

Influence of organic selenium food supplements and fasting on oxidative damage in different tissues of broiler chickens

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The influence of organic selenium food 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 were investigated. Seven days old chickens were randomly divided into two groups of 20 broilers each as following: standard diet fed control group and experimental group, pair-fed with the same diet supplemented with 0.3 ppm organic selenium (Sel-Plex[®], Alltech, Inc., KY). At the age of 42 days, ten chickens of control and ten chickens of experimental group were sacrificed and liver, kidney and small intestine were removed. Other chickens were deprived of food for 48 hours with free access to tap water. After the period of fasting, ten chickens of control and ten chickens of experimental group were also sacrificed and the same tissues were removed. Liver, small intestine and kidney tissue homogenates were analysed spectrophotometrically for lipid peroxide concentrations measured as thiobarbituric-acid reactive substances (TBARS) and protein carbonyl content (PCC). A complex pattern of changes was observed. The PCC in kidney and small intestine of experimental chickens at the end of fattening period was higher than in control broilers ($P<0.05$). As a result of the food deprivation, lower PCC was found in liver of both control and experimental group ($P<0.05$). Simultaneously, the values for experimental group was significantly lower than in the control group ($P<0.05$). Kidney PCC was increased after fasting only in control birds ($P<0.05$). The TBARS concentration in kidney at the end of fattening was significantly higher in chickens fed diet supplemented with organic selenium ($P<0.05$). Kidney and small intestine tissue TBARS concentrations were decreased after food deprivation of both control and experimental group ($P<0.05$). At the same time TBARS concentration in liver of experimental group was increased ($P<0.05$). Results of this study demonstrated that dietary organic selenium supplements increased lipid peroxidation in kidney and protein oxidation in kidney and small intestine at the end of fattening. Food deprivation resulted in decreased tissue oxidative damage.

Keywords: chicken; organic selenium; food deprivation; lipid peroxidation; protein oxidation

Introduction

Selenium is an essential trace element that regulates antioxidative mechanisms in all living cells incorporated as selenocysteine in glutathione peroxidase (GSH-Px) and thioredoxin reductase and many other selenoproteins. There are two mayor sources of selenium for poultry organic selenium, mainly in the form of selenomethionine, and inorganic selenium, mainly selenite or selenate, which are widely used for dietary supplementation (Surai, 2002). Organic selenium has an advantage in reducing oxidative stress in comparison with inorganic forms (Mahmoud and Edens, 2003) and incorporates 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). Furthermore, selenomethionine oxide is easily reduced back to

selenomethionine by glutathione and oxidative damage of selenomethionine is reversible. On the basis of this observation, selenomethionine and glutathione were suggested to act as an antioxidant system, protecting cells against oxidants such as peroxynitrite (Schrauzer, 2000).

Approximately half or 40% of whole-body selenium is in GSH-Px and its presence increases enzyme activity 100-1000 fold (Burk, 2002). Enzyme GSH-Px with 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 (Michiels et al., 1994). Oxidant injury can result from the increased generation of reactive oxygen species and/or from decrease in antioxidant defense (Ivanova and Ivanov, 2000). Reactive oxygen species interact with a number of cellular components. The damage manifests as the peroxidation of membrane polyunsaturated fatty acid chains and disrupts the cohesive lipid bilayer arrangement and structural organization (Yu, 1994).

Amino acids, the building blocks of peptides and protein macromolecules, are also targets of free radical attack. It results in modification of DNA and carbonylation 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, and in the case of enzymes they are converted to catalytically inactive or less active, more thermolabile forms (Stadtman and Oliver, 1991).

Nutritional factors can influence the sensitivity of tissues to oxidative stress and 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 was associated with 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 Wohhaieb, 1988; Di Simplicio et al., 1997). Thus, the aim of this study was to determine the influence of organic selenium food supplements on lipid peroxidation and protein oxidation in liver, kidney and small intestine in response to 48 hours food deprivation.

Material and methods

The experiment was performed on Ross 308 chickens. One hundred newly hatched broiler chickens were allocated in pens. Ambient temperature of the experimental room was set at 32 °C at the time of placement, and over 6 weeks growing period, ambient temperature was reduced to 20 °C. Lights were on continuously. Feed and water were provided for *ad libitum* consumption. The diets consisted of the starter diet – 12.85 MJ/kg ME, 22.5% 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 days old chickens were randomly divided into two groups: standard diets (containing min. 0.15 ppm selenium as sodium selenite) fed control group and 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 of control and ten chickens of experimental group were sacrificed and liver, kidney and small intestine 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 of control and ten chickens of experimental group were also sacrificed and the same tissues were removed. All tissues were frozen at - 80 °C until analyzed.

After centrifugation of the homogenates (10 000 g, 15 min., 4 °C) in supernatants lipid peroxide concentration, protein carbonyl content and protein concentrations were determined.

Lipid peroxide concentration measured as thiobarbituric acid reactive substances (TBARS) was performed according to the method of Trota et al. (1982). Absorption peak was measured at 532 nm and concentration was calculated using molar extinction coefficient of 1.5×10^5 (Placer et al., 1966). TBARS concentration was expressed per g of protein.

Protein carbonyl content (PCC) was measured spectrometrically using the 2,4-dinitrophenylhydrazine (DNPH)-labelling procedure (Levine et al., 1991). Aliquots of homogenate (~ 1 mg protein) were incubated for 1 hour at 50 °C with DNPH. The hydrazone derivatives were sequentially extracted with 10% (w/v) trichloroacetic acid and washed three times with ethanol/ethyl acetate 1/1 (vol/vol) and reextracted with 10% trichloroacetic acid. The final precipitate was dissolved in 6 M guanidine hydrochloride. The difference spectrum between DNPH-protein in guanidine

hydrochloride and guanidine hydrochloride-protein blank was used to calculate nmol DNPH incorporated per mg of protein. The reference absorptivity of 21.0 mM⁻¹cm⁻¹ was used. Protein concentration was determined by the method of Lowry et al. (1951).

All results are presented as mean ± SD. Statistical analyses was performed with Student's t test. A probability level of P<0.05 was considered statistically significant.

Results and discussion

Lipid peroxidation arising from the reactions of free radicals with lipids is considered prevalent, 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 (Rikans and Hornbrook, 1997).

Organic selenium possesses antioxidant properties (Schrauzer, 2000). Surai (2000) found lower lipid peroxide accumulation in the liver of 1 day old and 5 day old chicks hatched from eggs of broiler breeder hens fed diet supplemented with organic selenium than in chickens hatched from eggs of broiler breeder hens fed standard diet. Organic selenium supplementation resulted in increased selenium concentration both the yolk and the albumen of the egg (Surai, 2000). Selenium accumulated in the egg can be transferred to the developing embryo.

In present study TBARS concentration in liver of experimental group of chicken at the end of fattening period was not significantly different in comparison with control (p>0.5, Table 1). Our data were similar to previous finding in plasma of broiler chicken given a dietary supplement of organic selenium (Arai et al., 1994).

Food deprivation has diverse effects on tissue and plasma components; duration of deprivation has an important influence. In this investigation 48-hours fasting resulted in significantly higher lipid peroxide concentration in liver of experimental group (P<0.05) in comparison with values at the end of the fattening period (Table 1). Hidalgo et al. (1990) also observed increase of lipid peroxide concentration in rat liver after 18 hours food deprivation and Domenicalli et al. (2001) after 18 and 36 hours fasting. The same results were reported in liver of fish *Sparus aurata* after 46 days starvation (Pascual et al., 2003). On the other hand Marczuk-Krynicka et al. (2003) observed unaltered content of lipid peroxides in rat liver exposed to 36-hours food deprivation.

Table 1 Changes in lipid peroxide concentration (µmol/g protein) in chicken liver, kidney and small intestine before and after 48 hours fasting for control group (standard diet) and experimental group (organic selenium supplementation)

	before 48-h fasting		after 48-h fasting	
	Control	Experimental	Control	Experimental
LIVER	0.67 ± 0.17	0.55 ± 0.16	0.75 ± 0.12	0.79 ± 0.17**
KIDNEY	0.68 ± 0.16	0.97 ± 0.17 ^a	0.55 ± 0.05*	0.56 ± 0.06**
SMALL INTESTINE	1.879 ± 0.20	1.749 ± 0.29	1.208 ± 0.35*	1.122 ± 0.27**

Values are expressed as means ± standard deviation; *Significant difference between the control groups (P<0.05); **Significant difference between the experimental groups (P<0.05); ^aSignificant difference between control and experimental group (P<0.05)

Fasting for 24 or 72 hours in chickens reduces body temperature. Most of this reduction occurred during the first 24 hours. Food deprivation for 72 hours causes further decrease in body temperature but of much lesser magnitude (Ait-Boulashen et al., 1989).

Like temperature changes, alterations in food availability and composition cause decrease of triiodothyronine (McNabb, 2000; Power et al., 2000). Starvation lowered plasma corticosterone level and brain Na⁺K⁺-ATPase activity in rats (Shaheen et al., 1996). Such a response of animals to food deprivation is an adaptive mechanism to suppress the stress-induced rise in catabolic hormones, saving energy expenditure.

In present study organic selenium food supplement resulted in significantly higher lipid peroxide concentration at the end of fattening period in kidney of experimental chickens in comparison to control group ($P<0.05$) (Table 1). Kidney and small intestine tissue TBARS concentrations were decreased after food deprivation of both control and experimental group ($P<0.05$) suggesting that food deprivation decrease metabolic rate and 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 intestine are the first line of defence against all those toxic elements. Food deprivation has a positive effect on lipid peroxidation because intestine is not challenged by dietary lipid hydroperoxides as well as other toxins.

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 rates of oxidation and the rate of degradation of oxidized proteins, the accumulation of oxidized protein is a complex function of the numerous factors that govern the synthesis and oxidation of proteins and activities of various proteases that selectively degrade the oxidized forms (Stadtman and Oliver, 1991).

Table 2 Changes in protein carbonyl content (nmol/mg protein) in chicken liver, kidney and small intestine before and after 48 hours fasting for control group (standard diet) and experimental group (organic selenium supplementation)

	before 48-h fasting		after 48-h fasting	
	Control	Experimental	Control	Experimental
LIVER	113.63± 19.74	131.01± 29.78	80.21 ± 12.14*	66.47 ± 12.64** ^a
KIDNEY	87.42 ± 11.07	113.55 ± 14.86 ^a	113.64 ± 17.38*	132.80 ± 30.40
SMALL INTESTINE	76.13 ± 8.53	87.63 ± 11.06 ^a	89.32 ± 25.40	94.53 ± 25.19

Values are expressed as means ± standard deviation; *Significant difference between the control groups ($P<0.05$); **Significant difference between the experimental groups ($P<0.05$); ^aSignificant difference between control and experimental group ($P<0.05$)

In present study the PCC in kidney and small intestine of experimental chickens at the end of fattening period was higher than in control broilers ($P<0.05$) (Table 2). As a result of the food deprivation, lower PCC was found in liver of both control and experimental group ($P<0.05$) suggesting that proteins may be more resistant than lipids to the oxidative stress related to food deprivation. Kidney PCC was increased after fasting only in control birds ($P<0.05$). In the same time liver protein carbonyl content (Table 2) when birds were subjected to 48-hours fasting for experimental group was significantly lower than in control group ($P<0.05$) suggesting that in the liver organic selenium has protective effect on protein oxidative damage.

In conclusion, starvation and organic selenium supplementation is associated with alterations in oxidant injury that differ from tissue to tissue. This data provide the basis for further studies of tissue antioxidative system and indicators of tissue oxidative status.

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