Histological Investigations on an Intestinal Level in Common Carp, One Summer Old, Fed with Organic Selenium (Selplex) Supplemented Fodder

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Abstract

The goal of this research was to analyze the effect of organic Selenium (SelPlex) on the intestinal mucosa and the impact on the growth and consumption performances in one summer old common carp (Cyprinus carpio), Lausitz and Galitian varieties. The research were conducted on a number of 392 fish, Lausitz variety divided in 2 groups and on 192 fish, Galitian variety, divided into 2 groups. The fish were raised in 2 ponds (one pond for the control group and one pond for the experimental group), in a semi-intensive polyculture system. The rearing conditions were identical for the 2 groups. The fodder for the experimental group was supplemented with Selenium (0.03mg/kg fodder). At the end of the experiment, the Selenium supplementation had a positive influence on the height of the intestinal villi. The experimental group (both varieties) showed significant differences compared with the control group. The mean height of the intestinal microvilli from the experimental group, Lausitz variety, was 2.328µm, 0.95% bigger compared to the same variety of the control group. The Galitian variety showed a mean height of the microvilli of 2.227µm, 0.72% biger than the same variety of the control group. This development of the villi structures in the experimental groups led to an increase in the absorption surface of the intestine which explains the accelerated growth and the improvement of the feed conversion ratio.

Keywords: carp, consumption, growth, microvilli, organic selenium, villi.

1. **Introduction**

Selenium is a trace mineral found in 1817 by Swedish chemist Jons Jakob Berzelius [1] is accepted as a necessary element in food until 1957 [2]. It is generally accepted that selenium in biological system is part of the protein is a component of amino acids: cysteine, methionine and derivatives, respectively selenocysteine selenomethionine. Selenium is available in various forms. Selenite is organic forms of selenium and selenocysteine and selenomethionine are organic forms. Organic form, selenium is chemically bonded to a chelating agent, or ligant, represented by amino acids or peptides. Hence the name of mineral and trace element and protein [3]. Selenium is essential for animals, including fish. It was found that the selenium is an integral part of enzyme glutathioneperoxidase (GSH-Px) [4], which acts as an antioxidant in cells. This enzyme oxidizes glutathione and thus remove hydrogen peroxide that occurs in cells transforming it into the water. It is a process of detoxification of cells, hydrogen peroxide peroxides produced by oxidation in turn threatening to polyunsaturated fatty acids of cell membranes [5]. GSH-Px is the most common antioxidant protein that contains selenium. GSH-Px family is best characterized in mammals, where to find the number of 5 members, but their investigation has not yet been carried out on fish. GSH-Px family consists of: the so-called classical GSH-Px tiozolică; glutathione peroxidase phospholipid hydroperoxide (PH-GSH-Px); glutatio peroxidaza plasma (pl-GSH-

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Px), glutathione peroxidase gastrointestinal (GI-GSH-Px) and the glutathione peroxidase sperm nuclei (SN-GSH-Px), [6].
The fish is covered with multiple intestinal mucosa that increase the surface area of chorionic intestinal absorption. Carp (Cyprinus carpio) raised to a temperature of 10 º C is the size of villi lower than that grown at 30 ° C, resulting in an increase in surface mucosa by 58% without changing the total number of villi [7]. Microvilli are microfilaments composed of polysaccharides [8]. Microvilii carp have a cylindrical shape and a rounded, their length varies depending on age and weight 250g/exemplar to have a length of 1.2 µm and a diameter of 0.12 µm [9].
In this context the goal of this research was to analyze the effect of organic Selenium (SelPlex) on the intestinal mucosa and the impact on the growth and consumption performances in one summer old common carp (Cyprinus carpio), Lausitz and Galitian varieties.

2. Materials and methods

The biological material was represented by two variety of carp (Cyprinus carpio), and Galician Lausitz has been elevated to the status of juvenile fish consumption by summer.
The experiments were performed on a total of 392 carp, Lausitz variety, divided into two groups (194 copies / control group and 198 copies / experimental group) and 192 specimens, divided into two groups Galician variety, (94 copies / control group and 98 copies / experimental group). Population groups was carried out in two pools, a pool and one control groups for the experimental groups, was applied to a in polyculture farming semi system (Figure 1.).
Growth conditions were identical in the two groups, except food. Food fed pelleted compound feed was based on nutritional value of which is as follows: 38% protein, 5% fat, 9% moisture and 3.5% cellulose. The combined experimental pelleted feed the structure adding organic selenium (SelPlex) from the company Alltech USA, powder form, in an amount of 0.03 mg / kg feed. Feed grain was 3-5 mm which was maintained throughout the experiment.
During the experiments was monitored developments in the main indices of growth and consumption, feed consumption.
For histological examination, samples were taken from the gut. Samples of tissue (intestinal mucosa) were placed in fixative (10% buffered formalin, pH 7) for 24 hours. After this time samples were processed after the inclusion technique in paraffin sections of 4-5 microns were made using microtome Leica RM 2125 RT and then were subjected to hematoxylin-eosin staining as follows: dewaxing and hydration gradual alcohol and water 5-10 minutes staining in hematoxylin solution, rinsed with distilled water until it turns blue sections; differentiation 5-10 seconds in acid-alcohol solution, if the nuclei supracolorare, rinse with distilled water until Sections are blue, 10-minute dye eosin solution, washing with tap water 1-5 minutes, gradually dehydrated alcohol 96 and then with absolute alcohol; clarification 1:1 xylene-absolute alcohol and finally in xylene, mounted in Canada balsam.
Preparations were then examined under a microscope Olympus BX 51. The images were taken with an Olympus SP 350 digital camera and analyzed with an Olympus DP-soft program properly.
The data were statistically analyzed using GraphPad Instat 3.0 program.

3. Results and discussion

At the end of the experiment both varietys of carp from a summer average experimental group has a higher weight (1258.8 ± 18.23 g and 1191 ± 014.21 Lauistz variety variety Galician g) compared with controls (917.78 ± 18.29 g and 908.67 ± 17.78 Lauistz variety g Galician variety) (Table 1).
At the end of the experimental period, between experimental and control groups in both strains there were significant differences (*** P <0.001%) in favor of experimental groups compared with control groups in terms of body weight.
Effect of organic selenium on growth and consumer indices registered for a summer carp is presented in Table 2.
The main indices of growth and consumption are influenced by the addition of organic selenium favorable (at a dose of 0.03 mgSelPlex / kg feed) in common carp to a summer. There is an improvement in growth of 41.06% increase in Lausitz and 41.32% for the Galician variety of experimental group compared with controls, the

same varieties. Growth rate (g body weight / day) had the highest values in the experimental group: the best growth rate is recorded Galician variety of experimental group 4.53 g / day, 41.32% higher than the same variety control group. Lausitz variety of experimental group registered a higher growth rate of 41.06% in the control group. Analyzing the feed conversion ratio in Lausitz variety is found in the index improved by 2.7% in the experimental group (1,79:1) compared with controls (1,84:1). Addition of organic selenium resulted in an improvement of feed conversion ratio of 8.85% for Galitian variety of experimental compared with the control group, the same variety. Improving feed conversion ratio of the two varieties of carp in the experimental group confirms the positive effect of organic selenium has on the index, which is demonstrated in other species of fish [10], [4].

To highlight the effects of organic selenium in the gut and to show favorable results obtained demonstrate progress on growth and consumer indices, histological investigations were performed, which consists in measuring height of intestinal villi and microvilli.

Villi height value from the intestinal to a summer common carp are presented in Table 3, statistically in Table 4 and their appearance in Figure 2. Analyzing the intestinal villi height in the two varieties of carp in the summer is noticed that the experimental group variety Galician (2002.9 ± 78.36 µm) show higher by 1.77% compared to the variety of Lausitz (1967.4 ± 354.90 µm), which explains the faster growth to the variety of Galician.

Microvilli height value from the intestinal to a summer common carp are presented in Table 5 and their appearance in Figure 2.

The average height of the carp intestinal microvilli a summer in Lausitz variety of experimental group is 2.328 ± 0.403µm, with 0.95% more than the same variety in the control group. Galician variety of experimental group shows an average height of 2.227 ± 0.320µm intestinal microvilli with 0.72% more than in the control group.

The development of structures in experimental viliare surface increased the absorption of nutrients circulating through the intestinal, which explains the faster growth rate in the experimental groups compared with controls. With the increase in height of villi and intestinal microvilli, organic selenium is an antioxidant character of the intestinal through the active participation of glutathione peroxidase intestinal (GI-GSH-Px), [4].

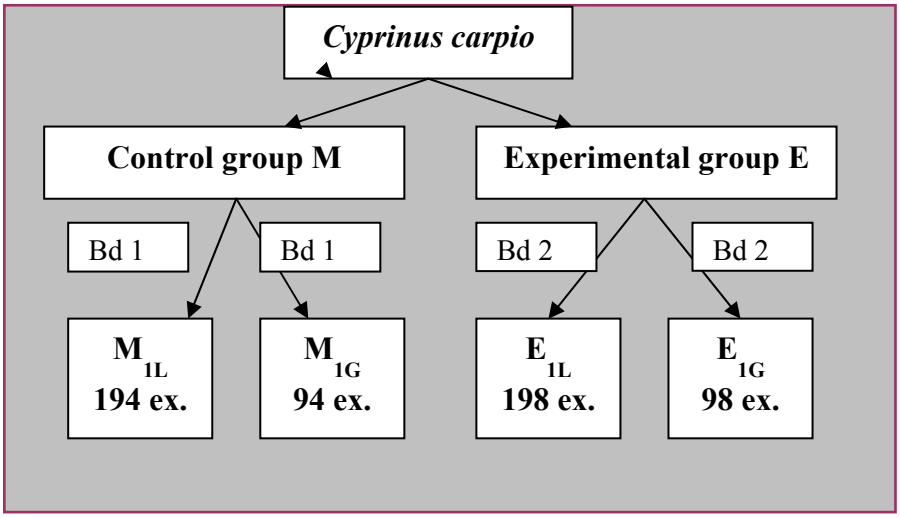

Figure 1. Experimental groups

Table 1. Statistical parameters of body weight of carp at the end of the summer experiment

Specification	Control group		Experimental group	
	Lausitz (M_{1L})	Galician (M_{1G})	Lausitz (E_{1L})	Galician (E_{1G})
n	189	90	198	97
$\overline{X} \pm S\overline{x}$	917.78±18.29	908.67±17.78	1258.8±18.23***	1191±014.21***
V%	9.6	9.3	7.5	7.7

*** P <0.001% - very significant; V% - variability; n - number of individuals; $S\overline{x}$ – standard deviation; $\overline{X}$ - average.

Table 2. Values of growth and consumption at the end of a summer experimental carp

Specification	Measuring unit	Control group		Experimental group	
		Lausitz	Galician	Lausitz	Galician
Spor growth	g	640.97	642.28	904.18	907.73
	%	100	100	141,06	141,32
Specific growth rate	g body weight / day	3.20	3.21	4.52	4.53
	%	100	100	141,06	141,32
Feed conversion rate	kg feed / kg body mass	1.84:1	1.85:1	1.79:1	1.70:1
	%	100	100	97.28	91.89

Table 3. The values of intestinal villi height (μm) at a summer carp

Specification	Control group		Experimental group	
	Lausitz (M_{1L})	Galician (M_{1G})	Lausitz (E_{1L})	Galician (E_{1G})
n	18	19	20	19
$\overline{X} \pm S\overline{x}$	1490.7±118.2	1734.7±283.50	1967.4±354.90***	2002.9±78.36***
V %	7,93	16.32	18.03	3,91

*** P <0.001% - very significant, V% - variability; n - number of individuals; $S\overline{x}$ – standard deviation; $\overline{X}$ - average.

Table 4. The statistical significance of differences between the two groups recorded the height of the villi

Comparison	The average difference	q	P
M_{1L} vs M_{1G}	-244.01	3.678	nsP>0.05
M_{1L} vs E_{1L}	-476.70	7.028	***P<0.001
M_{1L} vs E_{1G}	-512.27	7.113	***P<0.001
M_{1G} vs E_{1L}	-232.69	3.874	***P<0.001
M_{1G} vs E_{1G}	-268.27	4.143	***P<0.001
E_{1L} vs E_{1G}	-35.576	0.5367	nsP>0.05

***P < 0,001– very significant; ns P>0.05 – not signifcant.

Table 5. Intestinal microvilli height values (μm) at a summer carp

Specification	Control group		Experimental group	
	Lausitz (M_{1L})	Galician (M_{1G})	Lausitz (E_{1L})	Galician (E_{1G})
n	21	23	15	17
$\overline{X} \pm S\overline{x}$	2.306 ± 1.138	2.211±0.256	2.328 ±0.403ns	2.227±0.320ns
V %	7,93	16.32	18.03	3,91

nsP>0.05 – not significant, V% - variability; n - number of individuals; $S\overline{x}$ – standard deviation; $\overline{X}$ - average.

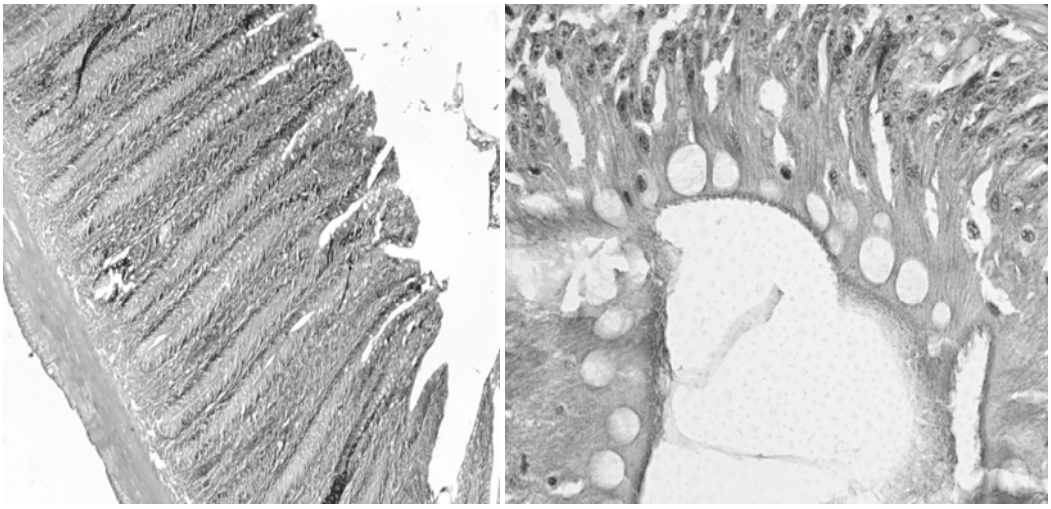

Figure 2. The appearance of intestinal villi (left) and intestinal microvilli (right) in carp (*Cyprinus carpio*) a summer (mm) 1000x.

4. Conclusions

Due to its action at intestinal level organic selenium (SelPlex) positively influenced viliare and microvilliar structures, resulting in better absorption of fodder, which led to the achievement of higher growth indices in the experimental group in both groups and varieties. Analyzing the intestinal villi height of both varieties of carp is found a summer of significant differences (*** P <0.001%) in favor of the experimental group compared with the control group. This development has increased the surface structures viliare absorption of nutrients circulating through the body, which explains the faster growth in a variety of both experimental group fed with 0.03 mgSelPlex / kg feed, resulting in a higher body mass. Analyzing the height of the carp intestinal microvilli a summer experimental group is an increase of 4.53% for the Lausitz. Organic Selenium (SelPlex) at a dose of 0.03 mgSelPlex / kg feed operating at increasing intestinal nutrient absorption surface by increasing the length of the villi and reduction of oxidative processes at this level resulting a faster growth rate in a period short time, so we recommend the use of organic selenium in food carp.

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