Influence of organic selenium food supplements on age-related changes on antioxidant system in thigh muscle of broiler chicken

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Abstract

The influence of organic selenium food supplements on activities of antioxidant enzymes, reduced glutathione (GSH) concentrations and lipid peroxidation in thigh muscle (*m. biceps femoralis*) of Ross heavy line chickens were investigated. At the age of 2, 4 and 6 weeks ten chickens from standard diet fed (control) group and pair-fed with the same diet supplemented with 0,3 ppm organic selenium (experimental) group were sacrificed. Thigh muscle homogenates were analysed for glutathione peroxidase (GPX), copper zinc superoxide dismutase (CuZnSOD), manganese superoxide dismutase (MnSOD) activities and glutathione (GSH) and lipid peroxide (TBARS) concentrations. GPX activity in experimental chickens significantly increased with age whereas MnSOD activity and concentrations of GSH and TBARS decreased. During fattening period control six weeks old group showed the highest GPX and the lowest CuZnSOD, MnSOD activity and GSH concentration. Organic selenium food supplements resulted in higher GPX and MnSOD activities with simultaneous decrease of CuZnSOD activity, GSH concentration and lipid peroxidation intensity.

Introduction

Selenium is an essential element that regulates antioxidative mechanisms in all living cells incorporated as selenocysteine in glutathione peroxidase (GPX) and thioredoxin reductase and many other selenoproteins. Approximately half or 40% of whole-body selenium is in GPX and its presence increases enzyme activity 100-1000 fold (Burk, 2002). Enzyme GPX with catalase and superoxide dismutase and nonenzymatic molecules (glutathion, vitamines A, E, C, uric acid, bilirubin, etc.) defend for lipids and nucleic acids from oxidative damage and play important role in cell membrane structure protection (Michiels et al., 1994).

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). During chicken fattening requirement for selenium increase suggesting that muscle development is very intensive, so the aim of this study was to investigate influence of organic selenium food supplements on age-related changes on antioxidant system in tight muscle of broiler chicken.

Materials and Methods

The experiment was performed on Ross heavy line chickens maintained under standard conditions with free access to food and water. Chickens were randomly divided into two groups: standard diet fed control group and experimental group, pair-fed with the same diet supplemented with 0.3 ppm organic selenium (Sel Plex™, Alltech). By the end of 2, 4 and 6 weeks of age ten chickens of control and ten chickens of experimental group were sacrificed and thigh muscle tissue (*m. biceps femoris*) were removed and stored at - 80 ^{o}C until analyses.

After centrifugation of the homogenates (1500 g, 10 min. 4 ^{o}C) activities of CuZnSOD and MnSOD, GSH, TBARS and protein concentrations were determined. GPX activity and protein concentration were assayed in homogenates centrifuged 10000 g/15 min at 4 ^{o}C.

The concentration of glutathione was determined by the method of Beutler et al. (1963). Concentrations of TBARS were performed according to the method of Trotta et al.

(1982) using molar extinction coefficient of 1.5 x 10^5 (Placer et al., 1966). Total SOD activity (E.C. 1.15.1.1) was measured using commercial kit (RANSOD, "Randox", UK). MnSOD was assayed after incubation with 1 M KCN and CuZnSOD activity was calculated. GPX activity (E.C. 1.11.1.9) was measured using commercial kit (RANSEL, "Randox", UK). 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 $p<0.05$ or as described in the text was considered statistically significant.

Results and Discussion

Activity of GPX in muscles is affected by the amount of selenium in the feed (Daun and Akesson, 2004). The data from this investigation (Table 1) show a clear trend toward increasing tight muscle GPX activities in experimental chickens from two to six weeks of age ($p<0.01$), whereas in six weeks old control chickens was observed increased activities compared with two ($p<0.05$) and four weeks ($p<0.001$) old chickens. Organic selenium food supplement resulted in significantly higher GPX activity in thigh muscle of experimental chickens four and six weeks old in comparison to control group ($p<0.01$). Our data on GPX activity were similar to previous findings (Mahmoud and Edens, 2003; Arai et al., 1994)

Table 1 Effect of selenium food supplement and age of broiler chicken on thigh muscle GPX, CuZnSOD, MnSOD, GSH and TBARS

	Two weeks		Four weeks		Six weeks	
	C	Sel Plex™	C	Sel Plex™	C	Sel Plex™
GPX (U/g protein)	248.82 ± 45.22^{a}	249.29 ± 50.02^{A}	220.17 ± 56.31^{a}	312.51 ± 49.45^{B*}	312.99 ± 78.15^{b}	430.23 ± 95.37^{C*}
CuZnSOD (mU/mg protein)	5.48 ± 1.47^{a}	4.41 ± 1.67^{A}	4.56 ± 0.81^{a}	2.98 ± 0.92^{B*}	3.61 ± 1.12^{b}	3.80 ± 0.52^{A}
MnSOD (mU/mg protein)	4.81 ± 0.71^{a}	6.27 ± 1.14^{A*}	5.03 ± 0.56^{a}	4.81 ± 0.85^{B}	3.75 ± 0.78^{b}	4.12 ± 0.30^{C}
GSH (mol/g protein)	0.20 ± 0.03^{a}	0.22 ± 0.05^{A}	0.22 ± 0.03^{a}	0.17 ± 0.03^{B*}	0.12 ± 0.04^{b}	0.12 ± 0.03^{C}
TBARS (μmol/g protein)	0.59 ± 0.20^{a}	0.67 ± 0.20^{A}	0.28 ± 0.15^{b}	0.33 ± 0.09^{B}	0.37 ± 0.07^{b}	0.39 ± 0.06^{B}

Values are expressed as means±standard error of 10 chickens

^{abc}Means of control values in the same row with different superscripts differ significantly ($p<0,05$),

^{ABC}Means of experimental values in the same row with different superscripts differ significantly ($p<0,05$)

*Significantly differs from control ($p<0,05$)

The CuZnSOD activity decreased in six weeks old control chickens compared with two ($p<0.01$) and four ($p<0.05$) weeks old chickens. Thigh muscle from chickens four weeks old fed selenium showed lower CuZnSOD activity than two and six weeks old chicken ($p<0.05$). The CuZnSOD thigh muscles activity was significantly lower in four week old experimental chicken than in control group ($p<0.001$) due to intensive metabolism and higher body weight gain in experimental chicken in that period.

Thigh muscles are oxidative muscle and have more mitochondria and a higher content of myoglobin than the glycolytic ones. MnSOD is a critical antioxidant enzyme because mitochondria are the major source of reactive oxygen species (Ibrahim et al., 2000). The

MnSOD activity in both control and experimental chickens reaches its lowest ($p<0.05$) level at the 6 weeks of age. Thigh muscle from chickens fed selenium showed higher MnSOD activity ($p<0.01$) at the 2 weeks of age. CuZnSOD and MnSOD activities seem to be result of intensive metabolism and compensatory effect of increased GPX activity.

The GSH concentration decreased ($p<0.01$) in thigh muscle Sel Plex™ chickens as the chickens grew older. In six weeks old control chickens GSH concentration showed significant decrease compared with two and four weeks old chickens ($p<0.001$). Thigh muscle from four weeks old chickens feed organic selenium showed higher GSH concentration ($p<0.01$) compared to those fed standard diet. Mahmoud and Edens (2003) suggest that GPX-facilitated GSH oxidation was greater in organic selenium supplemented chickens.

Oxidative muscles are prone to lipid oxidation because of their high content of fat but in present study thigh muscle TBARS concentrations decreased in both control and experimental group as the chicken grew older ($p<0.01$).

The results of this study demonstrate that the organic selenium food supplement has a positive effect of broiler chicken GPX activity in thigh muscle.

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