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The risk-based control of the safety and quality of freshwater fish for sale in the agri-food market

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ABSTRACT

Scientifically substantiated and experimentally proven the feasibility of conducting proper risk-based control of the safety and quality of freshwater fish in the Kyiv region's agro-food markets following the regulatory document's requirements, developed by express, improved methods for determining freshness and microstructural examination of muscle tissue. At organoleptic assessmentUkrainian scaly carp, crucian carp, and pike perch were fresh, and rotan was of dubious freshness. Regarding pH value, Nesler number, and qualitative reaction to the content of ammonia and ammonia salts with 'Nesler's reagent, the meat of Ukrainian scaly carp, crucian carp, and pike perch corresponded to fish of a fresh degree. For rotan meat, its dubious freshness was established. In the photometric determination of the studied fish's freshness, the optical density of the supernatant correlated with the quality indicators for the content of ammonia and ammonia salts. An improved benzidine test with a filtrate from the gills of the mouth confirms the doubtfulness of the freshness of the fish. The studied fish samples corresponded to the standard indicators according to microscopic indicators and the number of mesophilic aerobic and facultative anaerobic microorganisms. By determining the chemical parameters of the studied fish, it was found that the mass fraction of water in meat was the highest in rotan (78.30 ±0.13%) and was accompanied by the smallest mass fraction of dry matter (21.70 ±0.09), the proportion of proteins (16.96 ±0.06), indicators of fat in meat (3.01 ±0.06%) and formed the lowest indicator of its relative biological value – 92.5%. In benign fish with organoleptic indicators, a microstructural study of muscle tissue revealed significant changes in its structure with atrophy of individual muscle fibers and growth in these areas of connective, mainly fatty tissue.

Keywords: fish, carp, pike perch, rotan, risk-based control, muscle, atrophy, agri-food market

INTRODUCTION

In Ukraine, fish and fish products are included in the list of strategically important food products. According to the World Food Organization (FAO), fish products are the third largest food producers in the world.

Aquaculture plays an important role in ensuring countries' food and nutrition security. Thus, the fish farming industry is an important component of the economy not only in Ukraine but also in the countries of Central and Eastern Europe. At the same time, the production of aquatic biological resources in Ukraine over the past decade has decreased to 90 thousand tons, which has led to an increase in the import of fish and fish products **[5]**, **[24]**, **[55]**.

Fish and fish products are necessary for complete human nutrition, as they are a source of complete proteins, vitamins, macro-, microelements, and other nutrients **[10]**, **[31]**, **[38]**, **[46]**, **[74]**. The development and globalization of import-export relations between countries against the background of technological progress in the field of production, intensification, and commercialization of the fishing industry, processing and redistribution of food from fish, increase the level of consumer requirements for their safety and quality **[2]**, **[19]**, **[20]**, **[84]**, **[85]**. Therefore, the priority task for veterinary medicine specialists is to strengthen the proper risk-based control of the safety and quality of fish and fish products put into circulation **[1]**, **[13]**, **[15]**, **[22]**, **[23]**, **[26]**, **[32]**, **[49]**, **[52]**, **[53]**, **[85]**.

Satisfactory microbiological indicators must confirm the safety of fish products, and the residual amounts of salts of heavy metals (cadmium, zinc, copper, arsenic, mercury; decarboxylation products of amino acids – histamine and nitrosamines; pesticides, radionuclides) must not exceed the maximum allowable levels (MAL) **[7]**, **[28]**, **[34]**, **[40]**, **[45]**, **[50]**, **[63]**, **[69]**, **[82]**, **[83]**. A wide range of indicators to confirm the good quality of fish products is explained by the fact that fish is often the cause of serious food poisoning, sometimes fatal **[11]**, **[29]**, **[54]**. According to the literature data, freshwater fish caught from water bodies contaminated with untreated sewage and organic matter can be affected by pathogenic and conditionally pathogenic microflora. As a rule, signs of the disease in such fish are absent. Still, the presence of microorganisms leads to its rapid deterioration with the accumulation of histamine during storage and sale **[8]**, **[25]**, **[54]**, **[85]**. It is known that fish can be a carrier of pathogens of Asian human cholera, swine fever, erysipelas, tuberculosis, and Escherichia coli, very dangerous for humans, toxic infections, and toxicosis caused by *Clostridium botulinum, Clostridium perfringens*, bacteria of the genus *Salmonella, E. coli*, *Proteus,* leptospira, various coca microflora, etc. **[6]**, **[15]**, **[26]**, **[30]**. Harmful infections can occur when a product contains more than 106 cells of live toxigenic bacteria per 1 cm^3. Fish toxic infections include diseases caused by bacteria of the *Escherichia coli* group*, Salmonella*, *Bacillus cereus, and Clostridium perfringens*, typical representatives of the Proteus genus **[8]**.

The occurrence of food intoxication is associated with consuming fish products with enterotoxins secreted by certain types of microorganisms (coagulase-positive staphylococci, *Clostridium botulinum*). At the same time, they themselves may be absent, for example, after heat treatment. Recently, there have been more frequent reports of toxic food infections caused by opportunistic microflora, constantly found in water bodies and the body of fish. This is due to many circumstances, in particular, a violation of ecological relationships within bacterial associations, a change in the existing balance between the normal microflora in the human body, a decrease in the level of natural immunity, and the widespread use of antibiotics, to which many opportunistic bacteria are resistant. The possibility of occurrence of food-toxic infections caused by spores of bacteria of the genera *Citrobacter, Klebsiella, Pseudomonas, Aeromonas, Hafnia, Vibrioparahaemolyticus* has been proved **[6]**, **[12]**, **[21]**, **[70]**, **[78]**.

Therefore, when conducting a study of fish products, it is necessary to thoroughly and reliably confirm their compliance with the requirements established in regulatory documents to ensure their safety for life, health, property of citizens, and the environment **[52]**, **[53]**, **[54]**. Today, this is especially relevant due to the loss of validity from 01/01/2018 of the Resolution of the Cabinet of Ministers of Ukraine dated 05/10/2018, 1993, No. 46-93 "On standardization and certification" and "List of products subject to mandatory certification in Ukraine", approved by order of the State Consumer Standard of Ukraine dated 01.02. 2005, No. 28, registered with the Ministry of Justice of Ukraine on May 04, 2005, 2005, No. 466/10746. This means that as of May 17, 2021, food products are not subject to mandatory certification in the State Certification System of Ukraine.

Thus, the nature of the dangers associated with fish consumption is global and falls within the scope of professional interests not only of veterinary and sanitary experts but also of human medicine doctors and all those associated with the production, processing, and sale of freshwater fish. Therefore, the purpose of the research is to assess the compliance of the studied freshwater fish (Ukrainian scaly carp, crucian carp, pike perch, rotan) from different producers in the Kyiv region, in terms of safety and quality, with the requirements of the State Standard of Ukraine (SSTU) 2284:2010 and approbation of modern express methods to identify the degree of its freshness.

Scientific Hypothesis

Will risk-based fish safety and quality control be ensured by improved express methods in the conditions of the agro-industrial market?

MATERIAL AND METHODOLOGY

The work was carried out from 2020 to 2021 at the Research Laboratory of Veterinary and Sanitary Expertise of Livestock Products, the Laboratory of the Department of Veterinary and Sanitary Expertise of the Institute for Postgraduate Education of Managers and Specialists of Veterinary Medicine, a research laboratory for complex ichthyopathological studies, laboratory of the Department of Safety and Quality of Food Products, Raw Materials and Technological Processes of the Bila Tserkva National Agrarian University.

Samples

The material for the study was freshwater fish, which was supplied for sale in the agro-food markets (department No. 1-3) in the Bila Tserkva, Kyiv region. The fish of the following species were studied: scaly carp, crucian carp, pike perch, and rotan. Freshwater fish was fresh. The selection of medium samples of freshwater fish has been carried out following the requirements of SSTU 7972:2015 "Fish and fish products. Acceptance rules, sampling methods" **[36]**.

Chemicals

Nesler's reagent - pure for analysis, manufacturer "Ural Plant of Chemical Products", Russia; hydrogen peroxide, benzidine hydrochloric acid - pure for analysis, manufacturer "Inter-Synthesis, Ukraine; potassium iodide, iodine crystalline, crystal purple, ammonium oxalate, sour fuchsin, hematoxylin, eosin – pure for analysis, Farmakom, Ukraine; immersion oil, glycerol, xylene, neutral formalin (10%), concentrated hydrochloric acid (p = 1.19 g/cm^3), sodium hydroxide, sodium hypochlorite, sodium sulfate, ammonia sulfate, ethyl alcohol with a mass concentration of 96%, "Farmak", Ukraine; Essential oil Clove (Oleum Caryophylli) 100%, natural, PC "Zolotonosha PCF", Ukraine; PCA media (Plate Count Agar); XLD (Xylose-Lysine Deoxycholate Agar; PALCAM agar; Endo, Beard-Parker agar medium, HiMedia, India

Animals, Plants and Biological Materials

Carp *(Cyprinus carpio),* Crucian carp *(Carassius gibelio),* Pike perch *(Sander lucioperca),* Rotan *(Perccottus glenii),* gills and muscle tissue.

Instruments

Potentiometer pH-meter NI 8314; photoelectric photometer series - Washer IW−8, FRIMED, Romania; binocular microscope MICROmed, KRÜSS, INVESTLAB, Germany; binocular microscope Euromex BioBlue S/N – EC 1800836 with Euromex Microscope Camera CMEX-5 PRO USB 3.0, Holland; sledge microtome MS-2 Primed, Russia; laboratory weights FEN-300-S, Helpix, Ukraine; water bath VB-4, Soxhlet apparatus, homogenizer for undermining shots, Laboratorna Tekhnika, Ukraine; drying oven SNOL-24/200 Thermo, "Thermoengineering", Ukraine.

Laboratory Methods

Organoleptic assessment of freshwater fish: general condition, appearance, colour, smell, taste - according to DSTU 2284:2010 "Live fish. General technical requirements" and GOST 7631-85 "Fish, marine mammals, invertebrates and products of their processing. Acceptance rules, organoleptic quality assessment methods, sampling methods for laboratory research" **[46]**.

Determining freshwater fish's physical and chemical characteristics (degree of freshness). The pH value was set according to DSTU ISO 2917-2001 **[60]**, the ammonia and salts content, and the Nesler number - GOST 7636-85. Fish freshness was determined using the photometric method **[18]** and an improved benzidine test **[17]**, and the water-retaining capacity of fish meat was determined using an improved method **[16]**.

Determination of microscopic and microbiological characteristics. Microscopy of smears-prints from the surface layers of fish muscle tissue was carried out according to DSTU 4895:2007 **[37]**; quantity of MAFAnM – DSTU ISO 4833:2006 **[66]**; count of coagulase-positive staphylococci (*Staphylococcus aureus*) – DSTU ISO 6888-1:2003 **[65]**, bacteria of the genus *Salmonella* – DSTU ISO 6579:2006 **[67]**, *Listeria monocytogenes* – DSTU ISO 11290-1:2003 **[64]**.

In the meat of the studied fish, mass particles of water and dry matter were determined according to DSTU ISO 1442:2005 **[61]**, protein – DSTU ISO 937:2005 **[59]**, fat – DSTU ISO 1443:2005 **[62]**, ash – GOST 26226–1995 **[35]**, energy value – following the guidelines of the State Research Institute of Laboratory Diagnostics and Veterinary Sanitary Expertise, relative biological value – using the test object of the ciliate Tetrahymena pyriformis **[68]**.

Histological studies (tissue sampling, fixation, wiring, placement in a compacting medium, making histological sections, their staining, and making histological preparations) of freshwater fish muscle tissue were carried out using modern histological methods **[48]**.

Description of the Experiment

Sample preparation: The fish of each species, in the amount of 30 individuals, was anesthetized, cleaned of scales, the skin in the back was cut off, muscle tissue was cut out with scissors in the amount of 15.0 ±0.5 g, then a combined sample of fish meat was obtained, and a point sample was isolated from it in the amount of 200 ±0.5 g for testing; for histological studies, pieces of muscle tissue were taken together with skin 1x1 cm in size in the area of the dorsal lateral muscle and intercostal oblique muscles.

Number of samples analyzed: 30 fish samples of each species were analyzed.

Number of repeated analyses: 30.

Number of experiment replication: 3.

Design of the experiment: The experiment planned to determine the quality and safety indicators of fish: organoleptic; physicochemical methods for determining freshness by generally acceptable methods and developed express ones; microscopic and microbiological; quality (water content, dry matter, protein, fat, energy value, relative biological value); morphological.

Statistical Analysis

The results obtained were calculated by the methods of variation statistics using an ASUS personal computer using MS Excel software packages, STATISTICA 7.0 (Statsoft) software. We determined the arithmetic mean (M) and the statistical error of the arithmetic mean (m), the probability of the difference between the arithmetic means of two variational series according to the probability criterion (p) and Student's tables. The difference between the values was considered probable p <0.05; 0.01 and 0.001.

RESULTS AND DISCUSSION

When organoleptic evaluation (general condition, appearance, colour, smell, taste of meat and broth) of freshwater fish (Ukrainian scaly carp, crucian carp, pike perch, rotan) from different producers of the Kyiv region, it was found that it met the requirements of SSTU 2284:2010. In the studied fish, the scales are shiny, with a mother-of-pearl tint, it is difficult to pull out, and the mucus is transparent. The fish of the studied species is characterized by the natural colouration inherent in the Ukrainian scaly carp, crucian carp, pike perch, and rotan. The skin is elastic, and the fins are solid. Gill covers tightly close to the gill cavity. The eyes are convex, and the cornea is transparent and dirty grey. The muscle tissue is tight and tightly attached to the bones. In the cross-section, it has a characteristic colour for fish of each species. The abdomen is not deflated, and the anus is not protruded. When cooking, the smell and taste are specific for each type of fish, without putrefactive or other foreign odours and flavours. The broth is transparent, with drops of fat on the surface of the appropriate size, depending on the age and type of fish. The smell is pleasant, specific, and fishy, the muscle tissue is well divided into muscle bundles, and the taste of the broth and fish is pleasant, without bitterness and mustiness. According to organoleptic assessment, Ukrainian scaly carp, crucian carp, and pike perch corresponded to fresh fish. The results obtained coincide with the technical approaches of the authors Magas et al., according to the assessment of the main features of the organoleptic indicators of freshwater fish **[56]**. The authors point to a possible difference in the quality of carp meat in terms of organoleptic indicators, which depends on feeding, type of nutrition and environmental factors **[56]**. The rotan broth is somewhat cloudy, with a sour smell, which testified to the dubious degree of freshness of the fish of this species. Improved methods are being developed to determine the freshness of freshwater fish meat, Xiao and Zheng proposed a system for identifying freshwater fish meat of different freshness, based on multi-sensor synthesis using the BP artificial neural network method, providing a high degree of recognition **[86]**. Such studies often include the analysis of PH values, electrical conductivity, and odour, which are determined, combined and analyzed by fuzzy theory in comparison with reference samples **[87]**.

In implementing risk-based control of food safety and quality, effective studies should be applied to determine their suitability for consumption **[9], [72]**. Therefore, we have developed express methods for determining the degree of freshness of fish using a photometric method, an improved benzidine test, and determining the water-holding capacity of meat. These methods provide high reliability of research to control the safety and quality of fish sold in the Kyiv region's agro-food markets.

The method for determining the degree of freshness of fish was carried out by the photometric method using 2.0-2.2 g of a crushed sample of fish meat and infusion of the meat extract for 12-15 minutes. At the next stage, 1.0-1.2 cm^3 of Nesler's reagent was added to the filtered meat-and-breed extract (4.0-4.2 cm^3), kept in a tripod for 4-5 min, then centrifuged for 1-2 min at 2000 rpm, followed by measurement of the optical density of the colour intensity of the supernatant on a photoelectric photometer (in a cuvette absorbing light thickness of 1 cm at a wavelength of 455-460 nm).

When determining the water-retaining capacity of fish meat, fish meat samples were used in the amount of 100.0-150.0 g, ground in an electric meat grinder, and thoroughly mixed, preventing the loss of meat juice, ground minced meat was taken in the amount of 0.3-0.4 g, was placed on a polyethylene circle, then transferred to a circle of filter paper placed on a glass plate so that the minced meat sample was covered with a glass plate. A press weighing 1.0 kg was placed on it and kept for 9-10 min, after which a sample of fish meat was freed from filter paper and polyethylene disks, placed in pre-calibrated weighing bottles, weighed and dried in an oven at a temperature of 105-106 °C in within 4-5 minutes. The formula calculated the water-holding capacity of fish meat as a percentage.

When setting up an improved benzidine test, 2.0-2.2 cm^3 of a filtered extract from fish gills was used (the ratio of gills and distilled water was 1:5). The extract was infused for 12-14 minutes, 0.4-0.5 cm^3 of an alcohol

solution of benzidine with a mass fraction of 0.3% and 0.20-0.25 cm^3 of a hydrogen peroxide solution with a mass fraction of 2% were added. The colour intensity of the filtrate from the gills was determined: if it is of intense blue-green colour, and after 2-3 min it becomes dark brown, the fish is fresh; if it slowly turns into a light bluish-green colour, and after 3-4 minutes it becomes dark brown – the fish is of dubious freshness; if it remains unchanged, but after 5-6 minutes it acquires a dark brown colour – the fish is stale.
The results of the study of the physicochemical parameters of freshwater fish from various producers of the Kyiv region are presented in Table 1.

Table 1 Physical and chemical indicators of the meat of the studied fish.

Indicator	**Type of fish**			
	Carp *(Cyprinus carpio)*	**Crucian carp** *(Carassius gibelio)*	**Pike perch** *(Sander lucioperca)*	**Rotan** *(Perccottus glenii)*
pH value	6.65 ±0.02	6.59 ±0.02	6.88 ±0.03	7.15 ±0.03*
Nesler number	0.80 ±0.01	0.98 ±0.01	1.02 ±0.01*	1.25 ±0.01*
Qualitative reaction to the content of ammonia and ammonia salts	Colour			
	olive	olive	olive	intense yellow
The optical density of the colour intensity of the supernatant extract from fish meat, Bel	0.195 ±0.024	0.218 ±0.014	0.289 ±0.019*	0.684 ±0.032*
The water-holding capacity of fish meat, %	69.35 ±0.26	72.61 ±0.34	70.53 ±0.21	62.13 ±0.17*
Benzidine test (qualitative reaction for peroxidase)	colour			
	intense blue-green	intense blue-green	intense blue-green	light-blue-green

Note: *M*±*m*, *n*=30.* p<0.05 to carp indicators.

Analyzing the data in Table 1, according to the studied indicators, carp, crucian carp, and pike perch corresponded to fish of a fresh degree. Thus, the pH value of the meat-water extract of this fish was within the established standards – 6.65 ±0.02 units, 6.59 ±0.02, and 6.88 ±0.03 units, respectively; in the meat of rotan – 7.15 ±0.03 units (p <0.05), which indicated a violation of the shelf life of this fish. According to the Nesler number, carp, crucian carp, and pike perch corresponded to fresh-grade fish according to the requirements of regulatory documents (up to 1.0). However, in rotan, this indicator was 1.25 (p <0.05), which did not correspond to fresh-grade fish. Indicators of ammonia and salts' content correlated with optical indicators of the colour intensity of the supernatant using Nesler's reagent (in Bel). When conducting a study on a photoelectric photometer, the reliability of tests was 99.8%.

The water-holding capacity of rotan was the lowest – 62.13 ±0.17% (p <0.05), in the studied fish of other species – in the range from 69.35 ±0.26 to 72.61 ±0.34%, the reliability of the test results was 99.4%.
When the improved benzidine test was performed, the filtrate of the gills of carp, crucian carp, and pike perch was of intense blue-green colour, turning dark brown in 2-3 minutes (fresh fish); the filtrate from the gills of rotan turned slowly into a light bluish-green colour and after 3-4 min acquired a dark brown colour (fish of dubious freshness). The reliability of the test results was 99.5%.

Microscopic and microbiological characteristics of the studied freshwater fish

Microscopic examination of the superficial muscles of the fish (under the skin, in the region of the spine), in 10 fields of view of the 1st smear-imprint, stained according to Hram in the modification of Khuker, the number of microorganisms was counted, and the average value was obtained arithmetically.
The results of microscopic and microbiological studies are presented in Table 2.

Table 2 The results of microscopic and microbiological parameters of the studied fish of different species.

Indicator	Type of fish			
	Carp *(Cyprinus carpio)*	**Crucian carp** *(Carassius gibelio)*	**Pike perch** *(Sander lucioperca)*	**Rotan** *(Perccottus glenii)*
The number of microbial cells in 1 field of view of the microscope	6.0 ±2.0	7.0 ±2.0	8.0 ±2.0	14.0 ±2.0*
Quantity of MAFAnM, CFU/cm^3	(1.34 ±0.21) x 10^2	(1.74 ±0.25) x 10^2	(1.12 ±0.09) x 10^2	(2.84 ±0.16) x 10^3*
Bacteria of the *Escherichia coli* group (coliforms), in 0.001 g	not detected	not detected	not detected	not detected
Coagulase-positive staphylococci, in 0.01 g	not detected	not detected	not detected	not detected
Pathogenic microorganisms, including bacteria of the genus *Salmonella* and *Listeria monocytogenes*, in 25 g	not detected	not detected	not detected	not detected

Note: M ±m, n = 30. The standard amount of MAFAnM in fish meat is 5x10^4 CFU/cm^3; *p <0.05 to carp indicators.

The results of microscopic examination of smears-prints from the surface layers of the muscles of the studied carp, crucian carp, and pike perch corresponded to fresh fish - the number of microbial cells in the 1st field of view of the microscope was from 6 ±2.0 to 8 ±2.0. Relative to rotan, this indicator was 14.0 ±2.00 (p <0.05) microbial cells, which confirmed the conclusion about a possible violation of the shelf life of this fish, so rotan meat must be subjected to bacteriological examination to resolve the issue of its food use.

The amount of MAFAnM in the meat of carp, crucian carp, and pike perch was within the normative indicators, according to SSTU 2284:2010 – (1.34 ±0.21) x 10^2, (1.74 ±0.25) x 10^2, (1.12 ±0.09) x 10^2 CFU/g, respectively. However, in rotan meat, this indicator was somewhat higher and amounted to (2.84 ±0.16) x 10^3 CFU/cm^3 (p <0.05)

In the studied freshwater fish (carp, crucian carp, pike perch, rotan), no bacteria of the Escherichia coli group (coliform), coagulase-positive staphylococci, pathogenic microorganisms (*Salmonella, Listeria monocytogenes*) were found. Similar results were obtained by Onishchenko, who noted that the quality of fresh fish carcasses obtained in terms of bacteriological parameters, the number of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAM), contamination with bacteria of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* depends on the condition of the fish on sale. According to the results of the studies, it was found that the meat of fresh fish sold on the agro-food market, according to bacterial indicators, is of high quality and does not pose a risk to the consumer. From the muscle tissue of such fish, mesophilic aerobic and facultative anaerobic microorganisms were isolated in 6.6% of the studied carcasses. Also, bacteria of the group *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* were not detected. From fish meat of dubious freshness, the QMAFAM indicator was above the norm in 20.6% of samples, bacteria of the *Escherichia coli* group – 10.3% of *Salmonella* – in 6.6%, *Staphylococcus aureus* was not isolated **[73]**.

According to Khan, studies using polymerase chain reaction have established the prevalence of hemolytic *L. monocytogenes* in isolates from fish meat in 4.0% of the studied samples **[51]**. The presence of microorganisms in fish isolates that are openly sold on the market is also indicated by Marijani, who found the presence of six types of bacteria: *E. coli* – 40.0%, *Klebsiella* spp. – 26.0, *Salmonella* spp. – 24.0, *Shigella* spp. – 6.7, *Citrobacter* spp. – 6.5 and *Pseudomonas* spp. – 2% **[57]**.

Also, no live helminths and their larvae, dangerous to humans, were found in the studied fish.

The chemical composition of fish meat is not constant and can change depending on various factors. The chemical composition, energy, and relative biological value of the meat of the studied freshwater fish were determined. The results of the study are presented in Table 3.

Table 3 Chemical indicators and biological value of fish of different species.

Indicator	Type of fish			
	Carp *(Cyprinus carpio)*	**Crucian carp** *(Carassius gibelio)*	**Pike perch** *(Sander lucioperca)*	**Rotan** *(Perccottus glenii)*
Mass fraction of water, %	74.50 ±0.12	71.34 ±0.09	72.21 ±0.08	78.30 ±0.13*
Mass fraction of dry matter, %	25.50 ±0.08	28.64 ±0.07	27.79 ±0.06	21.70 ±0.09*
Mass fraction of protein, %	19.15 ±0.06	20.63 ±0.06	21.33 ±0.06	16.96 ±0.06*
Mass fraction of fat, %	4.84 ±0.06	5.99 ±0.06	4.31 ±0.06	3.01 ±0.06*
Mass fraction of ash, %	1.24 ±0.08	2.02 ±0.08	2.15 ±0.08	1.73 ±0.08*
Energy value, kJ	527.25 ±2.08	546.09 ±3.11	540.12 ±2.45	465.02 ±2.64*
Relative biological value, %	99.8	99.5	98.9	92.5*

Note: M ±m, n = 30. *p <0.05 to carp indicators.

Table 3 data indicate that the highest indicator of the mass fraction of water is in the meat of rotan 78.30 ±0.13% (p <0.05), the lowest is crucian carp (71.34 ±0.09%), in the meat of Ukrainian carp scales – 74.50 ±0.12%. Accordingly, the mass fraction of dry matter in rotan is 21.70 ±0.09% (p <0.05), crucian carp and pike perch – 28.64 ±0.07 and 27.79 ±0.06%, Ukrainian scaly carp – 25.50 ±0.08%. The obtained values of indicators of the mass fraction of water coincide with the results of studies by Martsenyuk indicating that the muscle tissue in three-year-old small-scaled carp contains 77.54% water and in two-year-olds – 75.1%.At the same time, the author notes that two-year-olds' muscle tissue is characterized by a higher fat content and confirms an interdependence between moisture content and fat **[58]**. Golovko obtained similar results of the water content in muscle tissue the mass fraction of which averages 75% **[47]**. According to Blazhekovikj-Dimovska, the meat of common carp from open water contains 76.03% water **[14]**.

When determining the mass fraction of proteins, it was found that their lowest indicator was in the meat of rotan (16.96 ±0.06%; p <0.05), and the highest was in pike perch (21.33 ±0.06%), in carp meat, it was 19.15 ±0.06%. Other researchers have obtained similar values. So, according to Golovko, protein content in spring-caught carp meat ranges from 16% to 18.8% **[47]**. Skibniewska et al. point to similar values of the indicator, the level of which depends on the technology of carp breeding and can range from 16.9 to 18.6% **[79]**. The mass fraction of fat in rotan was the smallest among the studied fish and amounted to 3.01 ±0.06%. In crucian carp, it was the highest (5.99 ±0.06%). Accordingly, the energy value of crucian meat is 546.09 ±3.11 kJ, rotan – 465.02 ±2.64 kJ (p <0.05). Blazhekovikj-Dimovska obtained similar values of the mass fraction of protein. The results of the studies show that the meat of common carp from open water contains 2.92% fat and 1.06% ash **[14]**. At the same time, Martsenyuk points to excellent values of the indicator, according to which the fat content in meat is 6.39 ±0.69 for two-year-olds and 3.09 ±0.57 for three-year-old carp (p >0.99) **[58]**. Authors Golovko et al. noted that carp meat's mass fraction ranges from 3.1% to 8%, classifying it as a medium-fat fish **[47]**.

An important indicator of the quality of freshwater fish is the relative biological value, which depends on the level of assimilation of meat proteins. Olifirenko et al. also point out the need for constant quality control of the resulting commercial fish and determining the biological value, which will provide a solution to the problems of modern fish farming: selection of fish species, stocking density, development of recommendations on technology and bionorms of cultivation fish, determining the timing of fishing, transportation and storage of fresh marketable pond fish **[71]**. The highest relative biological value of fish meat was in the Ukrainian scaly carp, which amounted to 99.8%, and the lowest in rotan (92.5%; p <0.05).

Thus, the research results indicate that the chemical composition of fish meat depends on the species and feed ration.

The energy value of fish meat directly depends on the chemical composition, especially the content of fats and proteins. In particular, the indicator of the mass fraction of proteins in the meat of pike perch and carp is 21.33 ±0.06 and 19.15 ±0.06%, respectively. The relative biological value of carp was 99.8%, and that of pike perch was 98.9%. This indicates that fish's energy and relative biological value depends on its species, food supply, and protein content. According to Martsenyuk, the calorie content of carp meat is 130.26 ±6.11 and 103.29 ±5.58 kcal/100 g for two and three-year-olds, respectively **[58]**.

Microstructural analysis

Fish meat is represented by muscle and connective tissues. The skeletal muscles of fish are formed by striated muscle tissue, the structural and functional unit of which is the muscle fiber. Muscle fibers are combined into myomers, separated by myosepta **[39]**, **[76]**, **[77]**. The muscle fibre composition includes sarcoplasm, myofibrils, and numerous nuclei surrounded by sarcolemma. For myofibrils, individual sections with different structures and optical properties, a transverse banding is characteristic, forming the actual banding of the fibers **[33]**. Actin myofilaments form light stripes (isotropic disks) with single characteristic refraction, and dark (anisotropic disks) are formed by myosin and, partially, actin myofilaments – with birefringence.

The muscle fibers of the skeletal muscles of fish differ in colour, shape, structure, and functional activity and, as a result, are divided into red and white. Red muscle fibers are located in the muscles superficially above the white ones. The taste of fish and its nutritional and biological value depends on the degree of adipose tissue development. Adipose tissue is formed from loose connective tissue containing fat cells – adipocytes. In the body of fish of different types, the localization of adipose tissue is somewhat different (under the skin, near the fins, in the tail, liver, in most fish – in the thickness of the muscles).

Fish oil largely determines adults' resistance to the effects of persistent fat-soluble toxins and can accumulate harmful substances **[81]**. Such hydrobionts pose a danger to humans when consumed.

The most valuable part of the fish is its body (from the head to the beginning of the anal fin), its main muscles: the longissimus dorsi, superficial and deep lateral muscles, and intercostals, in particular, the internal oblique muscle. Therefore, samples of these muscles were taken for microstructural analysis.

For histological examination, 30 samples of striated muscle tissue 0.5-1 cm^3 in size were taken from the superficial and deep lateral muscles, the internal oblique (intercostal) muscle of freshly caught freshwater fish. Muscle tissue was fixed with a neutral formaldehyde solution with a mass concentration of 10% at room temperature for 24 hours. After fixation, the material was washed with running water, dehydrated in alcohols of increasing concentration, and poured into celloidin. Sections 5-10 μm thick were made on a microtome and stained with hematoxylin and eosin by the Van Hizon method (picrofuchsin dye, which contains picric acid, has a differential property), according to modern histological methods **[48]**.

Microscopic examination of celloidin histological preparations of fish muscles was performed using a binocular microscope with a built-in video camera.

According to the results of their histological examination, it was revealed that the muscle tissue of the lateral muscles of the scaly carp (*Cyprinus carpio*) on fixed longitudinal sections of the superficial and deep muscles is characterized by a parallel arrangement of muscle fibers. Muscle fibers had a thin sarcolemma and well-defined transverse striation. There are no adipocytes in the sarcoplasm, but a small number were found in the perimysium. On transverse sections, both superficial and deep lateral muscle fibres had a rounded, oval, triangular shape with rounded edges, tightly adjacent. Staining of fixed histological preparations from muscle tissue with Weigert's hematoxylin and picrofuchsin occurred evenly. Microscopy at a magnification of 10 x 40 revealed the existing granularity in the structure of muscle fibers, due to the placement of myofibrils over the entire area of the fiber (Figure 1a). On transverse sections of muscle tissue in the perimysium of muscle bundles of the II order, single adipocytes were observed.

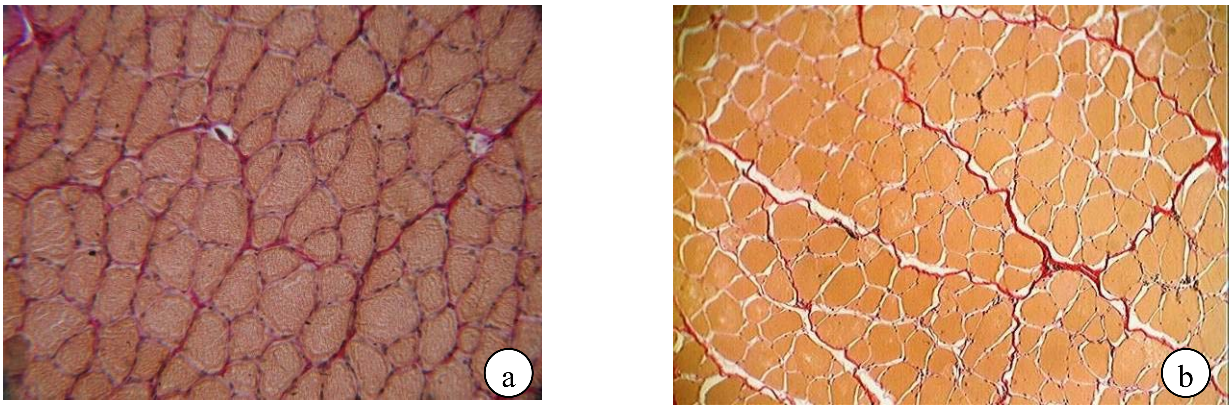

Figure 1 The structure of muscle tissue is normal. Note: a – superficial lateral muscle; b – internal oblique muscle. Cross-section, Van Hizon, Magnification: 400×.

According to the results of a microstructural study of the internal oblique muscle (intercostal), it was found that the areas bordering the deep lateral muscle had the usual structure of muscle tissue. Muscle fibers were round and polygonal in shape, placed close to each other, had a uniform colour, and the nuclei were localized on their periphery. The oblique muscle fibres formed well-defined bundles (Figure 1b).

Consequently, no changes in their structure were noted during the study of some muscle samples. That is, it was characteristic of the fish of this species **[80]**.

However, histological examination of the muscle tissue of individual samples revealed some changes. So, in scaly carp, classified as a quality fish according to organoleptic parameters, significant structural changes were noted in the internal oblique muscle (intercostal). On longitudinal sections, curvature, thinning of muscle fibers, compaction, and stratification were noted. In some areas, the thickening of muscle fibers and their ruptures were observed. Such fibers had no transverse striation. In addition, some fibers were lysed. In such areas, large voids were noted due to the growth of adipose tissue (Figure 2), lymphocytes, fibroblasts, collagen fibers, and a large amount of intercellular fluid.

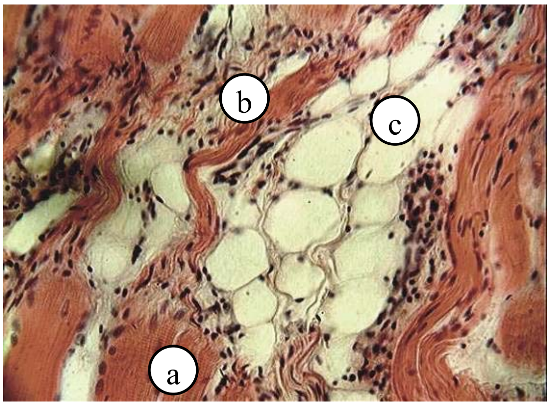

Figure 2 Replacement of the internal oblique muscle fibres with connective tissue. Note: a – muscle fibers; b – atrophied muscle fibers; c – connective tissue (longitudinal section, hematoxylin-eosin; Magnification: 400×).

On transverse sections of muscle tissue in muscle fibers, a loose placement of myofibrils was observed, and in some areas – voids (Figure 3a), endomysium, and perimysium – thickened. Muscle fibers had a heterogeneous colour: inside the fiber – light and dark on its periphery.

Many destroyed fibres were noted in the bundles of muscle fibers (Figure 3b), inside - with dystrophic and destructive changes, and fibers with the rest of myofibrils next to the sarcolemma (Figure 3c).

The perimysium between the fiber bundles is massive and thickened, with dense collagen strands and many adipose tissue cells. Scientists have also noted changes in muscle fibers' structure in other fish species, particularly zander. In studies of striated muscles by the authors Grushko found an increase in the distance between muscle fibers and fixed fibers in which the transverse banding barely appeared. At the same time, the deformation and weakening of muscle fibers were established. The reasons for deviations from the norm in histological preparations were: weakening of muscle fibers – 50%, necrosis and violation of the structure of muscle fibers – 10% **[43]**. Thus, with qualitative organoleptic indicators of fish, significant structural changes can occur in its muscle tissue.

In particular, dystrophic and destructive changes lead to the atrophy of individual muscle fibers. There is a scattered placement of thinned, atrophied fibers among fibers with a relatively preserved structure or hypertrophied ones. The areas of muscle tissue that have undergone lysis are filled with connective tissue, mainly adipose tissue. Therefore, it can be assumed that changes in the structure of fish muscle tissue are due to the influence of unfavourable growing conditions, in particularly stressful situations **[4]**, the presence of harmful toxic substances, in which there are violations of the regulation of metabolic processes **[75]**, which leads to a decrease in biosynthetic processes and increased fat formation **[81]**, violation of the hydrochemical regime **[41]**. The study by Grushko et al. also confirms, pointing to the establishment of a wide range of pathologies identified during histological studies of the internal organs of fish, which is probably associated with persistent environmental pollution due to human economic activity **[44]**.

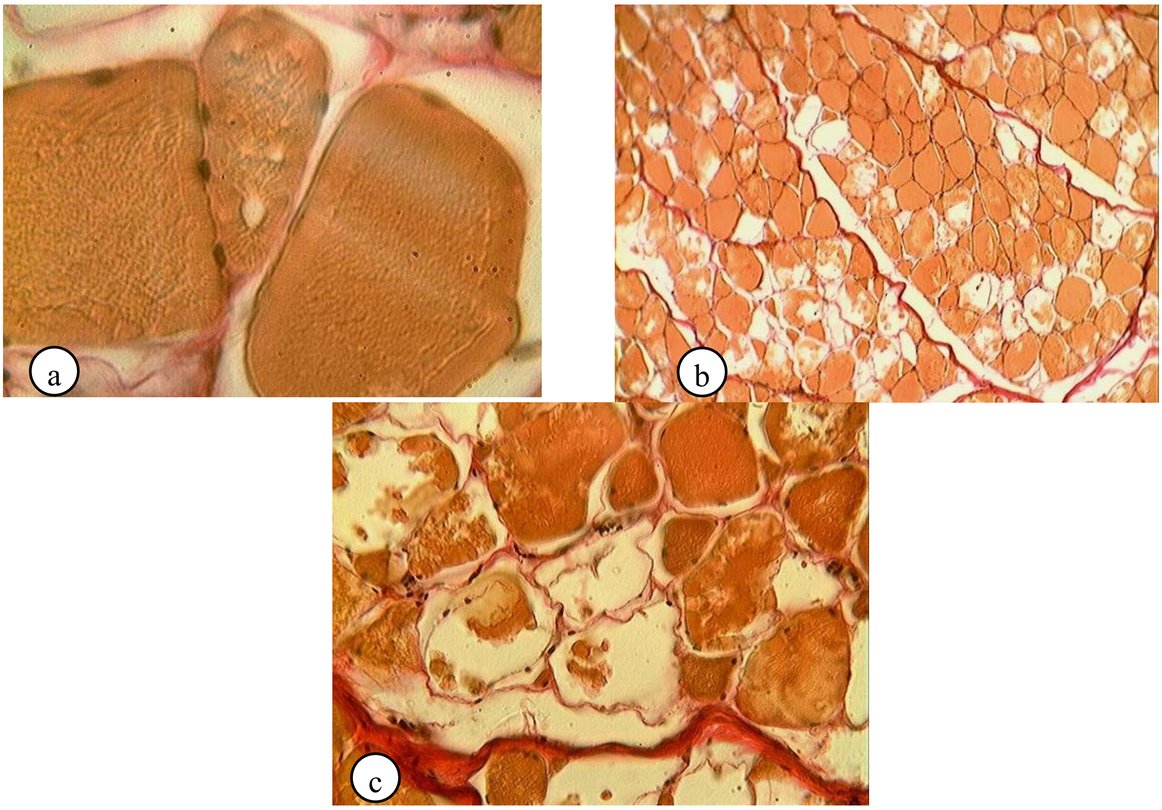

Figure 3 Structural changes in the intercostal muscles of scaly carp. Note: a – voids between myofibrils (cross-section, Van Hizon; Magnification: 1000×); b, c – atrophied muscle fibers (cross-section, Van Hizon; Magnification: 100× and 400×).

The possibility of the influence of the ecological situation, especially chronic intoxication of aquatic organisms with organochlorine, oil and other pollutants, can cause serious physiological changes in various body systems. In this regard, it is essential to assess the current state of fish according to biological criteria, the most important of which is histological **[42]**. Damage to the organs and tissues of fish can be observed without external signs of intoxication; in such cases, pathomorphological changes are the only indicator of the harmful effects of toxic substances **[3]**. The histological method of research allows, at the cellular and tissue level, to find out the depth of the pathological process in each fish and assess the damage to the entire herd in the reservoir **[27]**.

CONCLUSION

According to organoleptic (general condition, appearance, colour, smell, taste of fish and broth) and physicochemical parameters (pH value, Nesler number, qualitative reaction to ammonia and ammonium salts, optical density, the water-holding capacity of fish meat, improved benzidine test), scaly carp, crucian carp, pike perch corresponded to the indicators of fresh fish, and rotan – dubious. Ukrainian scaly carp, crucian carp, and pike perch met the requirements of regulatory documents (DSTU 2284:2010) in terms of microscopic indicators and the number of MAFAnM; in rotan, the indicators of the number of microbial cells in the field of view of the microscope and the number of mesophilic aerobic and facultative anaerobic microorganisms were slightly higher. When determining the chemical parameters and biological value of fish of various types, it was found that the largest mass fraction of water was found in the meat of rotan (78.30 ±0.13%), the smallest in crucian carp (71.34 ±0.09%), and the average values had the meat of the Ukrainian scaly carp (74.50 ±0.12%). In rotan, the mass fraction of dry matter was 21.70 ±0.09%, crucian carp, pike perch – 28.64 ±0.07 and 27.79 ±0.06%, respectively, carp – 25.50 ±0.08%. The mass fraction of proteins is the smallest in rotan meat (16.96 ±0.06%), the largest – pike perch (21.33 ±0.06%), and in carp meat, this figure was 19.15 ±0.06%. The lowest fat content was in rotan meat (3.01 ±0.06%), and crucian carp (5.99 ±0.06%) was the highest. The energy value of crucian meat was the highest and amounted to 546.09 ±3.11 kJ, in rotan – the lowest (465.02 ±2.64 kJ). The relative biological value of Ukrainian scaly carp meat was 99.8% (the highest indicator), rotan – was 92.5% (the lowest indicator). In the muscle tissue of fish, recognized by organoleptic indicators as benign, histological examination of its structure revealed significant changes with atrophy of individual muscle fibers and growth, mainly of adipose tissue. In risk-based control of the safety and quality of freshwater fish, state veterinary inspectors were asked to use the developed express methods for determining the degree of its freshness (photometric determination of optical density, determination of the water-holding capacity of fish meat, staging an improved benzidine test), reliability 99.8%, 99.4 and 99.5% respectively.

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