

SELENIUM SUPPLEMENTATION IN FISH NUTRITION

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Abstract

Selenium is a microelement necessary for normal growth, development and reproduction. In our research on carp at intensive breeding, we followed up the effects of selenium supplementation on the activity of the selenoenzyme glutathione peroxidase (in erythrocytes and in liver) and type I deiodinase (in serum). The experiments were performed in experimental pools in the fish farm "Boshava" during 2010, by introduction of inorganic (sodium selenate) and organic selenium (selenium starter) in supplemental food for young carps (0+). That way, at the end of the experiment a statistically significant length and weight growth was noticed in the youngsters' groups fed with selenium supplemented food, compared to the control group fed without selenium supplement. Low mortality in the pools where selenium enriched food was implemented must be particularly pointed out. Mortality in the control group was 17.5%, in the group with inorganic selenium supplement 12.3%, and in the group with organic selenium supplement only 0.7%. In laboratory conditions we followed up the influence of cadmium in water (1.5 mg/l) on the activity of the enzyme glutathione-S-transferase, an enzyme important in the biotransformation of various pollutants. The activity of this enzyme is in correlation with the activity of the selenium-dependent enzyme, glutathione peroxidase. In relation to the control group, in the groups supplemented with selenium an increase of glutathione peroxidase activity in erythrocytes and liver was found, with simultaneous decrease of glutathione-S-transferase activity in carps' plasma and liver.

Keywords: activity of enzymes, fish nutrition, selenium.

INTRODUCTION

It is necessary to take in more than 20 elements in the form of various inorganic compounds into the organism of a vertebrate for its normal functioning. These inorganic nutrients are classified in two groups - macroelements needed in larger amounts (calcium, phosphorus, magnesium) and microelements, needed in milligramic and microgramic amounts (iron, iodine, zinc, copper, selenium, nickel, cobalt, molybdenum). The elements of both groups have different functions within the cell and the organism, and are necessary for the survival of each live cell.

The importance of selenium for live organisms

Selenium is a microelement necessary for normal growth, development and reproduction. Chemically similar to sulphur, selenium is present in many proteins in the form of selenocysteine or selenomethionine. Though the role of all these proteins is not completely elucidated yet, the role of selenium as a

cofactor of a few enzymes (selenoenzymes) is known. Selenium-dependent glutathione peroxidase (GSH-Px) and iodothyronine deiodinase, an enzyme important to thyroid hormone synthesis, are among the most important selenoenzymes.

Glutathione peroxidase was discovered in 1957 (Mills, 1957) and is present in almost all the cells of the vertebrates. Its spread in invertebrates is not completely determined yet. However, it is known that insects don't contain it (Pritsos et al., 1988; Grubor-Lajsic et al., 1996; Grubor-Lajsic et al., 1997). It is also absent in high-life plants and bacteria (Halliwell et Gutteridge, 1989). Glutathione peroxidase is part of the enzymatic system of antioxidant protection, which has developed during the evolution with the purpose of elimination and mitigation of the negative impact of reactive oxygen particles (free oxygen radicals and molecules of great reactivity, like the hydrogen peroxide) that are produced in each aerobic cell. Glutathione peroxidase catalyses the reduction of numerous organic hydroperoxides as well as of hydrogen

peroxide, and the reduced form of glutation is a cofactor of this enzyme.

Exposure of fish to oxidative stress

The concentration of oxygen in the atmosphere is relatively constant, but in aquatic environments it often comes to fluctuations, first of all because of temperature variations. For that reason fish are especially exposed to the danger of oxidative stress, originated at abrupt oxygen concentration changes. Good antioxidative protection of these organisms is necessary because of the prooxidative action of various organic xenobiotics and heavy metals, often present in aquatic environments. Beside that, lipids, especially those with poly-unsaturated fatty acids, present in fish tissues in higher percentage, are particularly liable to oxidative changes. The existence of nuclei and other membranous organelles in fish erythrocytes, makes fish erythrocytes particularly sensitive to free radicals' action. And the haemoglobin too, which is additional source of generating reactive oxygen particles, is relatively high in fish erythrocytes. Therefore it is no surprise that one of the highest values of glutation peroxidase activity values is seen exactly in fish erythrocytes (Matkovics et al., 1977).

Although the World Health Organization (WHO) as early as 1987 recommended fish meat as a source of selenium in nutrition, there are very few data on selenium application in nutrition of fish and the role of this microelement in their metabolism.

Selenium absorption depends on its chemical form. It is known that inorganic selenium forms (sodium selenite and sodium selenate) quickly show their effect by increasing the amount of selenium in plasma and the activity of selenium-dependant peroxidase, but this effect is short-lasting. The selenium contained in organic chemical compounds (starter cultivated on selenium containing bed, so called selenium starter) has slower but prolonged effect on the selenium level and the activity of selenoenzymes.

In our research on carp at intensive breeding, we followed up the effects of selenium supplementation on the activity of the selenoenzyme glutation peroxidase (in erythrocytes and in liver) and type I deiodinase

(in serum). Via determining the activity of other antioxidative protection enzymes (superoxide-dismutase, catalase, glutation-S-transferase), we tried to perceive their characteristics and mutual relations within this complex system. Via determining the basic biometric and haematologic parameters, we followed up the health condition of fish, their increment and survival.

MATERIALS AND METHODS

The experiments were performed in experimental pools (1.0 ha in area) in the fish farm "BOSHAVA" during 90 days, by introduction of inorganic (sodium selenate) and organic selenium (selenium starter) in supplemental feed for young carps (0+). Proximate composition of the basal diet was 38% crude proteins, 4% crude fat and moisture 9%. Inorganic selenium (sodium selenate) and organic selenium (selenium starter) were added to the experimental basal feed at the rate of 0.5 mg/kg feed. During the feeding trial, the carps were counted and weighed the body mass and body length individually, ten per group. Blood samples were withdrawn from the caudal vein often fish from each treatment. Aliquots of blood samples were used to determine the selenium glutathione peroxidase (GSH-Px) activity. 6 carps were collected randomly from each experimental group at the end of the experiment, sacrificed and the liver was removed. The selenium glutathione peroxidase (GSH-Px) activity in the blood was determined spectrophotometrically according to the modification of the technique of Paglia and Valentine (1967). The survival rate was monitored as well. The parameter values were statistically processed.

RESULTS AND DISCUSSIONS

Biometric and haematological parameters

Based on biometric parameters, a significant yield increase in the conditions of intensive production was found. That way, at the end of the experiment (after 80 days) a statistically significant length and weight growth was noticed in the youngsters' groups fed with selenium supplemented food, compared to the

control group fed without selenium supplement.

The average standard length on the control group units at the end of the experiment was 119 mm, the total length 148 mm, and average weight 69 g. The experimental group units with inorganic/organic selenium had average standard length of 139 mm/137 mm, total length of 172 mm/169 mm, and average weight of 104 g/105 g. Low mortality in the pools where selenium enriched food was implemented must be particularly pointed out. Mortality in the control group was 17.5%, in the group with inorganic selenium supplement 12.3%, and in the group with organic selenium supplement only 0.7% (Table 1).

Table 1. Average values of body weight, increment and mortality of young carps in their first (0+) and second year (1+) of life

Specification		body weight		mortality
		X [g]	Increment [%]	[%]
0+	control	69A		17.5A
	inorganic Se	104B	50.7	12.3A
	organic Se	105B	52.2	0.7B
1+	control	555		
	inorganic Se	692	24.7	

A, B - values in the same column with no common superscript differ significantly ($P < 0.01$).

Similar experiment was performed during 2012, using inorganic selenium supplementation (sodium selenate) in carps' feed in the second year of their life (15 mg selenium/kg premix, constituting 1% of the total mass of supplementary food) in same experimental conditions ("Boshava" fish farm pools). At the end of the experiment, after three months of intense breeding, haematological analysis of carps' blood was performed as an indicator of the condition and the overall state of the fish. Compared to the control group (without addition of selenium in food), the results obtained in the experimental group demonstrated that selenium supplementation of the food favourably influences all the haematological parameters (increase of the number of erythrocytes, greater haematocrite, greater haemoglobin concentration, greater average haemoglobin charge of erythrocytes

and greater average haemoglobin concentration in erythrocytes), as well as reduction of the number of leucocytes in the blood of these fish. Our results coincide with that of the results of the growth performances observed in the research of Zhou and al. (2009) which experiment was done with supplemented selenium nanoparticle and selenium methionine and Ani et al. (2009) which study was done with organic selenium (Sel-plex).

The influence of selenium on the antioxidative system of erythrocytes and liver of carps

Selenium supplementation significantly influences the increase of the selenium-dependent enzyme glutathione peroxidase activity in the erythrocytes of carp youngsters in the first year of life (0+). Inorganic selenium in the form of sodium-selenate has similar effect on the increase of the activity of this enzyme and on the antioxidative protection in carps in the second year of their life (1+) (Jovanovic et al., 1995; Jovanovic et al., 1997; Zhou et al., 2009).

The increase of glutathione peroxidase activity at adequate provision of selenium influences other antioxidative protection enzymes as well. This is of particular importance for fish which because of their ecological niche are very exposed to the influence of numerous organic xenobiotics and heavy metals (Kleinow et al., 1987; Zhou et al., 2009).

In laboratory conditions we followed up the influence of cadmium in water (1.5 mg/l) on the activity of the enzyme glutathione-S-transferase, an enzyme important in the biotransformation of various pollutants. The activity of this enzyme is in correlation with the activity of the selenium-dependent enzyme, glutathione peroxidase. In relation to the control group, in the groups supplemented with selenium an increase of glutathione peroxidase activity in erythrocytes and liver was found, with simultaneous decrease of glutathione-S-transferase activity in carps' plasma and liver (Table 2).

By our investigations, we have shown that the addition of selenium in food for fish has important biochemical effect which reflects on the resistance to diseases, on the survival and the increment, and makes this micronutrient

necessary in carps' nutrition during intense breeding.

Table 2. GSH-Px activity in erythrocytes and liver of control and experimental group carps (with inorganic and organic bound selenium) in the first year and inorganic selenium in the second year of their life

Specification		<i>erythrocytes</i>	<i>liver</i>
0+	<i>control</i>	4.7±0.4A	14.0±2.1
	<i>inorganic Se</i>	5.3±0.8A	16.1±1.4
	<i>organic Se</i>	8.3±0.7B	17.7±1.8
1+	<i>control</i>	21.2±3.4A	11.7±3.6
	<i>inorganic Se</i>	37.3±6.2B	13.4±1.7

A, B – values in the same column with no common superscript differ significantly (P<0.01).

CONCLUSIONS

In conclusions, this study had demonstrated that different sources of selenium (inorganic and organic) supplemented in basal diet could significantly improve the final weight, GSH-Px activity and decreased the mortality rate. This could contribute to the productivity of the fish farms and to their better economical profit.

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