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Research article

EFFECTS OF DIFFERENT PREPARATIONS CONTAINING SELENIUM ON SELENIUM STATUS AND REPRODUCTIVE PERFORMANCE OF COMMON CARP *(Cyprinus carpio L.)* BROODSTOCK

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This study evaluated the efficiency of different Selenium supplements on blood Se status and reproductive performance of carp broodstock. Female carp (n=120) were distributed in 4 groups supplemented with Se as follows: control (non-supplemented), sodium selenite 200 μg/kg feed, sodium selenite i/m injection (160 μgSe/kg body-weight), and selenized yeast 200 μg/kg feed. Blood was sampled on days 1, 20, and 30. On day 30, samples of roe were taken from females, and samples of blood and milt from 10 unsupplemented males. Larvae samples were taken after hatching. Plasmatic Se concentration was 51.38±3.77 μg/kg (day 1) and did not vary significantly. The erythrocyte Se concentration increased from 14.04±3.05 μg/kg (day 1), reaching 90.58±12.43 (day 20), to 212.38±57.87 μg/kg (day 30). Plasmatic glutathione peroxidase activity in supplemented groups increased from 4.41±1.15 to 7.73±2.26 μkat/L and plateaued until day 30. A similar pattern was observed for erythrocyte glutathione peroxidase activity, ranging from 54.51±15.45 μkat/L (day 1) to 80.72± 6.82 μkat/L (day 30). Se concentration and selenoenzyme activities in males did not differ from the female control group. Roe Se content ranged from 48.26±17.53 μg/L in control to 65.68±34.39 μg/L in fish supplemented selenized yeast, and in the pooled larvae samples, ranged from 26-43 μg/L. The Se concentration in the milt was 24.78±4.27 μg/L. Results suggest that 250 μgSe/kg feed was sufficient to meet the requirements of mature carp, sodium selenite being more efficient in elevating blood Se concentrations and selenoenzymes activities, while selenized yeast being more efficient in roe and larvae.

Keywords: carp broodstock, selenium status, reproductive performance.

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INTRODUCTION

Selenium is an essential micronutrient in the diet of animals, including fish. It performs its physiological role through selenoenzymes, where it is co-translatory integrated as a part of a non-standard amino acid – selenocysteine. The prominent roles of selenium are reflected in the antioxidant protection of the body and the metabolism of thyroid hormones [1]. Fish selenium-dependent glutathione peroxidase (GPx, EC 1.11.1.9) is a family of isoenzymes that remove hydroperoxides from the cytosol and nuclei of animal cells and blood plasma. There are evolutionary differences in blood GPx isozymes between mammals and fish. The main GPx in mammalian erythrocytes is cytosolic GPx1, while GPx3 is active in the blood plasma. Corresponding GPxs in fish are GPx 1/2, active in the cytosol and nuclei, and DRGPx3 in blood plasma [2].

The interest in investigating the biological role of selenium in fish is generally twofold. First, the effect of selenium on fish's health, growth, and production performance. Additionally, fish meat is an excellent natural source of selenium in the human diet [3,4].

Most of the selenium enters the body of animals through the diet and, after resorption, is distributed in tissues, starting with blood plasma, through the liver and whole blood, down to all tissues. Therefore, the status of selenium in animal organisms will depend on the concentration and bioavailability of selenium in the diet. This general rule is confirmed by the relatively few experiments with fish that have been carried out so far.

Overall, plant feedstuffs grown in Serbia are deficient in selenium. The deficit can be considered marginal in the northern, lowland areas (50 μg/kg dry weight). Still, it increases in the southerly direction, so south of the Sava and Danube rivers, the selenium concentration in cereals is highly deficient at <30 μg/kg dry weight. On the territory near the experimental fishery, the concentration of Se in wheat grain measured from 50.94 ± 22.25 μg/kg dry weight [5]. It should not be forgotten that fish can absorb selenium through the gills [6]. However, since selenium concentrations in freshwater ecosystems are low, this source, if not negligible, cannot meet the needs of fish for selenium. Dangić et al. [7] measured selenium concentrations of 0.23±0.13 μg/L in 11 samples of Danube water.

Data provided by FAO [8] reveal that the predominant freshwater fish cultures in the US and Western European countries are salmonids, such as rainbow trout (*Oncorhynchus mykiss*). At the same time, carp (*Cyprinus carpio L.*) is primarily cultivated in Central and Eastern European countries (including Serbia) and Asia. While there is some, albeit insufficient, literature data on the physiology of selenium in salmonids, they are pretty scarce on carp production. The rapid development of freshwater fisheries on the Asian and African continents in recent decades is based on cyprinid cultures, which is why research on this topic has been revived.

When studying the physiological processes in the body of fish, it should always be borne in mind that these are poikilothermic animals, in which the intensity of

metabolic processes depends largely on the temperature of the external environment. This can complicate the comparison of results, both within and between species or in comparison with higher classes of vertebrates. The optimum temperature for the growth and development of carp in the summer months is around 28 °C. When it drops below 14 °C at the end of autumn and during the winter, the metabolism slows down and stops at a temperature below 4 °C [9]. A critical period in the annual cycle in cyprinid fish occurs at the end of winter and in early spring, especially in the broodstock, because then begins an intensive metabolism in the gonads, characterized by a significant turnover of lipids, primarily for the formation of vitellus. These lipids are presumably synthesized from bodily amino acids [10] since feed intake during winter and early spring is minimal.

In the semi-intensive way of carp farming, characteristic of fisheries in Serbia [11], the basis of the diet is natural food from ponds (zooplankton and zoobenthos), and feeding is done with feed mixtures with a significant share of cereals, primarily wheat and/or corn [12]. Adding selenium to compound feed in a suitable chemical form is the most rational supplementation for young and mature fish. It is possible to incorporate the parenteral (intramuscular, i/m) addition of selenium preparations into the technological procedures of manipulating broodstock in early spring before spawning.

The selenium requirements of fish, based on optimal growth and maximum GPx activity in tissues, were determined experimentally for rainbow trout at 0.15 – 0.38 mgSe/kg [13] and for channel catfish at 0.25 mgSe/kg + 30 IU vitamin E [14,15]. The authors conclude that organic forms of selenium have a higher bioavailability than sodium selenite. Jovanović et al. [16] found that selenium supplementation of growing carp, in the form of selenized yeast, significantly increased the activity, not only of GPx in the erythrocytes but also increased the activity of superoxide dismutase (SOD) and glutathione-S-transferase (GST) in the liver.

Manifest pathological disorders can occur due to nutritional deficiency of selenium, typically in the form of nutritional muscular dystrophy and, in severe cases, Zenker's muscle degeneration [13,17]. It is likely, however, that in most cases, selenium deficiency leads to a decrease in production performance, often without discernible clinical symptoms [18]. Thus, Gatlin and Wilson [14] showed that channel catfish (*Ictalurus punctatus*) fed a diet containing selenium below 0.25 mg/kg exhibited a reduced growth rate, but no pathological changes were observed.

Fish tissues are rich in lipids, especially n-3 polyunsaturated fatty acids, an adaptive mechanism that allows cell membrane systems to function normally at lower temperatures [19]. Moreover, fish erythrocytes have a nucleus, i.e., a relatively larger surface area of biological membranes, compared to higher vertebrates. Unlike in the atmosphere, oxygen concentration in aquatic ecosystems can vary significantly in 24 hours and throughout the year. All the abovementioned factors elevate the risk of *in*

vivo peroxidation and a need for robust defense mechanisms against reactive oxygen species [20].

The aim of this study was to evaluate the effects of different methods of selenium supplementation as a) sodium selenite, administered in feed, or b) sodium selenite, administered as i/m injection, and c) selenized yeast, administered in feed, on selenium status in blood, roe, milt, and larve of common carp (*Cyprinus carpio L.*) broodstock.

MATERIAL AND METHODS

Technological characteristics of the fishery

The experiment was conducted on a commercial carp fishery with a spawning facility 70 km north of Belgrade. The standard operating procedure of production is based on the technology model standard for the mass production of juvenile carp in Eastern Europe [9,12]. The pond water temperature, pH, and dissolved oxygen were monitored daily.

Experimental animals and feeding method

The experiment involved 120 clinically healthy females and 10 males of the parent carp brood. The females were 4-5 years old, with an average body weight of 6.49 ± 1.62 kg, and the males were 3-4 years old, with an average body weight of 6.21 ± 1.22 kg. Females and males were kept until spawning in specially prepared wintering ponds equipped with aerators.

The primary feed for fish was natural food (zooplankton and zoobenthos). A standard carp broodstock mixture with a basal selenium content of 48.37 μgSe/kg was used for additional feeding. It had been prepared from locally produced feedstuffs, following the technological recipe of the fishery (Tables 1 and Table 2).

Table 1. Composition of the supplementary mixture (%)

Feedstuffs composition		Chemical composition	
	% mixture		% mixture
Feed meal	53.0	**Protein**	30.64
Soybean meal	20.0	**Cellulose**	4.37
Fish meal	5.0	**Calcium**	1.42
Meat meal	15.0	**Phosphorus**	0.77
Dehidrated Alfalfa meal	3.0	**Metabolic energy (MJ/kg)**	13.53
Animal yeast	3.0	**Lysine**	1.75
Vitamin and mineral premix	1.0	**Methionine + cystine**	0.99

Basal selenium content was 48.37 mg Se/kg feed.

Table 2. Composition of the vitamin and mineral premix

Ingredient	amount	Ingredient	amount
Vitamin A, IU/kg	1000000	**Cholin chloride, mg/kg**	200000
Vitamin D_3, IU/kg	200000	**Biotin, mg/kg**	100
Vitamin E, mg/kg	4000	**Vitamin C, mg/kg**	8000
Vitamin K_3, mg/kg	300	**Cu, mg/mg**	1000
Vitamin B_1, mg/kg	1000	**Fe, mg/kg**	4000
VitaminB_2, mg/kg	500	**Zn, mg/kg**	4000
Vitamin B_6, mg/kg	600	**Mn, mg/kg**	6000
Vitamin B_{12}, mg/kg	2	**J, mg/kg**	200
Niacine, mg/kg	4000	**Co, mg/kg**	100
Folic acid, mg/kg	100	**Antioxidant BHT, mg/kg**	10000
Ca-pantotenate, mg/kg	4000	**Carrier**	Ad 1 kg

Experimental design

After receiving the pituitary extract (April 24th), broodstock was placed in the single wintering pond for 8 days to stabilize the metabolism (adaptation phase) and fed according to the above prescription without selenium supplementation.

The selenium supplementation phase lasted 30 days (May 3rd to June 2nd). The broodstock was randomly divided into four experimental groups of 30 individuals each. Each group was housed in a separate wintering pond. Selenium was added to the experimental groups of female broodstock according to the following schedule:

1. The control group was not supplemented.
2. Na-selenite (feed): 200 μg/kg in complete feed, days 1-30.
3. Na-selenite (i/m): 1 mL solution (160 μg/kg body weight), once, on day 1
4. Selenized yeast (feed): 200 μg/kg in complete feed, days 1-30.

The group of 10 unsupplemented male broodstock was randomly selected on the day of spawning (day 30) for blood and milt sampling.

The experimental design, handling of broodstock fish, and specimen sampling were planned and executed according to the technology SOP of the fish farm, based on the guidelines provided by FAO [9]. No morbidity or mortality incidents were recorded among animals during and after the experiment.

Collection, processing, and storage of samples

Blood for analysis (6 mL) was collected from 10 fish from each group by random sample, by puncture of the caudal vein, into heparinized vacutainers three times during the experiment according to the following scheme: day 1, immediately before the start of selenium supplementation, day 20, and day 30, during spawning. Day 30 was the last day of Se supplementation.

Blood samples for analysis were divided into two parts: 1 mL was left as whole blood for hematological analysis, and from the rest, blood plasma and erythrocytes were separated as follows: whole blood was first centrifuged at 1500 × g for 15 minutes at 4 °C, after which the supernatant was aspirated, leaving behind an erythrocyte pellet. The pellet was washed 3 times in phosphate buffer solution – PBS (1X, pH 7.4).

On the thirtieth day (during spawning), samples of unfertilized eggs were taken from female broodstock and left in 4% formalin for counting. Blood was taken from the males for analysis, as well as samples of milt (sperm). After fertilization, the eggs of each experimental group were placed in Zug incubators, and three days after hatching, a sample of larvae was taken from each group. Due to technological limitations, fertilized eggs and larvae were pooled by experimental groups, and results were expressed as means without variation indicators.

Roe, larvae, and sperm for selenium content determination were diluted in PBS in a volume ratio 1:5 (1X, pH 7.4) and homogenized by triple treatment in a homogenizer with a Teflon piston, with cooling. The homogenates were centrifuged at 1500 × g for 15 minutes at 4 °C, after which the liquid contents were aspirated and frozen at -20 °C until analysis.

Analytical procedures

Samples intended for measuring selenium concentrations were stored at -20 °C. All other analyses were carried out from fresh samples kept at 4 °C for no longer than 24 hours.

Selenium concentration was measured in blood plasma, erythrocytes, roe, milt, and larvae using the hydride technique on an atomic absorption spectrophotometer (Thermo electron Solar AA, Series 4) with a hydride module and electrical heating of quartz cuvettes in an EC 90 furnace. Quality control was performed using the referent material BCr 185 (irMM, Belgium). The obtained values in the replicate were within the range of the certified values.

Glutathione peroxidase activity was measured in blood plasma (DRGPx3) and erythrocytes (GPx1/2) samples using a coupled test [21,22]. All chemicals were obtained from Sigma Aldrich. Blood samples were hemolysed using Drabkin's reagent (1.6 mM KCn, 1.2 mM $K_2Fe(Cn)_6$ and 0.023 M $NaHCO_3$). The final concentrations of the reagents used were 100 mM phosphate buffer (pH 7.4), 4 mM EDTA, 6 mM GSH, 0.375 IU/mL glutathione reductase, 0.3 mM NADPH, and 1.575 mM TBH. The decrease in NADPH concentration was measured continuously for 3 min at λ=366 nm using a Cecil Ce2021 spectrophotometer with a Peltier thermostat unit

(25 °C). The activity of GPx1/2 and DRGPx3 were expressed in microkatals per liter (μkat/L).

Relative fertility was determined for individual fish by counting the number of unfertilized eggs in 1 g of roe spread over a glass plate and **hatching rate** as a percentage of larvae (pooled by experimental groups) hatched from eggs 3 days after fertilization.

Red blood cell count (RBC) was determined from whole blood, under the microscope, using Neubauer's chamber, and **hematocrit** (packed cell volume, PCV) was measured after centrifugation of microcapillary tubes.

Statistical data processing

Statistical analyses were conducted using MS Excel 2021 and Graph Pad Prism 5 statistical software packages.

All results were expressed as mean ± SD. The significance of the differences between experimental groups was evaluated using two-way ANOVA followed by Bonferoni's test and, where appropriate, Student's t-test. In all cases, results were deemed statistically significant if $p<0.05$.

Correlations between plasma Se concentrations and GPx activities were tested by linear regression analysis.

RESULTS

Water temperature, oxygen concentration, and pH

The mean daily temperature of the water in the pools gradually increased during the experimental period (May) and ranged from 14.5 °C to 21.4 °C. The mean daily oxygen concentration in the pools during the experimental period ranged from 6 to 7 mg/L, and the pH of the water ranged from 6.4 to 6.9.

Indicators of selenium status in the blood of broodstock

The selenium concentration in the blood plasma of broodstock females of the control and experimental groups did not increase significantly from day 1-20. From day 20 to day 30, a significant decrease was observed in the control group ($p < 0.05$), while in the selenium-supplemented groups, plasma concentrations did not change significantly compared to the previous sampling. The highest plasma concentrations of selenium were recorded on day 30 in the group receiving sodium selenite in food (Table 3).

The selenium concentration in the broodstock erythrocytes increased continuously during the experimental period, both in the control group of fish and in the groups

that received selenium preparations. In this respect, the exception is the sodium-selenite i/m group, where no increase in selenium concentration in erythrocytes was observed from day 20 to day 30 (Table 3).

Table 3. Selenium concentration (μg/L) in the blood of broodstock (n=10).

	Day 1 (all groups)	Day 20	Day 30
Blood plasma			
Control	51.38 ± 3.77A	54.09 ± 4.38B	41.70 ± 6.83A.B
Na-selenite (feed)		52.33 ± 5.57	58.31 ± 12.25a
Na-selenite (i/m)		54.52 ± 10.62	50.97 ± 7.35a
Selenized yeast		52.93 ± 5.92	53.47 ± 7.72a
Males (nonsupl.)			45.78 ± 10.48
Erythrocytes			
Control	14.04 ± 3.05A	51.97 ± 10.78A.B	146.83 ± 27.42A.B
Na-selenite (feed)		59.51 ± 10.98A.B	212.38 ± 57.87A.B.a
Na-selenite (i/m)		90.58 ± 12.43A.B	133.03 ± 42.06A.B
Selenized yeast		76.94 ± 9.82A.B	149.52 ± 37.53A.B
Males (nonsupl.)			130.49 ± 30.60

The same capital superscript letters represent significance ($p<0,05$) between sampling dates; small letters represent significance ($p<0,05$) between Se supplemented groups and control at any given date; no letters represent no significance.

The activity of selenoenzyme DRGPx3 in the blood plasma (Table 4) stagnated in the control group throughout the experiment. In contrast, in the selenium-supplemented groups, it increased from day 1 (4.41 ± 1.15 μkat/L) to day 20 (7.40 ± 1.23 to 7.73 ± 2.26 μkat/L) and then stagnated on day 30 (5.93 ± 1.09 to 6.55 ± 1.69 μkat/L).

The activity of selenoenzyme GPx1/2 in the erythrocytes of the broodstock on day 1 was 54.51 ± 23.49 μkat/L and did not change significantly during the experimental period. At the second sampling (day 20), supplemented groups significantly increased selenoenzyme activity. From day 20 to day 30, GPx activity slightly decreased, and only the sodium selenite group retained a significant difference compared to control (Table 4).

Table 4. GPx activities (μkat/L) in the blood of broodstock (n=10).

	Day 1 (all groups)	Day 20	Day 30
Blood plasma (DRGPx3)			
Control	4.41 ± 1.15A	5.45 ± 1.39	5.21 ± 1.09
Na-selenite (feed)		7.73 ± 2.26A,a	5.93 ± 1.09A
Na-selenite (i/m)		7.40 ± 2.00A.B,a	6.34 ± 0.81A.B,a
Selenized yeast		7.40 ± 1.23A,a	6.55 ± 1.6
Males (nonsupl.)			5.43 ± 1.61
Erythrocytes (GPx1/2)			
Control	54.51 ± 15.45A	52.07 ± 10.27	48.40 ± 13.21
Na-selenite (feed)		83.64 ± 22.79A,a	80.72 ± 16.82A,a
Na-selenite (i/m)		76.65 ± 24.82A,B,a	44.93 ± 10.31B
Selenized yeast		78.20 ± 22.76A,a	65.72 ± 15.08
Males (nonsupl.)			35.81 ± 8.64

The same capital superscript letters represent significance ($p<0,05$) between sampling dates; small letters represent significance ($p<0,05$) between Se supplemented groups and control at any given date; no letters represent no significance.

The bioavailability of different forms of added selenium on indicators of blood selenium status is shown as a percentage (Table 5), with sodium selenite defined as 100 %.

Table 5. Bioavailability (%) of experimental Se sources, estimated by their impact on the indicators of blood Se status.

Indicators	Selenium source	Day 20	Day 30
Se concentration in blood plasma	Na-selenite (feed)	100	100
	Na-selenite (i/m)	104	87
	Selenized yeast	101	92
Se concentration in erythrocytes	Na-selenite (feed)	100	100
	Na-selenite (i/m)	152	63
	Selenized yeast	129	70
GPx activity in blood plasma	Na-selenite (feed)	100	100
	Na-selenite (i/m)	96	107
	Selenized yeast	96	110
GPx activity in erythrocytes	Na-selenite (feed)	100	100
	Na-selenite (i/m)	92	56
	Selenized yeast	93	78

A linear correlation (y=ax+b) between selenium concentration and DRGPx3 activity in maternal blood plasma was observed on the 1st, 20th, and 30th day of the experiment (Table 6), with the correlation curve slope (a) and the dependency coefficient (R^2) decreasing with each subsequent sampling.

Table 6. Correlation between selenium concentration (μg/L) and DRGPx3 activity (μkat/L) in the blood plasma.

Parameter	Day 1 (n=10)	Day 20 (n=40)	Day 30 (n=40)
Formula	y = 0.23x – 7.75	y = 0.20x – 3.12	y = 0.09x + 1.14
Correlation coefficient	R = 0.69	R = 0.65	R = 0.55
Dependency coefficient	R^2 = 0.48	R^2 = 0.42	R^2 = 0.30
Significance	p<0.001	p<0.001	p<0.001

The number of erythrocytes in the blood of broodstock was constant throughout the experiment and ranged in all groups in a narrow range of 1.81 ± 0.29 to 2.27 ± 0.22 × 10^{12}/cm^3, as well as **hematocrit**, in the range of 26.0 ± 1.79 to 30.20 ± 3.83 %. On day 30, the number of erythrocytes in males was 3.17 ± 0.27 ×10^{12}/cm^3), and the hematocrit was 42.80 ± 4.02%, which was significantly higher than that of female control.

Selenium concentration in roe, milt, and larvae, relative fertility, and percentage of larvae hatched

Selenium concentrations in broodstock roe ranged from 48.26 ± 17.53 μg/L in the control group to 65.68 ± 34.39 μg/L in broodstock receiving selenized yeast. The variation within all groups was very high, so it was impossible to determine the significance of the differences between them. The selenium concentration in the milt of the broodstock males was 24.78 ± 4.27 μg/L.

The selenium concentration in the larvae body ranged from 26-43 μg/L. The concentration was determined in an aggregate sample (larvae from all broodstock from one group were kept together), so it was impossible to determine the variation measure. However, it is noticeable that higher concentrations of selenium in larvae in the groups that received selenized yeast and sodium selenite i/m correspond to higher concentrations measured in eggs (Table 7).

Table 7. Selenium concentrations (μg/L) in eggs, larvae and milt on the day of spawning (day 30).

	Roe (n=10)	Larve (pooled by experimental group)
Control	48.26 ± 17.53	27.20
Na-selenite (feed)	49.75 ± 20.11	26.00
Na-selenite (i/m)	57.52 ± 48.26	40.30
Selenized yeast	65.68 ± 34.39	43.00
	Milt (n=10)	
Males (nonsupl.)	27.78 ± 4.72	

Total number of eggs in one gram of roe (30^{th} day) ranged from 924.20 ±169.46 to 1026 ± 136.90, and the **percentage of hatched larvae** ranged from 87.9–90.3%. No differences were observed between the selenium-supplemented and control groups.

DISCUSSION

According to Van't Hoff's equation, a temperature increase of 10 °C in poikilothermic organisms doubles the intensity of the physiological processes. However, it turns out that the equation is insufficient to explain the effect of temperature on the complicated metabolic systems of different fish species [23]. By measuring the impact of temperature changes on oxygen consumption, it has long been established that carp's metabolic rhythm is relatively constant at temperatures ranging from 14-28 °C [24]. Goldspink [25] showed that changes in the expression of specific genes underlie adaptation to changes in external temperature in carp. Our experiment was conducted in mid-spring for 30 days when the water temperature in the pond rose steadily from 14.5 to 21.4 °C, allowing the fish to gradually adapt to changes.

The selenium concentration in the broodstock's blood plasma ranged from 50.97 ± 7.35 to 58.31 ± 12.25 μg/L throughout the experimental period and did not change significantly (Table 3). An exception in this respect was the control group on day 30, in which plasma Se concentrations decreased significantly (41.70 ± 6.83 μg/L). At first glance, this seems like a paradox, but it should be noted that plasma Se concentration has a dynamic character. Blood plasma is considered the principal distributor of selenium in the body. Most of the Se in plasma is incorporated into selenoprotein P [26], produced in the liver by incorporating selenium of all chemical forms, which arrives in the body through food. An increase in the need for tissues for Se during the spring does not allow a significant increase in the concentration of Se in plasma. Moreover, plasma concentration will decrease in animals undersupplied with Se, as shown in the control group in our experiment.

In support of the above, **the concentration of Se in the broodstock erythrocytes** increased continuously during the experimental period, from 14.04 ± 3.05 μg/L on day 1 to a maximum of 212 ± 30.95 μg/L on day 30 in the sodium selenite group. This value was significantly higher ($p<0.05$) than the control group.

The concentration of Se in the blood plasma and erythrocytes of non-supplemented males on day 30 did not differ significantly from the control group of females, indicating no sexual differences in selenium concentration in mature carp (Table 3).

Bell et al. conducted two experiments on growing trout and found that blood plasma selenium concentration ranged from 32 to 157 μg/L [17] and 31 to 219 μg/L [26]. The lowest values were expressed by the fish not receiving selenium in the diet. It must be borne in mind that the experiments of the above authors were done with trout, which live under different environment temperatures and metabolic regimes, so their results cannot be uncritically compared with ours.

The broodstock **plasma selenoenzyme activity (DRGPx3)**, observing the maximum values achieved, were higher than those expressed in trout fed a diet with comparable doses of selenium [13, 14] and slightly lower than those measured in canal catfish [15]. As our experiment was carried out during the spring, temperatures did not reach the summer optimum. Therefore, we assume that the GPx activity in carp broodstock during the summer would be comparable to the values recorded in channel catfish.

Selenoenzyme activity in erythrocytes (GPx1/2) steadily increased in Se-supplemented groups from day 1-20 and retained the plateau until day 30. The exception was the group receiving sodium selenite i/m in which GPx1/2 dropped to the control level on day 30, indicating the depletion of the parenteral supplement given initially on day 1.

GPx activity in the blood of males for 30 days did not differ significantly from the control group of females, indicating no sex differences in GPx activity in carp.

It is generally established that a strong **correlation between Se concentration and GPx activity** indicates a low Se status in animals. Correlation dependence in our trial decreased steadily from day 1-30 between selenium concentration and DRGPx3 activity in blood plasma (Table 6). In our experiment, the sum of naturally occurring and supplemented selenium was approximately 250 µgSe/kg complete feed on a dry weight basis. Therefore, taken together with a plateau of selenoenzyme activity on days 20-30 (Table 4), it indicates that this amount of Se may be adequate to meet the needs of broodstock carp.

The distribution patterns of Se content and GPx activities show a distinctive difference (Figure 1). The Se content of erythrocytes on day 1 was approximately 20%; on day 20, it varied from 40-60%, and on day 30, it reached 70-80% due to the erythrocyte Se content increase. On the other hand, DRGPx3 (plasma) and GPx1/2 (erythrocytes) activities changed in unison, and the fixed distribution rate of 10:90 % between plasma and erythrocytes was maintained.

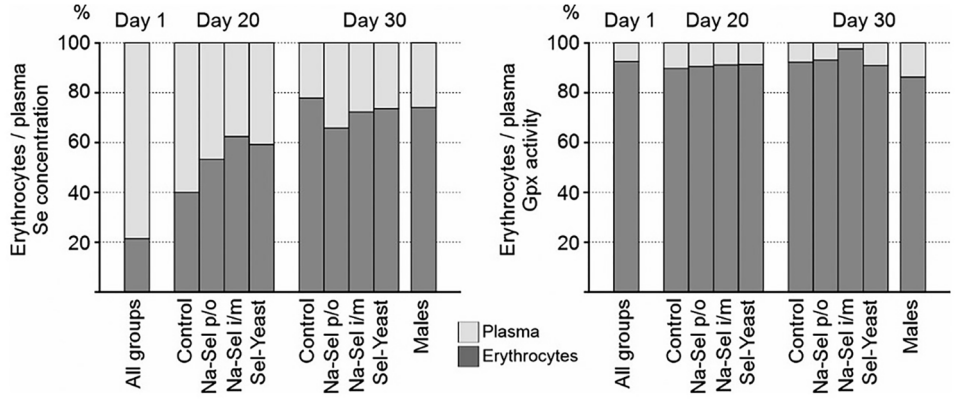

Figure 1. Pattern distribution of selenium concentration, and GPx activity between blood plasma and erythrocytes.

According to Witeska [27], the life span of fish erythrocytes can be considerably longer (up to 500 days) compared to mammalian (120 days). The circulating erythrocytes of teleost fish contain nuclei and express proteins while in circulation. The protein production decreases during the lifespan of erythrocytes [28] but is responsive to temperature stress. Fifteen most abundant active genes in erythrocytes of a rainbow trout (*Oncorhynchus mykiss*) are connected to hemoglobin functions [29]. Still, they account for only about 10-30% of overall protein production. Whether fish erythrocytes are capable of producing selenoproteins is unknown. The fact that changes in selnoenzymes activities in our experiment took considerably less time than the erythrocytes' half-life could be an indicator. However, due to a complicated synthesis mechanism and selenocysteine supply, it is much more probable that the selenoprotein production is limited to the period of erythropoiesis, and the pace of change in GPx1/2 reflects the regular erythrocytes turnover rate.

The bioavailability of Se from experimental supplements can be assessed if the results are expressed as a percentage of the effect observed for sodium selenite as a reference standard. Of course, it is crucial to consider the general biological context in which the experiment took place. Table 5 shows that for days 1-20, selenized yeast (129%) and sodium selenite given i/m (152%) had a more substantial effect on the increase in Se concentration in erythrocytes than sodium selenite. This is consistent with the data for the channel catfish [15] and cyprinid species [16,30,31]. However, on day 30 of our trial, sodium selenite was the most effective source of supplemental selenium. The most likely explanation for this difference is that the abovementioned trials were conducted on young, growing fish, while ours was performed on mature carp. There are indications that, in fish, the requirement for selenium decreases with the age of the animals because of the growing efficiency of selenium utilization mechanisms later in life [32]. Sodium selenite given i/m has proven to be a slightly more efficient source of selenium than selenized yeast on day 20 because it was administered parenterally and did not undergo the process of resorption in the digestive tract.

Considering GPx activity in plasma and erythrocytes, it was observed that from day 1-20, there were no significant differences in the effectiveness of supplements. In contrast, on day 30, sodium selenite in the diet was the superior supplemental source. The explanation for this should be sought in the rhythm of selenium uptake and synthesis of selenoenzymes in tissues. GPx1/2 is synthesized during erythropoiesis, while renal tissue is considered the principal DRGPx3 synthesis site [2].

It is logical to expect that the status of selenium in eggs, milt, and larvae is biologically significant because, in fish eggs, there is a large amount of vitellus, which is predominantly composed of lipids prone to peroxidation, and because in fish, fertilization takes place in the external environment, so fertilized eggs and larvae develop in open water. Despite this, data showing physiological concentrations of selenium in the gamets and fish larvae are virtually non-existent in the literature. Wherever mentioned, they are considered in the context of toxicity [33, 34]. These data show that fish eggs can uptake selenium from water. However, as in the case of adult fish, this cannot be a

reliable support for supplying this microelement in regular conditions. More probably, it is the accumulation of selenium in roe during the maturation of the eggs.

Data on selenium status in gamets and carp larvae are shown in Table 7. Individual selenium concentrations in broodstock eggs ranged from 23.10-76.35 μg/L. The highest average concentration was measured in the eggs of broodstock receiving selenized yeast (65.68 μg/L). Selenium concentration in the larvae was the highest (43 μg/L) in a group descended from broodstock supplemented with selenized yeast. These results should be taken only as indicative since the variability within the groups was very high, which made it impossible to make a reliable statistical comparison. The likely reason for this is the high prevalence of vitellus, which does not contain selenium [35]. Despite this, it is observed that, in both eggs and larvae, selenized yeast has shown greater efficiency in the supply of selenium.

The number of erythrocytes in the blood, as well as the **hematocrit** of broodstock, was constant throughout the experiment regardless of the selenium supplementation method.

Our data do not indicate any effect of selenium supplementation on the **number/ size of unfertilized eggs** produced or the **larvae survivability**. This could eventually change if broodstock fish had been supplemented with selenium before winter and in the spring. The **selenium concentration in the milt** of (unsupplemented) males was 24.78 ± 4.27 μg/L, almost twice as low as the concentration in the eggs of the control group of females.

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Author's contributions

JIB conceptualization, study design, data preparation and analyses, project administration, manuscript writing, MS blood sampling, laboratory analyses, draft sections of the manuscript, GD, laboratory analyses, statistical data analysis, VO laboratory analyses, draft sections of the manuscript prepared manuscript for submission, corresponding author. All authors have read and approved the manuscript.

Competing interests

The authors declare that they have no competing interests.

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REFERENCES

1. Behzad M, Beggs AH: Selenoproteins and their impact on human health through diverse physiological pathways. Physiology (Bethesda) 2006, 21:307–315.
2. Margis R, Christophe D, Teixeira FK, Margis-Pinheiro M: Glutathione peroxidase family – an evolutionary overview. The FEBS Journal 2008, 275, 1742-464X:3959-3970.
3. Anttolainen M, Valsta LM, Alfthan G, Kleemola P, Salminen I, Tamminen M: Effect of extreme fish consumption on dietary and plasma antioxidant levels and fatty acid composition, Eur J Clin Nutr 1996, 50:741-746.
4. Wen HY, Davis RL, Shi B, Chen JJ, Chen L, Boylan M, Spallholz JE: Bioavailability of selenium from veal, chicken, beef, pork, lamb, flounder, tuna, selenomethionine, and sodium selenite assessed in selenium-deficient rats. Biol Trace Elem Res 1997, 58:43-53.
5. Jovanović IB, Pešut O, Mihailović M, Kosanović M: Selenium content in feedstuffs from Vojvodina (Serbia), Acta Veterinaria 1998, 48:339-44.
6. Hilton JW, Hodson PV, Slinger SJ: Absorption, distribution, half-life and possible routes of elimination of dietary selenium in juvenile rainbow trout (Salmo gairdneri). Comp Biochem Physiol C Comp Pharmacol. 1982, 71C(1):49-55.
7. Dangić A, Rakočević P, Dangić J, Ocokoljić, V: Selenium in natural waters of Serbia. Conference on Selenium, Proceedings of the Scientific Meeting, 22-23 June 1993. Ed: Maksimović, ZJ, Petrović VM, Spužić IV, Gašić MJ, SANU 1995, 78, 53-64.
8. Bartley DM: World Aquaculture 2020 – A brief overview. FAO Fisheries and Aquaculture Circular 2022 No. 1233. Rome, FAO.
9. Horváth L. Tamás G. Coche, AG: Common Carp 2: Mass Production of Advanced Fry and Fingerlings in Ponds, FAO 1985, Rome, Italy, 44. Available online: www.fao.org/docrep/X0086E/X0086E00.htm
10. Wiegand MD: Some aspects of lipid metabolism in Cyprinid fish, In: Aquaculture of Cyprinids, Ed. Billard R, Marcel J, INRA 1986, Paris, 71-80.
11. Marković ZZ: Aquaculture in Serbia, (Article in Serbian) Veterinarski Glasnik 2022, 76, 168-175. https://doi.org/10.2298/VETGL201124017M
12. Horvath L, Hegyi A, Lefler KK, Csorbai B, Kovacs E, Szabo T, Muller T, Urbanyi B: Review of Central-Eastern European Propagation and Larvae Nursing Method for Common Carp (*Cyprinus carpio L.*). Life 2023, 13, 2334.
13. Hilton JW, Hodson PV, Slinger SJ: The Requirement and Toxicity of Selenium in Rainbow Trout (Salmo Gairdneri), J Nutr 1980, 110:2527-2535.
14. Gatlin DM 3rd, Wilson RP: Dietary selenium requirement of fingerling channel catfish, J Nutr 1984, 114:627-633.
15. Lovell RT, Wang C: Comparison of organic and inorganic sources of selenium for growth and health of channel catfish. In: Biotechnology in the Feed Industry, Proceedings of the 13th Annual Symposium, Ed: Lyons, TP, Jacques, KA. Nottingham University Press 1996, Longborough, Leies, UK, 165-179.
16. Jovanović A, Grubor-Lajšić G, Djukić N, Gardinovački G, Matić A, Spasić M: The effect of selenium on antioxidant system in erythrocytes and liver of the carp, Crit Rev Food Sci Nutr 1997, 37:443-448.
17. Bell JG, Cowey CB, Adron JW, Shanks AM; Some effects of vitamin E and selenium deprivation on tissue enzyme levels and indices of tissue peroxidation in rainbow trout (Salmo Gairdneri). Brit J Nutr 1985, 53:149-157.

18. Wang KY, Peng CZ, Huang JL, Huang YD, Jin MC, Geng Y: The pathology of selenium deficiency in *Cyprinus carpio L*, J Fish Dis 2013, 36:609-15.
19. Bell JG, Cowey CB, Youngson A: Rainbow trout liver microsomal lipid peroxidation the effect of purified glutathione peroxidase, glutathione S-transferase and other factors. Biochimica et Biophysica Acta (BBA) – Lipids and Lipid Metabolism 1984, 795:91-99.
20. Wdzieczna J, Zalesna G, Bartkowiak A, Witas H, Leyko, W: Comparative studies on superoxide dismutase, catalase and peroxidase levels in erythrocytes of different fish species, Comp Biochem Physiol 1981, 68b:375.
21. Günzler WA, Steffens GJ, Grossmann A. Kim, SMA, Ötting F, Wendel A, Flohé, L: The Amino-Acid Sequence of Bovine Glutathione Peroxidase. Biological Chemistry 1984, 365:195-212.
22. Sankari S: Plasma glutathione peroxidase and tissue selenium response to selenium supplementation in swine. Acta Vet Scand 1985, 81 (Suppl.):1–127.
23. Tsukuda H: Temperature dependency of the relative activities of liver lactate dehydrogenase isozymes in goldfish acclimated to different temperatures. Comp Biochem Physiol 1975, 52:343-345.
24. Schmeing-Endberding F: Die Vorcugstemperaturen einiger Knochfische und ihre physiologishe Bedeutung. Z Fisch 1953, N.F., 2:125.
25. Goldspink G: Adaptation of fish to different environmental temperature by qualitative and quantitative changes in gene expression. J Therm Biol 1995, 20:167-174.
26. Kryukov GV, Gladyshev VN: Selenium metabolism in zebrafish: multiplicity of selenoprotein genes and expression of a protein containing 17 selenocysteine residues, Genes to Cells 2000, 5:1049-1060.
27. Bell JG, Pirie BJS, Adron JW, Cowey CB: some effects of selenium deficiency on glutathione peroxidase (EC 1.11.1.9) activity and tissue pathology in rainbow trout (Salmo gairdneri). Brit J Nutr 1986, 55:305–311.
28. Witeska M: Erythrocytes in teleost fishes: a review. Zool Ecol 2013, 23:275–281.
29. Lund SG, Phillips MC, Moyes CD, Tufts BL: The effects of cell aging on protein synthesis in rainbow trout (Oncorhynchus mykiss) red blood cells. J Exp Biol 2000, 203, 14:2219-2228.
30. Götting M, Nikinmaa MJ: Transcriptomic analysis of young and old erythrocytes of fish. Front Physiol 2017, 12:1046.
31. Saffari S, Keyvanshokooh S, Zakeri M, Johari SA, Pasha-Zanoosi H: Effects of different dietary selenium sources (sodium selenite, selenomethionine and nanoselenium) on growth performance, muscle composition, blood enzymes and antioxidant status of common carp *(Cyprinus carpio)*. Aquacult Nutr 2017, 23:1353-5773.
32. Zhu C, Wu Z, Liu Q, Wang X, Zheng L, He S, Yang F, Ji H, Dong W; Selenium nanoparticles in aquaculture: Unique advantages in the production of Se-enriched grass carp (Ctenopharyngodon idella), Animal Nutrition 2024, 16:189-201.
33. Leonzio C, Focardi S, Bacci E: Complementary accumulation of selenium and mercury in fish muscle. Sci Total Environ 1982, 24:249-254.
34. Huckabee JW, Griffith NA: Toxicity of mercury and selenium to the eggs of carp *(Cyprinus carpio),* Trans Amer Fish Soc 1974, 4:822-824.
35. DeForest DK, Gilron G, Armstrong SA, Robertson EL: Species sensitivity distribution evaluation for selenium in fish eggs: considerations for development of a Canadian tissue-based guideline. Integr Environ Assess Manag 2012, 8:6-12.

EFEKTI RAZLIČITIH PREPARATA KOJI SADRŽE SELEN NA STATUS SELENA I REPRODUKTIVNE PARAMETRE MATICA ŠARANA *(Cyprinus carpio L.)*

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Procenjivana je efikasnost različitih suplemenata selena na status selena u krvi i reproduktivne performanse matica šarana. Sto dvadeset ženki šarana je raspoređeno u 4 grupe i suplementirano Se na sledeći način: **kontrola** (nesuplementirana), **natrijum selenit** 200 μg/kg hrane, **natrijum selenit i/m** injekcija (160 μgSe/kg telesne mase) i **selenizirani kvasac** 200 μg/kg hrane. Krv je uzorkovana 1., 20. i 30 dana. Tridesetog dana, uzeti su uzorci ikre od ženki, a uzorci krvi i mleča od 10 nesuplementiranih mužjaka. Uzorci larvi uzeti su nakon izleganja. Koncentracija Se u krvnoj plazmi je bila 51,38±3,77 μg/kg (1. dan) i nije značajno varirala. Koncentracija Se u eritrocitima je rasla od 14,04±3,05 μg/kg (1. dan), do 133,03±42,06 i 212,38±57,87 μg/kg (30. dan). Aktivnost plazmatske glutation peroksidaze porasla je sa 4,41±1,15 na 7,73±2,26 μkat/L nakon čega se uspostavio plato do 30. dana. Sličan obrazac primećen je kod aktivnosti glutation peroksidaze eritrocita, u rasponu od 54,51±15,45 μkat/L (1. dan) do 80,72±16,82 μkat/L (30. dan). Koncentracije selena i aktivnosti selenoenzima u krvi mužjaka nisu se razlikovale od kontrolne grupe ženki. Sadržaj Se u ikri se kretao od 48,26±17,53 μg/L kod kontrole do 65,68±34,39 μg/L grupi hranjenoj seleniziranim kvascem, a u pulovanim uzorcima larvi, od 26-43 μg/L. Koncentracija Se u mleču je bila 24,78±4,27 μg/L. Rezultati ukazuju da je ukupna koncentracija od 250 μg Se/kg hrane bila dovoljna da zadovolji potrebe odraslih šarana. Natrijum selenit dat kroz kompletnu smešu bio je najefikasniji u podizanju koncentracije selena i aktivnosti selenoenzima u krvi, dok je selenizirani kvasac bio efikasniji u ikri i larvama.