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Age-related antioxidant enzyme activities and lipid peroxidation in heart muscles of broiler chickens fed with supplementary organic selenium

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ABSTRACT

The effect of organic selenium feed supplements on the activities of antioxidant enzymes and on lipid peroxidation was investigated in chickens' heart muscles during growth. The experiment was carried out on Ross 308 broiler chickens of both sexes, either on standard diet (control) or receiving organic selenium supplementation in the standard diet (experimental). After two, four and six weeks of growth, ten chickens from each group were sacrificed by decapitation and heart muscles were collected for analyses. In the heart muscle the activities of glutathione peroxidase (GSH-Px), catalase (CAT), cooper zinc superoxide dismutase (Cu,Zn-SOD), manganese superoxide dismutase (Mn-SOD), reduced glutathione (GSH) and lipid peroxide (TBARS) were determined. During the experiments, age-related differences of antioxidant enzyme activities and GSH and TBARS concentrations were obtained in control and in experimental chicken. At the end of growth, after the 6th week of age, a significant increase of GSH-Px, Cu,Zn-SOD, and CAT activities ($P<0.001$; $P<0.001$; $P<0.05$) with a significant decrease of Mn-SOD activity and GSH concentration ($P<0.01$; $P<0.01$) were obtained in the heart muscle of the experimental chickens. In the control chickens, significant increases of GSH-Px, Cu,Zn-SOD and TBARS ($P<0.001$; $P<0.001$; $P<0.01$) with a significant decrease of GSH concentration ($P<0.05$) were obtained in the heart muscle after six weeks of age. After the fourth week of age, chickens with organic selenium supplementation had a significantly higher activity of GSH-Px, Cu,Zn-SOD, and Mn-SOD ($P<0.001$; $P<0.05$; $P<0.05$) than the control chickens of the same age. In conclusion, chickens with organic selenium supplementation in the diet maintained their antioxidant systems in the heart muscle more effectively, with enhanced antioxidant defence.

Key words: chickens, organic selenium, heart muscle, antioxidant enzymes, lipid peroxidation

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Introduction

Selenium is a constituent element of the entire defence system that protects the organism from harmful free radical action. 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 damage 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 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 mostly a cytosolic enzyme. In small quantities, it is found in mitochondrial membranes and in endoplasmic reticulum. Another important enzyme in the antioxidant system 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 depends on the quantity of copper and selenium in the diet (BOZCAYA et al., 2001). The activity of Cu, Zn-SOD, for the largest part a cytosolic enzyme, depends on the quantity of copper in the diet. AYDEMIR et al (2000) found its higher activity in chicken erythrocytes when copper is supplemented in the diet. According to the reports by SURAI (2000), over the first few days after hatching, chicks mainly depend on selenium supplies stored in the liver. From the seventh day after hatching and 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 activity of the mentioned enzymes and an adequate supply of 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 investigate the effect of supplementary organic selenium

in the animal diet on prooxidative and antioxidative characteristics in the chicken heart muscle during growing.

Materials and methods

The experiment was performed on Ross 308 broiler chickens. One-day old chicks 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 the experiment the room temperature was gradually decreased. The room temperature was around 24 °C at the fifth week, and around 20 °C at the end of the experiment. The chickens were kept under a whole-day light regime with access to feed and water *ad libitum* during the experiment.

The chickens were divided into two groups. The control group received a standard diet, in three phases (starter diet until the 14th day of age, grower diet until the 29th day of life, and finisher diet until the end of growing). This standard diet had a concentration of 0.15 mg of inorganic selenium per kilogram feed. The experimental group was given 0.3 ppm of supplementary organic selenium (Sel-Plex™, Alltech, USA) added in standard feed mixtures from the 7th day of age to the end of the growing period. After the 2nd, the 4th and after the 6th week of growing, ten randomly selected chickens from the control and ten from the experimental group were sacrificed by decapitation. 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 tissue samples were homogenised in 0.14 mol/L KCl in the ratio 1 : 5 (w/v) on ice, with a Schüthomogen^{plus} Teflon glass homogeniser at 2800 rotations per minute for 120 seconds. The tissue homogenates were centrifuged at 1500 g for 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 for 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 measured using the PAGLIA and VALENTINE (1967) spectrophotometry method with cumene hydroperoxide, and total SOD (E.C. 1.15.1.1) was determined spectrophotometrically, using commercial kits by Randox Laboratories Ltd., Crumlin, Co. Antrim, UK. The activity of Mn-SOD was assessed after incubation with 1 M KCN, whereas Cu,Zn-SOD activity was calculated. The activity of CAT (E.C. 1.11.1.6) was determined spectrophotometrically according to the method of AEBI (1983), using H_2O_2 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 measured spectrophotometrically

using the method described by TROTTA et al. (1982). The absorption coefficient 1.5×10^5 was used for 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, and coefficient of variability, and were presented in tables as the mean value ± SD. The significance of the differences between the results was verified using the Student *t*-test and the Statistica 6.1 computer programme.

Results

Age-related differences in antioxidant enzyme activities and GSH and TBARS concentrations in the heart muscle of control and experimental chickens were presented in Table 1.

Table 1. Effect of organic selenium supplements in the diet on antioxidant enzymes activities, and concentrations of GSH and TBARS in heart muscles of chickens during growth.

	2 weeks of age		4 weeks of age		6 weeks of age	
	Control	Experimental	Control	Experimental	Control	Experimental
GSH-Px (U/g protein)	84.09 ± 21.20	87.58 ± 18.55	131.86 ^{c} ± 22.69	178.56 $^{**, C}$ ± 26.57	1 270.73 ^{c} ± 218.69	1 413.76 ^{C} ± 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 ^{A} ± 0.004
Cu,Zn-SOD (U/mg protein)	1.35 ± 0.91	0.99 ± 0.59	2.11 ± 0.99	3.95 $^{*, B}$ ± 2.18	16.16 ^{c} ± 4.71	17.78 ^{C} ± 5.44
Mn-SOD (U/mg protein)	14.11 ± 2.10	15.48 ± 3.03	22.29 ^{c} ± 4.32	26.49 ^{*,C} ± 4.20	18.78 ± 4.32	21.54 ^{B} ± 2.83
GSH (mol/g protein)	0.34 ± 0.05	0.36 ± 0.05	0.31 ± 0.06	0.32 ± 0.06	0.26 ^{a} ± 0.08	0.22 ^{B} ± 0.07
TBARS (μmol/g protein)	0.44 ± 0.10	0.44 ± 0.09	0.40 ± 0.12	0.48 ± 0.07	0.60 ^{b} ± 0.17	0.56 ± 0.26

The values were expressed as mean ± SD. The significance of difference during growing (2 *vs* 4 weeks and 4 *vs* 6 weeks of age): in control group: ^{a} P<0.05, ^{b} P<0.01, ^{c} P<0.001; in experimental group: ^{A} P<0.05, ^{B} P<0.01, ^{C} P<0.001.The significance of difference between control and experimental group: * P<0.05; ** P<0.001.

In the heart muscle of the chickens fed with supplementary organic selenium a significant increase in the activities of GSH-Px (P<0.001), Cu,Zn-SOD (P<0.01) and Mn-SOD (P<0.001) was obtained after the 4th week of age. At the end of the growth, after the 6th week of life, a further increase in the activities of GSH-Px (P<0.001) and Cu,Zn-SOD (P<0.001) as well as an increase in CAT activities (P<0.05) in the heart muscle of

the experimental chickens was determined. At the same time, a significant decrease in Mn-SOD ($P<0.01$) activity and GSH concentration ($P<0.01$) were obtained in the heart muscles of the experimental chickens.

In the heart muscles of the control chickens, the activities of GSH-Px and Mn-SOD significantly increased ($P<0.001$) after the 4th week of age. After the 6th week, a significant increase of GSH-Px ($P<0.001$), and Cu,Zn-SOD activities ($P<0.001$) were noted, with a simultaneous increase in TBARS concentration ($P<0.01$). At the same time, the concentration of GSH in the heart muscles of the control group decreased significantly ($P<0.05$).

After the 4th week of age, chickens that received organic selenium in their diet had significantly higher GSH-Px ($P<0.001$), Cu,Zn-SOD ($P<0.05$) and Mn-SOD ($P<0.05$) activity in the heart muscle than the birds that were fed with standard feed mixtures containing inorganic selenium (Table 1.). Contrary to these enzymes, during the chickens' growth, no significant differences were found in CAT activities and GSH and TBARS concentrations between the experimental and control chickens.

Discussion

During growth, age-related differences in antioxidant enzyme activities and GSH and TBARS concentrations were obtained in the heart muscles of control and experimental chickens. Heart muscle is highly oxidative tissue that produces more than 90% of its energy from mitochondrial respiration (VENTURA-CLAPIER et al., 2003). Since the superoxide radicals are created in the mitochondria in comparatively high concentrations, Mn-SOD has an important role in the overall antioxidative protection. The biological role of SOD is to remove the superoxide radical, formed *in vivo* in concentrations that increase with exposure to oxygen (FRIDOVICH, 1997). The age-related differences of Cu,Zn-SOD activity obtained in the heart muscles of the control and experimental chickens were in agreement with the earlier data on the greater activity of Cu,Zn-SOD in tissues of older sheep (PAYNTER and CAPLE, 1984). 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 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, the safe removal of hydrogen peroxide is attained through the joint action of GSH-Px and catalase. In the present investigation, higher activity of GSH-Px was obtained in the heart muscles of older chickens. Simultaneously,

GSH concentration in both chicken groups fell significantly in the 6th week of life. It is known that GSH concentration depends on the animal species, age and the investigated organ (WANG et al., 1998). Taking into account that reduced glutathione (GSH) stabilises free radicals and act as a co-factor of the GSH-Px, the reduction of GSH concentration in the chicken heart muscle upon completion of growth could be explained by the increased GSH-Px activity, but also by a different organic distribution of GSH excreted from the liver into the blood flow.

According to the results of the present investigation, the chickens aged 4 weeks that received organic selenium in their diet had significantly higher GSH-Px activity, and Cu,Zn-SOD and Mn-SOD in the heart muscle than the control chickens (Table 1). The obtained results corroborate the existing knowledge on the positive effect of selenium on GSH-Px in chicken erythrocytes, blood, muscles, and in the liver (ARAI et al., 1994; KURICOVÁ et al., 2003; PIRŠLJIN et al., 2008). Resorbed selenomethionine, which has not been immediately used for selenoprotein synthesis, is incorporated into the structural proteins of the muscles, the gizzard, heart and other organs, and becomes an important reservoir of selenium (SCHRAUZER, 2000). 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 enzyme's active site increases its activity up to a thousand times (BURK, 2002). In selenium deficiency, selenoprotein concentration with antioxidative characteristics decreases. The higher activity of GSH-Px in the heart muscle of experimental chickens speaks in favour of better resorption, better metabolising and a higher storage level of organic selenium in the tissues, along with an increase in the GSH-Px activity.

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's disease and many others (POLLACK and LEEUWENBURGH, 1999). 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, may have indicate better antioxidant protection, which reduces the risk of the development of cardiovascular diseases.

Oxidative damage develops when antioxidant potential is reduced and/or when factors contributing to oxidative stress increase (IBRAHIM et al., 2000; POLJIČAK-MILAS et al., 2004; MILINKOVIĆ-TUR et al., 2007). Although no significant differences in TBARS concentrations between the experimental and the control group were confirmed in this investigation, a significant increase of TBARS was assessed in the heart muscles of the controls after the 6th week of life. This finding suggests more abundant lipid peroxidation processes in the heart muscles of the control chickens. At the same time, in the chickens fed

with organic selenium, such a significant increase of TBARS in the chicken myocardium during growing was not observed.

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

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SAŽETAK

Istražen je učinak dodavanja organskoga selena u hranu na aktivnost antioksidativnih enzima i na intenzitet lipidske peroksidacije u srčanom mišiću pilića tijekom tova. Istraživanja su načinjena na pilićima hibridne linije Ross 308 koji su tijekom tova hranjeni standardnim smjesama (kontrolna skupina) i na pilićima kojima je tijekom tova u standardnu smjesu dodano 0,3 ppm organskoga selena (pokusna skupina). Po deset nasumce odabranih pilića kontrolne i deset pilića pokusne skupine žrtvovano je dekapitacijom u dobi od dva, četiri i šest tjedana. Odmah po žrtvovanju životinja uzeti su uzorci srčanoga mišića u kojima su određene aktivnosti glutation peroksidaze (GSH-Px), katalaze (CAT), bakar cink superoksid dismutaze (Cu,Zn-SOD) i manganske superoksid dismutaze (Mn-SOD) te koncentracija reduciranog glutationa GSH) i intenzitet lipidske peroksidacije (TBARS). Tijekom tova pilića utvrđene su značajne razlike aktivnosti antioksidativnih enzima te koncentracija GSH i TBARS kod obje skupine pilića. Po završetka tova, u dobi od šest tjedana, utvrđen je značajan porast aktivnosti GSH-Px, Cu,Zn-SOD i CAT ($P<0,001$; $P<0,001$; $P<0,05$) uz značajno smanjenje Mn-SOD i GSH ($P<0,01$; $P<0,01$) u srčanom mišiću pokusne skupine pilića. Istodobno je u srčanom mišiću kontrolne skupine pilića utvrđen značajan porast GSH-Px, Cu,Zn-SOD i TBARS ($P<0,001$; $P<0,001$; $P<0,01$) te značajno smanjnje koncentracije GSH ($P<0,05$). Značajno viša aktivnost GSH-Px, Cu,Zn-SOD i Mn-SOD ($P<0,001$; $P<0,05$; $P<0,05$) u srčanom mišiću pokusne skupine pilića u dobi od četiri tjedna upućuje na pozitivan učinak organskoga selena, koji u konačnici može rezultirati boljom antioksidativnom zaštitom srčanoga mišića.

Ključne riječi: pilići, organski selen, srčani mišić, antioksidativni enzimi, lipidska peroksidacija