 0.05
ABTS [5]	8.58 cd	9.55 de	8.13 bc	10.7 e	7.75 abc	7.35 abc	7.26 abc	7.56 abc	6.61 a	6.88 abc	7.92 abc	0.270	< 0.05
FRAP [6]	0.105 d	0.097 ab	0.148 f	0.100 bc	0.112 e	0.101 cd	0.095 a	0.110 e	0.102 cd	0.111 e	0.114 e	0.003	< 0.05
TBARS [7]	5.32 bc	4.59 a	5.27 bc	5.50 c	5.55 c	4.68 ab	6.41 d	4.32 a	9.18 f	6.32 d	7.91 e	0.312	< 0.05

[1] Glutathione peroxidase expressed in U/g of wet tissue; [2] Superoxide dismutase, expressed in U/g of wet tissue; [3] Catalase, expressed in U/g of wet tissue; [4] Total antioxidant activity measured by DPPH assays. DPPH are expressed in µmol equivalents of Trolox.; [5] Total antioxidant activity measured by $ABTS^+$ assays. $ABTS^+$ units are expressed in µmol equivalents of Trolox.; [6] Total antioxidant activity measured by Ferric reducing antioxidant power (FRAP); expressed as µmol of Fe^{2+} equivalents/g of tissue

Catalase (CAT) activity

The enzyme activity in breast muscle groups of the control group of chickens fed a standard diet averaged 273 U/g. Modifications to the chicken diet had varied effects on CAT activity in breast muscles. Greater ($p < 0.05$) catalase activity was found in the muscles obtained from chickens fed a diet supplemented with 0.5 mg/kg of Se yeast, and as well in the groups with added methionine at 8.2 g/kg and 9.7 U/g. Analysis of the individual breast muscles *pectoralis major* and *pectoralis minor* confirmed these results (Table 2). The antioxidant activity of catalase present in the leg muscles of the control group was more than twice higher than in breast muscle.

The lowest CAT activity was determined in leg muscles of chickens fed the diets containing 9.7 g/kg methionine and 0.38 mg/kg organic selenium. A significant ($p < 0.05$) effect of diet modification on catalase activity was also observed in the individual leg muscles (Table 3). The highest CAT activity in the control group was analyzed for m. *semimembranosus* and m. *sartorius* (more than 700 U/g), with the lowest for m. *iliotiobalis* (less than 200 U/g). Supplementation with 0.5 mg of either form of selenium to broiler feed resulted in increased ($p < 0.05$) CAT activity in all analyzed individual leg muscles. Still, the lowest enzyme activity was measured in m. *gastrocnemius* (average 400 U/g), with the highest for m. *semimembranosus* (more than 900 U/g). Increasing methionine levels in the feed to 11.2 g/kg resulted in greater CAT activity in relation to the control in most of the analyzed muscles, with the exception of m. *gastrocnemius* (reduction of 51.0 U/g) and m. *semimembranosus* (reduction of 117 U/g). In general, as previously established, catalase has a higher activity in in oxidative than in glycolytic muscle [24].

DISCUSSION

Antioxidant characteristics of broiler breast muscles

Antioxidants are multifunctional and in complex heterogenous foods such as meat their activity cannot be evaluated by a single method. Thus, many procedures including radical scavenging assays were required to investigate the antioxidative potential of muscle tissues [25,26]. Scavenging of DPPH radicals permits evaluation of the hydrogen-donating potency of antioxidative compounds, while scavenging of ABTS radicals determines their single electron-transfer capabilities [15,27]. The Fe+3 probe in a FRAP assay reflects the reductive antioxidant power and finally TBAR can serve as a marker of lipid oxidative damage and reflects the intensity of lipid oxidation [18,28]. Total antioxidative capacity measured by the ABTS method was generally greater than by the DPPH method. This indicates the greater effect of hydrophilic than hydrophobic substances in the tissues on the antioxidative potential. This is in agreement with Sacchetti et al. (2008), who reported that the hydrophilic extract was most effective in the establishment of the antioxidant capacity in chicken meat [14]. Moreover, DPPH is likely more selective than $ABTS^{+}$ in the reaction with H-donors

which could explain the lower TEAC values obtained by DPPH compared to ABTS assays [26,29].

Antiradical activity against $ABTS^+$ radicals indicated a more effective antioxidant action of dietary organic selenium comparing to the inorganic form. Wang and Xu (2008) also showed a greater impact of organic Se on broiler antioxidant status [30]. It is known that organic Se has a higher bioavailability than inorganic Se for Se deposition in the tissues. This is probably due to their different absorption mechanisms: inorganic Se is passively absorbed from the intestine by a simple diffusion process, competing with a number of mineral elements for the same absorption route, whereas organic Se is actively absorbed through the amino acid transport mechanisms [31]. Selenium application in the chicken diet did not significantly change the FRAP values for the wings and breast muscles; however, a significant increase in the iron reduction ability was noted for both sets of the leg muscles, and back muscles, which can be related to the higher deposition of the selenium in the part richer in lipids. The individual *pectoralis major* and *minor* muscles exhibited significantly greater DPPH scavenging ability than the whole breast tissue, especially in supplementation with selenium compounds (Table 2). This showed that for better understanding of the relationships between dietary components and antioxidative status, individual muscles should also be analyzed. Previously, Korzeniowska and coworkers (2018) showed that selenium supplementation is more effective in the leg muscles compared with breast meat, and that the organic form of selenium is deposited to a greater extent than the inorganic form [32]. Authors explain this by the differences in metabolic pathways between organic and inorganic forms of selenium since organic selenium is actively absorbed through an amino-acid transport mechanism, whereas inorganic selenium is passively absorbed from the intestine by a simple diffusion process [31].

Se supplementation to chicken diets causes a significant increase in the iron reduction ability for both sets of the leg and back muscles, which can be associated with the higher Se retention in the lipids-rich parts. Assuming that both TEAC and SOD activity were greater in the leg than in breast muscles and GPx activity in leg muscles was more affected by the addition of dietary selenium, it can be stated that chicken leg muscles could be more effectively stabilized against oxidation by such a feeding modification. Namely, chicken fat contains relatively high amounts (25-30%) of polyunsaturated fatty acids for which is known to contain long chain fatty acids with a higher number of double bonds, which increases the susceptibility of meat to oxidation process. This is similar to Kikusato and Toyomizu (2019) results, who found that the TBARS value was higher in m. *gastrocnemius* than in m. pectoralis in chickens in the thermoneutral zone [33–35]. A relationship was observed between muscle morphology and the degree of oxidation. Selenium supplementation decreased the extent of oxidation processes in most of the muscles with predominant red (slow twitch) fibers, such as *biceps femoris, semimembranosus* and *gastrocnemius*, while for some muscles with a greater amount of white fibers, like *peroneus longus* and *iliotiobialis*, this relation was not distinctive. Thus, this could explain the lack of strong effect of Se supplementation on oxidation processes in the whole group of leg muscles.

Enzymatic activity in individual broiler breast and leg muscles

Antioxidant enzymes constitute an intracellular barrier against free radicals. In skeletal muscles the most important enzymes are catalase, superoxide dismutase and glutathione peroxidase. Superoxide dismutase is an important antioxidant defense in nearly all cells exposed to oxygen and catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide while CAT and GPx catalyze the dismutation of hydrogen peroxide into water and oxygen. GSH-Px can additionally decompose lipoperoxides formed during lipid oxidation [36–38]. It is generally considered that oxidative muscles show higher activities of antioxidative enzymes than glycolytic muscles such as GSHPx [22]. The effect of selenium supplementation in poultry diets on antioxidative enzymes, especially glutathione peroxidase, has been intensively studied, but only on whole groups of breast and leg muscles. The results of the antioxidative enzymes activity of individual and whole groups of chicken breast and leg muscles are presented in Tables 2-4. Greater superoxide dismutase activity was found in the Flex chickens' leg muscle groups compared to their breast muscle groups, which could be explained by different types of metabolism given that SOD activity is correlated with the number of mitochondria [24].

Glutathione peroxidase (GPx) activity

Glutathione peroxidase is an essential component of the integrated enzymatic antioxidant defense system and has the ability to remove hydrogen peroxide from the system as well as to detoxify lipid peroxides formed during oxidation processes [39]. Although the addition of selenium compounds did increase the enzyme activity in the breast and leg muscles, there was no discernible effect of dietary methionine on GPx activity in those muscles. This corresponds to the majority of available data on selenium supplementation in poultry, since selenium is essential for catalytic functions of GSH-Px [40]. In the present study the highest effect was observed for the highest content of Se in the diet regardless of the form of the microelement, while Wang et al. (2011) observed that the inorganic form of Se was more effective [41].

Similar to SOD activity, the lowest GPx activity was observed in m. *gastrocnemius* and m. *iliotibialis*, indicating that selenium supplementation had more negligible effect on antioxidant enzymes activity in these muscles. This may be related to the type of muscle fibers and/or less selenium deposition in these muscles. GSH-Px activity is higher in oxidative muscles, due to different metabolism and, therefore, different susceptibility towards oxidative damage [42]. Tissues containing higher amounts of antioxidative enzymes would be expected to be more stable towards lipid peroxidation but, because they contain more fats and iron that could be pro-oxidative, they are more susceptible to peroxidation [42]. Therefore, supplementing feed with selenium can delay the process of lipid peroxidation.

Catalase (CAT) activity

Catalase, as an enzyme capable of degrading hydrogen peroxide, protects living cells from oxidatively induced damages. Unlike white muscles, CAT activity in the chicken leg muscles decreased as a result of feed supplementation with selenium and methionine. This can be related to high GPx activity in this type of the muscle. These differences in CAT activity might have been be due to the metabolic differences between muscle types [43].

CONCLUSIONS

The total antioxidative potential of broiler chicken leg muscles was greater than breast muscles. Supplementation of bird diets with selenium and methionine (especially greater levels of addition) resulted in greater total antioxidative capacity of individual breast and leg muscles, while for the whole groups of these muscles the relation was less evident. In contrast, antioxidative enzymes (SOD and GPx) were more active, both in whole muscle groups and in individual muscles, when greater concentrations of Se and Met were added to the chickens' diet. Dietary selenium slowed down the oxidation processes in individual broiler chicken muscles, as monitored by TBARS. Antioxidative enzymes were more active in the leg muscles than in the Flex chicken breast muscles. Summing up, it can be stated that supplementation of chicken diets with selenium and high concentrations of methionine positively influences the antioxidative potential of individual muscles, and could lead to greater quality and extended shelf-life of fresh meat.

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Authors' contributions

MK and WK created the idea and designed of study. MK carried out the experiment and analyses, cured the results, drafted the manuscript. BK and DK calculated and interpreted the results and helped to improve the manuscript. AR graphical presentation of the results and help to improve the manuscript. All authors read and approved the final manuscript.

Declaration of competing interests

The authors declare that they have no competing interests.

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POBOLJŠANJE ANTIOKSIDATIVNE AKTIVNOSTI MIŠIĆA BROJLERA NAKON DJETETSKE MODULACIJE SELENOM I METIONINOM

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Cilj istraživanja bio je da se uporedi antioksidativni kapacitet muskulature pilećih grudi i nogu brojlera nakon modulacije ishrane sa selenom (Se) i metioninom (Met). Aktivnosti uklanjanja slobodnih radikala (ABTS, DPPH) i redukcije gvožđa (FRAP) su određene kao ukupni antioksidativni potencijal (TEAC), kao i aktivnost enzima katalaze (CAT), superoksid dismutaze (SOD) i glutation peroksidaze (GPx), u odnosu prema koncentracijama krajnjih produkata peroksidacije lipida (TBARS). Analize su rađene na *m. pectoralis superficialis* i *profundus* pojedinačno i zajedno.

Proučavani mišići nogu uključivali su *biceps femoris*, *gastrocnemicus*, *iliotiobialis*, *peroneus longus*, *sartorius*, *semimembranosus*, *semitendinosus* i sve mišiće nogu zajedno. Flex brojlerski pilići su hranjeni hranom sa dodatkom 6,7, 8,2, 9,7 i 11,2 g DL-metionina/kg hrane i Se kao natrijum selenita i selenizovanog kvasca u količini od 0,26, 0,38 i 0,50 mg Se/kg. Veće aktivnosti TEAC i enzima primećene su u mišićima nogu nego u grudnoj muskulaturi. Selen nije promenio TEAC u mišićima, ali je poboljšao antiradikalni kapacitet u velikom i malom pektoralisu, sartorijusu i bicepsu. Najviši nivo metionina je povećao TEAC u pojedinačnim mišićima nogu. Selen i metionin u najvišim koncentracijama povećavaju aktivnost SOD u celoj grupi i pojedinačnim mišićima, dok Se povećava aktivnost GPx. Zaključno, dijetarna suplementacija selenom i visokim koncentracijama metionina imala je veći uticaj na antioksidativni potencijal pojedinih mišića nego na ceo skup mišića pilećih prsa i nogu. Pozitivni efekat proučavane modulacije ishrane mogao bi da unapredi kvalitet i produži rok trajanja svežeg pilećeg mesa.