Veterinary-sanitary evaluation of fish when applying the Akwa-Biot-Norm nutrient feed additive

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Abstract. The paper presents the data of veterinary-sanitary assessment of the Lena sturgeon grown using the nutrient feed additive Akwa-Biot-Norm. It has been revealed that organoleptic characteristics of fish samples correspond to the benign products. The chemical composition of the muscle tissue of the experimental and control groups of fish was almost the same. The cadmium level in both fish groups was 0.007 mg/kg; the mercury level was 0.00019 mg/kg and 0.00018 mg/kg in the control and in the experimental group respectively, which does not exceed the maximum permissible concentrations. Microbiological studies of Lena sturgeon meat have shown that the number of mesophilic aerobic and facultative anaerobic microorganisms (NMAFAnM) in fish samples does not exceed the safety requirements and nutritional value of food products. Coliform bacteria (BGKP), Staphylococcus, Salmonella, and Listeria monocytogenes were not isolated from all selected samples (control and experimental). The results of the veterinary-sanitary assessment of the Lena sturgeon grown with the use of the nutrient feed additive Akwa-Biot-Norm indicate that the fish meets the requirements of the technical regulations of the Eurasian Economic Union "On the safety of fish and fish products" (EEU TR 040/2016) and is safe for the consumer.

1. Introduction

Fish and products of its processing are a source of many nutrients necessary for the person and, first of all, full-fledged proteins, fats, carbohydrates, mineral elements, and vitamins. Lena sturgeon is characterized as oily fish. Its fat is easily digestible, has a beneficial effect on lowering cholesterol levels in the blood. Regular consumption of its meat contributes to the prevention of heart and vascular diseases [1, 2].

In recent years, the fishery of a valuable food fish has declined significantly. This was primarily concerned sturgeons. The water engineering, poaching and other negative anthropogenic factors have led to the catastrophic decline in population of these valuable species of fish [3].

In modern terms, a reliable source of increasing the volume of food fish production is aquaculture. In a connection with the increase in the rate of production of valuable fishery products, accelerating growth of fish has assumed great significance. With this purpose various growth factors, containing complexes of amino acids, vitamins and microelements are already used in the practice of domestic and foreign fish industry [4].

Protein deficiency in the feed causes great damage to the development of industrial aquaculture. With the industrial growing, artificial feeding becomes the only means of creating a sustainable and
guaranteed food source for fish. In such conditions, the use of biologically active substances becomes relevant for enriching the diet with nutrients and improving fish growth [5].

Guseva Y.A. et al. are presented materials about studying the influence of pancreatic hydrolysate of soy protein on productivity, survival rate, chemical and amino acid composition of the muscle tissues in the Lena sturgeons grown in cages at natural temperature of the 4th fishery zone of the Russian Federation. The research was aimed at increasing productivity of the Lena sturgeon grown in cages. Analysis of the obtained research data shows that the content of crude protein in the muscle tissues of bions in the experimental group was higher by 8.6% (P>0.95) than that in the reference group [6].

Zimens Y.N. et al. present the results of an experiment on growing of Lena sturgeon (Acipenser baerii stenorrhynchos A. Nikolsky) juveniles using iodized yeast as a dietary supplement. The experiment was conducted in a recirculation installation facility in research laboratory "Technology of fish feeding and rearing" of Saratov State Agrarian University. The additive used contained iodine in a ration of 2 % by weight to test the relationship between the amount of iodine in food and the level of thyroxine in the blood. Based on the analysis of the dynamics of growth of weight of Lena sturgeon optimal degree of introduction of iodine was determined in the feed, providing growth of fish. The greatest weight gain of Lena sturgeon was observed for use in feeding iodinated yeast with iodine 200 micrograms per 1 kg of fish [7].

Poddubnaya I.V. et al. applied feed additive "Abiopeptide" in a chemical bonds with the organic iodine in the feeding of the Lenà sturgeon. It was carried out a technology of adding of iodinated feed additive "Abiopeptide" into the feed fed with the purpose of studying the effects of iodine on the productivity and functional status of the fry of the Lenà sturgeon [8].

Given the increasing demand for commercial sturgeon products, one of the objectives of the fisheries industry is to increase the production of these species in aquaculture. At the present time in the composition of feed for animals, including fish, biologically active substances are introduced for more growth of body mass, enhance digestibility and absorption of feed, stimulation of nonspecific immunity and saving feed cost [9-11]. One of these biologically active substances is the biogenic feed additive Akwa-Biot-Norm, developed by scientists of the Chuvash State Agricultural Academy on the basis of the Saccharomyces cerevisiae polysaccharide complex of yeast cells, which has no analogs. The use of the feed additive activates the links (cellular and humoral) of nonspecific resistance, stimulates the growth and development of the fish organism, and contributes to the prevention of infectious and invasive diseases [12, 13].

The food safety of our country should be guaranteed by the release of high-quality and safe products by domestic producers, and the requirements for ensuring its safety in veterinary-sanitary and sanitary-hygienic relations should comply with domestic and international requirements [14].

Veterinary and sanitary examination of fish is a complex veterinary measure aimed at ensuring the quality and safety of fish products [15].

The purpose of this work is the veterinary-sanitary assessment of the Lena sturgeon grown using the nutrient feed additive Akwa-Biot-Norm.

2. Materials and methods
The experimental part of the research work was carried out in the fish farm of UTAS LLC of the Chuvash Republic (Russia), and the material was processed in the accredited testing laboratory - Chuvash republican veterinary laboratory of the State Veterinary Service of the Chuvash Republic and at the Department of Epizootiology, Parasitology and Veterinary-Sanitary Expertise Chuvash State Agricultural Academy in the period of 2017-2018. Fish farm “UTAS” is a warm-water basin farm that receives warm and cold water from the Cheboksary Thermal Power Center (CHPP-2).

The objects of research were individuals of the Lena sturgeons (Acipenser baerii Brandt, 1869). Two groups of fish were formed, 500 copies each, experimental and control, according to the principle of groups of analogs, taking into account the clinical and physiological state and body weight. The selected fish copies of both groups were placed in separate ICA-2 trays with a capacity of 2.5 m³. The conditions of holding and feeding fish of both groups were identical. The biogenic feed additive
Akwa-Biot-Norm was additionally introduced into the diet of fish from the experimental group, in two courses lasting 5 days with a break of 2 days. During the experiment, fish were fed twice a day: at 8:00 a.m. and at 18:00 p.m. by full-ration mixed. When feeding the fish, an extruded combined food "ESIKO Sigma 811" manufactured by Biomar was used.

The biogenic feed additive Akwa-Biot-Norm is a suspension of 50 parts by weight of 1% gelatin solution, 1 part by weight of a concentrate of a purified polysaccharide complex derived from Saccharomyces cerevisiae microbial cells and 3 parts by weight of levomisole. Gelatin solution ensures preservation of initial biological activity and eliminates loss of preparation as a result of water washing.

The duration of the experiment was 240 days. At the end of the experiment, the fish was subjected to control slaughter and veterinary-sanitary assessment (5 copies from each group). Fish weighing 940-955 g and biological length 59±1 cm was used for the assessment.

In organoleptic studies, the appearance and potency of fish, the condition of mucus, scales and outer cover, eyes, gills colors were evaluated, and the odour from the surface of the carcass and from the depth of muscles was determined. During the autopsy of the fish, attention was paid to the condition of the internal organs.

In order to determine the qualitative composition of the muscle tissue of the grown ribbon sturgeon its chemical composition (water, dry matter, fat, ash) was determined. The moisture content was determined from the weight loss of the test samples when they were dried - the drying temperature was 100-105 °C. The determination of the fat content was carried out by Soxlet method. The method is based on multiple extraction of fat with a solvent from the dried analyzed sample in a Soxlet extractor followed by removal of the solvent and drying the recovered fat to a constant weight. The test sample was dried on an hour glass or in a petri dish in an oven at (103 ± 2) °C for 1 hour. The dried assay sample was quantified into a cartridge made of filter paper, on the bottom of which a piece of wool was placed. The watch glass or petri dish was strained with a wool wetted in a solvent (hexane, diethyl ether or petroleum ether), which was also placed in a liner. The sleeve was thoroughly closed and placed in a Soxlet extractor. Pre-dried extraction flask of the Soxlet apparatus was filled with a solvent (diethyl or petroleum ether, hexane) at about 2/3 of the flask volume. The extraction flask was placed in a water bath. Duration of extraction was from 5 to 7 hours at multiplicity of extract drains 5-8 for 1 hour. The completeness of degreasing was checked by applying a drop of extract from the extractor to the filter paper. There should be no fat spot on paper. After the extraction was complete, the solvent from the extraction flask was distilled off. Extraction flask with fat remaining after extraction was dried in drying cabinet at temperature (103 ± 2) °C to constant mass. Then results were processed.

Studies on heavy metals in fish were carried out on the atomic absorption spectrometer "MGA-1000" (Lumex, Russia).

The method of determining the amount of mesophilic aerobic and optional anaerobic microorganisms sowing into agarised nutrient media is based on sowing of product overhang into the nutrient medium and counting of all grown visible colonies after the incubation.

Veterinary and sanitary assessment of fish was carried out in accordance with Technical Regulations of the Eurasian Economic Union "On safety of fish and fish products" (TP OF EEU 040/2016), TR CU 021/2011 "On safety of food products" and the reference books [16, 17].

Determination of quality indicators of fish meat was performed according to the interstate standard (GOST 7631-2008 "Fish, non-fish objects and products from them. Methods for the determination of organoleptic and physical parameters").

Determination of physical and chemical parameters of fish meat was carried out in accordance with GOST (Interstate Standard) 7636-85 "Fish, marine mammals, marine invertebrates and products thereof. Method of analysis."

Studies on the content of toxic elements in the muscle tissue of fish were carried out according to the Interstate Standards GOST R 51183-2008 "Food products. Determination of trace elements. Determination of mercury by atomic absorption spectrometry of cold steam with preliminary
mineralization of the sample under pressure" and GOST 31707-2012 (EN 14627:2005) "Food products. Determination of trace elements. Determination of mercury by atomic absorption spectrometry with the generation of hydrides with preliminary mineralization of samples under pressure", MG (Methodical instructions) 4.1.986-200 "Method of measuring the mass fraction of lead and cadmium in food and food raw materials by electrothermal atomic absorption spectrometry" (developed by the Federal Center of Gossanepidnadzor of the Ministry of Health of Russia).

Microbiological studies of fish were carried out according to the Interstate Standards GOST 10444.15-94 "Food products. Methods for determining the number of mesophilic aerobic and facultative anaerobic microorganisms", GOST 31747-2012 "Food products. Methods of detection and determination of the number of coliform bacteria (coliform bacteria)", GOST 31659-2012 (ISO 6579:2002) "Food products. Methods of detection of bacteria of the genus Salmonella", GOST 31746-2012 (ISO 6888-1:1999, ISO 6888-2:1999, ISO 6888-3:2003) "Food. Methods to identify and quantify coagulase-positive Staphylococcus and Staphylococcus aureus", GOST 32031-2012 (ISO 3166) 004-97 "Food Products. Methods of detection of bacteria Listeria monocytogenes".

Parasitic cleanliness was determined according to SanPiN (Sanitary rules and regulations) 3.2.3215-14 "Prevention of parasitic diseases in the territory of the Russian Federation".

Experimental data were processed by the method of variable statistics on the validity of the difference of the compared indicators (P < 0.05-0.01) using the Microsoft Office Excel 2007 software complex.

3. Research results
Organoleptic properties of the experimental fish in both groups were determined by the state of mucus, eyes, gills, muscle tissue, abdomen, the smell of gills and cooking test. It was established: transparent mucus, skin elastic, whole fins, natural color. Gill covers tightly cover the gill cavity, the gills are covered with transparent mucus, dark red, eyes are light, bulging, transparent cornea, abdomen not swollen. Muscle tissue is elastic, tight to the bones. When boiling is sampled, the broth is clear, there are large drops of fat on the surface, the smell is fishy, pleasant, the meat is well divided into muscle bunches.

Thus, organoleptic characteristics of fish in control and experimental groups did not differ and meet the requirements of benign fish.

However, organoleptic indicators cannot always be a reliable source of information about the safety of a product. For an accurate safety assessment, additional laboratory tests are required. This is primarily due to the fact that fish is a carrier and source of various parasitic infections, toxic infections, toxicosis, etc.

The chemical composition of fish meat, which determines its nutritional value and taste qualities, is characterized primarily by the content of water, dry matter, fat, etc. The chemical composition of the muscle tissue of the fish in control and experimental groups did not have significant differences (table 1).

| Indicator              | Group         |
|-----------------------|---------------|
|                       | control       | experimental  |
| Mass fraction of moisture, % | 70.5±0.36    | 70.4±0.31    |
| Dry matter mass fraction, % | 29.5±0.14    | 29.6±0.12    |
| Mass fraction of fat, %    | 10.44±0.08   | 10.47±0.08  |
| Mass fraction of ashes, %  | 1.30±0.08    | 1.3±0.09    |

Thus, the use of the biogenic feed additive Akwa-Biot-Norm did not affect the chemical composition of fish muscle tissue.

Given that the content of harmful substances in aquatic ecosystems is slowly but constantly increasing due to their constant supply and accumulation, penetrating into the food chain, the final link
of which are fish, they accumulate in them and, ultimately, the food chain fall into the human body (consumer). In order to ensure chemical safety, we have studied the content of toxic elements and their quantitative characteristics. The content of toxic elements in the muscle tissue of fish is presented in table 2.

Table 2. Content of toxic elements, mg/kg.

| Name of toxic substances | The value of the characteristics | control group | experimental group |
|-------------------------|---------------------------------|---------------|-------------------|
|                         | permissible level               |               |                   |
| lead                   | 1.0                             | not detected  | not detected      |
| arsenic                | 1.0                             | not detected  | not detected      |
| cadmium                | 0.2                             | 0.007         | 0.007             |
| mercury                | 0.3                             | 0.0019        | 0.0018            |

Based on the studies it was found that in the samples of the muscle tissue of the fish of the control and experimental groups the content of cadmium and mercury did not exceed the maximum permissible concentrations, and such elements as arsenic and lead were not found.

Pesticides (HCH (alpha, beta, gamma isomers), DDT and its metabolites) were not found in muscle tissue samples of experimental fish of both groups.

Microbiological studies of Lena sturgeon meat have shown that the number of mesophilic aerobic and facultative anaerobic microorganisms in fish samples does not exceed the safety requirements and nutritional value of food products. Coliform bacteria (CB), Staphylococcus, Salmonella, and L. monocytogenes were not isolated from all selected samples (control and experimental).

In the study of fish samples, pathogens of parasitic diseases were not found. Fish corresponding to the presented product samples is not dangerous for humans.

4. Conclusion

The results of veterinary-sanitary assessment of the Lena sturgeon grown using the nutrient feed additive Akwa-Biot-Norm showed that the introduction of this additive in the diet of fish does not adversely affect the quality. Fish meat meets veterinary, sanitary and hygienic requirements of the technical regulations of the Eurasian Economic Union "On the safety of fish and fish products" (EEU TR 040/2016) and is safe for the consumer.

It is advisable to use biogenic fodder additive Akwa-Biot-Norm on the basis of polysaccharide complex of yeast cells Saccharomyces cerevisiae for stimulation of growth and development of Lena sturgeon, prevention of their morbidity in industrial technology of fish cultivation.

It is recommended to feed fish with combined fodder enriched with Akwa-Biot-Norm, two courses for 5 days with a break of 2 days, 2 times a day.

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