The influence of organic selenium feed supplement and fasting on oxidative damage in different tissues of broiler chickens

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ABSTRACT
The influence was investigated of organic selenium feed supplements on lipid peroxidation and protein oxidation in different tissues of Ross 308 broiler chickens of both sexes in response to 48 hours food deprivation. Chickens were randomly allocated into a standard diet fed control group (N = 50) and an experimental group, pair-fed with the same diet supplemented with 0.3 ppm organic selenium (Sel-Plex®, Alltech, Inc., KY; N = 50). At the age of 42 days and after 48 hours food deprivation, ten chickens from the experimental group and ten chickens from the control were sacrificed and the liver, kidneys and small intestines were removed. Liver, small intestine and kidney tissue homogenates were analysed for lipid peroxide concentrations (LPO) and protein carbonyl content (PCC). The PCC in kidney and small intestine of experimental chickens at the end of the fattening period was higher than in the control broilers (P<0.05). As a result of the food deprivation, lower PCC was found in the liver of both groups (P<0.05). Simultaneously, the values for the experimental group were lower than in the control group (P<0.05). Kidney PCC was only increased after fasting in the control birds (P<0.05). The LPO in the kidneys at the end of fattening was higher in chickens fed diet supplemented with organic selenium (P<0.05). Kidney and small intestine tissue LPO of both groups was decreased after fasting (P<0.05). At the same time the LPO in the liver of the experimental group was increased (P<0.05). The results of this study demonstrate that dietary organic selenium supplements increase oxidative damage in the kidneys and small intestines at the end of fattening. Food deprivation resulted in decreased tissue oxidative damage due to the lower metabolic rate.

Key words: chicken, organic selenium, food deprivation, lipid peroxidation, protein oxidation

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Introduction

Selenium is an essential trace element that regulates antioxidative mechanisms in all living cells incorporated as selenocysteine in glutathione peroxidase and thioredoxin reductase and many other selenoproteins. There are two major sources of selenium for poultry feed supplementation: organic selenium, mainly in the form of selenomethionine, and selenite or selenate as inorganic selenium. The inorganic form is widely used for dietary supplementation (SURAL, 2002). Organic selenium has an advantage in reducing oxidative stress in comparison with inorganic forms (MAHMOUD and EDENS, 2003; VINSON et al., 1998). After absorption it is incorporated into skeletal muscles, kidney, liver and gastrointestinal mucosa proteins as selenomethionine and selenocysteine. This selenium allows reversible release by normal and intensive metabolic processes (SCHRAUZER, 2000).

Approximately half or 40% of whole-body selenium is in glutathione peroxidase and its presence increases enzyme activity 100-1000 fold (BURK, 2002). Enzymes glutathione peroxidase, catalase and superoxide dismutase and nonenzymatic molecules (glutathione, vitamins A, E, C, uric acid, bilirubin, etc.) are major determinants of tissue susceptibility to oxidant injury (MICHELS et al., 1994).

Oxidant injury may result from the increased generation of reactive oxygen species and/or from a decrease in antioxidant defence (IVANOVA and IVANOV, 2000). Reactive oxygen species interact with a number of cellular components. The damage manifests as peroxidation of membrane polyunsaturated fatty acid chains and disruption of the cohesive lipid bilayer arrangement and structural organisation (YU, 1994).

Amino acids, the building blocks of peptides and protein macromolecules, are also targets of free radical attack. This results in modification of DNA and carboxylation, and loss of sulfhydryls in proteins, among other changes. Carbonyl modifications of proteins occur in certain amino acid residues present near transition metal-binding sites. After oxidative modification, the protein becomes highly sensitive to proteolytic degradation, which, in the case of enzymes, leads to their conversion into catalytically inactive or less active, more thermolabile forms (STADTMAN and OLIVER, 1991).

Nutritional factors may influence the sensitivity of tissues to oxidative stress and the effects tend to be most marked in the case of nutritional deficiencies, which are generalized in nature or involved in the biochemical processes which determine tissue antioxidant status. Starvation has been associated with a complex pattern of antioxidative enzyme and nonenzymatic molecule alterations, the nature of which varied with the particular tissue studied (CHO et al., 1981; GODIN and WOHAIEB, 1988; DI SIMPLICIO et al., 1997). Thus, the aim of this study was to determine the influence of organic selenium feed supplements on lipid peroxidation and protein oxidation in the liver, kidneys and small intestines, in response to 48 hours food deprivation.
Materials and methods

The experiment was performed on Ross 308 chickens. One hundred newly hatched broiler chickens were allocated to pens. Ambient temperature of the experimental room was set to 32 °C at the time of placement, and was gradually reduced to 20 °C over a 6 week period. Lights were on continuously. Feed and water were provided ad libitum. The diets consisted of starter diet -12.85 MJ/kg ME, 22.5% crude protein (CP, 1-10 days of age); grower diet -12.85 MJ/kg ME, 18.5% CP (11-29 days of age); and finisher diet -12 MJ/kg 16% CP (30-42 days of age).

Seven-day-old chickens were randomly allocated into two groups: the standard diet (containing min. 0.15 ppm selenium as sodium selenite) fed control group and the experimental group, pair-fed with the same diets supplemented with 0.3 ppm organic selenium (Sel Plex™, Alltech, Inc., KY). At the age of 42 days, ten chickens from the control and ten from the experimental group were sacrificed and the liver, kidneys and small intestines were removed. The rest of the animals were subjected to 48-h fasting with free access to tap water. After the period of fasting, ten chickens from the control and ten from the experimental group were also sacrificed and the same tissues removed. All tissues were frozen at -80 °C until analyzed.

Tissues were homogenised in 0.14 mol/L KCl with Schüthomogen™ homogenizer (Schütt labortechnik, Germany) at 2800 rpm, 60 s on ice and centrifuged (10 000 g, 15 min., 4 °C; Sigma 3 K 15 Germany). Lipid peroxide concentration, protein carbonyl content and protein concentrations were determined by spectrophotometry on Thermospectronic Helios delta (Unicam, Cambridge, UK) in supernatants.

Lipid peroxide concentration (LPO), was measured according to the method of OHKAWA et al. (1979). It detects malondialdehyde and other thiobarbituric acid-reactive substances, decomposition products of polyunsaturated fatty acid hydroperoxides. The quantification of LPO was carried out by comparing the absorption at 532 nm to the standard curve of MDA equivalent generated by hydrolysis of 1,1,3,3 tetramethoxy propane.

Protein carbonyl content (PCC) was measured using the 2.4-dinitrophenylhydrazine (DNPH)-labelling procedure (LEVINE et al., 1990). Samples (protein concentration <1 g/L) are derivatized with DNPH, followed by precipitation with TCA (trichloroacetic acid). Protein pellets are dissolved in guanidine hydrochloride. The absorbance of protein-hydrozone is measured at 360 nm. The reference absorptivity of 21.0 mM−1cm−1 was used to calculate mmol DNPH incorporated per mg of protein.

Protein concentration was determined by the method of LOWRY et al. (1951). The LPO and PCC concentrations were expressed as μmol per g of protein.

The obtained data were evaluated by the Statistica 9.1 program (StatSoft, Inc., USA). The data were checked for normality (Kolmogorov-Smirnov and Shapiro Wilk’s W
Descriptive statistics were performed and the results presented as mean ± standard deviation (mean ± SD). The results were processed by the Mann Whitney test or Student’s \( t \) test. A probability level of \( P<0.05 \) was considered statistically significant.

**Results**

Values of lipid peroxide concentrations in the chicken livers, kidneys and small intestines before and after 48 hours fasting are presented in Table 1. Lipid peroxide concentrations in the kidney of chickens given organic selenium after the fattening period and after 48-h fasting was significantly higher compared to the control group (\( P<0.05 \)). The fasting resulted in a significant decrease in the lipid peroxide levels in the kidneys and small intestines of both groups of chickens (\( P<0.05 \)). Simultaneously, in the livers of Sel Plex chickens increased peroxide generation was noticed (\( P<0.05 \)).

Table 1. Changes in lipid peroxide concentrations (\( \mu \text{mol/g protein} \)) in chicken livers, kidneys and small intestines before and after 48 hours fasting for the control group (standard diet) and the experimental group (organic selenium supplementation)

<table>
<thead>
<tr>
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<th>Before 48-h fasting</th>
<th>After 48-h fasting</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Experimental</td>
</tr>
<tr>
<td>Liver(^1)</td>
<td>0.67 ± 0.17</td>
<td>0.55 ± 0.16</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.68 ± 0.16</td>
<td>0.97 ± 0.17(^a)</td>
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<tr>
<td>Small intestine(^1)</td>
<td>1.88 ± 0.20</td>
<td>1.75 ± 0.29</td>
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Values are expressed as means ± standard deviation; \(^1\) Data presented at Beer Ljubić et al., (2012); \(^a\) Significant difference between the control and experimental groups (\( P<0.05 \); **Significant difference between the experimental groups (\( P<0.05 \); \( \mu \text{mol/g protein} \))

Table 2. Changes in protein carbonyl content (\( \mu \text{mol/g protein} \)) in chicken livers, kidneys and small intestines before and after 48 hours fasting for the control group (standard diet) and the experimental group (organic selenium supplementation)

<table>
<thead>
<tr>
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<th>before 48-h fasting</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Experimental</td>
</tr>
<tr>
<td>Liver</td>
<td>113.63 ± 19.74</td>
<td>131.01 ± 29.78</td>
</tr>
<tr>
<td>Kidney</td>
<td>87.42 ± 11.07</td>
<td>113.55 ± 14.86*</td>
</tr>
<tr>
<td>Small intestine</td>
<td>76.13 ± 8.53</td>
<td>87.63 ± 11.06*</td>
</tr>
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Values are expressed as means ± standard deviation; \(^a\) Significant difference between the control and experimental groups (\( P<0.05 \)); \( \mu \text{mol/g protein} \))
Table 2 shows PCC in chicken livers, kidneys and small intestines before and after 48 hours fasting. Organic selenium feed supplement resulted in higher PCC concentrations in the kidneys and small intestines at the end of the fattening period (P<0.05). As a result of the food deprivation, lower PCC was found in the livers of both groups (P<0.05). Simultaneously, the values for the experimental group were lower than in the control group (P<0.05). Kidney PCC was only increased after fasting in the control birds (P<0.05).

**Discussion**

Organic selenium possesses antioxidative properties (SCHRAUZER, 2000). The selenium in Sel-Plex includes selenomethionine, selenocysteine and related compounds suited to digestion and metabolism. It has been suggested that selenomethionine and glutathione act as an antioxidant system, protecting cells against free radicals, such as peroxynitrite (SCHRAUZER, 2000).

**Lipid peroxidation.** Lipid peroxidation arising from the reactions of free radicals with lipids is considered a prevalent and important feature of the cellular injury brought about by free radical attack (YU, 1994). Lipid peroxidation potential is mainly a reflection of the degree of unsaturation of the fatty acids present in the membranes and is species, strain and gender dependent (PIRŠLJIN et al., 2006; RIKANS and HORNBROOK, 1997).

In the present study LPO in the livers and small intestines of the experimental group of chickens at the end of a fattening period was not significantly different in comparison with the control group (P>0.5, Table 1). At the same time the kidney LPO was significantly higher in chickens fed organic selenium supplemented feed (Table 1). Organic selenium feed supplementation results in a higher level of selenium in the eggs and liver of newly hatched chicks (SURAI, 2000). SURAI (2000) found lower liver lipid peroxide accumulation in 5 day old chicks hatched from the eggs of broiler breeder hens fed a diet supplemented with organic selenium, than in chickens hatched from the eggs of broiler breeder hens fed a standard diet. ARAI et al. (1994) found no influence of organic selenium on plasma lipid peroxide concentrations in broiler chickens given a dietary supplement of organic selenium. The protective effect of organic selenium feed supplementation on lipid oxidation diminishes during the fattening period.

Food deprivation has diverse effects on tissue and plasma components; the duration of deprivation has an important influence. Our previous study has shown that fasting resulted in lower levels of reduced glutathione and lipid peroxide in the blood of cockerels and pullets (PIRŠLJIN et al., 2006) as well as total antioxidant status (MILINKOVIĆ TUR et al., 2007).

Fasting for 24 or 72 hours in chickens reduces body temperature (AIT-BOULAHSEN et al., 1989). Like temperature changes, alterations in food availability and composition cause a decrease of triiodothyronine (McNABB, 2000; POWER et al., 2000). Starvation...
lowers plasma corticosterone levels and brain Na\(^+\)K\(^-\)-ATPase activity in rats (SHAHEEN et al., 1996). This response by animals to food deprivation is an adaptive mechanism to suppress the stress-induced rise in catabolic hormones, saving energy expenditure.

The liver LPO after food deprivation in the present study was elevated only in the experimental group of chicken. This is in agreement with HIDALGO et al. (1990) and DOMENICALI et al. (2001). The authors observed an increase of lipid peroxide concentrations in rat livers after 18 and 36 hours’ food deprivation. The same results were reported in the livers of the fish Sparus aurata after 46 days’ starvation (PASCUAL et al., 2003). On the other hand MARCZUK-KRYNICKA et al. (2003) observed the unaltered content of lipid peroxides in rat livers exposed to 36-hour food deprivation.

The kidney and small intestine tissue LPO decreased after food deprivation in the present investigation, suggesting that food deprivation lowers metabolic rates and the production of free radicals. The intestinal mucosa is constantly challenged by diet-derived oxidants and lipid hydroperoxides, as well as endogenously generated reactive oxygen species (AW et al., 1992; AW, 1999). In such conditions antioxidant protective mechanisms in the intestines are the first line of defence against all those toxic elements. Food deprivation decreases lipid peroxidation, because the intestines are not challenged by dietary lipid hydroperoxides as well as other toxins.

**Protein oxidation.** Oxidative inactivation of enzymes and oxidative modification of proteins by metal-catalyzed oxidation reactions are accompanied by the generation of protein carbonyl derivatives that can react with DNPH to form protein hydrazone derivatives (OLIVER et al., 1990). The accumulation of oxidized proteins may be an early indication of oxygen radical mediated tissue damage. Since the intracellular level of oxidized proteins reflects the balance between the rate of their oxidation and the rate of degradation, the accumulation of oxidized protein is a complex function of the numerous factors that govern the synthesis and oxidation of proteins and the activities of various proteases that selectively degrade the oxidized forms (STADTMAN and OLIVER, 1991).

In the present study, protein carbonyl content (PCC) in the kidneys and small intestines of chickens given organic selenium at the end of the fattening period, was higher than in the control broilers (Table 2).

As a result of the food deprivation, lower PCC was found in the livers of both groups, suggesting that liver proteins may be more resistant than lipids to the oxidative stress related to food deprivation. At the same time, the liver PCC of the experimental group (Table 2) was significantly lower than in the control group. Kidney PCC after fasting only increased in the control birds. Presumably, organic selenium has a protective effect on protein oxidative damage in the liver and kidneys.
We found no data on PCC in food-deprived chickens in the available literature, so our results may be the first to point to certain dynamics of changes in protein carbonyl concentrations in chickens. The role of this oxidative injury indicator needs to be investigated.

**Conclusion.** We assessed the concentrations of LPO and PCC, as indicators of tissue oxidative injury, in chickens given a dietary supplement of organic selenium. Starvation and organic selenium supplementation are associated with alterations in oxidant injury, that differ from tissue to tissue. Organic selenium supplementation had no beneficial effect on the lipid oxidation of tissues studied in this experiment. After food deprivation biomarkers of oxidative damage generally decreased, suggesting a decrease in metabolic rate and the production of free radicals. This data provide the basis for further studies of the tissue antioxidative system and indicators of tissue oxidative status.

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**References**


povećana (P<0,05). Rezultati istraživanja pokazuju da dodatak organskog selena u hrani uzrokuje oksidativna oštećenja u hubrezima i tankom crijevu pilića na kraju tova. Gladovanje smanjuje stupanj oksidativnih oštećenja zbog smanjenja intenziteta metabolizma.

**Ključne riječi:** pilići, organski selen, gladovanje, lipidna peroksidacija, oksidacija proteina