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Carbonyl and sulfhydryl groups of chicken meat proteins after dietary modulation with selenium

1 Introduction

Over the past decade, protein oxidation and its implications has been an innovative topic of increasing interest among researchers. Protein oxidation is characterized by greater complexity and a larger variety of oxidation products than lipid oxidation [1-2]. Protein oxidation is responsible for many biological modifications such as protein fragmentation or aggregation, decreased protein solubility, and decreased amino acid bioavailability [3-4]. Meat proteins are susceptible to oxidative chemical modifications and those oxidized proteins may have an impact on meat quality. The chemical nature some of the particular protein oxidation products is still indefinite and the potential effect of oxidized meat proteins on meat quality or human health remain not fully recognized [1,5].

Protein oxidation is a covalent modification of protein induced either directly by reactive oxygen species, or indirectly by reaction with secondary products of oxidative stress. Different amino acid groups are sensitive to oxidation [6-8]. Depending on the target and the oxidizing agent, protein oxidation propagates and terminates according to multiple mechanisms and the consequences include: reduced tryptophan fluorescence, the loss of sulfhydryl groups, the formation of protein carbonyls, the formation of cross-links and the modification of aromatic amino acids, among others [2,4,9].

Carbonylation is generally recognized as one of the most remarkable chemical modifications in oxidized proteins. Basic amino acids like lysine, histidine and arginine are oxidized with the resultant products bearing carbonyl groups in their side-chains, which can then react with free amino groups to form amide bonds. Four major pathways to generate protein carbonyls include: fragmentation of backbones through the α-amidation pathway and β-scission, binding of non-protein carbonyl compounds from lipid peroxidation by Michael addition (4-hydroxy-2-nonenal and malondialdehyde) to protein amino acid side chains including histidine imidazole, cysteine sulfhydryl and lysine amino groups [10-12], direct oxidation of amino acid side chains including...
arginine, lysine, proline and threonine [13], as well as the addition of reactive carbonyl derivatives (ketoamines, ketoaldehydes, and deoxyosones) generated by reducing sugars and their oxidation products after reacting with lysine [9,14].

Oxidation and nitrosylation of amino acid sulfhydryl groups can lead to the generation of disulfide bonds resulting in the formation of cross-linked proteins [15]. The sulfhydryl group of cysteine residues may be oxidized to disulfide, as well as to sulfenic and other derivatives [16]. Tyrosine can give dityrosine bridges by the inner reaction between tyrosyl radicals or with tyrosine residues to form several stable biphenolic compounds. The 2,2′, biphenol bityrosine appears to be the major product of this reaction [5,17].

The formation of protein carbonyls from particular amino acid side chains contribute to impair the conformation of myofibrillar proteins leading to denaturation and loss of functionality. However, the significance of protein carbyonation in meat systems is still poorly understood. Beyond their role as markers of protein oxidation, specific protein carbonyls, such as α-aminoadipic (AAS) and γ-glutamic semialdehydes (GGS), are active compounds that may be implicated in several chemical reactions with relevant consequences on meat quality [1,5].

The complex composition of chicken muscle tissue offers advantages and health benefits, but it also creates susceptibility to oxidation, which quickly deteriorates meat quality. In muscle foods, oxidative reactions continue postmortem and are a leading cause of quality deterioration during processing and storage. There are also limited numbers of studies available in the literature on protein oxidation in chicken meat [18]. The measurement of sulfhydryl and carbonyl content are an interesting way to evaluate free radical attack on proteins in muscles [17]. Some authors described the efficacy of certain antioxidant strategies against meat protein oxidation [19]. Therefore, incorporation of dietary antioxidant such as selenium in chicken food has been implemented to prevent protein oxidation.

Selenium (Se) is a trace mineral that performs many functions in the body, it is important for good animal performance and for cell regulation of the anti-oxidant system. It is a part of the enzyme glutathione peroxidase as selenocysteine, and it is readily ionisable at physiological pH. It protects the cell against free radicals in mammalian species that use oxidative metabolism, therefore, it is crucial for cell survival by reducing hydrogen peroxide and lipid hydroperoxides [20].

In summary, the aim of this study was to investigate the effects of selenium modulation on the protein carbonyl and sulfhydryl groups alteration in chicken breast and leg meat.

2 Materials and methods

2.1 Muscles collection and preparation

Breast and leg meat samples were collected from 42-day old male Flex broiler chickens (45 birds in total) (Hubbard, Poland) previously as a one-day old chicks allocated to 7 dietary treatments; group I was fed with control (standard) feed mixture (negative control); groups II to IV with basal diet supplemented with inorganic selenium (sodium selenite, Sigma-Aldrich) to reach the levels of 0.26 mg kg⁻¹, 0.38 mg kg⁻¹ and 0.50 mg kg⁻¹, respectively. Treatments V to VII were fed with diet containing organic selenium (selenized yeast) at the same levels as for inorganic selenium. All diets contained feed vitamin premix. In the period from 1 to 14 days the chicks were supplied with starter, and then from 22 to 42 days with grower feed mixtures. The crude protein content in the starter diet was about 220 g kg⁻¹, whilst in grower about 200 g kg⁻¹.

Energy value was calculated at the mean level of 12.2 and 12.7 MJ kg⁻¹, respectively. After 42 days of the feeding study all birds were slaughtered by spine dislocation. Breast and leg muscles were harvested manually, immediately chilled on ice and analyzed as fresh meat. Other parts of chicken breast and leg muscles were chilled on ice, then stored for seven days at 4°C (chilled samples). Frozen samples were obtained by freezing the meat directly after slaughter to internal -18°C and storing at -20°C for three months. Directly before analyses frozen muscles were defrosted to -2°C.

2.1.1 Carbonyl group content

Carbonyl group (CO) content was evaluated by the derivatisation reaction with DNPH (2,4-dinitrophenylhydrazine) [21,22]. Meat was thawed, minced and homogenized 1:10 (w/v) in pyrophosphate buffer (pH 7.4) (PB) consisting of 2.0 mM Na₃P₂O₇, 10 mM tris-maleate, 100 mM KCl, 2.0 mM MgCl₂ and 2.0 mM EGTA (ethylene glycol tetraacetic acid) using an ultraturrax homogenizer for 30 s. The homogenates were divided in two equal aliquots of 0.1 mL. Then, proteins were
Selenium on meat protein carbonyls and sulfhydryls

Precipitated in both aliquots by adding 1 mL of 10% TCA (triacetic acid) and centrifuged for 5 min at 5,000 rpm. Finally, the supernatants were removed and one pellet was treated with 1 mL 2 N HCl (for quantifying protein concentration) and the other one with an equal volume of 0.2% (w/v) DNPH in 2 N HCl (for carbonyl concentration measurement). Both samples were incubated for 1 h at room temperature. Then, samples were precipitated with 1 mL of 10% TCA and washed twice with 1 mL of 1:1 ethanol/ethyl acetate (v/v), mixed and centrifuged for 5 min at 10,000 rpm. The pellets were then dissolved in 1.5 mL of 20 mM sodium phosphate buffer (pH 6.5) containing 6 M guanidine hydrochloride, stirred and centrifuged for 2 min at 5,000 rpm to remove insoluble fragments. Protein concentration was analyzed spectrophotometrically at 280 nm (Evolution 160 UV/VIS, ThermoScientific, USA) using BSA as standard. The absorbance of the second aliquot was measured at 370 nm (Evolution 160 UV/VIS, ThermoScientific, USA) and expressed as nmol of carbonyl per mg of protein using the adsorption coefficient for the protein hydrazones (22.0 mM$^{-1}$ cm$^{-1}$).

2.1.2 Sulfhydryl group content

Total free sulfhydryl groups (SH) content was determined by reacting with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) [23-24]. Meat samples were thawed, minced and dissolved in 20.0 mL urea-SDS solution (8.0 M urea, 3% SDS, 0.1 M phosphate, pH 7.4) by shaking at room temperature for 8 h. Then 1 mL of the protein solution was incubated with 0.3 mL DTNB reagent (10 mM DTNB in 0.1 M phosphate buffer, pH 7.4) at room temperature for 15 min. Absorbance of the solutions was read at 412 nm (Evolution 160 UV/VIS, ThermoScientific, USA) and expressed as nmol of carbonyl per mg of protein using the adsorption coefficient for the protein hydrazones (13,600 M$^{-1}$ cm$^{-1}$).

2.1.3 Selenium content

Selenium concentration was determined according to PN-EN 13805:2003 and PN-EN 14084:2004. Hydride generation atomic spectroscopy (Spectra AA240FS, Varian Analytical Instrument) was carried out of the acid digest samples [25]. The measured parameters follow: 280 V of negative high voltage, 80 mA of the current of hollow cathode lamp, 7 mm of electrothermal atomizer height, pure argon as a carrier gas, 800 mL min$^{-1}$ of carrier flow, 1.0 mL of injecting sample.

Total antioxidative capacity (TAC)
The hydrophilic fraction of the samples was prepared by homogenization of muscles with redistilled water (Mixer B-400 Buchi, Switzerland) and centrifugation [26], then used for the total antioxidative capacity assays (ABTS, DPPH, FRAP, TBARS).

2.1.4 ABTS$^+$ assay

The total antioxidant capacity of the hydrophilic fraction of chicken breast and leg muscles was analyzed by Trolox-equivalent antioxidant capacity (TAC) assay [27]. The formation of ABTS$^+$ radical cation 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 persulphate, followed by incubation in the dark at room temperature for 12–16 h. Prior to analysis the absorbance of the ABTS$^+$ solution was measured at 734 nm and adjust 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 read at 734 nm (Evolution 160 UV/VIS, ThermoScientific, USA), followed up by second measurements taken after 6 min of incubation at 30°C. The percentage inhibition of the blank absorbance was calculated for Trolox standard reference and meat sample, respectively.

2.1.5 DPPH$^+$ assay

Scavenging activity of the muscle samples was analyzed towards 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) (Sigma–Aldrich, Poland) [28]. 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 centrifugation at 5,000 × g for 15 min. The supernatant was mixed with ethanol and DPPH radical solution and incubated at room temperature without light exposure for 10 min before taking the absorbance measurement at 517 nm (Evolution 160 UV/VIS, ThermoScientific, USA). The ability to scavenge the DPPH radical was expressed as µM Trolox per g wet muscle tissue.
2.1.6 FRAP assay

Ferric reducing antioxidant power (FRAP) assay [29] was carried out on meat homogenates. Roughly, the meat samples were homogenized for 2 min at 3,000 rpm (B-400 Buchi, Germany) in potassium phosphate buffer (pH 7.2) and centrifuged at 5,000 × g for 15 min, then supernatant was collected. 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$_2$Cl$_3$ (Sigma–Aldrich, Poland) added to 300 mM acetate buffer. The absorbance was measured immediately after mixing at 593 nm (Evolution 160 UV/VIS, ThermoScientific, USA). A standard curve was prepared with FeCl$_2$ (Sigma–Aldrich, Poland). The antioxidant power of the samples was expressed as μM of Fe$^{2+}$ per 1 g wet muscle tissue.

2.5 Statistical analysis

Collected data were statistically evaluated by one-factorial ANOVA using StatSoft Statistica® Software (2009). The differences between treatments for all parameters were tested according to the following statistical model:

\[ Y_{ij} = \mu + a_i + e_{ij} \]  \hspace{1cm} (1)

where \( Y_{ij} \) is variance associated with parameter \( a \), \( \mu \) is the overall mean, \( a_i \) is the treatment effect and \( e_{ij} \) the error term. The individual measurements for chicken muscles were treated as the experimental units and differences between treatments means were analyzed for significance (\( P < 0.05 \)) using Tukey’s test. The data are presented as an average value and accompanied by SEM (standard error).

All procedures that were carried out on animals have been approved by the Local Ethic Commission for animal experiments.

3 Results and discussion

3.1 Selenium content in chicken breast and leg meat

Selenium is an essential micro nutrient in chicken diet. Concentrations of selenium in broiler chicken breast and leg meat averaged from 10.5 µg 100 g$^{-1}$ to 12.8 µg 100 g$^{-1}$ for leg and breast meat respectively (Fig. 1). The supplementation of birds’ diet with both inorganic (sodium selenite) and organic (selenized yeast) selenium, significantly (\( p \leq 0.05 \)) increased the selenium level in chicken breast and leg meat. Moreover, the supplementation of birds’ diet with organic selenium was more efficient compared to its inorganic form. Choct et al. [30] found that an increasing supplementation rate of Se from 0.10−0.25 mg kg$^{-1}$ increased the breast muscle selenium concentration to 0.23−0.28 mg kg$^{-1}$ for both selenium sources (organic and inorganic). They reported that the amount of Se available for assimilation by the tissues was dependent on the source and concentration of the element while organic Se is deposited in the body tissues more efficiently than inorganic selenium. This
is probably due to different absorption mechanisms for the organic and inorganic forms of selenium. Inorganic selenium is passively absorbed from the intestine by a simple diffusion process, whereas organic selenium is actively absorbed through the amino acid transport mechanisms [31-32]. Spears et al. [33] reported that broiler chickens that were fed 0.15 ppm Se-Met had increased breast Se concentrations compared with those that were fed sodium selenite. Dietary selenium is converted in living organisms, via various metabolic pathways, to selenite, selenodiglutathione, methylselenol, selenomethionine and Se-methylselenocysteine \((\text{CH}_3\text{SeCH(NH)COOH})\) [34], whose molecular action are related to redox changes in cells, leading to the inhibition of proliferation and enhancement of apoptosis [35]. The other role of selenium in diets is the modulation of antioxidant enzyme glutathione peroxidase (GSH-Px) activities. It was suggested that GSH-Px contributes to the total muscle antioxidant defense system, decreasing tissue susceptibility to oxidation accomplished by organic Se supplementation of the poultry diets [36].

### 3.2 Changes in meat protein carbonyls after modulation with selenium

Carbonyl group concentration is a primary indicator of meat protein oxidation. As muscle tissue is rich in substances initiating oxidative reactions, like metal ions, free radicals generated during lipid oxidation and other pro-oxidants, proteins are susceptible to oxidative alteration, especially during long lasting storage and processing [18,37]. Amino acid residues are modified as a direct consequence of the oxidative damage leading to the generation of oxidized derivatives, such as protein carbonyls and the formation of cross-links and aggregates [37]. The concentration of carbonyl groups in fresh Flex chicken averaged 2.41 nM g\(^{-1}\) proteins in breast and 1.25 nM g\(^{-1}\) protein in leg muscles, respectively (Table 1). No significant \((p \leq 0.05)\) effects of organic selenium supplementation on protein carbonyl content were noted in breast muscles, whilst in leg muscles an increased level of carbonyls was observed. Selenized yeast contains both forms of organic selenium, mainly selenomethionine and small level of selenocysteine. Whereas selenomethionine is randomly fused into the protein structure in place of methionine, selenocysteine incorporation into proteins requires a complicated translation mechanism [16]. Selenomethionin is easily oxidized to the corresponding selenoxide, which can be reduced non-enzymatically by two equivalents of glutathione [38]. Therefore, selenomethionine could play an important role in antioxidative mechanisms and protect proteins from oxidative damage due to the suppression of repairable over non-repairable sites of damage, which also require proteolytic degradation of the protein [16]. In opposition to the organic Se form, selenite decreased the concentration of CO groups in fresh breast muscles. However, it was not effective in the protection against protein oxidative alteration in leg muscles. The reason may be due to the fact that inorganic selenium, which is incorporated into proteins in the form of selenocysteine, can enhance specific formation of selenoproteins. One of these is selenoprotein W, occurring at high levels in muscles from selenium-supplemented animals, but almost undetectable in cases of white muscle disease associated with a lack of selenium in diet, when most of the selenoproteins are not created [39]. Chilled storage of the meat did not modify protein carbonyls in control samples, however, strong antioxidant action was observed in breast muscles collected from birds supplemented with organic selenium (0.38 mg kg\(^{-1}\)) and sodium selenite (0.38 mg kg\(^{-1}\) and 0.50 mg kg\(^{-1}\)). Antioxidant action of dietary selenium expressed by a lower dynamic of carbonyl group formation, was also observed by Wang et al. [36]. In contrast, in leg muscles, more carbonyl groups were detected after dietary modulation with selenium (for 0.26 mg kg\(^{-1}\) and 0.50 mg kg\(^{-1}\) for inorganic and organic selenium, respectively), which could be explained as a pro-oxidative action of the microelement. Hence, in high concentrations selenium compounds are toxic by their pro-oxidant catalytic activity to produce superoxide \((\text{O}_2^-)\), hydrogen peroxide, and very likely other cascading oxyradicals [40], which can accelerate lipid and protein oxidation in meat. Three-month freezing storage resulted in a significant \((p \leq 0.05)\) increase in the formation of protein carbonyls in control breast and leg muscles (up to 3.0−3.5 nM g\(^{-1}\) protein), indicating the extent of oxidation processes in relation to fresh and chilled meat. Both forms of selenium effectively \((p \leq 0.05)\) protected chicken breast and leg muscle proteins against oxidatively induced alterations during frozen storage. Moreover, in breast muscles the lowest changes in CO groups were found with higher levels of inorganic selenium (0.38 mg kg\(^{-1}\) and 0.50 mg kg\(^{-1}\)), which corresponds to the relation observed in fresh and chilled meat. Furthermore, in leg muscles the effect for both forms of selenium was detrimental. Petrovic et. al. [41] found a differentiated effect of organic and inorganic Se forms on oxidation processes in frozen stored breast and thigh muscles. Inorganic selenium was more effective in the protection of muscle tissue components against oxidation, expressed by peroxide value, whereas oxidized lipid derivative formation was
Table 1: Carbonyl and sulphydryl groups content in breast and leg chicken muscles after dietary modulation with selenium.

|          | Breast |         | Leg   |         |
|----------|--------|---------|-------|---------|
|          | CO     | SH      | CO    | SH      |
| Fresh    |        |         |       |         |
| control  | 2.41 defg | 64.74 h | 1.25 ab | 96.10 e |
| Sen0.26  | 2.25 abcd | 63.30 gh | 1.22 ab | 94.93 de |
| Sen0.38  | 2.16 abc | 62.53 gh | 1.33 abc | 95.13 de |
| Sen0.5   | 2.05 a   | 62.13 gh | 1.16 a | 95.48 de |
| Seo0.26  | 2.40 defg | 63.18 gh | 1.53 bcd | 94.64 de |
| Seo0.38  | 2.41 defg | 63.60 gh | 1.62 cdef | 92.15 cde |
| Seo0.5   | 2.30 bcdef | 63.05 gh | 1.74 def | 92.68 cde |
| SEM      | 0.03 | 0.18 | 0.049 | 0.311 |
| Chilled  |        |         |       |         |
| control  | 2.54 g | 63.97 gh | 1.34 abc | 93.20 cde |
| Sen0.26  | 2.41 defg | 62.41 gh | 1.72 def | 92.89 cde |
| Sen0.38  | 2.28 bcde | 59.25 ef | 1.55 bcde | 89.99 bcde |
| Sen0.5   | 2.15 ab | 57.17 de | 1.37 abc | 89.19 bcde |
| Seo0.26  | 2.48 efg | 62.48 gh | 1.51 bcd | 91.51 cde |
| Seo0.38  | 2.29 bcde | 61.51 fg | 1.55 bcde | 90.25 bcde |
| Seo0.5   | 2.38 cdefg | 61.73 fg | 1.74 def | 90.37 bcde |
| SEM      | 0.03 | 0.42 | 0.033 | 0.359 |
| Frozen   |        |         |       |         |
| control  | 3.49 h | 49.12 b | 3.02 g | 70.29 a |
| Sen0.26  | 2.36 bcdefg | 54.65 d | 1.87 ef | 81.80 b |
| Sen0.38  | 2.30 bcdef | 42.96 a | 1.90 f | 86.14 bcd |
| Sen0.5   | 2.27 abcde | 42.00 a | 1.64 cdef | 88.91 bcde |
| Seo0.26  | 2.53 g | 51.95 c | 1.74 def | 83.92 bc |
| Seo0.38  | 2.53 g | 48.39 b | 1.79 def | 68.97 a |
| Seo0.5   | 2.51 fg | 47.96 b | 1.90 f | 69.79 a |
| SEM      | 0.08 | 0.84 | 0.085 | 1.826 |

p Storage 0.000 0.000 0.000 0.000
Feeding 0.000 0.000 0.000 0.000
FxS 0.000 0.000 0.000 0.000

a,b,c... statistically significant differences $p \leq 0.05$
equally inhibited by both organic and inorganic selenium. Higher dynamic of carbonyl group formation during freezing in relation to fresh and chilled muscles was observed in leg rather than breast chicken muscles. This was similar to the results of Soyer et al. [42] and could be explained by differences in chemical composition, especially in regard to lipids and iron. Incorporation of selenium compounds (both organic and inorganic) to chicken diet increased selenium levels in breast and leg meat (Fig. 1), which significantly (p ≤ 0.05) reduced the formation of protein carbonyls during frozen storage.

3.3 Changes in meat protein sulfhydryls after modulation with selenium

Radical-mediated oxidation of proteins leads to the conversion of sulfhydryl groups into disulfides and other oxidized species [43]. Selenium incorporated into the chicken diet did not significantly (p ≤ 0.05) change the sulfhydryl group concentration in breast and leg muscles (Table 1). Storage of meat at chilling conditions up to seven days was found not to influence (p ≤ 0.05) SH content, unless, in leg meat, sodium selenite was added to the chicken diet. Molecular oxygen and trace metals are important reagents in the spontaneous generation of cysteine sulfenic acid, which can serve as a means of regulating protein activity by helping to absorb oxidative insults [44]. However, inorganic forms of selenium can react with tissue thiols to create selenotrisulfides which are prone to reaction with other thiols, generating free radicals by redox catalysis [45] and accelerating oxidation. Longer storage generally led to a decrease in muscle proteins sulfhydryl group content [46]. Reduction in SH content was observed for all analyzed samples after three-months of frozen storage. A significant (p ≤ 0.05) decrease in SH groups occurred in frozen breast muscles after dietary modulation with sodium selenite exceeded 0.38 mg of Se per kg diet. It was already found that selenite and methylselenol, even at micromolar level, are able to facilitate intramolecular S–S bond formation in the cysteine-containing catalytic subunit of protein kinase C, leading to enzyme inactivation in precancerous cells [47]. However, inorganic selenium was effective in the protection of SH groups against alteration by oxidation processes during frozen storage of chicken leg meat. Some explanation of inhibitory mechanisms of Se compounds relating to the modification of cysteine residues in proteins, can involve both the formation of Se adducts/intermediates of the selenotrisulfide (S-Se-S) or selenenylsulfide (S-Se) type and oxidation of thiol groups, as well as reduction of disulfide bonds [48]. However, on the other hand, thiols do not spontaneously oxidize one another to generate disulfide bonds in the absence of an oxidizing reagent [49].

3.4 Total antioxidative potential of meat after modulation with selenium

The measurement of TAC is a useful tool in evaluating the anti-oxidative role of the investigated compounds. To specify a radical scavenging capacity of the analyzed samples, two or more assays are required to investigate heterogeneous samples, since each assay involves different chemicals and may reflect different aspects of their antioxidant properties [50]. The following assays including 2,2-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging methods, as well as ferric reducing antioxidant power (FRAP) have been frequently used to estimate antioxidant capacities. Even though DPPH and ABTS tests are radical scavenging assays, and they can be used to determine both hydrophilic and hydrophobic antioxidant capacities, the dynamic of the oxidation processes inhibition will vary due to different methods of radical and sample preparation, as well as stability of the radicals [51-52]. The total anti-oxidative potential of chicken breast and leg meat after modulation with selenium is presented in Table 2. Dietary selenium modulation did not affect the total antioxidant power of fresh and chilled breast chicken meat. Organic selenium, which is considered to be a more available form of micronutrient [53], increased the DPPH radical scavenging capacity of broilers leg muscles after chilling storage. A higher total anti-oxidative capacity of chicken breast meat after supplementation with selenium was also reported by Wang et al. [54-55]. The other authors showed that the selenium yeast alone, and in combination with sodium selenite, significantly increased (p ≤ 0.05) the Se content and TAC in breast muscle. After 12 days of storage at 4°C TAC remained stable. It was concluded that selenium yeast was more effective than sodium selenite in increasing TAC and the oxidative stability of chicken breast meat [56]. Similar results were obtained in a study involving geese, where dietary selenium yeast also improved the antioxidant capacity of goose breast muscle [57]. No significant (p ≤ 0.05) effects of selenium addition were noted on the total anti-oxidative potential of frozen leg meat, whereas TAC of breast meat after freezing increased significantly (p ≤ 0.05) when selenium was present in chicken diet. Differences in chemical
Table 2: Total antioxidative status of breast and leg chicken muscles after dietary modulation with selenium

|          | Breast          |          | Leg           |          |
|----------|-----------------|----------|---------------|----------|
|          | DPPH ABTS FRAP  | DPPH ABTS FRAP |
| Fresh    |                 |           |               |          |
| Control  | 6.09 a          | 10.155 de | 0.681 b       | 14.67 abc| 8.78 bc  | 1.29 ab |
| Sen0.26  | 6.21 a          | 10.203 de| 0.687 b       | 15.69 bcde| 8.60 abc | 1.30 ab |
| Sen0.38  | 6.13 a          | 9.521 d  | 0.658 b       | 12.36 a  | 7.08 a   | 1.17 a  |
| Sen0.5   | 6.54 a          | 9.123 d  | 0.682 b       | 13.01 a  | 9.52 bc  | 1.20 ab |
| Seo0.26  | 6.29 a          | 9.341 d  | 0.740 b       | 17.49 defgh| 9.27 bc  | 1.24 ab |
| Sen0.38  | 6.70 a          | 10.250 de| 0.746 b       | 13.78 ab | 7.09 a   | 1.14 a  |
| Sen0.5   | 6.47 a          | 11.500 e | 0.686 b       | 14.91 abcd| 9.77 c   | 1.36 b  |
| SEM      | 0.09            | 0.210    | 0.012         | 0.389    | 0.254    | 0.020   |
| Chilled  |                 |           |               |          |
| control  | 12.00 bcd       | 2.594 c  | 0.165 a       | 15.01 abcd| 8.47 abc | 1.25 ab |
| Sen0.26  | 11.50 b         | 2.138 abc| 0.123 a       | 16.67 cdefg| 8.40 abc | 1.27 ab |
| Sen0.38  | 12.12 bcd       | 2.302 abc| 0.129 a       | 16.13 bcdefg| 8.11 abc | 1.25 ab |
| Sen0.5   | 13.72 de        | 2.616 c  | 0.157 a       | 15.84 bcdef| 8.55 abc | 1.22 ab |
| Seo0.26  | 11.62 b         | 2.128 abc| 0.136 a       | 16.70 cdefg| 8.65 abc | 1.22 ab |
| Seo0.38  | 12.31 bcd       | 2.251 abc| 0.144 a       | 18.08 efgh| 8.62 abc | 1.22 ab |
| Seo0.5   | 13.56 cde       | 2.481 bc | 0.159 a       | 19.70 hi  | 9.73 c   | 1.23 ab |
| SEM      | 0.20            | 0.210    | 0.003         | 0.315    | 0.114    | 0.009   |
| Frozen   |                 |           |               |          |
| Control  | 5.74 a          | 1.485 abc| 0.154 a       | 17.94 efghi| 8.26 abc | 1.13 a  |
| Sen0.26  | 11.19 b         | 1.744 abc| 0.133 a       | 18.00 efghi| 8.29 abc | 1.20 ab |
| Sen0.38  | 11.73 b         | 1.122 ab | 0.132 a       | 18.29 efghi| 7.93 ab  | 1.21 ab |
| Sen0.5   | 12.81 bcd       | 1.204 abc| 0.138 a       | 18.47 fghi| 8.32 abc | 1.22 ab |
| Seo0.26  | 11.80 bc        | 0.954 a  | 0.136 a       | 18.13 efghi| 8.58 abc | 1.16 a  |
| Seo0.38  | 12.55 bcd       | 0.973 a  | 0.145 a       | 18.68 ghi | 8.37 abc | 1.16 a  |
| Seo0.5   | 14.86 e         | 0.861 a  | 0.142 a       | 20.19 i   | 9.11 bc  | 1.22 ab |
| SEM      | 0.52            | 0.067    | 0.002         | 0.164    | 0.087    | 0.010   |
| p        |                 |           |               |          |
| Storage  | 0.00            | 0.000    | 0.000         | 0.000    | 0.352    | 0.003   |
| Feeding  | 0.00            | 0.008    | 0.192         | 0.000    | 0.000    | 0.016   |
| FxS      | 0.00            | 0.001    | 0.282         | 0.000    | 0.006    | 0.029   |

a,b,c... statistically significant differences p ≤ 0.05
composition, distribution of chemical substances and, as well, protein polymorphism (aerobic and anaerobic metabolism of muscle fibres) are considered to determine the susceptibility of muscle tissue to oxidation processes [22]. Supplementation of chicks diet with selenium compounds was proved to enhance muscles and the organ’s antioxidant status [58], also with depleting levels of other natural antioxidants like vitamin E.

4 Conclusions

Selenium was effective in protection against protein reactive groups alteration to carbonyls during frozen storage of both types of chicken muscles i.e. breast (with white fibre domination) and leg (with red fibre domination). Sodium selenite effectively inhibited the loss of protein sulphydryl groups in leg muscles during frozen storage. Selenium compounds can be used in broilers nutrition as a protein anti-oxidizing agent, especially in perspective of the long storage of meat under freezing conditions.

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