



Using a mixture of natural forage in growing of yearlings of carp under conditions of recirculation installations

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ABSTRACT

The basic fish-breeding and biotechnological and biological indicators of yearlings of scaly carp grown in recirculation installations using forage mixture of natural food objects were researched. It was established that the introduction of natural fodder for feeding carp in controlled conditions positively affect the growth of fish. Using histological studies it was determined that in terms of growing fish in closed water plants, feeding fish with natural feed ingredients have positive effect on the metabolism in the body of the fish.

Keywords: carp; recirculation installations; feeding of fish; natural feed; liver

1. INTRODUCTION

Fish farming has always played a significant role in ensuring the country's food, supply of raw materials to related industries of the national economy and increase of employment level [1,2]. The deep structural deformation and a significant backlog from developed countries are observed in fisheries over the past decades. Unfortunately, the tendency of reduction of the consumption of fish products of Ukraine's population is observed annually that is caused by the decline of fish catch and production of fish products, inhibiting the use of new technologies in fish farming.

Pridniprovsk region has a favorable basis for the development of fisheries. To solve the problem of further development of the fishery is possible only through an integrated approach in the development of modern fishery technologies using Dnieper reservoirs. Implementation of advanced European technologies to process fish breeding in our country will help change the situation in the fishing industry and ensure the sustainable development of fisheries. Improving the use of fishery technologies in Dnieper reservoirs will increase the yield of marketable fish production and restore natural ichtiocomplex of reservoir [3-5].

In currently existing technologies of growing of carp planting material are focuses on the use of feed with natural origin, completely contain a whole set of necessary substances for fish [6,7]. Researches in this area has always been and continue to be relevant as natural feed is the only reliable source of intake of fish essential amino acids, unsaturated fatty acids, vitamins, minerals and other components necessary for the growth and development of fish, they often lack enough amount in artificial feed, commonly used for feeding of carp [8,9].

For growing of young carp not only specialized feed is used, but also natural foods are used. This optimal proportion of natural forage in the diet of carp yearlings depends on the quality of artificial feed and ranges from 15 to 40 % of the total [6,10].

Under artificial rearing of carp in closed water plants proportion of natural forage in the diet can be increased by feeding them with nutritionally valuable invertebrates, which are proposed for the cultivation by many methods [7,8,11].

Currently effectiveness of cultivation of carp yearlings under controlled conditions with the activities to enrich their diet with natural foods is understudied in this regard, the study is important.

The aim of work was to study the efficiency of growing of carp yearlings in installations of close water supply during a set of measures to improve the content of natural forage in their diet, for further stocking of Dnieper Reservoir in modern hydroecological conditions.

In accordance with the objectives of the work there were following tasks:

- To examine the development of zoobenthos in Dnieper Reservoir in environmental conditions;
- To examine the indicators of growth rate of carp yearlings by using natural feed;
- To examine the impact of feed factor on the growth rate of carp yearlings;
- To justify and develop recommendations on the use of technology of growing of young carp for stocking Dnieper reservoir to improve the resistance of stocking material.

2. MATERIALS AND METHODS

Researches were conducted in Dnieper Reservoir – the multi-purpose reservoir during 2015. The reservoir is located in the South-west of Ukraine, in the territory of the agro-industrial zones and been under strong anthropogenic influence (Fig. 1).

Benthos samples were taken in 2015 with use of Ekman-Burge's grab by the standard method [13,14]. Samples were fixed in 4 % formalin solution. Soil was washed through the fine-meshed mill gauze. Weighting was conducted on torsion balance by groups. Determining of species composition was performed using microscopes MB-1 and MBS-1.

Experiment with growing of scaly carp (*Cyprinus carpio* (Linnaeus, 1758)) yearlings with natural feeding components held in the recirculation installations with a working volume

of 500 liters in two versions. The first (I) variant (control) yearlings of carp are fed only with specialized feed. In the second case (II) enrichment of the diet of carp yearlings was conducted with natural foods using forage mixture: Gammarus (*Gammarus pulex* (Linnaeus, 1758)) – 30 %, Daphnia (*Daphnia magna* (Straus, 1820)) – 30 %, Common duckweed (*Lemna minor* (Linnaeus, 1753)) – 30 %, Tubifex (*Tubifex tubifex* (Müller, 1774)) – 10 %. These components were selected because they imitate natural forage of shallows of Dnieper reservoir encountered in the spectrum of carp diet and have enough nutrients (Table 1).



Figure 1. The scheme of Dnieper Reservoir and the site of samples location.

Table 1. The chemical composition of food objects, % of absolute dry weight [7,11,12]

Feed component	Humidity	Dry matter	Protein	Fat	Carbohydrates
<i>Gammarus pulex</i>	83.2	16.8	8.1	1.2	3.0
<i>Daphnia magna</i>	90.0	10.0	4.5	1.8	1.8
<i>Tubifex tubifex</i>	82.7	17.3	10.5	1.9	3.7
<i>Lemna minor</i>	78.0	22.0	26.0	5.0	14–43

The daily amount of artificial feed was determined under recommendations of Y. P. Bobrov and others [11,12] and it was 10 % of weight of fish. To study the effect of feed additives in fish tissues and organs histological research methods were used. The tissues and organs of individuals from control and experimental group were selected and fixed in 4 % formalin followed by treatment by conventional histological methods [15-18]. To produce sections microtome tobogganing MS-2 was used. Sections were dyed using hematoxylin-eosin. Photos of preparations were made using a digital camera «Sciencelab T500 5.17 M», which is joined to the microscope of company "Biolam 70". Description of histological structures was produced using fish histology atlases.

All digital data received in the work were processed by computer software Microsoft Excel, Statistica 10. Statistical significance of differences was determined by Student t-test.

3. RESULTS AND DISCUSSION

3. 1. Assessment of food supply by features of zoobenthos

In 2015, it was found 98 species of benthos belonging to 12 groups as part of zoobenthos of Dnieper reservoirs. There were registered: 35 species among chironomid larvae, 23 species of oligochaetes, 15 species of clams, 9 species of crustaceans, 4 species of leeches and 22 species of other groups. Species composition of benthofauna of reservoir is characteristic for the Dnieper cascade of reservoirs.

The representatives of the Ponto-Caspian sector dominated in zebra mussel fouling and habitats of the middle part of the reservoir. The number of species in the stations ranged from 6 to 27, the smallest number of species was registered in profundal zone and in the littoral contaminated sites, and the largest number was registered in the bush littoral zone.

In faintly muddy sand there were observed biocenoses of two species of zebra mussel (*Dreissena bugensis*, *Dr. polymorpha*), which is consumed by roach to some extent. In the middle of the reservoir in winter soft benthos biomass was average 20.1 g/m², and in the summer and autumn decreased to 3.2–5.5 g/m², which is caused by an active nutrition of fish.

At the lower part of reservoir a significant siltation is observed that affects both the species composition of benthic groups and figures of biomass of feed organisms. Below the Stari Kodaky village to Volosske village zebra mussel met infrequently because of sludge accumulation and the total biomass of benthos in summer increased from 4.3 to 14.2 g/m² with the dominance of chironomid larvae (*Chironomus semireductus*, *Ch. Plumosus*). Below the Voyskove village dominance passed to oligochaetes *Limnodrilus hoffmeisteri*, *Tubifex tubifex*, which is caused by significant sludge accumulation.

The average biomass of benthos of profundal of middle part of reservoir in summer was 2.4 g/m², which was almost 5 times lower than benthos biomass at the lower part, where zoobenthos biomass reached 11.8 g/m². Average benthos biomass in autumn decreased in the profundal of middle part of reservoir to 1.5 g/m², at lower part to 9.4 g/m².

Considering the indicators of benthos development during 2015 and in the littoral part the average biomass of soft zoobenthos in Dnieper Reservoir reached 9.8 g/m². This corresponds to reservoirs with high nutrition supply. On the assumption of the P/B ratio of benthos which is 6, we have found that the production of benthos in Dnieper reservoirs is 630 kg/ha, which corresponds to 45.2 kg/ha of potential fish productivity by carp.

3. 2. Results of the research of growing juvenile carp in installations of close water supply

The rate of growth of carp yearlings in recirculation installations is affected by complex factors that can be controlled: hydrochemical composition of water, oxygen saturation, temperature, availability of food, feed composition and duration of artificial feeding [19-21].

The growth rate of young carp, after their transfer from the ponds to the recirculation installations and before feeding a mixture of natural origin feed was the same. During the feeding of carp yearlings, under the same conditions of recirculation installations it was found that a higher growth rate of yearlings was observed in the second version of the experiment in which young fish were fed a mixture of natural food (Fig. 2).

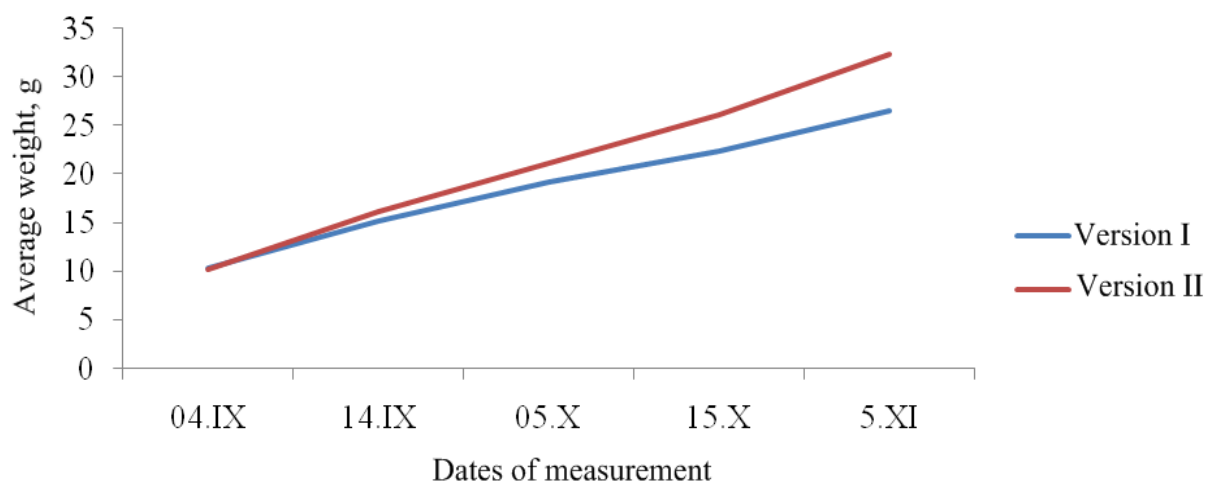


Figure 2. Dynamics of growth of carp yearling: Version I – research, version II – control.

At the end of the experiment in the experimental recirculation installations of second version average weight of carp yearlings was higher than latest of the first version of experimental recirculation – 32.4 g to 26.5 g. Statistically significant difference between control and experimental group of fish reached 22.2 % ($p < 0.05$).

Thus, to improve cultivation of carp yearlings and improving their fishery indicators in recirculation installations it is necessary to enrich their diet with natural foods throughout the growing period.

During experimental studies it was conducted assessing the impact of natural forage mixture on histological parameters of liver and intestine of carp yearlings grown in recirculation installations. In the first experiment optimum growing conditions were provided (the control version). The second (version II) experiment yearlings were fed using forage mixture: Gammarus – 30 %, Daphnia – 30 %, Common duckweed – 30 %, Tubifex – 10 %.

Introduction to the diet of carp yearlings the natural feed mixtures significantly affected the height of intestinal microvilli of experimental fish, the fish, to which diet the natural forage was added had a significant decrease in intestinal microvilli height. Intestinal villus height in the experimental group did not change compared to the control (Table 2).

Table 2. Results of morphological histological analysis of intestinal of carp yearlings.

Version of research	Microvilli height, μm	Width of microvilli (1/2) Height of microvilli, μm	The thickness of the muscle layer, μm
Version I (control)	6.821 ± 0.037	2.516 ± 0.018	0.015 ± 0.0022
Version II (research)	$3.757 \pm 0.042^*$	$1.701 \pm 0.023^*$	0.015 ± 0.0017

Note: * – the difference is statistically significant.

State of intestinal epithelium of carp can be characterized as satisfactory on histological sections abnormalities were not observed. It was determined that the height of intestinal villi of scaly carp in the control was average $6.821 \pm 0.037 \mu\text{m}$ and a height of microvilli was $2.516 \pm 0.018 \mu\text{m}$. The thickness of the muscle layer reached $0.015 \pm 0.0022 \mu\text{m}$ (Fig. 3).

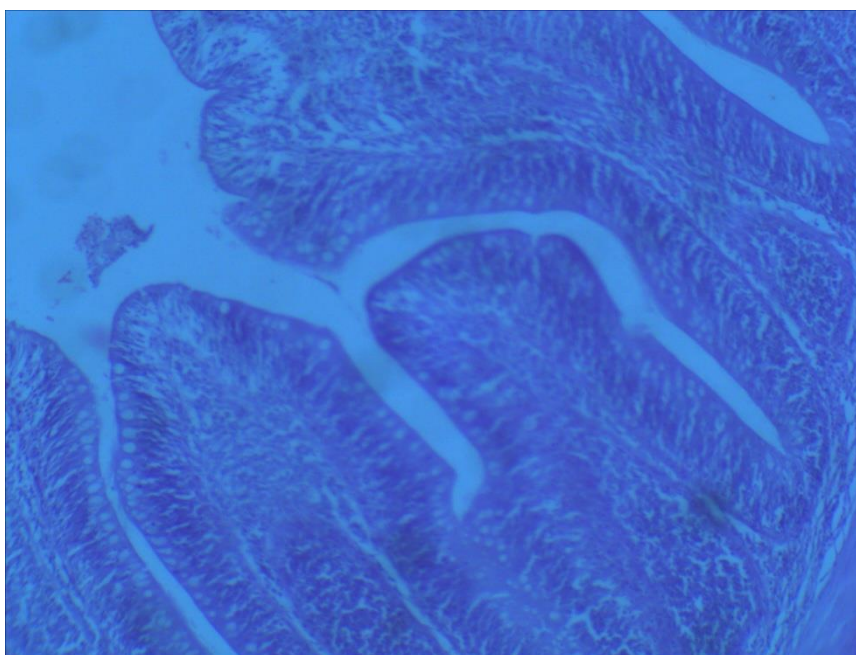


Figure 3. Intestinal epithelium of carp, control.

It was found that while feeding of carp yearlings on natural feed fish intestinal villi height was significantly decreased almost 2 times the height of microvilli significantly below 1.5 times. In terms of the thickness of the muscle membrane significant difference was observed. The height of the intestinal villi of carp in the second experiment was $3.757 \pm 0.042 \mu\text{m}$ and a height of microvilli was $1.701 \pm 0.023 \mu\text{m}$ (Fig. 4).

The number and length of the outgrowths of epithelial cells of the intestine of carp largely depend on the nutritional value of food and biological substances. Under the low nutritional value of feed the surface area of the intestine folds increases, providing more surface for absorption of nutrients. In conditions of enough nutrients surface area of the intestine is optimized and reaches physiological norm. Thus, it was determined that feeding carp fish on natural foods positively affects the digestive system of carp which is displayed on histological morphology of intestine. So, using of forage mixture of natural ingredients positively impact on fish breeding in recirculation installations.

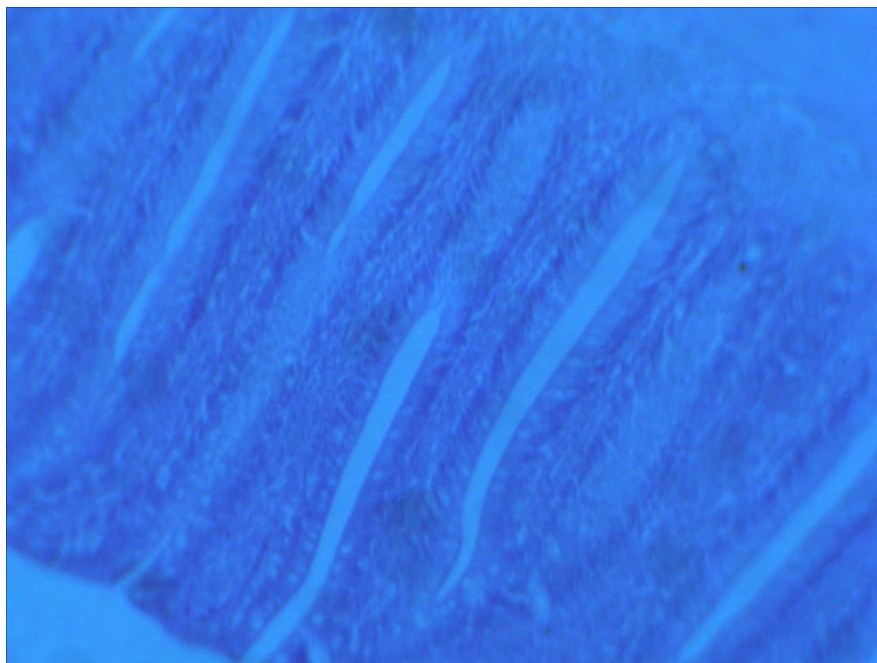


Figure 4. Intestinal epithelium of carp fed on mixture of natural feed.

Parenchyma of carp's hepatopancreas was stained a grayish-purple, blood vessels were dark purple, the nucleus of hepatocytes were dark blue. Around the central veins of the liver pancreas cells, called pancreatocytes were located that adhere to each other. The number of cells around the hepatic veins in the two study groups did not differ significantly and ranged from 7.45 to 7.92 pc. cells (Table 3).

Table 3. Results of morphological histological analysis of hepatopancreas

Version of research	The number of cells in the visual field, pcs./0.05 mm ²	The number of cells near blood vessels, pcs
Version I (control)	8.57±0.891	7.45±0.581
Version II (research)	10.20±0.326	7.92±0.014

The hepatopancreas of carp had lobed structure; hepatic lobes did not delimited between each other, because of the weak development of connective tissue and had no distinct boundary (Fig. 5). The structural morphofunctional unit of the liver is lobe, in the center of which is the central vein. The walls of central venous are built with endothelial cells of the inner layer, middle muscular layer, which in researched fish consisted of 2–3 layers of cells.

In all experimental samples the number of cells per 0.05 mm² (number of cells in the visual field) were counted. By the number of cells in the field researched samples did not differ statistically from the experimental samples. Averaged number of cells in sight ranged from 8.57±0.891 (version I) to 10.20±0.326 (version II).

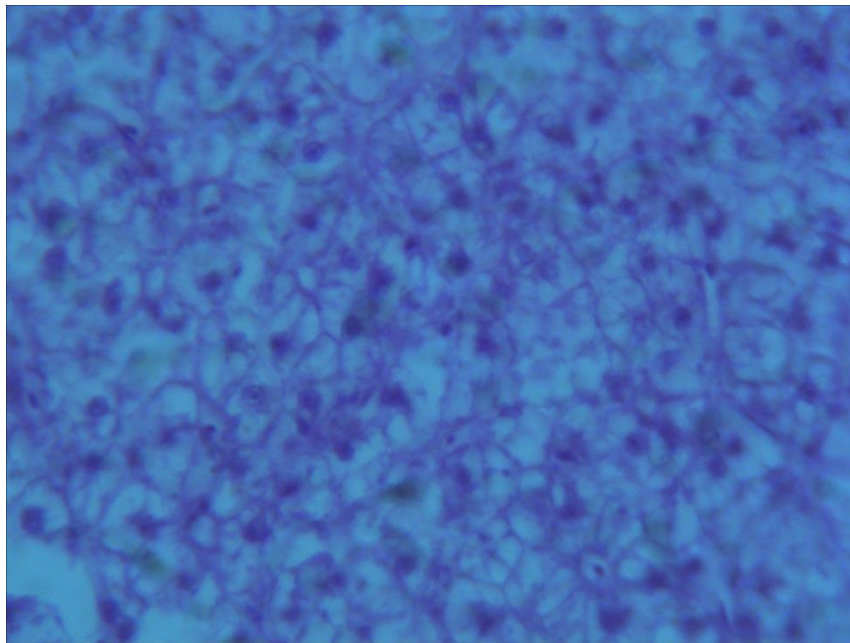


Figure 5. The histological picture of carp`s hepatopancreas, control.

All pancreatocytes of researched fish were stained more intensively than hepatocytes. Their cytoplasm around the nucleus was basophilic and homogeneous, and at the distance from the core, in the direction of duct it was acidophilus. It had a large amount of acidophilus grains. Nucleuses of pancreatocytes of carp were located near the basal membrane of the cell.

Hepatocytes of scaly carp had typical polygonal shape, containing one nucleus with clear nucleoli (Fig. 6). Each hepatocyte had two surfaces one was directed to the bile capillary, and another – to sinusoidal hemocapillary. In all researched tissues majority of hepatocytes had enlightened cytoplasm with acidophilus grit. The nuclei of hepatocytes were shifted to the periphery, as the bulk of cytoplasm was occupied by fat globules.

Hepatocyte nuclei were rounded and contained 1–2 nucleoli. In the center of nucleus there was condensed chromatin, from which darker strands of karioplasm diverged radially.

In control (version I) diameter of hepatocytes scaly carp`s yearlings reached an average of 1.517±0.057 μm (Table 4), but a significant difference in diameters of hepatocytes of experimental fish was not observed, but the area of liver cells varied significantly.

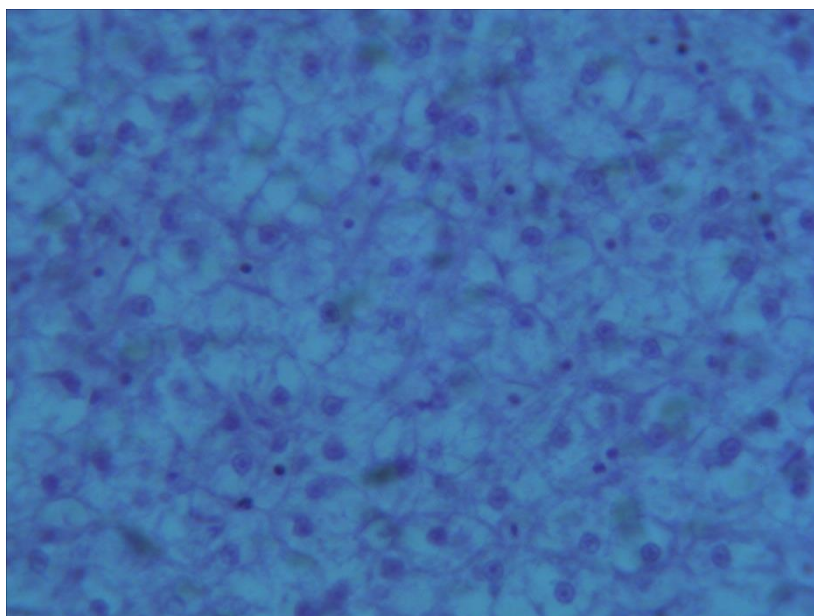


Figure 6. Hepatocytes of carp, experimental group.

Table 4. Morphohistological analysis of hepatocytes

Indicators	Version I	Version II
The area of nucleus, μm^2	1.841±0.001	2.631±0.002*
The area of cell, μm^2	20.268±0.018	20.454±0.017
Diameter of nucleus, μm	0.441±0.012	0.546±0.015*
Diameter of cell, μm	1.517±0.057	1.513±0.058

Note: * – the difference is statistically significant.

The average size of the nuclei of hepatocytes in control was $1.841 \pm 0.001 \mu\text{m}^2$. Wherein, figure coefficient of variation of this feature in control fishes amounted to 7.5 %. Nuclear-cytoplasmic ratio was 0.09 ± 0.005 , at the level of variability in the magnitude of 15 %.

In the experiment of feeding fish on a mixture of natural feed diameter of liver hepatocytes scaly carp's yearlings reached an average of $1.513 \pm 0.058 \mu\text{m}$, and the significant difference in diameters of hepatocytes of experimental fish were not observed. Area of liver cells while feeding on natural foods did not differ from area of hepatocyte cells of the control group.

The average size of nuclear of the fish hepatocyte from researched installation of closed water supply was $2,631 \pm 0,002 \mu\text{m}^2$ with diameter of $0.546 \pm 0.015 \mu\text{m}$, which is 1.5 and 1.2 times significantly higher than the control. Nuclear-cytoplasmic ratio was 0.120 ± 0.006 , which is 1.4 times higher than in control. That is the nucleus area and diameter in the experiment was higher by almost 1.5 times at almost the same cross-section hepatocyte areas.

3. 3. Recommendations for stocking of Dnieper reservoir by carp.

Estimated average production of benthos in the Dnieper Reservoir on is 630 kg/ha. Potential growth of ichtiological mass by zoobenthos is 88.3 kg/ha. Total losses of ichtiological mass from natural mortality is 17.7 kg/ha. Growth of carp`s ichtiological mass during the growing season, taking into account natural mortality and competition with other fish is 31.7 kg/ha. Possible industrial fishing of carp is 30 % of the increase – 9.8 kg/ha. The difference between potential and actual catch of carp is:

$$9.8 - 0.5 = 9.3 \text{ kg/ha}$$

From here it follows that the density of stocking of carp with average growth during the season is:

$$0.5 \text{ kg} - 9.3/0.5 = 18.8 \text{ ind./ha (Table 5).}$$

Table 5. Recommended amounts of stocking of Dnieper Reservoir in 2016

Species, age of fish	Sample, g	Amount, thousand ind.			Total weight, tons		
		Dnieper region	Zaporozhye region	In all	Dnieper region	Zaporozhye region	In all
Carp, 1 ⁺	100–130	390	160	550	39–50	16–20	55–70

Stocking should be implemented in different parts of the reservoir to reduce mortality and increase the commercial return. Thus, for the rational use of forage resources of Dnieper Reservoir is stocking of ponds in the amount of 550 thousand ind. is appropriate to make pasture breeding conditions in the reservoir. The similar number of stocking can be possibly received through the implementation of measures of artificial cultivation of carp fish fry using specialized farms. Introduction of cultivation of young carp is appropriate and promising in the conditions of the installations of close water supply with fertilizing by natural foods.

4. CONCLUSIONS

The features of the current state of natural fodder and fisheries exploitation in Dnieper Reservoir in modern environmental conditions were researched. The basic biological fish-breeding and biotechnology indicators for scaly carp yearlings grown in experimental installations of close water supply with use of forage mixture of natural food objects were developed.

1. The average biomass of soft zoobenthos in Dnieper Reservoir reached 9.8 g/m². This corresponds to reservoirs with high food supply. Production of useful benthos in

Dnieper reservoirs is 630 kg/ha, which corresponds to 45.2 kg/ha for carp fish potential productivity.

2. To increase the efficiency of growing of mirror carp's yearlings and improving its fishery features during growing under controlled conditions it is necessary to enrich the diet of fish with ingredients of natural origin. It was found that the enrichment of the diet of carp fish with natural feed components occurring in the experimental installations of close water supply the average weight of carp was higher than in fish from the control variant – 32.4 g to 26.5 g. Statistically significant difference between weight of control and experimental group of fish reached 22.2 % ($p < 0.05$).
3. The use of natural fodder for feeding of flake carp's yearlings in terms of installations of close water supply leads to a moderate increase in the size of cells and nuclei of fish liver, which positively affects the metabolism in the body of the fish. In particular, the increase in size of liver cells provides the accumulation of fat and glycogen in tissue that is necessary for wintering of yearlings, and can be used by fish during the winter of starvation. Also supply of nutrients that accumulate in the liver can be used by yearlings of carp fish while adapting to the conditions of the reservoir after stocking. Pathological changes in liver structure were not observed, which gives reason to recommend the use of these feed mixture as a feed component for feeding of fish in installations of close water supply.
4. Introduction of natural feed components into the diet of carp yearlings significantly affect the intestinal microvilli height of experimental fish, the fish, which diet contained kelp in the amount of 5 % had a significant reduction in intestinal microvilli height. It was found that the feeding of carp's yearlings with natural feed fish intestinal villi height was significantly decreased almost 2 times the height of microvilli significantly below 1.5 times. By the indicators of the thickness of the muscle membrane significant difference was not observed.
5. Hepatopancreas of carp had lobed structure, hepatic lobes were not delimited between them, because of the weak development of connective tissue between partial and had no distinct boundaries. By the number of cells in the field prototypes were not statistically different from the experimental samples. Averaged number of cells in sight ranged from 8.57 ± 0.891 (version I) to 10.20 ± 0.326 (version II).
6. Experiment with fertilizing of fish by natural feed liver hepatocytes diameter of carp yearlings reached an average of $1.513 \pm 0.058 \mu\text{m}$, and the significant difference in diameters of hepatocytes of experimental fish was not observed. Area of liver cells under conditions of feeding with natural foods did not differ from area of hepatocyte cells of the control group. The average size of the fish hepatocyte nuclear from researched installation of close water supply was $2.631 \pm 0.002 \mu\text{m}$ with diameter of $0.546 \pm 0.015 \mu\text{m}$, which is 1.5 and 1.2 times significantly higher than the control.
7. The use of natural fodder in feeding of carp yearlings in terms of its growing in installation of close water supply causes the increasing of the size of cells and nuclei of fish liver, which positively affects the metabolism in the body of the fish. In particular, the increase in size of liver cells provides the accumulation of fat and glycogen in tissue and it is necessary for wintering of yearlings, and can be used by fish during the winter of starvation. Also, a significant supply of nutrients that

accumulate in the liver can be used by carp yearlings during the adapting to the conditions of the reservoir after stocking. Pathological changes in liver structure were not observed, which gives reason to recommend designed forage mixture as a feed component for feeding of fish under controlled conditions (installation of close water supply, pools, etc.).

8. For implementation of pasture breeding in the conditions of Dnieper reservoir it will be appropriate to conduct stocking of ponds by carp fry in the amount of 550 thousand. ind.

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