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THE INFLUENCE OF ZEOFISH ON THE HISTOMORPHOLOGICAL PROFILES OF THE FISH

This article presents the results of the influence of zeolites as feed additives on the histological profile of fish. Research was conducted for 63 days using rainbow trout from Turgen village (Kazakhstan). The studied material was zeolitic tuff from the Chankanay deposit as an additive to RGM-2M feed. They were fed with normal diet, and normal diet supplemented with 1%, 2%, 3% and 4% of natural zeolites. Pathomorphological and histological examinations of muscle tissue and internal organs of rainbow trout were carried out. The addition of natural zeolites to feed does not cause pathological changes in the liver, muscles and other organs of experimental fish, and no negative effect was determined.

Key words: feed additives, zeolite, fish, pathological changes, organs

Introduction

A number of studies have considered the use of zeolites in veterinary medicine. Zeolite supplemented diets are well tolerated by the animals; they support biomass production and improve the health status of the animals [1,2]. Malymin (2000) studied the effect of impurities introduced into the diet of cows (zeolite and humus coal) on the mineral and protein metabolism and on the parturition process. In another paper, Yarovan (2008) showed the possibility of using natural zeolites from Khonyn in order to prevent the development of oxidative stress, to cure disorders in the antioxidant system of cows, and to treat diseases of the reproductive system during unfavorable maintenance conditions and the winter-stall feeding period. The list of reports on the use of zeolites in feed for birds has been updated [3,4,5,6,7,8].

The following articles have been published on the use of zeolites in the fisheries sector [9]. One of these studies shows comprehensively studied patterns of nitrate and nitrite accumulation in water and fish products as well as ways of reducing their toxicity through the use of zeolites and preparations based on them. A combination of treatment and preventive measures has been developed and implemented in fisheries, including veterinary health requirements for growing fish in a polluted environment and recommendations for diagnosing, treating and preventing fish poisoning.

Studies that tested zeolites in fish husbandry have shown the possibility of their successful use as feed additives. There is a possibility that the use of zeolites and zeolite - type aluminum silicates can have a significant positive effect on fish that consume them. The addition of a natural zeolite of the clinoptilolite type to feed mixtures in low doses of about 1–2% has an influence on very important functions that have heretofore not been recorded for other natural compounds [10]. The addition of clinoptilolite to feed is assumed to have a similiar effect to that of antibiotics.

The purpose of the research is to perform a pathomorphological and histological examination of the muscle tissue and internal organs of rainbow trout fish while using zeolites from the Chankanay (Kazakhstan) deposit in their diet as feed additives.

The goal of research was to study the effect of zeolite feed additives on the morphological status of the muscle tissue and internal organs of fish.

Materials and methods

Investigations were carried out at the Department of Veterinary-Sanitary Examination and Hygiene, at the Kazakhstan-Japan Center of the Kazakh National Agrarian University and at the trout farm in Turgen village during 2011-2013 years.

The material of study was the zeolitic tuff of Chankanay deposit (Kazakhstan, Almaty region, Fig. 1). Chemical composition (%) of using zeolite: SiO_2 - 55.90, Al_2O_3 - 15.60, Fe_2O_3 - 5.90, CaO - 5.57, MgO - 2.54, Na_2O - 3.05, K_2O - 2.15, TiO_2 - 0.45.

For research was used feed by recipes of RGM-2M: fishmeal - 4.6%, meat and bone - 9%, blood - 5%, wheat - 11%, algal - 1%, hay - 2%, reverse dry - 9%, yeast hydrolysis - 4%, soybean meal (flour) 6%, sunflower meal (flour) - 2%, fish oil (vegetable oil) - 4%, premix - 1%. The fish fed 6–8 times a day.

In the first batch of experimental feed, 1%, 2%, 3% and 4% zeolite content (grit particle size 0.01 to 1 mm in diameter) was introduced by replacing from 1% to 4% of feed, respectively.

300 normal healthy *Oncorhynchus mykiss* were randomly divided into five groups. The studies were performed in triplicate. Tests were carried out for 63 days. The standard 10 m^3 cages have been used for this purpose. Density of fish stock, feeding rations and other parts of their growing biotechnology complied fish-breeding regulations for industrial fish farms. In each case age groups have been formed with fish that weren't significantly different from initial individual weight. Before and after the test all the fish in each cage were weighed, and in order to determine the average individual weight 20% of the weighted fish has been counted. Decadal average temperature in cages ranged from 22.5–22.9 ^oC, dissolved oxygen content was between 7.91–8.54 mg/l.

Sampling of fish meat was performed in compliance with GOST 23481 (1979). Fish were gutted, packed in ice and transported to the laboratory on the day of slaughter; all analyses were performed the next day. After being taken to the laboratory, fish were dissected and the liver, kidney and intestine of them were exposed.

Histological examination was carried out in accordance with GOST 19496 (1994) and GOST R 51604 (2000). Intestine samples (proximal, middle and distal parts, liver and kidney) were fixed in 10% formalin solution. After fixation, the samples were rinsed in water, dehydrated in graded levels of 50%, 70%, 90%, 95% and 100% ethyl alcohol for two minutes, cleared in xylene and embedded in paraffin. Dewaxed sections (5–7 μm) were stained for histological and histochemical purposes with haematoxylin and eosin (H&E), periodic-acid Schiff (PAS) and Alcian blue (PH 2.5) (Grethen 1979) and examined microscopically (Leica DM4000 B LED).

All data were subjected to one-way variance analysis (ANOVA) using the Statistica 8.0 software environment to test the effects of the experimental diets. Duncan's multiple range test and critical ranges were used to test differences among the individual means. The differences were regarded as significant when $P<0.05$. All of the results are expressed as the means±S.D.

Results and Discussion

Table 1 shows results of lipid, protein, fat and ash concentrations in the muscle tissue of fish in the control and experimental group. Results of amino acid content in meat are presented in Table 2. Fatty acid content in fish is shown in Table 3.

3.1. Histological analysis

Histological analysis of the digestive system is considered to be a good indicator of the nutritional status of fish. The intestine and liver are the most important organs involved in the digestion and absorption of nutrients from food, and therefore, monitoring of these organs is considered to be necessary.

The macroscopic structure of fish from the experimental groups did not have marked abnormalities of the internal organs. In macroscopic terms, the liver was not enlarged, the capsule was smooth, and the surface was flat, brown, with a normal consistency and mild hyperemia. A number of authors link the morpho-physiological condition of the liver with feeding (Ostaszewska et al. 2005). Our histological examination showed that livers of fish from the experimental group maintained their overall body plan, lobes and primary structural components. Radially arranged liver strands made up of polygonal liver cells diverge from the central vein. The cytoplasm of liver cells is round, located in the center of the cell. Clumps of chromatin are stained purple with hematoxylin. There are cells with two nuclei. Hepatic strands are closely intertwined with the sinusoidal capillaries which look like gaps between strands of hepatic cells when stained. The nuclei of the sinusoidal capillaries' endothelium are elongated, sometimes appearing in the lumen of the capillary. Interlobular bile ducts form triads between the liver lobules together with the ramifications of the portal vein and hepatic artery.

The skeletal muscle of the fish is represented by striated muscle tissue (Fig. 2). On the longitudinal sections of muscle fibers a strand of fiber is visible and has the form of a contour line. There are elongated nuclei with small clumps of chromatin under the sarcolemma on the fiber's periphery. The central part of the fiber is occupied by myofibrils, which give the fiber longitudinal striations that stand out differently in various fibers. The spaces between cross-striated muscle fibers are filled with layers of loose connective tissue, which are called the endomysium.

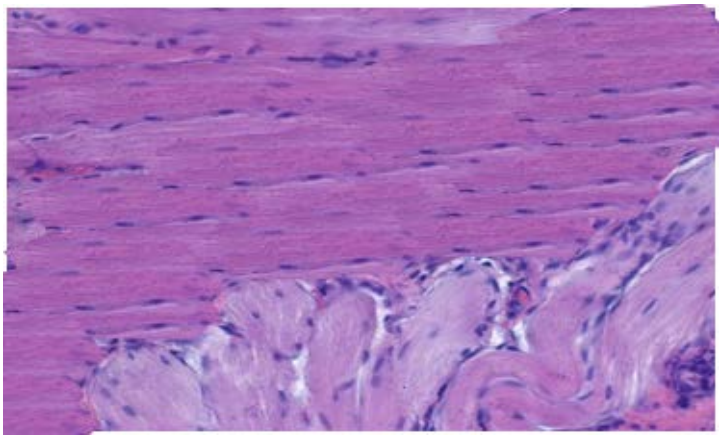
The addition of zeolites to the daily diet of fish for 63 days led to an increase of the thickness of the mucous membrane of the small intestine in comparison to the control group. The mucous membrane of the large intestine in the control group of fish is slightly thicker than in the experimental group, and the crypts in the mucosa of the experimental group of fish are larger. The ratio of goblet cells to other cells of the mucous membrane in the experimental group was higher than in the control group. As in all mammals, the intestinal wall of a fish consists of three layers. However, fish have no villi on the mucosa, there are only folds instead of them. The mucosa of the small intestine is shown as a single layer of prismatic epithelium, the cells of which are tall and narrow. The cytoplasm of the epithelium is stained pink by eosin. Striated edges are clearly visible at the apical end. The nuclei of cells, mainly oval in shape, lie closer to the basal end of the cells. Goblet cells are frequently observed between prismatic cells. Their cytoplasm is filled with mucus, and the nucleus is shifted to the basal part of the cell. The amount of goblet cells in the experimental group is increased compared to the control group.

The kidney is one of the first organs to be affected by contaminants (Iqbal et al. 2004). Histological investigation shows that in kidneys, the wall of the Bowman capsule is present in two layers. The outer layer of the capsule is visible, the nuclei of the cells are extended. The inner leaf of the capsule is difficult to discern, because it is closely fused with a ball of capillaries that grow into the capsule. The epithelial cells of tubules have a cubic shape, and nuclei are round in shape with distinct clumps of chromatin and a relatively large nucleolus. The cytoplasm of cells is cloudy with a hint of dark pink. A brush-shaped rim is well expressed at the apical end of the cells. The convolute tubules pass into a relatively short intercalated part. These are much thinner tubes lined with a low-columnar epithelium with oval nuclei. Their cytoplasm is bright, and there are no brush-type edges at the apical ends of the cells.

Table 1 shows a chemical profile of the muscle tissue of control and experimental groups of fish. Moisture, protein, lipid, and ash contents in the meat of experimental rainbow trout amounted to 73.96, 17.54, 5.46 and 1.44% on average, respectively. The results of the fish study showed that the use of natural zeolites in the diet of fish does not lead to significant changes of the chemical composition; however, a higher content of protein substances of about 2.73 g/100 g is observed in the muscle tissue of fish belonging to the experimental group of fish fed with feed with the addition of 4% zeolite. Lipid and ash content in all groups is nearly at the same level,

however the highest values are presented by the group of fish receiving feed with the addition of 4% zeolite (control group 5.30; 1.43 and experimental with 4% - 5.50; 1.45 g/100 g, respectively).

The presented values are favorably comparable with published reports on different salmonid species (Testi et al. 2006). Also, Gonzalez et al. (2006) reported higher lipid content (6.55%) and lower protein content (16.04%) in rainbow trout (*O. mykiss*) as compared to the findings of the present study. In the studies of Celik et al. (2008) moisture, protein, lipid and ash contents of rainbow trout meat were 1.65, 19.60, 4.43 and 1.36%, respectively. Similar values are presented in studies by Ozden (2005): 76.23, 18.57, 3.71 and 1.47%, for moisture, protein, fat and ash, respectively.



Transverly striated muscle tissue. 297x250 mm

Conclusion

The introduction of natural zeolites from the Chankanay deposit into the diet of fish in the amount of 1–4 % weight of the diet did not cause pathological changes in the liver, muscles and other organs of the fish in the experimental group. Due to this, obtained results are indirect proof that zeolites added to basic feed rations have no negative effect on the proteolytic enzyme systems of fish or on breeding, that is to say, they do not have a negative on the organisms of fish. Our studies showed that zeolites are a valuable mineral feed additive of natural origin that promotes the production of fish meat both qualitatively and quantitatively.

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ВЛИЯНИЕ ЦЕОФИШ НА ГИСТОМОРФОЛОГИЧЕСКУЮ СТРУКТУРУ МЯСА РЫБЫ

В данной статье изучали влияние кормовой добавки Цеофиш на гистоморфологическую структуру мяса рыбы. В результате исследовании было установлено, что в гистоморфологической структуре мяса рыб получавших Цеофиш с кормом патологических изменений не обнаружено.

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ЦЕОФИШТІҢ БАЛЫҚ ЕТІНІҢ ГИСТОМОРФОЛОГИЯЛЫҚ ҚҰРЫЛЫСЫНА ӘСЕРІ

Бұл мақалада Цефоиш азық қоспасының балық етінің гистоморфологиялық құрылысына әсерін зерттедік. Зерттеулер нәтижесінде азықпен бірге Цеофишті қабылдаған балық етінің гистоморфологиялық құрамында патологиялық өзгерістер байқалмады.

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THE MORPHOMETRIC INDICATORS AND THE DYNAMICS OF THE DEVELOPMENT PROCESS OF JAPANESE FEMALE QUAIL BREED GLANDULAR STOMACH IN POST INCUBATORY PERIOD

The article has devoted to an investigation work about the morphometric indicators and the dynamic development process of the Japanese female quail breed glandular stomach from 1 day to 290 days in post incubatory period. The largest linear growth indicators such as length, volume and thickness of the stomach wall switches are taken for purpose to investigate age differences of the quails groups. At the same time, at the dynamic developing process the