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EFFECTS OF HIGH BUT NON-TOXIC DIETARY INTAKE OF SELENIUM AND COPPER ON INDICES OF THE ANTIOXIDANT DEFENCE SYSTEM AND ON ACCUMULATION OF TRACE ELEMENTS IN CHICKS

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Lohman Brown chickens with age from the 1st to 35th day received the food with high doses of selenium (Se1 mg/kg), copper (Cu100 mg/kg), or both elements (Se1 + Cu100). Live weight increase of all three experimental chicken groups was by 9.3, 12.9 and 8.1%, respectively, in comparison with the control. The concentration of selenium in the blood of the Se1 group chickens was by 45.5, in liver by 63.4 and in kidney by 19.7% higher that in organs of control group chickens. Selenium accumulation in organs of Se1 group chickens was highly correlated with increase of glutathionperoxidase activity in blood (r = 0.90) and in liver (r = 0.85) and with decrease of glutathione concentration in liver. In Cu100 group chickens, copper concentration increased by 11.7 in blood, in liver by 23.7, and in kidney by 19.9%. Together with more intensive excretion of glutathione from hepatocytes, copper concentration in bile increased by 17.7% compared to that in control group chickens. Also wing feathers participated in the regulation of copper homeostatic balance, as copper concentration in feathers increased by 66.7%. The concentration of malondialdehide in liver of chickens from all groups was similar (43.5–45.2 μmol·g^{-1} wet wt.), indicating that overload of selenium and copper did not cause profuse production of oxyradicals in the organism. Increased accumulation of selenium and copper in chickens influenced biochemical regulation of iron, zinc and cadmium deposition in liver, kidney, tibia and feather, changing the relations between Se and Fe, Se and Cd, Cu and Fe, Cu and Zn, and Cu and Cd concentrations.

The analysis indicates increased tolerance of chicken to loads of selenium (1 mg/kg) and copper (100 mg/kg) doses.

Key words: *selenium, copper, glutathionperoxidase, malondialdehide, chicken.*

INTRODUCTION

Selenium and copper are essential trace elements through their function as cofactors in numerous biochemical reactions. Selenium plays an important role in the regulation of various metabolic processes in the body, and is an integral part of selenoprotein. About 50 selenoproteins that carry out nutritional functions of selenium have been identified (Behne *et al*., 2000, Gladyshev and Hatfield, 2010). The physiological role of selenium in organism is concentrated mainly on the function and activity of glutathionperoxidase (GSH-Px, glutathione: hydrogen-peroxide oxido-reductase, EC 1.11.1.9). Selenium is incorporated in the active site of this enzyme which acts as a free radical scavenger in the body protecting cellular membranes against oxygen and hydroperoxide induced lipid peroxidation (Bettger, 1993; Aydemir *et al*., 2000; Surai, 2006).

Copper is essential for erythropoesis, for the transport and utilisation of iron in the biosynthesis of hemoglobin, for normal growth and formation of bones (osteogenesis) and eggshell development (Harris *et al*., 1980). It participates in avian feather pigmentation, it is required as a specific cofactor in the antioxidant enzyme Cu, Zn-superoxide dismutase (superoxide: superoxide oxidoreductase, EC 1.15.1.1) (Stohs and Bagchi, 1995, Aydemir *et al*., 2000), and is incorporated into ceruloplasmin (Twomey *et al*., 2005) and cytochrome c oxidase (ferrocytochrome c: oxygen oxidoreductase, EC 1.9.3.1) (Engelking, 2004).

Metal interactions occur when administration or exposure to one metal changes the kinetics, critical doses or critical effects of other metal. The bioavailability or absorption of selenium and copper can be influenced by interacting constituents in the food (Aaseth and Ringstad, 1991). The interaction between selenium and copper in organism can be synergistic or antagonistic. It has been shown that selenium can counteract the toxic effect of copper (Kadiiska *et al*., 1993; Tatum *et al*., 2000) via creation of a metabolically inactive form (Dougherty and Hoekstra, 1982). On the other

hand, copper can affect the metabolism of selenium and modify selenium toxicity by interfering with selenium absorption and by causing accumulation of nontoxic compounds in tissue of chickens (Davis *et al*., 1996; Tatum *et al*., 2000).

Selenium and copper have dual roles in metabolic processes, as they act as antioxidants to protect against oxidative stress and as prooxidants to induce oxidative stress. Selenium and copper are essential components of enzymes such as glutathionperoxidase (Se) and superoxide dismutase (Cu), which are part of the body's first level antioxidative defence system for prevention of free radical formation (Surai, 2006). Among natural antioxidants in poultry feed, selenium and copper are needed in trace amounts. Overload intake of these trace elements leads to several disorders and toxicity mainly caused by synthesis of reactive oxygen species (ROS) *in vivo* (Aburto *et al*., 2001). Therefore, an optimal balance between selenium and copper in food and organism is essential for normal antioxidant status and health of poultry.

The allowed range of concentration of selenium in animal food is very limited. The Federal Register (Anonymous, 2002) has approved selenium as a feed additive for chickens and permits a level of supplemental selenium in diets at 0.1 mg/kg. The allowed limit in EU is 0.5 mg selenium per kg of chicken diet (Anonymous, 2004). Toxicity occurs when the concentration of selenium in chicken food is 5.0 mg/kg or higher (Атлавин и др., 1990) and LD_{50} is 15 mg per kg of body mass (Акулов, 1972).

Marked differences in copper requirements and tolerance exist among various animal species. The content of copper in poultry diet needs to be 5–10 mg/kg (Stef *et al*., 2006). In recent years the copper level in avian food sometimes is supplemented up to 100–150 mg/kg (Горобец, 2005; 2007). The maximum tolerable copper level for chickens is considered to be 250 mg Cu/kg diet, as limited by the National Research Council (U.S.) (2005). High copper dose addition to the diet is used to stimulate bird growth (Skrivan *et al*., 2001). Growth stimulation factors are involved in production of cytosolic reactive oxygen species (ROS) (Finkel and Holbrook, 2000). However, knowledge on the influence of enhanced levels of selenium and copper on chicken health is still fragmentary.

The aim of this work was to investigate the effect of high but non-toxic doses of selenium, copper, and their combination, in the diet on antioxidative substances in blood and liver, on kidney function markers, on selenium, copper, iron, zinc and cadmium accumulation in tissue and organs, and on gain of body mass in a chicken model.

MATERIALS AND METHODS

For the experiment, one-day-old Lohman Brown cockerels were used. The experiment lasted for 35 posthatching days. The chicken experiment was carried out in compliance with the Guidelines for laying hen care (Anonymous, 2003). The chickens were housed in cage units with free access to food and water. All chickens were divided into four similar groups of 25 heads in each. Groups of chickens were exposed to selenium or copper alone or a combination of these.

The control group chickens received full-value combined food (CF), balanced in all nutrients. Another group of chickens (Se1) were fed a diet containing the same CF with selenium as sodium selenite (Sigma), calculated to contain 1 mg selenium per 1 kg food. The third group of chickens (Cu100) received the same CF with copper as copper sulphate (Sigma), calculated to contain 100 mg copper per 1 kg food. The fourth group of chickens (Se1+Cu100) received diet containing CF with the same both trace elements as sodium selenite and copper sulphate in the respective doses.

At the end of the experiment, the 35-day-old cockerels were weighed and sacrificed by decapitation, using a guillotine in accordance with the Recommendation for Euthanasia of Experimental Animals of the European Convention (Close *et al*., 1998).

Whole blood, blood serum, liver, kidney, bile, tibia and feather were used for analysis. Selenium in blood, liver and kidney were estimated by fluorometry, applying 2,3-diaminonaphthalene reagent, according to the procedures of the AOAC (1997). Determination of trace elements (copper, zinc, iron and cadmium) in tissue samples was performed after dry ashing in an atomic absorption spectrophotometer Perkin-Elmer (model AAnalyst 700), according to the procedures of the AOAC (1999).

The antioxidant status was evaluated by measuring the level of the lipid peroxidation product malondialdehide, the activity of glutathionperoxidase and the amount of glutathione in blood and liver. The level of malondialdehide was measured in liver by thiobarbituric acid reaction (Surai *et al*., 1996). Glutathionperoxidase activity in the blood and liver homogenates was measured using the method described by Pinto and Bartley (Pinto and Bartley, 1969). Glutathione level was estimated spectrophotometrically in blood and liver by using 5,5-dithiobis-(2-nitrobenzoic acid) reagent (Beutler *et al*., 1963). In blood serum, to estimate kidney function, the marker uric acid was determined by the method of Eichhorn (Eichhorn *et al*., 1961) and creatinine level by using a commercially biochemical kit (Divi-Dent, Latvia).

All statistics were performed using the SPSS software. Results are expressed as means ± SE in individual groups and were tested by using the independent Student's t-test. Spearman's rank correlation coefficients between Se or Cu level and Zn, Fe or Cd concentration in chicken organs were calculated (Liepa, 1974). Statistical significance was assessed at the $P < 0.05$ level.

All of the experimental procedures were approved by the Animal Ethics Committee of the Food and Veterinary Service (Rīga, Latvia, authorisation reference number 13, from 22 December 2008).

RESULTS

Additional supplement of 1 mg/kg (Se1) selenium to the chicken diet significantly increased its concentration in blood by 45.5% ($P < 0.05$), in liver by 63.4% ($P < 0.01$) and in kidney by 19.7% ($P < 0.02$), in comparison with the control (Table 1). Copper addtition in food of the Cu100 group resulted in increased accumulation of selenium in blood, liver and kidney. The largest increase of selenium concentration was caused by simultaneous Se1 + Cu100 addition to the chicken food: up to 0.181 μg/ml ($P < 0.02$) in blood, 0.560 ($P < 0.01$) in liver and up to 0.716 μg/g ($P < 0.01$) in kidney.

The Cu100 group chickens had increased copper concentrations in peripheral blood by 11.7%, in liver by 23.7% ($P < 0.001$), in kidney by 19.9% ($P < 0.05$), in bile by 17.7% ($P < 0.01$), in tibia by 3.9% and in wing feather by 66.7% ($P < 0.001$), in comparison with the corresponding organs of control group chickens (Table 2). Separate Se1 addtition in chicken food did not significantly increase concentration of copper in tissue and organs. When chickens were fed food containing selenium and copper (Se1 + Cu100), copper concentration decreased in blood and liver, but increased in kidneys, bile and feather, in comparison with the corresponding indices of chickens receiving only copper addition to the diet (Cu100) (Table 2).

The effects of selenium and copper added to food on indices of lipid peroxidation is shown in Table 3. In the blood of Se1 group chickens, the activity of glutathionperoxidase in-

Table 1

CONCENTRATION OF SELENIUM IN TISSUE OF CHICKENS (mean ± SE)

Chicken tissue	Control group	Treated group		
		Se1	Cu100	Se1+Cu100
Blood, μg·ml^{-1}	0.121 ± 0.014	0.176 ± 0.016*	0.131 ± 0.011	0.181 ± 0.027**
Liver, μg·g^{-1} wet wt.	0.309 ± 0.027	0.505 ± 0.054***	0.383 ± 0.065	0.560 ± 0.045***
Kidney, μg·g^{-1} wet wt.	0.579 ± 0.051	0.693 ± 0.040**	0.604 ± 0.033	0.716 ± 0.052***

Significantly different from the control: * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$,

Control group — chickens received full value combined food (CF); treated groups: Se1 — chickens fed CF + Se (1 mg/kg); Cu100 — chickens fed CF + Cu (100 mg/kg); Se1 + Cu100 — chickens fed CF + Se (1 mg/kg) + Cu (100 mg/kg).

Table 2

CONCENTRATION OF COPPER IN TISSUE OF CHICKENS (mean ± SE)

Chicken tissue	Control group	Treated group		
		Se1	Cu100	Se1+Cu100
Blood, μg.ml^{-1}	1.20 ± 0.11	1.14 ± 0.20	1.34 ± 0.14	1.28 ± 0.21
Liver, μg.g^{-1} wet wt.	4.10 ± 0.12	4.04 ± 0.22	5.07 ± 0.15****	4.93 ± 0.12***
Kidney, μg.g^{-1} wet wt.	2.56 ± 0.19	2.28 ± 0.39	3.07 ± 0.17*	3.15 ± 0.94
Bile, μg.ml^{-1}	6.80 ± 0.30	7.00 ± 0,40	8.00 ± 0.21***	9.20 ± 0.61****
Tibia, μg.g^{-1} wet wt.	1.79 ± 0.08	1.84 ± 0.10	1.87 ± 0.09	1.89 ± 0.10
Feather, μg.g^{-1} wet wt.	0.45 ± 0.04	0.51 ± 0.02	0.75 ± 0.08****	0.92 ± 0.10****

Significantly different from the control: *$P < 0.05$, ***$P < 0.01$, ****$P < 0.001$

Control group — chickens received full value combined food (CF); treated groups: Se1 — chickens fed CF + Se (1 mg/kg); Cu100 — chickens fed CF + Cu (100 mg/kg); Se1 + Cu100 — chickens fed CF + Se (1 mg/kg) + Cu (100 mg/kg).

Table 3

EFFECT OF DIETARY SELENIUM AND COPPER ON INDICES OF LIPID PEROXIDATION IN BLOOD AND LIVER OF CHICKENS (mean ± SE)

Group of chickens	Blood		Liver		
	GSH	GSH-Px	GSH	GSH-Px	MDA
Control	1.93 ± 0.13	2.78 ± 0.16	4.86 ± 0.14	5.74 ± 0.52	45.2 ± 2.3
Se1	1.86 ± 0.15	4.26 ± 0.09****	4.30 ± 0.15*	7.13 ± 0.14***	43.5 ± 1.5
Cu100	1.99 ± 0.11	2.91 ± 0.35	4.43 ± 0.16*	5.94 ± 0.43	44.4 ± 1.9
Se1+ Cu100	1.87 ± 0.14	4.68 ± 0.21****	4.40 ± 0.30	7.30 ± 0.31***	44.2 ± 4.2

Significantly different from the control: * $P < 0.05$, *** $P < 0.01$, **** $P < 0.001$

GSH, glutathione, μmol·ml^{-1} in blood and μmol·g^{-1} wet wt. in liver; GSH-Px, glutathionperoxidase, μmolGSH·min^{-1}·ml^{-1} in blood and μmolGSH ·min^{-1}·g^{-1} in liver; MDA, malondialdehyde, μmol·g^{-1} wet wt.

Control group — chickens received full value combined food (CF); treated groups: Se1 — chickens fed CF + Se (1 mg/kg); Cu100 — chickens fed CF + Cu (100 mg/kg); Se1 + Cu100 — chickens fed CF + Se (1 mg/kg) + Cu (100 mg/kg).

Table 4

MEAN LEVELS OF SELECTED BLOOD AND BLOOD SERUM CONSTITUENTS OF CHICKENS (mean ± SE)

Constituents	Control group	Treated group		
		Se1	Cu100	Se1 + Cu100
Blood, haemoglobin, g /dl	7.97 ± 0.38	8.14 ± 0.18	8.67 ± 0.29	8.98 ± 0.19*
Blood serum, uric acid, mg%	1.85 ± 0.11	1.79 ± 0.45	2.12 ± 0.04**	1.84 ± 0.09
Blood serum creatinine, μmol.l^{-1}	65.7 ± 1.1	63.5 ± 2.2	60.8 ± 1.3	59.3 ± 2.2*
Blood serum ,Cu, μg %	23.2 ± 2.8	22.0 ± 1.6	38.8 ± 2.2***	34.4 ± 2.9*
Blood serum, Zn, μg %	352.0 ± 8.0	356.0 ± 7.5	408.0 ± 13.6***	340.0 ± 15.5
Blood serum, Fe, μg %	480.0 ± 30.0	500.0 ± 50.0	580.0 ± 20.0**	540.0 ± 31.0
Blood serum, Cd, μg %	5.2 ± 0.5	6.2 ± 0.5	6.4 ± 0.8	6.8 ± 0.6

Significantly different from the control:* $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$

Control group — chickens received full value combined food (CF); treated groups: Se1 — chickens fed CF + Se (1 mg/kg); Cu100 — chickens fed CF + Cu (100 mg/kg); Se1 + Cu100 — chickens fed CF + Se (1 mg/kg) + Cu (100 mg/kg).

creased by 53.2% ($P < 0.001$), and in blood of Se1 + Cu100 group chickens by 68.3% ($P < 0.001$), in comparison with enzyme activity of control group chickens. The activity of glutathionperoxidase in the liver of Se1 group chickens increased by 24.2% ($P < 0.01$), and in Se1 + Cu100 group chickens by 27.2% ($P < 0.01$) (Table 3). Cu100 addition to chicken food showed only a tendency to activate glutathionperoxidase in blood and liver (Table 3). In blood, the level of glutathione in all four chicken groups was practically the same and ranged between 1.86 and 1.99 μmol/ml. In liver, after addition of Se1, Cu100 and Se1 + Cu100, the concentration of glutathione decreased (Table 3). The level of malondialdehide in chicken liver did not statistically differ after addition of selenium, copper, and both elements, and ranged between 43.5 and 45.2 μmol/g (Table 3).

The lowest level of haemoglobin in peripheral blood (7.97 g/dl) was observed in control group chickens. The haemoglobin concentration increased up to 8.98 g/dl ($P < 0.05$) by addition of Se1 + Cu100 to chicken food (Table 4). Addition of 1.0 mg selenium to chicken did not affect kidney function markers (uric acid and creatinine) in blood serum (Table 4). In contrast, the concentration of uric acid of Cu100 group chickens in blood serum increased by 14.6% ($P < 0.02$). The concentration of uric acid in blood serum of Se1 + Cu100 group chickens did not significantly increase and was similar to that of the control group chickens — 1.84 mg% (Table 4). The amount of creatinine in chicken blood serum decreased by 7.5 ($P < 0.05$) and 9.7% ($P < 0.05$) after diet addition of Cu100 or Se1+Cu100, respectively (Table 4).

The copper concentration in blood serum of Cu100 group chickens increased by 67.2% ($P < 0.01$), zinc by 15.9% ($P < 0.01$) and iron by 20.8% ($P < 0.01$), in comparison with the control. A significant effect of Se1 on concentration of copper, zinc and iron in chicken blood serum was not observed. Separate addition of Se1 and Cu100, and simultaneous addition of both elements in food tended to increase concentration of cadmium in chicken blood serum, in comparison with the control (Table 4). An effect Se1 and Cu100 on accumulation of iron, zinc and cadmium in chicken tissue and organs was observed (Table 5). In liver of Se1 group chickens, iron concentration was lower by 9.1% ($P < 0.05$), zinc by 12.5% and cadmium by 32.8% ($P < 0.001$). The concentration of cadmium in feather was by 24.2% ($P < 0.05$) higher than that of control group chickens.

In liver of Cu100 group chickens iron concentration was lower by 7.2% and zinc by 14.5% ($P < 0.05$), but cadmium concentration increased by 15.5% ($P < 0.05$). In wing feather of the chickens of this group, zinc concentration was increased by 44.2% ($P < 0.001$) and cadmium by 28.7% ($P < 0.001$), in comparison with the corresponding indices of control group chickens (Table 5).

Table 5

IRON, ZINC AND CADMIUM CONCENTRATION CHANGES IN CHICKEN TISSUE INDUCED BY SELENIUM AND COPPER (mean ± SE)

Group	Organ	Fe, mg.g^{-1} ash	Zn, mg.g^{-1} ash	Cd, μg.g^{-1} ash
Control	Liver	6.84 ± 0.21	3.51 ± 0.22	17.4 ± 0.5
Se1		6.22 ± 0.04*	3.07 ± 0.18	11.7 ± 1.0****
Cu100		6.35 ± 0.16	3.00 ± 0.12*	20.1 ± 1.5*
Se1+Cu100		5.73 ± 0.19*	2.89 ± 0.11*	19.7 ± 0.2*
Control	Kidney	4.99 ± 0.22	2.34 ± 0.10	12.5 ± 0.4
Se1		4.94 ± 0.14	2.48 ± 0.97	13.6 ± 0.7
Cu100		4.90 ± 0.14	2.46 ± 0.12	12.2 ± 0.3
Se1+Cu100		5.05 ± 0.19	2.69 ± 0.11*	12.9 ± 0.3
Control	Tibia	0.31 ± 0.02	0.47 ± 0.03	4.7 ± 0.1
Se1		0.32 ± 0.04	0.39 ± 0.04	4.8 ± 0.2
Cu100		0.35 ± 0.02	0.49 ± 0.02	5.0 ± 0.2
Se1+Cu100		0.34 ± 0.01	0.40 ± 0.05	5.2 ± 0.1*
Control	Feather	4.87 ± 0.31	10.27 ± 0.70	44.3 ± 5.1
Se1		4.82 ± 0.23	9.08 ± 0.52	55.0 ± 4.5*
Cu100		5.22 ± 0.29	14.81 ± 0.61****	57.0 ± 5.3***
Se1+Cu100		4.92 ± 0.12	11.65 ± 0.40	54.6 ± 4.2*

Significantly different from the control: * $P < 0.05$, *** $P < 0.01$, **** $P < 0.001$

Control group — chickens received full value combined food (CF); treated groups: Se1 — chickens fed CF + Se (1 mg/kg); Cu100 — chickens fed CF + Cu (100 mg/kg); Se1 + Cu100 — chickens fed CF + Se (1 mg/kg) + Cu (100 mg/kg).

After feeding chickens simultaneously with Se1 + Cu100 the concentration of iron and zinc in liver was lower than that of the control group indices and when Se1 and Cu100 were added separately. The concentration of zinc in kidney of chickens of this group increased by 15.0% ($P < 0.05$), and the concentration of cadmium in chicken liver by 13.2% ($P < 0.05$), in tibia by 10.6% ($P < 0.05$) and in wing feather by 23.2% ($P < 0.05$), in comparison with the corresponding indices of control group chickens (Table 5).

The body mass of 35-day old chickens at the end of experiment were: control group chicken — 312.2 ± 11.6 g, Se1 — 341.2 ± 19.4 g, Cu100 — 352.5 ± 15.8 ($P < 0.05$) g and Se1+Cu100 group chicken — 337.6 ± 13.1 g.

DISCUSSION

The influence of selenium or copper on chicken growth depends on their quantity in the diet. Selenium in concentrations up to 4 mg per kg of food stimulates growth of chicken live weight (Атлавин и др., 1990; Sevčikova *et al*., 2006). The National Research Council (NRC) (1980) has accepted 5 mg selenium per kg diet as the toxic threshold for animals. In comparison with other species, the growth stimulating recommendations for copper addititon in chicken food for laying hens and broilers (Skrivan *et al*., 2001; Горобец, 2005) are a great deal larger — up to 260 mg of copper per kg food. The National Research Council (U.S.) and Committee on Minerals and Toxic Substances in Diets and Water for Animals (2005) have reported that addition of 250 mg copper per kg to broiler diet does not result in negative effects on animal health and performance. This can be explained by specifically increased tolerance of birds to intoxication with copper.

The addition of selenium, copper, and simultaneous addition of both these elements in food stimulated growth of chicken live weight, respectively, by 9.3, 12.9 ($P < 0.05$) and 8.1%, in comparison with the control. One kilogram of food of control group chickens (CF) contained 0.1 mg selenium and 10.0 mg copper. The chickens of the experimental groups (Se1, Cu100 and Se1 + Cu100) received 10 times larger doses of selenium or copper. Therefore, judging by live weight growth rates during the experiment, 1.1 mg of selenium and 110.0 mg of copper per food kg are non-toxic doses for chickens. In spite of this, to prevent pollution of the environment with metals, the EU Commission recommends 25 mg/kg as a maximum admissible level of copper use in the food of domestic fowl (Anonymous, 2003).

The enrichment of chicken diet with selenium or copper increased absorption and accumulation of selenium in organs and tissue. Selenium levels between 0.05 and 0.10 μg/ml in chicken blood are marginal, and greater than 0.10 are considered as adequate (Koller *et al*., 1986, Surai, 2006). Therefore, the concentration of Se 0.121 μg/ml observed in the blood of control group chickens was adequate to standard. Due to Se1 addition, its concentration in blood increased by 0.176 μg/ml ($P < 0.05$) and in liver by 0.505 μg/g ($P < 0.01$), which can be regarded as a harmless level for chicken health (Table 1). It was observed that the level of selenium in blood can reach 0.44 μg/ml without signs of selenosis in the organism (Koller *et al*., 1986).

Metal-metal interaction (Se1 + Cu100) can be mediated by interferences in the absorption process. In previous studies it has been observed that ^{75}Se-selenite absorption in small intestine was lower in its distal part (Apsite *et al*., 1994). However, after copper addition (254 mg/kg) to chicken food, selenite (^{75}Se) was most efficiently absorbed from ligated loops of *jejunum* and *ileum* (Атлавин и Апсите, 1982). The specialised carrier protein for absorption of copper in the intestinal wall is not always specific and can also transport selenium (Aaseth and Ringstad, 1991). It is possible that this specific synergism can explain the increased selenium concentration (by 10.9%) in liver of Se1+Cu100 group chickens, compared to that in Se1 group chickens (Table 1). Metal compounds undergo biotransformation in the body before they are excreted in urine or faeces. Supplemented selenium as selenite (Se1) can coact with glutathione to convert selenium to selenide. Selenide can be further methylated (Aaseth and Ringstad, 1991) and excreted through chicken kidney in a considerably higher proportion — 19.7% ($P < 0.02$) (Table 1).

The homeostatic balance of copper in the organism is regulated by the rate of element absorption as well as excretion (Горобец, 2005). The liver of chicken excretes excess copper from the body by biliary secretion of glutathione (Gregus *et al*., 1992; Wijmenga and Klomp, 2004). The enhance excretion of glutathione — 8.9% ($P < 0.05$) from hepatocytes (Table 3) takes place in association with raised hepatobiliary transport of copper into bile — 17.7% ($P < 0.01$) (Table 2). If there is overload of copper in the organism, also chicken wing feather are included in the regulation of copper homeostatic balance; concentrations of this element to feathers increased by 66.7% ($P < 0.001$) in comparison with the control (Table 2).

A direct relationship between glutathionperoxidase activity and selenium content of poultry tissue with selenium dietary supply has been reported (Payne and Southern, 2005; Surai, 2006). A highly significant correlation between selenium concentration and glutathionperoxidase activity in the Se1 group chicken blood ($r = 0.90$, $P < 0.001$) and liver ($r = 0.85$, $P < 0.01$) was found (Tables 1 and 3). Glutathionperoxidase reduces hydrogen peroxide, consuming a reduced form of the powerful antioxidant (glutathione) in chickens. Together with activation of selenium containing glutathionperoxidase, the consumption of the enzyme substratum (glutathione) increased in Se1 chicken liver, decreasing its concentration in this organ by 11.5% ($P < 0.05$) (Table 3).

Selenium in combination with copper showed a synergistic interaction on the activity of glutathionperoxidase. The highest concentration of selenium was in the liver of Se1 + Cu100 group chickens — 0.560 μ/g wet wt. (Table 1) and the concentration was correlated with the enzyme activity both in blood by 68.3% ($P < 0.001$) and in liver by 27.2%

($P < 0.01$) and negatively with concentration of glutathione in liver by 9.5% (Table 3). Similarly, in chicken erythrocytes, where glutathione of blood is concentrated, the activity of glutathionperoxidase increased by 35% after selenium addition and by 69 % after selenium and copper synergistic interaction (Aydemir *et al*., 2000, Bozkaya *et al*., 2001). In Cu100 group chicken liver, where the activity of glutathionperoxidase was practically the same (Table 3) and concentration of copper ion was elevated ($P < 0.001$) (Table 2), the reduced glutathione level was lower by 8.9% ($P < 0.05$) (Table 3). This might be associated with copper-catalysed oxidation of sulfhydryl groups of low-molecular-weight sulfhydryl compounds, such as glutathione (Doughert and Hoekstra, 1982).

Intensive accumulation of selenium and copper in chicken did not cause cytotoxic lipid peroxidation, characterised by the accumulation of malondialdehyde in tissue, as its concentration in liver of all four group chickens was practically the same (Table 3). This indicates stable peroxidation in liver of experimental chickens, that does not cause reactive oxygen species accumulation and imbalance between antioxidative and prooxidative substances.

Se1 addition in chicken food practically did not influence protein metabolism, as blood serum concentration of creatinine and uric acid, the final products of protein metabolism in birds, was similar to that of the control group chickens (Table 4). In contrast, after receiving a Cu100 diet, the level of creatinine in blood serum decreased by 7.5%, suggesting decrease of the intensity of muscle protein deterioration processes, resulting in chicken live weight increase by 12.9% ($P < 0.05$), compared to that of control group chickens. The simultaneous elevated accumulation of uric acid in blood serum by 14.6% ($P < 0.02$) suggests copper-induced disturbance of kidney excretion function associated with a change in purine metabolism intensity (Table 4). Although uric acid acts as an antioxidant and has a free-radical scavenging effect, when it accumulates to a high level in blood it can cause health problems (Mielcarz *et al*., 2006). Supplemented Se1 reduced the toxic effect of Cu100, indicated by a normal level of uric acid in blood serum of Se1 + Cu100 group chickens (Table 4).

Cu100 in combination with Se1 showed a synergistic interaction, as the concentration of haeomoglobin in peripheral blood increased by 12.7% ($P < 0.05$) (Table 4) and iron accumulations in liver decreased by 16.2% ($P < 0.05$), in comparison with the control. This suggests utilisation of liver iron in haemoglobin biosynthesis that is stimulated together by both elements, but not increased elimination of iron from the organism (Table 5).

An important finding of this study is that chickens fed with the selenium supplemented diet (Se1) had a significantly lower cadmium level in liver (Table 5). There are antagonistic relations between these two trace elements. Selenium can reduce the toxicity of absorbed cadmium by forming inert a cadmium-selenide complex (Badiello *et al*., 1996). Such inert complexes are excreted in urine (Aaseth and Ringstad, 1991). Thus, cadmium concentration in chicken liver decreased by 32.7% ($P < 0.001$) and the concentration was highly correlated ($r = 0.80$, $P < 0.02$) with that in wing feather and in kidney ($r = 0.21$, $P < 0.05$) (Table 5).

The Se1 and Cu100 supplement to chicken diet had no effect on tibia, and concentration of iron, zinc and cadmium was at normal levels (Table 5). Intake of the combination of both elements (Se1 + Cu100) caused an increase of cadmium concentration in tibia of chickens ($P < 0.05$). An increased cadmium concentration in tibia of Se1 + Cu100 chickens can induce bone damage by osteoporosis (Berzina *et al*., 2004).

The essential elements copper and zinc or copper and iron interact with each other. Excessive Cu100 supply affects hepatic zinc and iron metabolism in chickens. Reduction of zinc (14.5%, $P < 0.05$) and iron (7.2%) concentration in chicken liver was associated with increase of copper concentration in tissue (Tables 2 and 5). The Spearman's correlation coefficients between copper level in liver and iron, zinc and cadmium concentration in feather were $r = 0.28$ ($P < 0.05$), $r = 0.83$ ($P < 0.05$) and $r = 0.89$ ($P < 0.05$), respectively (Tables 2 and 5). The observed increase of copper, iron, zinc and cadmium in feather was associated with increased elimination of these trace elements, and possibly to the environment.

The results suggest that addition of 1 mg selenium and 100 mg per kg copper to the diet of chickens was not toxic, but it can not be recommended to use these levels in chicken feeding for commercial intentions.

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LIELU, BET NETOKSISKU SELĒNA UN VARA BARĪBAS PIEDEVU IETEKMES EFEKTS UZ ANTIOKSIDANTU AIZSARGSISTĒMAS RĀDĪTĀJIEM UN MIKROELEMENTU AKUMULĀCIJU CĀĻU ORGANISMĀ

Pētījuma mērķis bija noskaidrot antioksidantu aizsargsistēmas rādītāju izmaiņas cāļu asinīs un aknās un mikroelementu dinamiku organismā atkarībā no lielu selēna vai vara devu izbarošanas cāļiem. *Lohman Brown* cāļi no pirmās līdz trīsdesmit piektajai dienai saņēma barību ar lielu selēna (Se 1 mg/kg) vai vara (Cu 100 mg/kg), vai arī vienlaicīgu abu elementu (Se1 + Cu100) piedevu. Piedevu izbarošanas rezultātā palielinājās cāļu dzīvmasas pieauguma intensitāte attiecīgi par 9,3%, 12,9% un 8,1%, salīdzinot ar kontroli. Selēna koncentrācija Se1 grupas cāļu asinīs bija par 45,5%, aknās — 63,4% un nierēs par 19,7% lielāka kā kontroles grupas cāļu attiecīgos orgānos. Akumulētā selēna un glutationperoksidāzes aktivitātes palielināšanās asinīs un aknās, glutationa koncentrācijas samazināšanās aknās, kā arī šajā orgānā nemainīgais lipīdu peroksidācijas līmeņa rādītājs — malondialdehīds liecina, ka augstā Se1 deva nebija izraisījusi oksiradikāļu pastiprinātu produkciju ar oksidatīvā stresa situācijas veidošanos organismā un līdz ar to var uzskatīt, ka cāļiem šī koncentrācija nebija toksiska. Cu100 grupas cāļiem palielinājās vara akumulācija asinīs par 11,7%, aknās — par 23,7% un nierēs — par 19,9%. Šiem cāļiem reizē ar intensīvāku glutationa ekskrēciju no hepatocītiem žultī nonāca par 17,7% vairāk vara nekā kontroles grupas cāļu žultī. Būtiski, ka vara homeostatiskā balansa regulācijā iekļāvās spārna spalvas, palielinot vara koncentrāciju tajās par 66,7%. Konstatētās novirzes proteīna metabolismā — kreatinīna līmeņa pazemināšanās un urinskābes koncentrācijas palielināšanās asins serumā norāda uz Cu100 devas nelabvēlīgu ietekmi uz cāļu nieru ekskrēcijas funkciju. Selēna un vara palielināta akumulācija cāļu organismā ietekmēja dzelzs, cinka un kadmija deponēšanās bioķīmisko regulāciju aknās, nierēs, kaulos un spalvās, mainot korelatīvās attiecības starp Se un Fe, Se un Cd, Cu un Fe, Cu un Zn, Cu un Cd. Analizēto rādītāju kopums liecina par cāļu organisma palielinātu toleranci pret selēna (1 mg/kg) vai vara (100 mg/kg) lielu devu slodzi. Tomēr šīs devas nevar ieteikt lietot cāļu ēdināšanā komerciālos nolūkos.