

Effect of organic selenium supplementation on age-related antioxidant enzymes activities and on lipid peroxidation in heart muscle of broiler chickens

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The effect of organic selenium feed supplements on the activities of antioxidant enzymes and on lipid peroxidation was investigated in the chicken heart muscle during fattening. The experiment was carried out on Ross 308 broiler chickens of both sexes, either on standard diet (control group, n = 30) or receiving organic selenium supplementation (Sel-Plex™, Alltech) in the standard diet (experimental group, n = 30). After two, four and six weeks of fattening, 10 chickens from the control group and 10 chickens from the experimental group were sacrificed. In the heart muscles of the birds from both groups, the activities of glutathione peroxidase (GSH-Px), catalase (CAT), copper zinc superoxide dismutase (Cu,Zn-SOD), manganese superoxide dismutase (Mn-SOD), reduced glutathione (GSH) and lipid peroxide (TBARS) were determined. After the fourth week of age, the chickens that had been given organic selenium supplementation displayed a significantly higher activity of GSH-Px ($P < 0.001$), Cu,Zn-SOD ($P < 0.05$), and of Mn-SOD ($P < 0.05$) than the control group of the same age. During the experimental period, there were no statistically significant differences observed in CAT activity or in GSH and TBARS concentrations between the control and experimental groups ($P > 0.05$). During fattening, age-related differences of antioxidant enzymatic activities and GSH and TBARS concentrations were obtained in the control and in the experimental group. After four weeks of age, a significant increase in GSH-Px ($P < 0.0001$), Cu,Zn-SOD ($P < 0.01$) and Mn-SOD ($P < 0.0001$) activities was obtained in the heart muscles of the experimental chickens. As the chickens grew older, at six weeks of age, significant increases in GSH-Px ($P < 0.0001$), Cu,Zn-SOD ($P < 0.0001$), and in CAT ($P < 0.05$) activities were obtained in the experimental group. Simultaneously, at six weeks of age, a significant decrease in Mn-SOD activity ($P < 0.01$) and GSH concentration ($P < 0.01$) in the heart muscles of experimental birds was recorded. During the fattening, significant increases in GSH-Px ($P < 0.001$) and Mn-SOD ($P < 0.001$) activities were obtained in the control group after four weeks of age. After six weeks, significant increases in GSH-Px ($P < 0.0001$) and Cu,Zn-SOD ($P < 0.0001$) activities, with a significant increase in TBARS concentration ($P < 0.01$) were obtained in the heart muscles of the control chickens. At the same time, like in the experimental group, GSH concentrations in the heart muscles of the animals in the control group were significantly decreased ($P < 0.02$). According to the obtained results, during the fattening period, chickens with organic selenium supplementation in the diet maintained their antioxidant systems in the heart muscle more effective, with enhanced GSH-Px, Cu,Zn-SOD and Mn-SOD antioxidant defence.

Keywords: chickens; organic selenium; heart muscle; antioxidative enzymes; lipid peroxidation

Introduction

Selenium is a constituent element of the entire defence system that protects the body from harmful actions of free radicals. Organic selenium is more thoroughly resorbed and more efficiently metabolised than its inorganic equivalent, which is poorly resorbed and acts more as a prooxidant provoking glutathione oxidation and oxidative damages to the DNA (Levander, 1983; Schrauzer, 2000; Wycherly et al., 2004). Resorbed selenomethionine is primarily incorporated into the proteins of the muscles, erythrocytes, of the pancreas, liver, stomach, kidney and of the gastro-intestinal tract mucous membrane. Its metabolism is tightly connected with protein metabolism in the body (Schrauzer, 2000), and is accessible for the synthesis of glutathione and of other selenoproteins through which it displays its physiological action. The most important metabolic role of selenium is manifested in the activities of the selenoenzymes glutathione peroxidase (GSH-Px) and thioredoxin reductase. The enzyme GSH-Px, together with superoxide dismutase (SOD) and with catalase (CAT), protects cells from (hydrogen or lipid) peroxidation. Glutathione peroxidase is for the most part a cytosol enzyme. In small quantities, it is found in mitochondrial membranes and in endoplasmic reticulum. Another important enzyme in the system of antioxidative protection is SOD, whose presence in the cell allows a rapid dismutation of O_2^- into O_2 and H_2O_2 . For the major part, Cu,Zn-SOD is found in the cytosol, and Mn-SOD in the mitochondria (Fridovich, 1997). Catalase, acting together with SOD, transforms H_2O_2 into H_2O and O_2 (Michelis et al., 1994). Catalase activity, as well as the activity of other antioxidative enzymes, depends on the presence of antioxidants in the diet. Thus, the activity of GSH-Px in the blood of young chicks depends on the quantity of selenium (Kuricová et al., 2003), and the activity of catalase in chicken erythrocytes on the quantity of copper and selenium in the diet (Bozcaya et al., 2001). According to the reports by Surai (2000), over the first days after hatching, chicks mainly depend on selenium supplies stored in the liver. From the seventh day after hatching onwards, the first symptoms of selenium deficit begin to develop.

Selenium quantities in tissues as well as antioxidative enzyme activities depend on the animal's age, the type of the tissue and on the quantity and form of selenium in the feed. The action of the mentioned enzymes and an adequate supply with vitamin E and selenium protect chicks from numerous diseases, for instance, from encephalomalacia, exudative diathesis and from muscular dystrophy (Combs Jr., 1981; Hassan et al., 1990). Taking into account that in the conditions of commercial rearing, which are often associated with various stress factors, selenium requirements are increasing, the purpose of the present investigation was to monitor the effect of supplementary organic selenium in the animal diet on prooxidative and antioxidative characteristics in the chicken heart muscle during fattening.

Materials and methods

The investigations were conducted on Ross 308 broiler chickens. One-day old chickens (pullets and cockerels) were delivered into the trial room warmed up to the temperature of 32 °C, and over the first three days were put into metal cages with a grate-like floor and a cardboard bottom. During that period, the birds had *ad libitum* access to feed and water. From the day when the experiment animals were admitted into the trial venue, the room temperature was gradually decreased, until the fifth week of the study, when it was 24 °C. From this time until the end of the trial period the temperature was around 20 °C. Over the entire study period, the chickens were kept under a whole-day light regime.

The chickens were divided into groups according to the quantity and form of selenium they were to receive with the feed. The control group received a standard diet, in three phases (initial feed mixture, for chicken fattening up to the 14th day of age, the mixture for fattening of growing chickens, until the 29th day of life, and, from the 30th day of life until the end of fattening period, final feed mixture for chicken fattening). This diet had the concentration of 0.15 mg of inorganic selenium per kilogram feed. The study group was given 0.3 ppm of supplementary organic selenium (Sel-Plex[™], Alltech) added in standard feed

mixtures from the 7th day of age to the end of the fattening period. After the 2nd, the 4th and after the 6th week of fattening, ten randomly selected chickens from the control group and 10 from the trial group were sacrificed. Immediately upon sacrificing, samples of heart muscle were harvested, rinsed in cold physiological salt solution, dried with a paper napkin, weighed and stored at -80 °C until analysis. The analyses were performed within six months after freezing of the samples.

The tissue samples were homogenised in 0.14 mol/L KCl in the ratio 1 : 5 (w/v) and cooled, with a Schüthomogen^{plus} Teflon glass homogeniser at 2800 rotations per minute over 120 seconds (6 x 15 seconds with 5-second cooling intervals). The tissue homogenates were centrifuged at 1500 g over ten minutes at 4 °C. In the obtained supernatants, the activity of superoxide dismutase (SOD) was assessed, along with the concentrations of reduced glutathione (GSH), lipid peroxides (thiobarbituric acid reactive substances; TBARS) and of proteins. Further centrifuging at 10,000 g at 4 °C over 15 minutes yielded a supernatant in which glutathione peroxidase (GSH-Px) and catalase (CAT) activities were determined and protein concentration assessed.

The activity of GSH-Px (EC 1.11.1.9) was assessed using the Paglia and Valentine (1967) spectrophotometry method with cumene hydroperoxide, and total SOD (E.C. 1.15.1.1) was determined spectrophotometrically, using ready-made sets manufactured by Randox Laboratories Ltd., Crumlin, Co. Antrim, UK. The activity of Mn-SOD was assessed after the incubation with 1 M KCN, whereas Cu,Zn-SOD activity was calculated. The activity of CAT (E.C. 1.11.1.6) was spectrophotometrically determined, using H₂O₂ as the substrate. This activity was expressed as *k* (the constant). The concentration of GSH was spectrophotometrically measured using the Beutler et al. (1963) method. The concentration of TBARS was spectrophotometrically assessed using the method invented by Trota et al. (1982). The absorption coefficient 1.5×10^5 was used for the conversion into mols per litre (Placer et al., 1966). All the values were expressed per gram protein. Protein quantity in the supernatant was assessed using the Lowry et al. (1951) method.

The results were statistically analysed by calculating mean values, standard deviation, mean value and variability coefficient, and were presented in tables as the mean value \pm SD. The significance of the differences between the results was verified using student t-test and Statistica 6.1 computer programme.

Results and discussion

According to the results of the present investigation, the chickens aged 4 weeks that received supplementary organic selenium in the diet had a significantly higher GSH-Px activity ($P < 0.001$), Cu,Zn-SOD ($P < 0.05$) and Mn-SOD ($P < 0.05$) in the heart muscle than the birds that were fed with standard feed mixtures containing inorganic selenium (Table 1). The obtained results corroborate the existing knowledge on a positive action of selenium on GSH-Px in the chicken erythrocytes, muscles, the plasma and in the liver (Arai et al., 1994; Kuricová et al., 2003; Mahmoud and Edens, 2003). The quantity of selenium in the muscles is an important regulator of GSH-Px activity (Daun and Åkesson, 2004), whereas the presence of selenocysteine at the active enzyme's active site increases its activity up to a thousand times (Burk, 2002). In selenium deficiency, selenoprotein concentration with antioxidative characteristics decreases. A portion of resorbed selenomethionine, which has not been immediately used for selenoprotein synthesis, is incorporated into the structural proteins of the muscles, the gizzard, heart and of other organs. In this way, these tissues and organs become an important reservoir of selenium (Schrauzer, 2000).

The chickens in the present study that received supplementary organic selenium also presented a higher myocardial GSH-Px activity than the controls. After 4 weeks of age, this enzymal activity was even significantly higher. The obtained results speak in favour of an ampler resorption, a better metabolising and a higher storage of organic selenium in the tissues, along with an increase in the GSH-Px activity (Kuricová et al., 2003). Another important antioxidative enzyme is SOD. Its biological role is to remove the superoxide radical, formed *in vivo* in the concentrations increasing with the exposure to oxygen (Fridovich, 1997). The activity of SOD in the cells and in the extracellular fluid is very important in the

prevention of diseases closely associated with oxidative stress, for instance, cardiovascular diseases, Alzheimer's disease, Parkinson' disease and many others (Pollack and Leeuwenburgh, 1999). The activity of Cu,Zn-SOD, for the largest part a cytosol enzyme, depends on the quantity of copper in the diet; when copper is supplemented in the diet, its activity grows in chicken erythrocytes, too (Aydemir et. al., 2000). The significantly higher activities of Cu,Zn-SOD and Mn-SOD, also assessed after the fourth week of life in the heart muscle of the chickens fed with supplementary organic selenium, indicate a better antioxidative protection, which reduces the risk for the development of cardiovascular diseases.

Contrary to the mentioned enzymes, during the chicken fattening, no significant differences were found ($P>0.05$) in CAT activities and GSH and TBARS concentrations between the trial and control chicken groups (Table 1). Catalase is involved in the breakdown of H_2O_2 formed by β -oxidation of fatty acids. Catalase is present in the peroxysomes of the liver, kidney, hear, intestine, fatty tissue, muscles and of the brain. As reactive oxygen compounds are generally formed in mitochondria, where the activity of catalase is restricted, GSH-Px, which requires the presence of GSH for its activity (Mahmoud and Edens, 2003), plays an important role in the reduction of hydrogen peroxide.

Table 1 Effect of organic selenium supplements (Sel-Plex™) in the diet on antioxidant enzymes activities, and concentrations of GSH and TBARS in heart muscle of chickens during the fattening.

	2 weeks of age		4 weeks of age		6 weeks of age	
	Control	Sel-Plex	Control	Sel-Plex	Control	Sel-Plex
GSH-Px (U/g protein)	84.09 ± 21.20	87.58 ± 18.55	131.86 ± 22.69	178.56** ± 26.57	1 270.73 ± 218.69	1 413.76 ± 130.69
CAT (k/g protein)	0.013 ± 0.005	0.010 ± 0.004	0.014 ± 0.004	0.013 ± 0.004	0.014 ± 0.006	0.014 ± 0.004
Cu,Zn-SOD (U/mg protein)	1.35 ± 0.91	0.99 ± 0.59	2.11 ± 0.99	3.95* ± 2.18	16.16 ± 4.71	17.78 ± 5.44
Mn-SOD (U/mg protein)	14.11 ± 2.10	15.48 ± 3.03	22.29 ± 4.32	26.49* ± 4.20	18.78 ± 4.32	21.54 ± 2.83
GSH (mol/g protein)	0.34 ± 0.05	0.36 ± 0.05	0.31 ± 0.06	0.32 ± 0.06	0.26 ± 0.08	0.22 ± 0.07
TBARS (μ mol/g protein)	0.44 ± 0.10	0.44 ± 0.09	0.40 ± 0.12	0.48 ± 0.07	0.60 ± 0.17	0.56 ± 0.26

The significance of difference between control and Sel-Plex group: * $P < 0.05$; ** $P < 0.001$.

During the fattening, age-related differences in antioxidant enzymes activities, and GSH and TBARS concentrations were obtained in the control and experimental group. Thus, after the 4th week of age, a significant increase in the activities of GSH-Px ($P<0.0001$), Cu,Zn-SOD ($P<0.01$) and Mn-SOD ($P<0.0001$) was confirmed in the heart muscle of the chickens fed with supplementary organic selenium. At the end of the fattening, after the 6th week of life, a further increase in the activities of GSH-Px (0.0001) and Cu,Zn-SOD ($P<0.0001$) as well as an increase in CAT activities $P<0.05$) in the heart muscle of the experimental chickens was found. At the same time, in the control chicken group, a decrease in Mn-SOD ($P<0.01$) activity and a decrease in GSH concentration ($P<0.01$) were observed. After the completion of the 4th week of life, in the heart muscles of the birds in the control group, the activities of GSH-Px and Mn-SOD significantly increased ($P>0.001$). After the 6th week, the activity of GSH-Px kept increasing ($P<0.0001$). An elevation of Cu,Zn-SOD activities ($P<0.0001$) was noted with a simultaneous increase in TBARS concentration ($P<0.01$). During the study period, the concentration of GSH in the heart muscles of control group decreased significantly ($P<0.02$). The obtained results conformed with the earlier data on a greater activity of Cu,Zn-SOD in tissues of older sheep (Paynter and Caple, 1984). As Mn-SOD is found in mitochondria, its activity depends on the type of a tissue and on the mitochondrial

count in individual tissues. Superoxide radicals are created in the mitochondria in comparatively high concentrations; this is why Mn-SOD has an important role in the overall antioxidative protection. The results of the present investigation confirmed this.

The activity of catalase depends on the animal species, physical exertion, tissue type and the animal age. In pigs, β -oxidation and corresponding enzymes form quickly after the birth in liver and kidney peroxisomes, not, however, in heart peroxisomes (Yu et al., 1998). The differences in CAT activities in the liver, kidney and in the heart muscle need not only be a reflexion of H_2O_2 formation and antioxidative protection, but may also reflect various susceptibilities of tissues to H_2O_2 . While catalase is for the most part or entirely active in peroxisomes, GSH-Px displays its activity mainly in the cellular cytoplasm and only about ten per cent in the mitochondria (Halliwell and Gutteridge, 1999). In this way, a safe removal of hydrogen peroxide is attained through a joint action of GSH-Px and catalase. The elevation of the activities of the mentioned enzymes in the heart muscle of the chickens that had received supplementary organic selenium during fattening has confirmed the reports so far, on a positive action of selenium on the activity of these enzymes.

In the present investigation, GSH concentration in both chicken groups significantly fell in the 6th week of life. It is known that GSH concentration also depends on the animal species, age and the investigated organ. Thus, GSH concentration in the poultry liver is around 25 – 50 per cent lower than in the liver of mammals and is similar to the intestinal and renal concentration (Wang et al., 1998). Taking into account that the reduced glutathione (GSH) stabilises free radicals and is a co-factor of the enzyme GSH-Px, the reduction of GSH concentration in the chicken heart muscle upon the completion of fattening could be explained by the increased GSH-Px activity, but also by a different interorganic distribution of GSH excreted from the liver into the blood flow.

Oxidative damage develops when antioxidant potential is reduced and/or when factors contributing to oxidative stress increase (Ibrahim et al., 2000). Although no significant differences in TBARS concentrations between the study and the control group were confirmed in this investigation, a significant elevation of TBARS was assessed in the heart muscles of the controls after the completion of the 6th week of life. This suggests more abundant lipid peroxidation processes. At the same time, in the group of chickens fed with supplementary organic selenium, no significant increase in TBARS in the chicken myocardium during fattening was observed.

The results of the present study suggest a positive effect of supplementary organic selenium in chicken feed during fattening. This positive effect manifested in the higher activities of GSH-Px, Cu,Zn-SOD and Mn-SOD, which eventually resulted in a better antioxidant protection of the heart muscle.

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