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HISTOMORPHOLOGICAL ESTIMATION OF SOME INTERNAL ORGANS OF RAINBOW TROUT *ONCORHYNCHUS MYKISS* (WALBAUM, 1792) CULTIVATED ON EXPERIMENTAL FEED

Abstract. The article presents histological characteristics of gastrointestinal tract, liver and muscles of the rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) juveniles (5 specimens, control group) and yearlings (2 pilot groups, 15 specimens in each group) in the basins of "Chilik pond farm, LLP" (the Almaty region, Kazakhstan) fed with imported Denmark feed "Aller Aqua" and the feed produced by the local company "Kazkorm" (without immunomodulatory probiotic drugs and with immunomodulatory probiotic drug "Biocons", concentration 0.5%). The experiment was devided into 2 series; the first series lasted 45 days (yearlings, 15 specimens), the second series lasted 75 days (yearlings, 15 specimens). The total duration of the experiment made 75 days. In both series there were used 3 variants of feed: in the control group - "Aller Aqua", in pilot groups – feed without and with "Biocons" drug, concentration 0.5%. The histopathological analysis was conducted on termination of the first and second series. The histopathological changes were registered in all studies organs: the minimum changes were observed in the gastrointestinal tract; maximum changes were seen in the liver and somatic muscular system of fish that didn't take probiotic drug. The intensity of pathological processes grew with duration of taking feeds without probiotic drugs. In the liver of fish there were noted such pathologies as fatty degeneration, enlargement of the liver vessels, cell contact disorders, degradation and necrosis of hepatocytes, violation of the microcirculatory in the form of stasis of blood in the sinusoids. All studied individuals fed with experimental feed were observed with significant pathological changes in the structure of somatic musculature. The size/weight characteristics of fish fed without probiotic drugs were inferior to those in fish using probiotic drugs. The histopathological analysis of indicators of gastro-intestinal tract, liver and muscle, as well as the size/weight characteristics after using feed with probiotic drug showed that the feed can be recommended for practical use, as the feed is the most balanced and nutritious.

Key words: trout, histopathology, gastrointestinal tract, liver, muscles, feed, probiotics.

Introduction

Modern fish farming is based on growing fish in regulated conditions and urgently requires the most serious attention to the process of production and use of good quality and economically profitable feeds for all age groups of fish. With the increase of the controlled production balanced feeding and optimization of fish breeding conditions become the primary tasks of fish-breeding enterprises. In this relation, the fish health and the quality of feeds are decisive factors in the profitability of industrial fish farming.

Trout farming is an intensively developing branch of aquaculture in Kazakhstan. At present, there is a huge practical experience in growing trout, including a variety of intensive technological processes. To increase the development of trout farming it is necessary to create complex technology for trout growing, based on scientific achievements involving pathological-anatomical, physiological and biochemical research methods.

At artificial cultivation of such valuable aquaculture objects as trout it is required to have knowledge on their physiological development, somatic structures and, above all, musculature as the main food product.

To assess the quality of feed, an important condition is the monitoring of fish health controlled by various methods of research including histopathological analysis of digestive and locomotion organs.

Material and methods

The experiments were performed on 35 fish, grown in basin conditions on the basis of the CPF. The samples of gastrointestinal tract, liver and muscles were taken from 35 trout specimens to study the histological characteristics, 5 of which were taken at the beginning of the experiment (juveniles) as a control group, then the experiment was divided into two series, in the first series after 45 days were selected juvenile 15 specimens, in the second series of experiments after 30 days were taken 15 more individuals. The duration of the experiment as a whole was 75 days.

For the study fishes were fixed in 10% formalin, before pathomorphological analysis treatment biological and physiological features of the fish were taken. Subsequently, gastrointestinal tract, liver and muscles were selected for histological analysis, which were processed by traditional methods of microscopic technique [1-3]. For histological examination, were selected pieces of the studied organs no larger than 0.5 x 0.5 x 0.5 cm. After fixation, the material was washed in water and then dehydrated for 15 minutes in 6 shifts of ethyl alcohol. Xylene was used as intermediate stuff for pouring into paraffin. After paraffin embedding there were made serial slices 5-7 microns thick from the prepared blocks on a sliding microtome. The slices were smoothed out in a drop of warm water and stuck onto microscope slides treated with a protein-glycerin mixture. The resulting sections were dewaxed with xylene, dehydrated in alcohol of ascending concentration and dyed with hematoxylin and eosin. The dyed sections were put into Canada balsam under the cover glass.

A total of 35 fish species were processed; 105 blocks and 210 histological preparations were prepared. The obtained histological sections were studied on a light-optical microscope MicroOptix MX 300 (T) equipped with a digital camera. Microphotography of the obtained preparations was carried out at a 10x, 20x and 40x zoom.

Results of the research

Conducting the scientific research and evaluating the results of the experiment were carried out taking into account the control group of juveniles and three variants of yearlings growing. At the beginning of the experiment, the control group of fish was fed with feed No. 1, in the first and second series of the experiment the fish were divided into 3 groups: 1) the control group was kept on a diet of imported feed No. 1; 2) the experimental group was kept on the ration of the Kazakh feed No. 2; 3) the experimental group was kept on the Kazakh feed diet No. 3.

The results of the statistical analysis of size and weight parameters after 45 days are shown in Fig. 1, where, at the beginning of the experiment, the average body length of juvenile trout was 5.8 cm, the average weight - 4.6 grams. In the first series of experiments trout size and weight characteristics differed depending on the type of feed, the largest mass was noted in fish fed with feed No. 3, similar weight values were found in fishes fed with the feed No. 1, and the smallest characteristics were registered at those fed with feed No. 2.

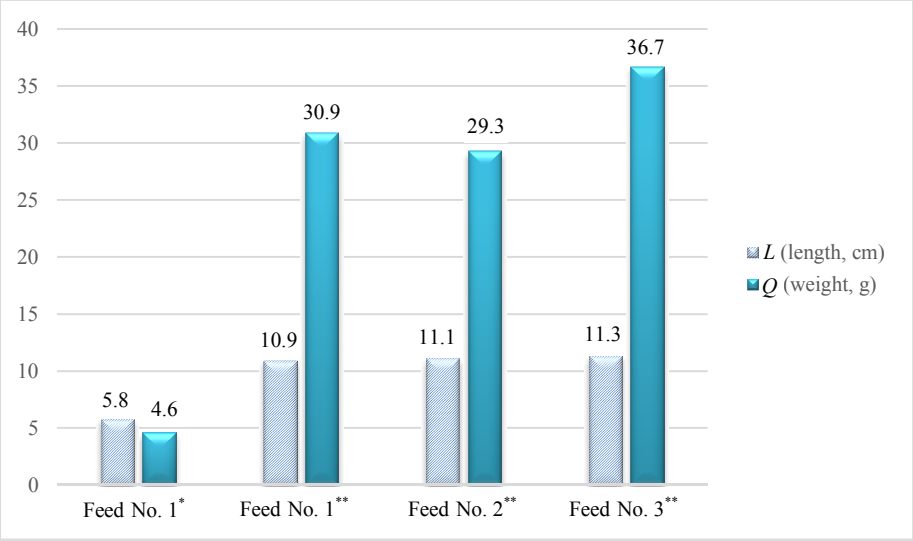
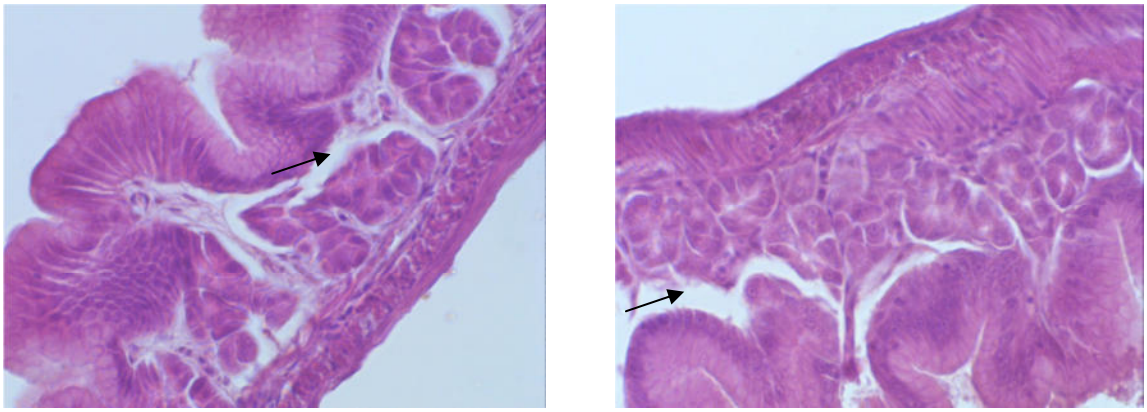


Fig. 1. Average values of the size and weight characteristics of trout during feeding with different feeds: * control group; ** the first series of experiments

In the second series of the experiments in 75 days trout in all samplings increased its size and weight indices; however, mean values of the size/weight indices in fish fed with feed No. 2 were lower (L - 14.1 cm, Q - 46 g), than when fed with feed No. 1 (L = 15.5 cm, Q = 57.2 g) and feed No. 3 (L = 15.4 cm, Q = 56 g).

Histological analysis of gastrointestinal tract of the trout

Morphological studies of the gastrointestinal tract showed that in the studied trout juveniles in control group the digestive tract was differentiated into the esophagus, stomach and intestine. The stomach has a larger diameter than the esophagus and the mucosa forms low simple folds lined with prismatic epithelium, cells of the apical part of which have a homogeneous cytoplasm without a clearly expressed mucus component. In the connective tissue stroma of the mucosa are located alveolar gastric glands formed by cubic cells. The muscular membrane of the stomach consists of only one layer of annular smooth muscles. There is observed swelling in the submucosa and destruction of the cells of the superficial epithelium of the stomach (Fig. 2).



A B

Fig. 2. Histological section of trout juvenile's stomach:
A - edema of the glandular mucosa of the stomach; B - rejection of the superficial epithelium.
Zoom 40x. Hematoxylin-eosin staining

The relief of the intestinal mucosa is well expressed and is represented by low zigzag folds. The connective tissue part of the mucosa participates in their formation, although it is less developed than in the stomach. Intestinal epithelium on all extent has a uniform structure and is formed by cylindrical limbic cells, among which there occur single goblet mucocytes. Differentiation of the muscular membrane of the stomach and intestine is manifested in the appearance of a very thin outer longitudinal layer of the musculature.

In the intestines of young trout, the mucosa is slightly differentiated; folds of the mucous membrane are low, they are lined with epithelium of various heights. In cells of the intestinal epithelium, the brush border is not expressed, and in their apical part the concentration of small vacuoles is observed, the content of which weakly stains on mucus. Among the cells of the intestinal epithelium there are practically no goblet mucocytes. The connective tissue base of the mucosa is developed in folds, whereas between folds it is practically not expressed. The proper muscular membrane of the intestine is thin, formed by annular smooth fibers. The intestine is covered with a thick layer of fat, which indicates good fatness of fish.

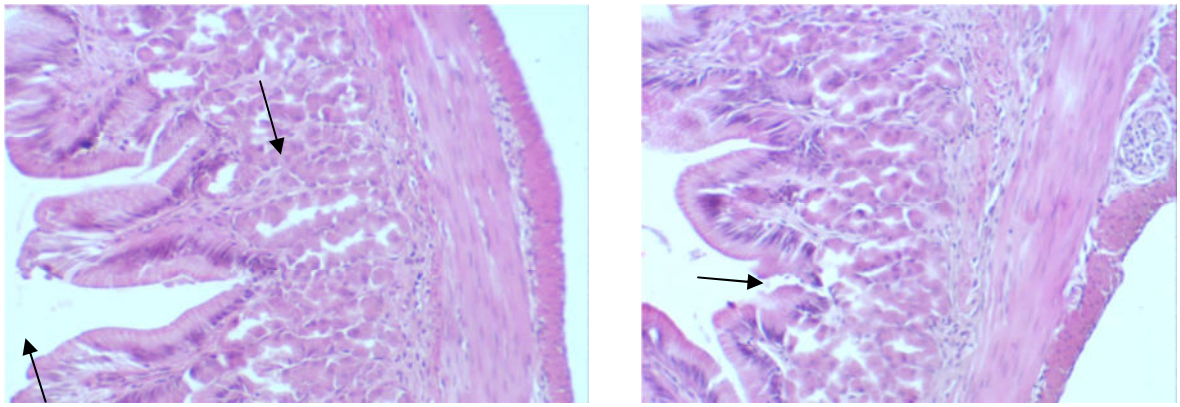
In histological structure of the intestine of all 5 trout specimens under study there are observed different pathological disorders to some extent: edema of mucosa, desquamation of epithelium, necrosis of epithelial cells in the folds.

Thus, in trout juveniles of the control group gastrointestinal tract did not reach the definitive state, which is symptomatic of underdevelopment of the pyloric appendixes, poor development of gastric glands, underdevelopment of folds of intestinal mucosa and muscular membranes of the entire gastrointestinal tract. At the same time, it should be noted that some pathological disturbances in the structure of the mucous membrane of the gastrointestinal tract are noted when feeding fishes with feed No. 1 which, apparently, are due to alimentary toxemia. These violations do not have complex consequences, because during the fish growth there are proliferative processes that update differentiating and developing tissues and organs.

The structure of the digestive tract of trout in 45 days since the beginning of the experiment corresponded to the definitive state of the yearlings. In the folds of the gastric mucosa there began to form folds of the second order, the tubes of the gastric glands lengthened, the connective tissue base repre-

sented by collagen fibers with various cellular inclusions took the shape. The wall of the muscular membrane of the stomach has become thicker; it is formed by the inner annular and outer longitudinal layers of smooth muscles. Outside, a thin layer of serosa covers the muscle shell.

In all trout species fed with feed No. 1 in the histological structure of the gastrointestinal tract there were observed minor pathologies. In some places there appeared destructive changes in the superficial epithelium, necrosis of the cells of the gastric glands, edema of the connective tissue base underlying the glandular section, and small swelling between the muscle fibers of the actual muscle membrane of the stomach (Fig. 3 A, B).

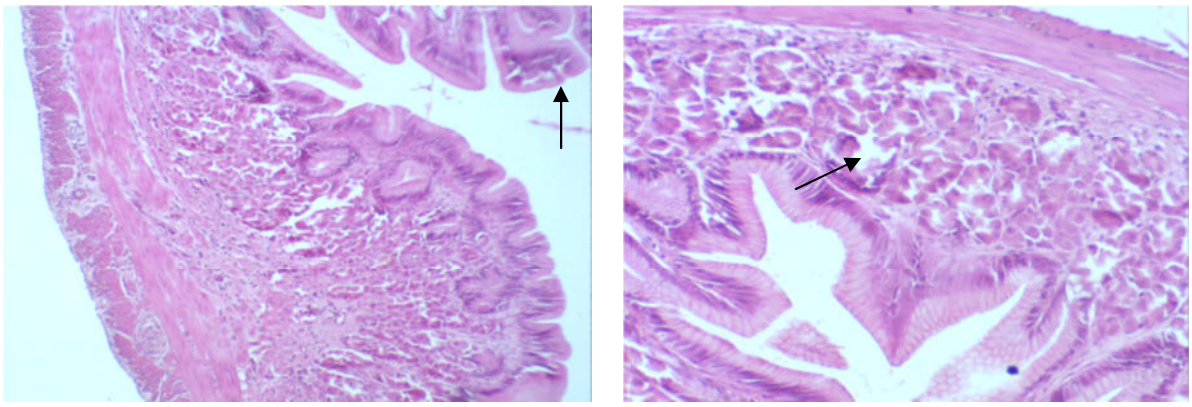


A B

Fig. 3. Histological section of the stomach of trout yearlings (feed No. 1):
A - destruction of superficial epithelium, necrosis of cells of the gastric glands;
B - swelling of the glandular part of the stomach. Magnification 40x. Hematoxylin-eosin staining

In all the studied trout species fed with different feeds, we did not find deviations and anatomical and morphological differences in the structure of the gastrointestinal tract.

The pathological analysis of trout fed with domestic feeds No. 2 and 3 showed (Fig. 4) that in the histological structure of stomach there are disorders similar to those described above (fed with feed No. 1).



A B

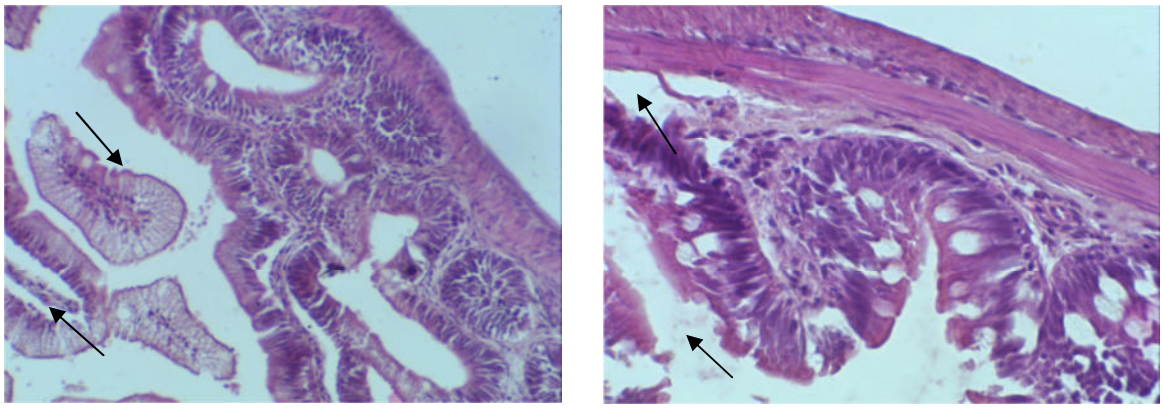
Fig. 4. Histological section of stomach of trout yearlings: A - destruction of the superficial epithelium (feed No. 2). 10x Zoom; B - necrosis of cells of the gastric glands and edema of the glandular part of the stomach (feed No. 3). 20x Zoom. Hematoxylin-eosin staining

During the experiment the length and mass of the fish increased markedly; respectively, further differentiation in the anatomical and histological structure occurred. At the beginning of the intestine, pyloric processes appeared, and the length of the intestine increased.

Histological analysis of the intestine and pyloric processes showed that the relief of the intestinal mucosa and pyloric processes has become more complicated. The folds of the mucous pyloric processes are simple, not high, covered with a single layer of highly prismatic epithelium. Epithelial cells in

the apical part form vacuoles leading to destruction of cells. The muscular membrane is very thin, the connective tissue is poorly developed and puffiness is observed in it. Outside, all pyloric processes were covered with fat tissue.

The relief of the intestinal mucosa is also complicated, folds anastomosing with each other are formed. In the depth of the folds proliferation of the intestinal epithelium is observed, large goblet mucocytes appear among the cells of the intestinal epithelium. In the apical part of the intestinal cells a formed cellular border on the border is clearly visible, which is accompanied by membrane digestion. All experimental fish exhibit pathological disturbances in the structure of the mucosa: vacuolization of intestinal cells (fatty degeneration of enterocytes), destruction and necrosis of epithelial cells (Fig. 5 A, B).



A B

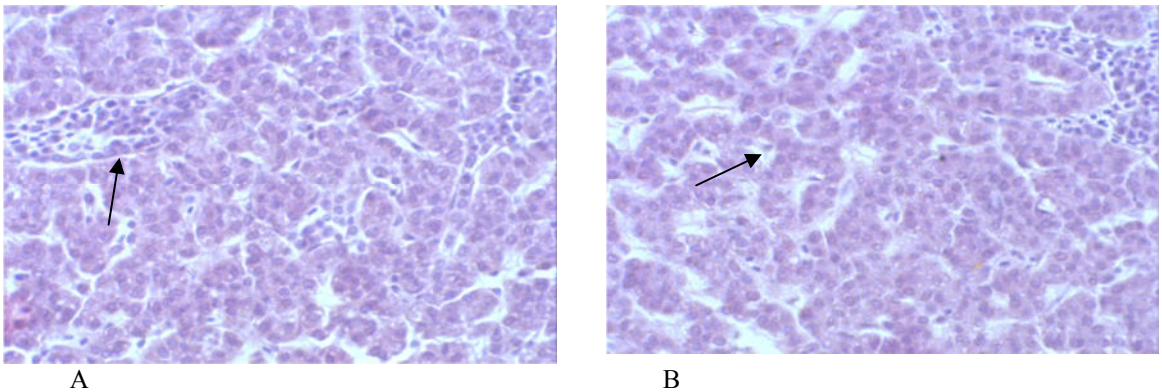
Fig. 5. Histological section through the intestines of trout yearlings:
A - vacuolization of enterocytes and mucosal edema. Magnification 10x;
B - necrosis of epithelial cells. Magnification 40x. Hematoxylin-eosin staining

The structure of submucosa base and the muscular membrane of the intestine do not have any significant disturbances, with the exception of a slight edema in the connective tissue base of the mucosa.

Thus, the results of histological analysis of the gastrointestinal tract in all experimental trout specimens did not reveal significant disorders in the structure of mucosa and muscle membranes. The revealed pathologies have a compensatory-adaptive character.

Histological analysis of the liver of trout

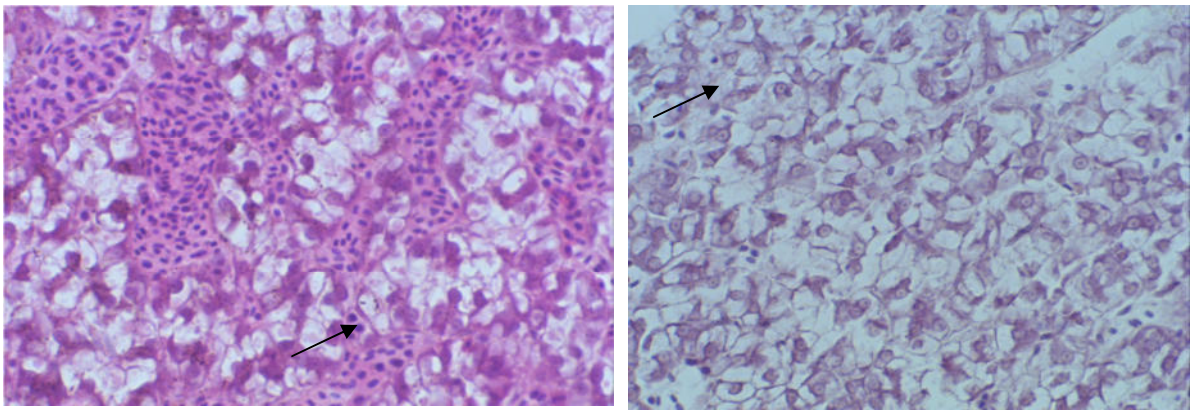
In the control group of young trout, the histological structure of liver was characterized by a well-defined tubular structure of the parenchyma with wide sinusoids. Hepatocytes are cubic and polygonal with large nuclei in the center of the cell. Occasionally there are dual-nuclear hepatocytes, and hepatocytes with large polymorphic nuclei. The cytoplasm of hepatocytes is homogeneous, but there are individual hepatocytes with small fat vacuoles. Some individuals show vasoconstriction and local expansion of sinusoids (Fig. 6 A, B).



A B

Fig. 6. Histological section of the liver of trout yearlings:
A - plethora of vessels and local expansion of sinusoids;
B - small fatty vacuoles. Magnification 40x. Hematoxylin-eosin staining

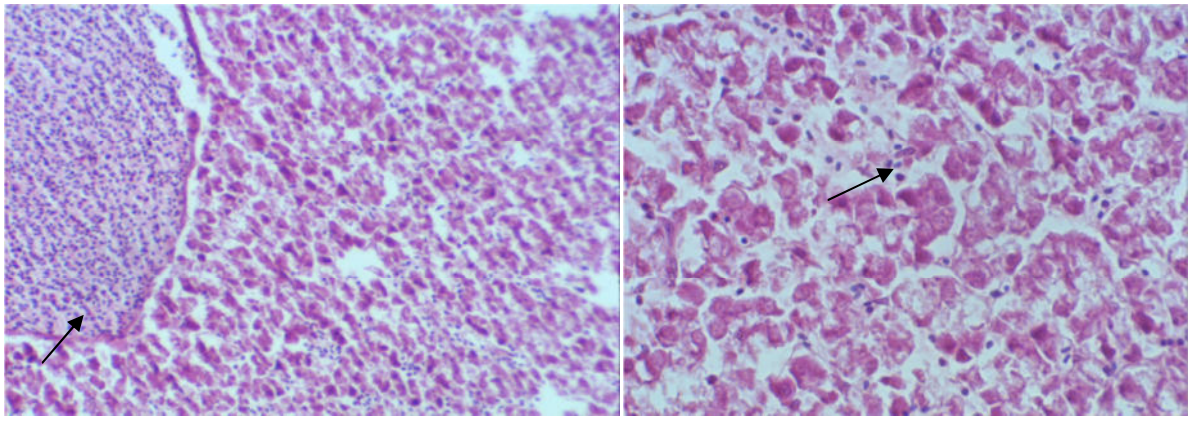
When feeding trout with feed No. 1 both in the control and in the experimental series, histopathological disorders of the liver were observed in all studied fish species. The majority of individuals showed a fatty degeneration of the parenchyma in liver. It should be noted that there is an increase in fatty dystrophy as the fish grows. Thus, in the first series of experiments, an increase in the size of hepatocytes is due to the accumulation of lipid inclusions in the cytoplasm for the entire fish sample. In the second series of experiments, many hepatocytes were strongly vacuolized and had nuclei displaced to the cell periphery. In some hepatocytes pyknotic nuclei were observed or they were completely absent, which led to necrotic changes. The liver of several individuals was characterized by a disturbance of the microcirculatory bed in the form of blood stasis in sinusoids and larger vessels. The main part of hepatocytes was characterized by fatty degeneration. There were cells with pycnotic nuclei in a state of necrosis (Fig. 7 B).



A B

Fig. 7. Histological section of the liver of trout yearlings:
A - fat degeneration and pycnosis of hepatocyte nuclei; B - necrotic changes, disturbance of the microcirculatory bed in the form of stasis of blood in sinusoids and larger vessels.
40x Zoom. Hematoxylin-eosin staining

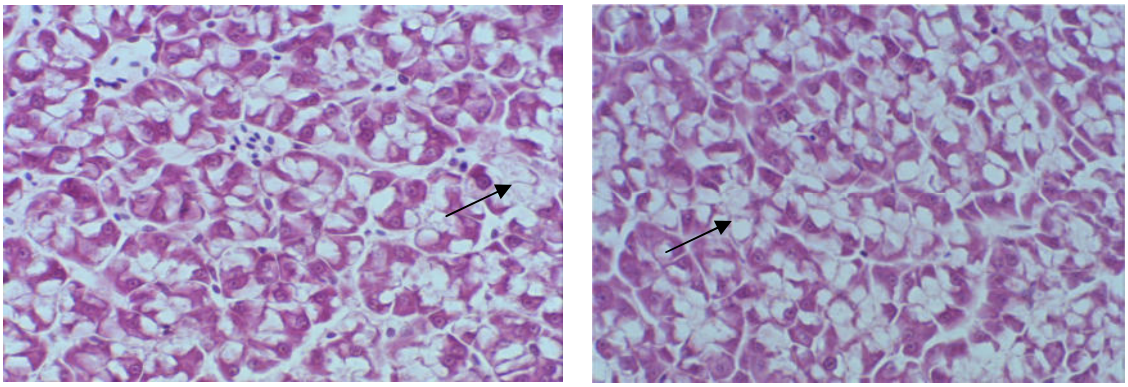
When using feed No. 2, there was registered enlargement of the liver vessels, cell damage, destruction and necrosis of hepatocytes, while proliferation of cells of the lympho-histiocytic series was observed in sinusoids (Fig. 8 A, B).



A B

Fig. 8. Histological section of the liver of trout yearlings:
A - widening of the blood vessels of the liver, disturbance of cellular contacts, destruction and necrosis of hepatocytes; B - proliferation of cells of lympho-histiocytic series in sinusoids.
40x Zoom. Hematoxylin-eosin staining

In all studied trouts fed with feed No. 3, the histological structure of the liver retained a tubular structure, accumulation of lipids was observed in the hepatocytes, and the nucleus in the cell was pushed to the vascular pole. In the sinusoids, single blood cells were observed (Fig. 9).



A B

Fig. 9. Histological section of the liver of trout yearlings:
A, B - accumulation of lipids and fatty degeneration. 40x Zoom. Hematoxylin-eosin staining

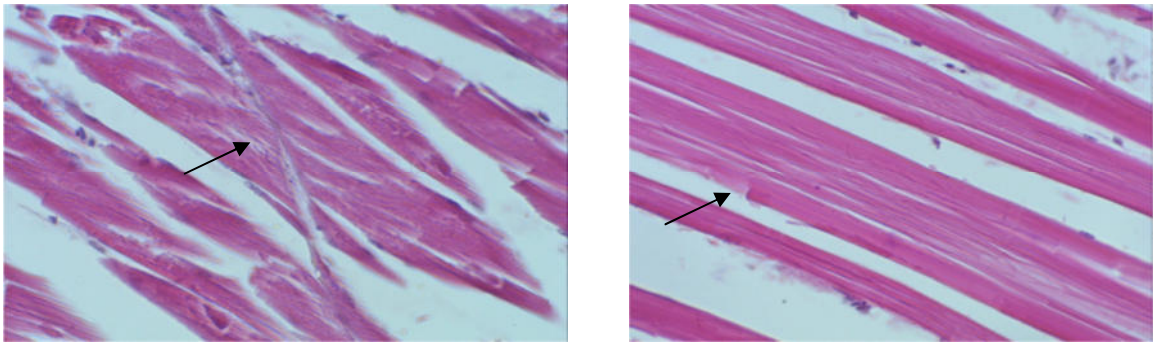
Thus, the study of trout liver fed with feeds No. 1 and 2, showed the development of fatty dystrophy and of vascular bed disorders in some individuals. More pronounced pathologies in the liver were observed in fish feeding them with feed No. 2. In this group of fish, liver defects in cell contacts, destruction, necrosis of hepatocytes, and proliferation of cells of lympho-histiocytic series in sinusoids were observed. Size/weight characteristics of fishes fed with feed No. 2 are also inferior to those of fishes that consumed feeds No. 1 and 3. Probiotics seem to perform a hepatoprotective function, which makes it possible to reduce the degree of endogenous intoxication, reducing the formation of toxic compounds.

Histological analysis of trout muscles

To analyze trout muscle tissue there were selected pieces with white muscles from the dorsal side of the body, above the lateral line, were selected.

Histological analysis of white muscles in the control group in trout juveniles showed moderate disconnection of muscle fibers and integrity of myofibrils of muscle fibers. The striation of transversal muscles was well defined. On longitudinal sections the fiber bundles are somewhat disconnected and some of them are characterized by a smaller thickness. In some muscle fibers breaks were observed, probably due to the stress factor at the time of capture and fixation of fish.

In all trout specimens (yearlings) examined in the first and second series of the experiment fed with feed No. 1 the skeletal muscles were characterized by dystrophy and cleavage of the muscle fibers, slight gusts of muscle fiber, while the transversal striation retained in all muscle fibers. In some parts of the muscles there was noted swelling of the nuclei (Fig. 10).

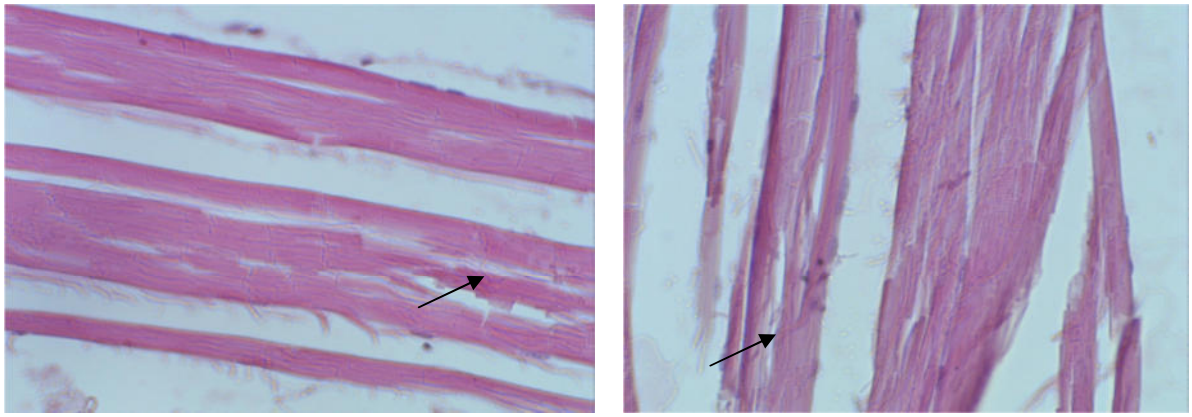


A B

Fig. 10. Histological section of muscles of trout yearlings:
A - dystrophy and dissociation of muscle fibers; B - cleavage of muscle fibers, minor gusts of muscle fibers.
40x-Zoom. Hematoxylin-eosin staining

According to the criteria for the evaluation of muscle pathology, developed by Yu. V. Altufiev for sturgeon species, the revealed pathologies of muscle tissues in the examined trout juveniles and yearlings corresponded to 2-3 points (moderate pathology), according to the proposed scale [4].

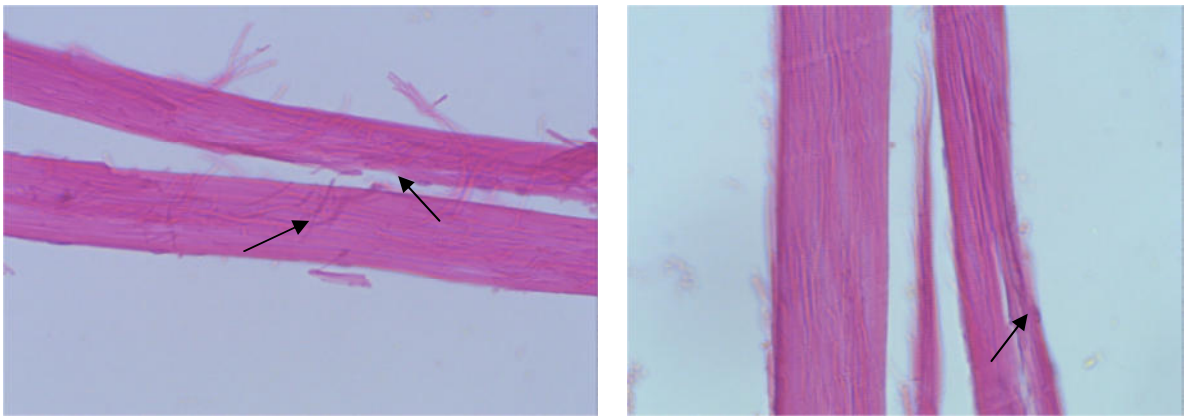
In the first and second series of the experiments there were observed pathological processes in muscle tissue of all studied trout fed with feed No. 2: skeletal muscles were characterized by a moderate, sometimes significant separation of the muscle fibers, by cleavage of the muscle fiber, by weakly expressed transversal striation of individual muscle fibers (Fig. 11 A). There was a separation of muscular fibers of the striated musculature leading to muscle atrophy and minor gusts of muscular fibers. Some species experienced edema in the intermuscular spaces, as well as small foci of inflammatory infiltration, slight defibration of myofibrils (Fig. 11 B). On longitudinal sections in some muscle beams there was atrophy of the sarcolemma. In general, all studied trout had varying degrees of expressed histopathological changes in muscle tissue, which we characterize as moderate pathologies and estimate at 2-3 points.



A B

Fig. 11. Histological section through the muscles of trout yearlings:
A - edema in the intermuscular spaces, foci of inflammatory infiltration; B - insignificant defibration of myofibrils, muscular fiber impulses. 40x Zoom. Hematoxylin-eosin staining

In all studied trout in the first and second series of the experiment fed with domestic feed No. 3 histopathological disorders of skeletal muscles are less pronounced than in those fed with feed No. 1 or No. 2. The skeletal muscles were characterized by moderate dissociation and looseness of the muscle fibers, cleavage of the muscle fibers, while the transversal striation was retained in all muscle fibers. In some parts of the muscles there was noted swelling of the nuclei and partial destruction of the fibers (Fig. 12).



A B

Fig. 12. Histological section of muscles of trout yearlings:
A, B - moderate dissociation and looseness of muscle fibers, cleavage of muscle fibers, destruction of myofibrils. 40x Zoom. Hematoxylin-eosin staining

Conclusion

In juveniles grown on all three feeds after 45 days, histopathological abnormalities in all studied organs were noted in the first series of experiments, and also after 30 days in the second series of experiments, the greatest pathological processes being registered in liver and muscles of fish consuming feed without probiotics (feed No. 2). Intensity of pathological processes increased with growth and duration of consumption of artificial feed.

Results of histological analysis of the gastrointestinal tract in all experimental trout specimens haven't shown any significant disturbances in the structure of the mucosa and muscle membranes. The revealed pathologies have a compensatory-adaptive character.

Liver, as an organ of depositing nutrients and detoxifying harmful metabolic products, is more susceptible to disorders. The studies of the trout liver fed with feeds No. 1 and No. 2, showed the development of fatty dystrophy and disturbance of vascular bed in some individuals. More pronounced pathologies in liver were observed in fish fed with feed No. 2. In this group defects in cell contacts in liver, destruction, necrosis of hepatocytes, and proliferation of cells of lympho-histiocytic series in sinusoids were observed.

Size/weight characteristics of fishes grown at the feed No. 2 are also inferior to those of fishes consuming feeds No. 1 and No. 3. Probiotics seem to perform a hepatoprotective function, which makes it possible to reduce the degree of endogenous intoxication, reducing the formation of toxic compounds.

In all the examined trout specimens (yearlings) in the first and second series of the experiment, when fed with feed No. 1, the skeletal muscles were characterized by degeneration and cleavage of the muscle fibers and minor gusts of muscle fibers. In the first and second series of the experiment, pathological processes in muscle tissue were observed in all the studied trout fed with domestic feed No. 2: edema in the intermuscular spaces, inflammatory infiltration sites, insignificant defibration of myofibrils. In all studied trout in the first and second series of the experiment fed with domestic feed No. 3, histopathological disorders of skeletal muscles were less pronounced, the skeletal musculature was characterized by a moderate dissociation and looseness of the muscle fibers.

The pathological processes mentioned above are not only associated with an alimentary factor, since a good balanced diet is a necessary condition for the growth and enhancement of fish biomass, but also, apparently, a large role in the manifestation of pathological processes belongs to the conditions of keeping and growing fish. High density of seeding, fluctuations in the oxygen regime of water and a high concentration of metabolic products in water cause the tension of adaptive mechanisms and reduce the mobility of fish. Feeding in accordance with the norms for each fish with their "crowding" lead to the non-use of all feeds and, as a consequence, to deterioration of living conditions and oxygen regime in the basins. The last factor results in the violation of blood microcirculation, gas exchange in tissues and affects the structural and functional characteristics in the liver and muscles.

Analysis of histopathological parameters of gastrointestinal tract, liver and muscle tissues after the application of domestic feed No. 3 allows us to recommend it for practical use. Kazakhstan's product - experimental feed "Kazkorm" including 0.5% of probiotic drug "Biocons" is the most adequate, balanced and nutritious for use in fish farms.

It should be noted that the proper choice of feeding technology is also a key to effective use of feed. Along with the receipt of nutritious and cheap feeds in economic terms, daily rhythm, rates and methods of feeding, depending on the age of the fish and their habitat, are of great importance. Timely quality control of feeds determines the effectiveness of the entire subsequent technological chain and makes planning the final product the most objective. It should be remembered that the use of poor-quality feeds often leads to very serious consequences, often causing massive loss of fish, which usually causes economic damage to both fisheries and public health.

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ГИСТОМОРФОЛОГИЧЕСКАЯ ОЦЕНКА НЕКОТОРЫХ ОРГАНОВ РАДУЖНОЙ ФОРЕЛИ *ONCORHYNCHUS MYKISS* (WALBAUM, 1792) ПРИ ВЫРАЩИВАНИИ НА ЭКСПЕРИМЕНТАЛЬНЫХ КОРМАХ

Исследовались гистологические характеристики желудочно-кишечного тракта, печени и мышц молоди (5 экз., контрольная группа) и сеголеток (2 экспериментальные группы по 15 экз.) радужной форели *Oncorhynchus mykiss* (Walbaum, 1792) при кормлении в бассейновых условиях на базе ТОО «Чиликское прудовое хозяйство» (Алматинская область, Казахстан) продукционным кормом датского производства «Aller Aqua» и кормами производства казахстанской компании «Казкорм» (без включения и с включением препарата пробиотического действия «Биоконс» 0,5 %). Эксперимент был разделен на две серии: первая – 45 суток (сеголетки, 15 экз.), вторая – 75 суток (сеголетки, 15 экз.). Продолжительность эксперимента в целом составила 75 суток. В обеих сериях использовались 3 варианта кормления: в контрольной группе – «Aller Aqua», в экспериментальных – корм без включения и с включением препарата «Биоконс» 0,5 %. Гистопатологический анализ проводился по окончании 1-й и 2-й серий. Гистопатологические изменения отмечались во всех исследуемых органах: наименее значительные – в структуре желудочно-кишечного тракта, наиболее значительные – в печени и в структуре соматической мускулатуры рыб, употреблявших корм без ввода пробиотиков. Интенсивность патологических процессов увеличивалась по мере длительности потребления кормов без пробиотических препаратов. В печени рыб отмечены такие гистологические патологии, как развитие жировой дистрофии, расширение сосудов печени, нарушения клеточных контактов, деструкция и некроз гепатоцитов, нарушение микроциркуляторного русла в виде стаза крови в синусоидах. Размерно-весовые показатели рыб, выращенных на корме без включения пробиотика, также уступали размерно-весовым показателям рыб, питавшихся кормом без пробиотиков. Анализ гистопатологических показателей желудочно-кишечного тракта, печеночной и мышечной ткани рыб и размерно-весовых показателей рыб, выращивавшихся на корме с включением препарата пробиотического действия,

позволяет рекомендовать его для практического использования в рыбоводных хозяйствах, т. к. этот препарат является наиболее сбалансированным и питательным.

Ключевые слова: форель, гистопатология, желудочно-кишечный тракт, печень, мышцы, корм, пробиотики.

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