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# ICHTHYOTROPHOLOGICAL AND ICHTHYOPATHOLOGICAL ANALYSIS OF BESTER (ACIPENCER NIKOLJUKINII)

The paper presents the results of ichthyotrophological and ichthyopathological studies of the bester-hybrid, which is of a significant importance among sturgeon fish. The purpose of the research work is to study the food spectrum of bester, bred in the pond and to study the external environment influence on the fish.

Sturgeon is a promising form of modern aquaculture. In addition, sturgeon fish currently undergo mass extinction and their population decline sharply every year, so the interest in their commercial breeding has increased. In 2001, four countries of the Caspian Sea – Kazakhstan, Turkmenistan, Azerbaijan, and Russia (except Iran) imposed a temporary ban on catching sturgeon. This ban was imposed by the UN requirement within the framework of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora). The problem was solved by the breeding of besters which are hybrid species produced from mating male sterlet and female huso. To carry out research bester hybrid fish was delivered from the Halyk Balyk pond farm in 2021. The absolute length of the studied 15 fish ranged from 12.7 to 30.1 cm, and their maximum weight ranged from 11.38 to 110.4 g. Ichthyological, ichthyotrophological and ichthyopathological analysis was carried out during the study. Standard numerical-weight and special histological methods were used for the study. Ichthyotrophological studies revealed that the fullness index was higher than 30.75%00. The studies showed a high level of nutrients in the reservoir and the absence of negative external environment influence on fish.

**Key words:** bester, ichthyotrophology, ichthyopathology, sturgeons, the Caspian Sea.

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## Бестер балығына (Acipencer Nikoljukinii) ихтиотрофологиялық және ихтиопатологиялық анализ

Мақалада бекіре тұқымдас балықтар арасында өте маңызды орын алатын гибрид- бестер балығының ихтиотрофологиялық және ихтиопатологиялық зерттеулерінің нәтижелері көрсетілген. Зерттеу жұмысының мақсаты – тоғанда өсірілген бестер балығының қоректену спектрін зерттеу және сыртқы ортаның балыққа тигізген әсерін зерттеу.

Бекіре тұқымдас балықтар қазіргі заманда аквамәдениеттің маңызы зор, бағалы өкілі болып есептеледі. Оған қоса, қазіргі уақытта бекіре тұқымдас балықтардың жаппай қырылуы және олардың санының жыл өткен сайын азаюы нәтижесінде, оларды тауарлық өсіруге деген қызығушылық өсуде. 2001 жылы Каспий теңізінің шекаралас төрт мемлекеті — Қазақстан, Түрікменстан, Әзербайжан және Ресей (Ираннан басқа) бекіре тұқымдас балықтарды аулауға уақытша тыйым салған болатын. Ол БҰҰ тарапынан CITES (қауіп төніп тұрған түрлермен халықаралық сауда жөніндегі конвенция) бойынша енгізілді. Бұл мәселенің шешімі бестерді өсіру болды, бестер аталық сүйрік пен аналық қортпаның шағылысуынан алынған гибрид түр болып саналады. Зерттеу жұмыстарына бестер балықтары 2021 жылы Halyk Balyk тоған шаруашылығынан жеткізілді. Зерттелген 15 дана балықтың абсолютты ұзындығы 12.7 см-ден

 

30.1 см арасында болса, үлкен салмағы 11.38 г-нан 110.4 г аралығындағы нәтиже көрсетті. Зерттеу барысында ихтиологиялық, ихтиотрофологиялық және ихтиопатологиялық анализ жүргізілді. Зерттеу жүргізуге стандартты сандық- салмақтық және арнайы гистологиялық әдістер қолданылды. Ихтиотрофологиялық зерттеулер бойынша толысу индексі 30,75%00 жоғары шамасын көрсетті. Зерттеу нәтижесінде суқойманың коректік қорының жоғары деңгейлігі және сыртқы ортадан балыққа теріс әсер болмағаны анықталды.

**Түйін сөздер:** бестер балығы, ихтиотрофология, бекіре тұқымдастар, ихтиопатология, Каспий теңізі.

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**Ихтиотрофологический и ихтиопатологический анализ бестера (Acipencer Nikoljukinii)**

В статье приведены результаты ихтиотрофологических и ихтиопатологических исследований бестера- гибрида, что имеет большое значение среди осетровых рыб. Целью научно-исследовательской работы является изучение спектра питания разводимой в пруду бестера и изучение влияния внешней среды на рыбу.

Осетровые – перспективная форма современной аквакультуры. Кроме того, в настоящее время осетровые рыбы подвержены массовому вымиранию и их численность с каждым годом резко сокращается, поэтому резко возрос интерес к их коммерческому разведению. В 2001 году четыре страны Каспийского моря – Казахстан, Туркменистан, Азербайджан, и Россия (кроме Ирана) ввели временный запрет на вылов осетровых. Он был введен под давлением ООН в рамках CITES (Конвенция о международной торговле видами, находящимися под угрозой исчезновения). Решением этой проблемы стало разведение бестеров, которые считаются гибридными видами, полученными от спаривания самцов стерляди и самок белуги. Абсолютная длина исследованных 15 рыб составляла от 12.7 до 30.1 см, а максимальная масса — от 11.38 до 110.4 г. В ходе исследования проведен ихтиологический, ихтиотрофологический и ихтиопатологический анализ. Для исследования использовали стандартные числово-весовые и специальные гистологические методы. В результате исследований установлено, что водоем отличается высоким уровнем питательных веществ и отсутствие негативного воздействия на рыб внешней среды.

**Ключевые слова:** бестер, ихтиопатология, ихтиотрофология, Каспийское море, осетровые.

## Introduction

Recently, biological diversity conservation in the Caspian Sea has become a big problem. Due to the endless fishing bioresources in the Caspian Sea, the number of valuable fish- sturgeon is sharply decreasing. According to the scientific data, over the past 10 years, the number of catching the sturgeon in the Ural-Caspian basin has decreased by 10 times. If in 1995-1996 the catching of the fish was 15-16 thousand tons, then in recent years this number has risen to 150-214 tons [1].

Sturgeons (Acipenseriformes) are relict fauna representatives, they appeared in the Lower Jurassic Period, 200 million years ago and survived various natural disasters of our planet. They are currently on the verge of extinction, some species have become extinct in several countries, where the measures to preserve these species were not taken [2,3]. The high marketable value of sturgeon is the main reason for their illegal catch that leads to a sharp decrease in their population [4,5].

The breeding of sturgeon started in the XIX century, when Russian scientists first began artificial breeding. Currently, the aquaculture of sturgeon is under industrial development which contributes to the preservation of endangered species. In the commercial breeding of sturgeon's interspecific hybrids are of a great significance. However, among interspecific hybrids bester plays the much more important role in the commercial breeding than any other species of this category of fish. There were many hybridizations between the Russian sturgeon, the sterlet, the beluga and the stellate sturgeon. The Huso Huso (beluga) was interbreed with the Acipencer ruthenus (sterlet), and the good result was not obtained due to the taxonomic distance and the huge differences between these species. However, as subsequent studies revealed, this hybrid gained an incredible successful and was widely used and, by the suggestion of the authors, it received the name "Bester" – consisting of the initial syllables of the parent species name: beluga and sterlet [6,7,8].

Bester has 5 rows of bony plates (scutes): 1 dorsal, 2 lateral and 2 ventrals. Lateral spines are 51-52. They have a flattened rostrum. There are two pairs of slightly flattened barbels. Bester has the characteristics of two fish. They are the beluga's rapid growth and the early maturation of the sterlet. It can reach up to 1.8 m long and weigh up to 30 kg. In aquaculture, due to the breeding in cages and pools the first-generation hybrids reached the mass of 1 kg or more in the second year [9,10].

Bester are trophologically carnivorous, they eat molluscs, small crustaceans, worms, and insects [11]. For ichthyotrophological research, the standard quantitative-weight method was employed. According to this method: the fish is cut from the abdominal section to the pharynx using scissors and a scalpel, the digestive system is removed, the digestive system is divided into 3 parts, the intestine is cut crosswise, and a nutrient node is made. After obtaining the nutrient node, the content is dried, weighed and systematically identified using a microscope.

Ichthyopathological studies were carried out using histological methods. The parts taken from the gills, muscles, liver gland and intestines of the fish are treated according to a histological method and paraffin incisions are made through a Microtome. The ichthyopathological changes in fish are observed using a microscope.

The relevance of the study is supported by the fact that sturgeon family representatives have been on the verge the extinction in recent years and many species were included in the Red Book. To preserve sturgeon, various hybrids were produced, including highly adaptable, high-quality bester. Bester matures and grows rapidly. So, this fish was chosen as the main object of our research because of its high commercial value.

The aim of the study is to determine the nutritional spectrum of the bester bred in the pond and to identify the pond conditions by bester histological studies in the presence of ichthyopathological changes.

## Materials and methods

In the fall of 2021, for the fish nutrition and histological studies the samples were taken from Halyk Balyk pond farm during the fall estimation period. 15 selected samples of the fish were placed in 10% formalin and were delivered to the laboratoty of Biodiversity and Bioresources of the Department of the Biology and Biotechnology faculty in Al-Farabi KazNU (Figure 1).

**Figure 1** − 15 bester samples taken for research

Ichthyological, ichthyotrophological and ichthyopathological studies of 15 bester fish taken for study were carried out. Ichthyological studies were carried out according to the Pravdin method. Ichthyological studies included the determination of large and small length, large and small weight of 15 bester samples, and their fatness according to Fulton and Clark formula. A caliper was employed to measure the research object length, and a MW-Micro Digital Computing Scale (Korea) was used to measure the weight. The following formulas were applied to calculate the fatness of samples [12,13]:

$$Fu = Q*100/ l^3$$
$$Cl = q*100/ l^3$$

Ichthyotrophological studies were carried out according to the standard quantitative – weight method and processing was carried out by the quantitative method. The ichthyotrophological study consists of steps. They are the isolation of the intestine, the extraction of the nutrient node, the drying, measurement, and the identification of nutrient organisms. During the study the fish was cut from the abdominal part to the pharynx with scissors and a scalpel, the digestive system was removed. The intestine was divided into 3 parts: the front, middle and back, respectively. A nutrient node was taken from the intestinal sections and was dried. The EP613C (Switzerland) torzion scale was employed to measure the nutritional node. Binocular magnifying glass (MBS-9 stereomicroscope (SCOPICA, Russian)) and microscope (MicroOptix light microscope (MicroOptx, Inc., Austria)) were used to identify organisms from the nutrient node. The relative values of nutrient organisms in the nutrition spectrum were estimated by the repetition frequency and the proportion of the individual components in the total composition of nutrient particles (in % of mass). During the studies fullness index of intestine (%) was calculated [14,15, 16,17].

According to ichthyopathological studies, a histological investigation was carried out to determine the physiological state of the fish. To carry out this investigation organs and special histological methods were employed. The taken organs parts were prepared for dehydration by placing the cut in cassettes. The dehydration process was carried out in the battery of butyl and ethyl alcohol of different concentration. It started at 70° of alcohol and was brought to 98°. The materials obtained from each solution were dried on filter paper, the exposure time to alcohol of different concentration was 35 minutes. After the immersion of the material into butanol-II solution, it was placed in melted paraffin. Then the material was put in the thermostat, at 56 °C, for a day and the tissue absorbed paraffin. When the tissue absorbed paraffin paraffin block was made. The material was solidified in paraffin, the microtome device ("MEP – 01 TECHNOM") was employed and paraffin sections 5 micrometers thick were prepared. The preparation was passed through xylene solutions and alcohol concentrations. It was dried and placed in hemotoxylin and eosin paints [18,19, 20]. The coloured samples were examined using an optical microscope (Motic BA-400 microscope (Motic Asia, Hong Kong, China)). Statistical data processing was carried out using standard methods, and statistical indicators were processed in Microsoft Excel.

## Results and discussion

Standard length and large and small weight were measured using ichthyological analysis. The total length value of fish samples was calculated to be from 12 cm to 30.1 cm, the average length was 19.1 cm, and the weight value ranged from 13.87 g to 119.4 g, the average weight was 43.6 g (Table 1).

**Table 1** – Length and weight values of the studied bester samples

| № | Q, g | q, g | L, cm | l, cm |
|---|---|---|---|---|
| 1 | 13.87 | 7.85 | 12.7 | 9.1 |
| 2 | 20.09 | 11.22 | 14.7 | 11 |
| 3 | 11.38 | 5.44 | 12 | 9.3 |
| 4 | 12.13 | 9.81 | 13.1 | 10.1 |
| 5 | 18.12 | 12.85 | 17.5 | 14.3 |
| 6 | 15.03 | 13.97 | 13.8 | 10.5 |
| 7 | 21.35 | 17.58 | 18.5 | 14.3 |
| 8 | 16.93 | 13.11 | 15.6 | 11.5 |
| 9 | 40.82 | 20.41 | 18.6 | 14 |
| 10 | 26.79 | 21.67 | 20.8 | 16.4 |
| 11 | 68.53 | 55.77 | 24 | 19.1 |
| 12 | 71.42 | 41.6 | 24.2 | 22.5 |
| 13 | 85.53 | 70.86 | 26.3 | 21 |
| 14 | 113.07 | 106.13 | 25 | 25.5 |
| 15 | 119.4 | 114.32 | 30.1 | 9.5 |

The table above demostrates the values of the total (L), standard (l) length and large (Q), small (q) weight of the studied 15 bester samples. The table shows that the correspondence of the length and the weight of the fish samples is a good indicator.

According to the Fulton and Clark formula the fish fatness was calculated, the interval of Fulton values was from 0.3 to 0.68, and the Clark interval was from 0.24 to 0.68 (Table 2).

According to Fulton and Clark results, the bester fish fatness was at a relatively good level, the samples with a low indicator have a small amount of nutritional node or empty intestine.

During ichthyotrophological studies, the fullness index of 15 bester samples was calculated. The overall fullness index was 461.3%00 and the average one was 30.75%00 (Figure 2).

**Table 2** − Fatness results of the studied fish samples by Fulton and Clark

| № | Fulton | Clark |
|---|---|---|
| 1 | 0.68 | 0.38 |
| 2 | 0.63 | 0.35 |
| 3 | 0.66 | 0.31 |
| 4 | 0.54 | 0.44 |
| 5 | 0.34 | 0.24 |
| 6 | 0.57 | 0.53 |
| 7 | 0.34 | 0.28 |
| 8 | 0.45 | 0.35 |
| 9 | 0.63 | 0.32 |
| 10 | 0.3 | 0.24 |
| 11 | 0.5 | 0.4 |
| 12 | 0.5 | 0.29 |
| 13 | 0.47 | 0.39 |
| 14 | 0.72 | 0.68 |
| 15 | 0.44 | 0.42 |
| Medium | 0.52 | 0.37 |

According to the fullness index, a very low indicator of samples № 3, 9, 10, 11 can be observed in the chart. These were the fish that had a small amount of nutrient node the came out of their intestines and a large number of fully digested food.

The study of the nutrition features of the obtained bester samples allowed determination of the pond nutritional base in the farm. The division of the intestine into 3 parts (front, middle and back) (Figure 3) was made using the methods employed in these studies.

The figures above illustrate the bester sample organs and the intestinal sections obtained by ichthyotrophological study and the nutrient node from each intestinal section.

According to the results of ichthyotrophological studies, mainly shrimp and undetectable digested food were found in the digestive tract of the studied samples (Figure 4-5).

Figures 4 and 5 present the main components which were found in the nutrient nodes of the bester fish samples, i.e., the main food of the bester was shrimps.

The repetition frequency of the fragments and the number of the fragments were determined using Microsoft Excel (Table 4) (figure 6-7).

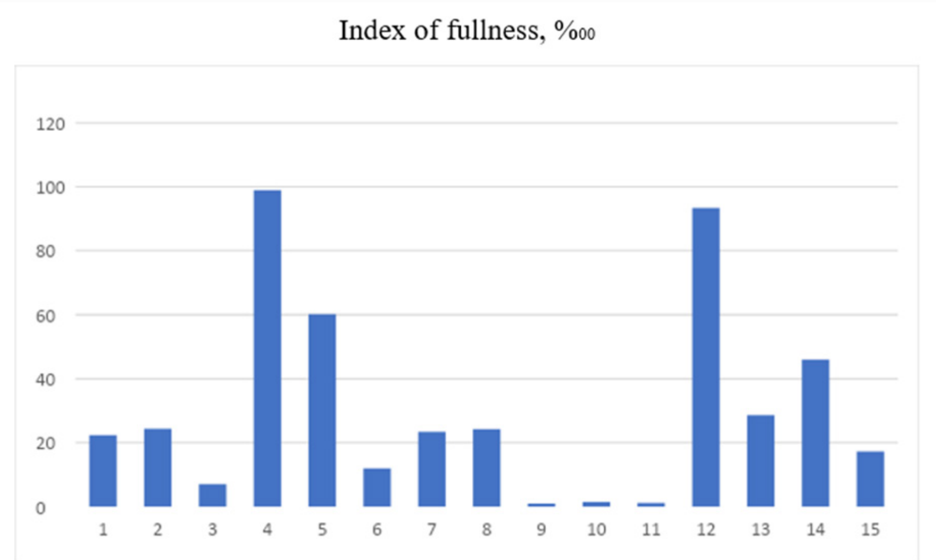



**Figure 2** − Fullness index of the studied bester

A B

**Figure 3** − Internal organs of sample, B-the digestive tract composition

A B

**Figure 4** − A, B- shrimp pieces from the intestines of bester

A

B

**Figure 5** – A, B – digested, undetectable food from the intestine of samples

**Table 3** – The repetition frequency and the number of components in the digestive tract of the bester

| Components | The number of components,% | The repetition frequency, % |
|---|---|---|
| shrimp | 87.8 | 70 |
| gammarus | 7.31 | 10 |
| mollusca | 2.43 | 10 |
| plants' seeds | 2.43 | 10 |

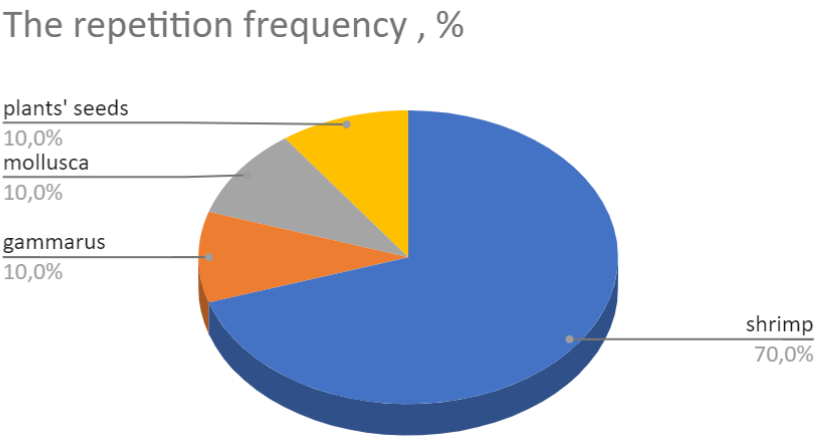



**Figure 6** – The repetition frequency of components in the digestive tract of the bester

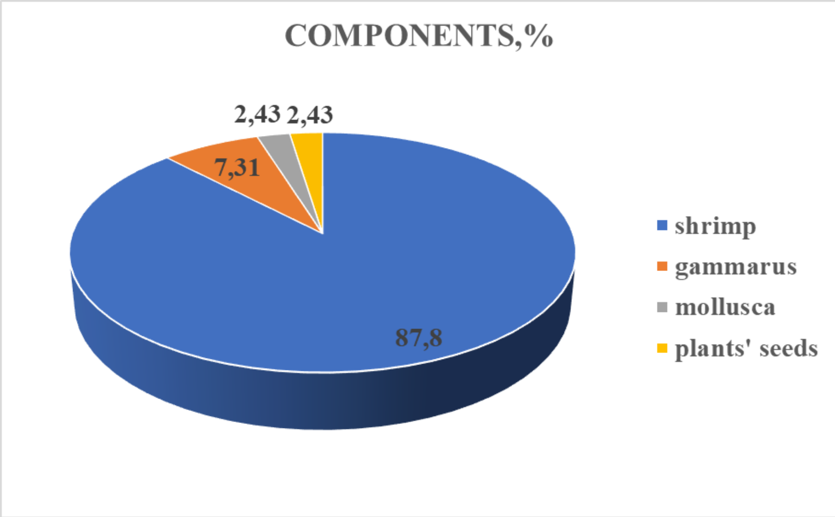



**Figure 7** – The number of the components in bester digestive tract

The diagrams above show the results of ichthyotrophological studies of 15 studied bester fish samples. The diagrams indicate that the main food of bester fish bred in the pond of the fish farm is shrimp. Gammarus and mollusc were identified as additional components, while plant grains were random components.

The structure of the fish gills studied by histological methods wasn't damaged, in general, the primary and secondary lamellas retain their normal structure. Bester gill's structure obtained in the study showed that, as in all fish, cartilaginous filaments are in the lamellas center of the 1st order and the 2nd order lamellas are horizontally attached to it (Figure 8).

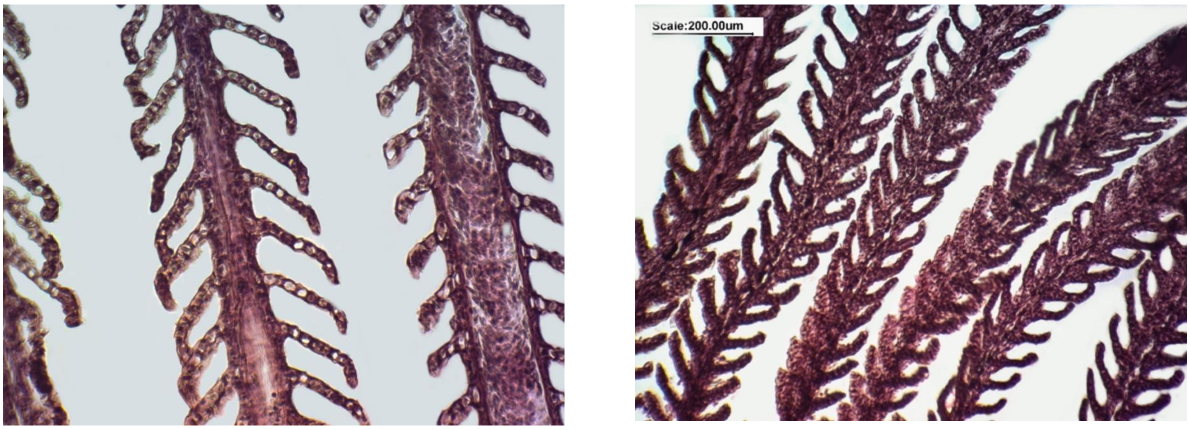
Some fish were observed to have hyperplasia of the primary and secondary lamellas (Figure 9).

According to a histological study, the skeletal muscle structure was shown to be normal. No pathological changes were found in histological samples of fish muscles. The muscles horizontal lines are well visible, as well as the sarcolemma is intact, the nuclei are located on the periphery (Figure 10).

When histological liver tests of fish samples were conducted, no pathologies were observed. Blood capillaries and stem cells looked good. Hepotocyte cells are intact. The liver capillaries are sufficiently filled with red blood cells. Hepatocyte cells and sinusoids looked good. The sinusoids can be observed to be filled with red blood cells (Figure 11).

Including the gills, liver, and muscles, we the digestive tract histological preparation of the studied bester were seen. Figure 11 shows clearly that intestines were full. The fragments of arthropods were found (Figure 12).

The front section of histological preparation of the digestive system of bester sample was examined. No dangerous pathologies were found here either. The walls are straight and intact. The figure demonstrates the normal state (Figure 12).




A B

**Figure 8** – The images of histological section of bester gills coloured by hematoxylin- eosin
A – 20x magnified, B – 10x magnified

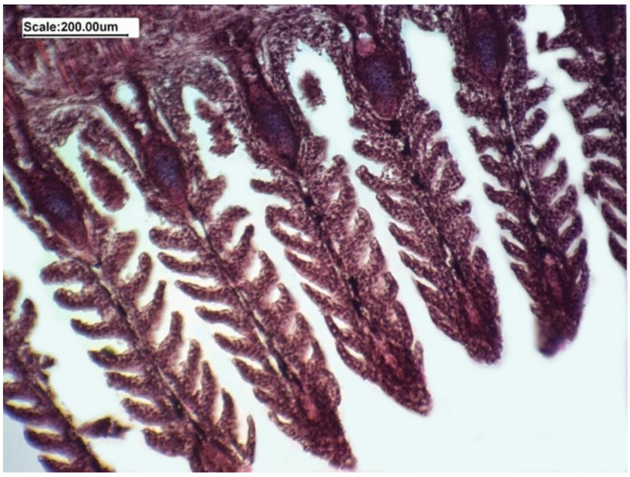
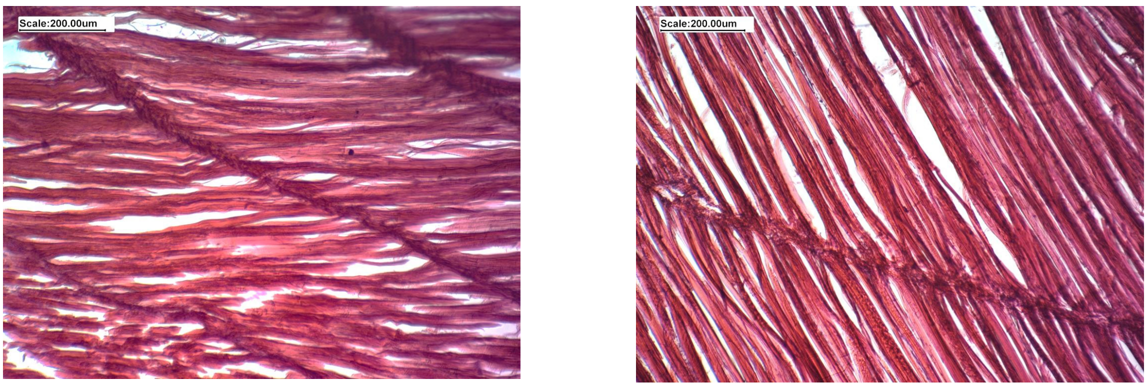
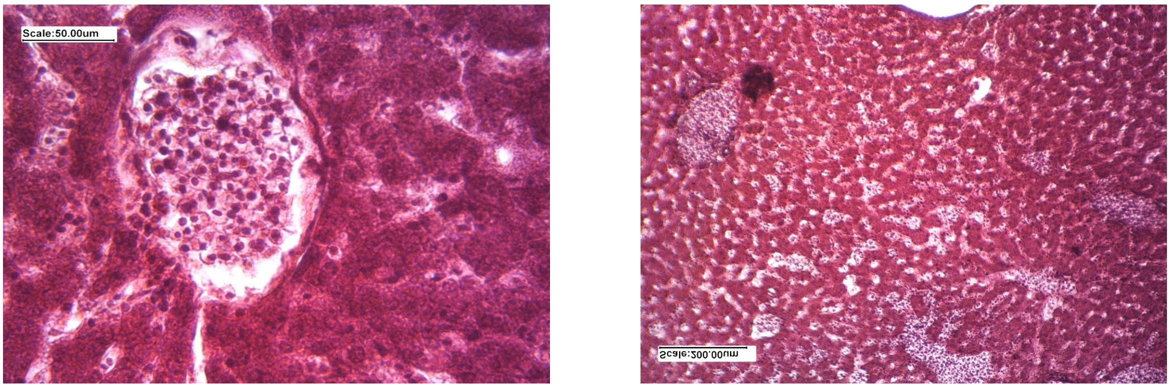



**Figure 9** – The images of histological section of bester gills coloured
by hematoxylin – eosin, 10x magnified




A B

**Figure 10** – The images of histological section of bester muscles coloured
by Hematoxylin- eosin A- 20x magnified, B- 10x magnified




A B

**Figure 11** – The Images of histological section of bester liver coloured by hematoxylin- eosin
A- 40x magnified, B- 10x magnified


**Figure 12** – The images of histological section of bester intestine coloured by hematoxylin- eosin, 20x magnified

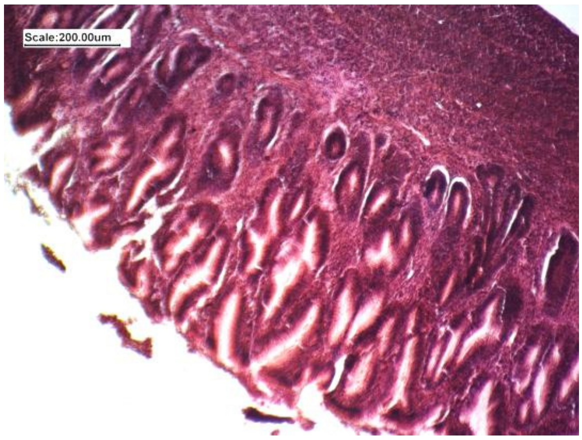



**Figure 13** – The images of histological section of bester intestine coloured by hematoxylin- eosin, 10x magnified

Ichthyopathological studies did not reveal any pathology in bester samples bred in pond conditions. Protozoa infestation and swellings have been observed in the fish gills. The structure of the liver, intestines, muscles wasn't damaged and was in a normal condition.

## Conclusion

The results of an ichthyotrophological study of bester (Acipencer nikoljukinii) bred in the fish farm "Halyk balyk" were obtained. The results showed that:

The quantitative analysis of nutrition showed that the repetition frequency was: 100% shrimp, 10% gammarus, 20% plant grains, 10% molluscs.

According to the qualitative analysis of nutrition: shrimp was 87.80%, Gammarus was 7.31%, plant grains were 2.43%, mollusks were 2.43%.

The results of bester histological studies showed the following:

The changes found in the gills were normal in most of them, hyperplasia was observed to occur because of the protozoa invasion in the gill lammellas and swelling of the secondary gill lamellas.

The structure of the liver gland was intact, and it was in a normal state, hepotocyte cells were in a columnar form.

The structure of the muscles was in a normal state and did not undergo changes.

The ichthyotrophological studies showed the sufficiency of the food supplies for bester samples bred in pond conditions and the good conditions of the pond nutritional base. According to ichthyopathological studies, no abnormalities were identified in the fish, and the conditions for fish breeding in the pond were discovered to be good.

Currently, the bester, which is of a major importance, is a valuable object for ichthyological research. The study of bester, the study of fish bred in the pond conditions promotes the preservation and breeding of sturgeon fish.

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