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The Influence of Chankanay Zeolites as Feed Additives on the Chemical, Biochemical and Histological Profile of the Rainbow Trout (Oncorhynchus mykiss)

Assel Paritova[1], Nurzhan Sarsembayeva[1], Bożena Łozowicka[2]*, Amangeldy Maulanov[3], Gulnur Kuzembekova[1], Aida Abzhalieva[1] and Piotr Kaczyński[2]

[1]Department of Veterinary Sanitary Examination and Hygiene, Kazakh National Agrarian University, Abaya 8, 050010 Almaty, Kazakhstan
[2]Institute of Plant Protection - National Research Institute, Chelmonskiego 22, 15-195 Bialystok, Poland
[3]Department of Biological Safety, Kazakh National Agrarian University, Abaya 8, 050010 Almaty, Kazakhstan

Abstract

This article presents the results of the influence of zeolites as feed additives on the chemical, biochemical and histological profile of fish. Research was conducted for 63 days using a rainbow trout from Turgen village (Kazakhstan). The studied material was zeolitic tuff from the Chankanay deposit as an additive to RGM-2M feed. The fish were fed with a normal diet, and the diet supplemented with 1%, 2%, 3% and 4% of natural zeolites. Pathomorphological and histological examinations of muscle tissues and internal organs of the rainbow trout were carried out. Additionally, lipid contents, FAs compositions and amino acid compositions were studied. The content of essential amino acids and proportion of essential amino acids to non-essential amino acids in the experimental group was higher than in the control group. Zeolite supplementation at the 4% inclusion level showed a high content of the amino acids. The results of this study confirmed that zeolites had a positive effect on the chemical, amino acid and fatty acid composition. The addition of natural zeolites to the feed does not cause pathological changes in the liver, muscles and other organs of the experimental fish, and no other negative effects were determined.

Keywords: Zeolite; Feed additives; Oncorhynchus mykiss; Kazakhs farm; Chemical composition

Introduction

Since the original discovery of zeolitic minerals in volcanogenic sedimentary rock, zeolitic tuffs have been found in many areas of the world. In past decades, natural zeolites have found a variety of applications in adsorption, catalysis, the building industry, agriculture, soil remediation, and energy [1,2].

A number of studies have considered the use of zeolites in veterinary medicine. Zeolite supplemented diets are well tolerated by animals; they support biomass production and improve the health status of the animals [3,4]. Malymin [5] studied the effect of impurities introduced into the diet of cows (the zeolite and humus coal) on the mineral and protein metabolism and on the parturition process. In other papers, Yarovan [6] 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 unfavourable maintenance conditions and the winter-stall feeding period. The list of reports on the use of the zeolites in the feed for birds has been updated [7-19].

Clinoptilolite in the diet for layer hens (50 g/kg) increased the numbers of laid eggs, stability of eggshell and efficiency of food utilisation. However, neither the onset of the egg laying cycle, nor the egg weight were affected [10]. Moreover, the zeolite supplementation of the diets with high contents of cholesterol exerts a hypocholesterolemic effect [20].

Kuramshina et al. [21] assess the effect of zeolites from Baimak and Sibai deposits added in the amount of 3% weight of chicken feed. The experimental group compared to the control group showed a significant increase of red and white blood cells, and hemoglobin. In the experimental group, egg yolks exhibited an increase of carotene, and vitamins A and B2 compared to the control group. Zedgenizova and Prosekina [13] also reported positive results of the experiments in which the zeolites were added to the chicken feed. In particular, when the zeolites were added in the amount of 5% weight of the feed, there was an increase of red and white blood cells, and hemoglobin. The feed additive also increased body weight without causing a negative impact on the overall condition of hens.

Lumbunov et al. [17] showed that zeolites with a particle size of 0.5–2.5 mm were good mineral supplements for adult birds. Their addition to the feed of hens (Sotnikovskoe poultry farm) in the amount of 3.6% increased egg production by 5-8% and improved egg shell quality. Furthermore, the use of the zeolites had a notable deodorizing effect. Similarly, such results were also found by Khaustov and Shadrin et al. [22].

Polyakov et al. [23,24] published a number of articles on the use of zeolites in the fisheries sector. One of their experiments showed comprehensively the studied patterns of nitrate and nitrite accumulation in water and fish products as well as ways of reducing their toxicity by means of the zeolites.

Studies that tested the zeolites in fish husbandry have shown the possibility of their successful use as feed additives. There is a possibility that the use of the zeolites and zeolite - type aluminum silicates can have a significant positive effect on the fish that consume them. The

***Corresponding author:** Bożena Łozowicka, Institute of Plant Protection - National Research Institute, Chelmonskiego 22, 15-195 Bialystok, Poland, Tel: +48 85 678 54 70; Fax: +48 85 675 34 19; E-mail: B.Lozowicka@iorpib.poznan.pl

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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 [25,26]. The addition of clinoptilolite to the feed is assumed to have a similar effect to that of antibiotics.

The fish are regarded to be a valuable source of animal protein and fat in human diets. It is known to be a source of protein that is rich in essential amino acids (isoleucyne, lysine, methionine, cysteine, threonine, and tryptophan). Omega-3 and omega-6 fatty acids (FAs), are essential compounds in fish lipids. These compounds play an important role in human nutrition and health promotion. The amount of amino acid and fatty acid content in the fish can be changed by diet.

Previously performed studies in this area can be characterized as fragmented and as not providing enough information to substantiate the use of tuff – type zeolites in the fish feed. Therefore, we were conducted research with the aim of defining the efficiency of using the zeolites as feed additives.

In general, the purpose of the research was to examine the influence of the feed additives containing the zeolites from the Chankanay (Kazakhstan) deposits on a rainbow trout.

The main goals of research were as follows. Firstly, it was to assess the effects of zeolite feed additives on the pathomorphological and histological status of the muscle tissue and internal organs of a rainbow trout (*Oncorhynchus mykiss*). Secondly, it aimed at analyzing the chemical and biochemical composition of the fish meat.

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, Figure 1). Chemical composition (%) of using zeolite: SiO2 - 55.90, Al2O3 - 15.60, Fe2O3 - 5.90, CaO - 5.57, MgO - 2.54, Na2O - 3.05, K2O - 2.15, TiO2 - 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.

Figure 1: Natural zeolite from Chankanay deposits (Kazakhstan, Almaty region).

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.

Normal healthy Oncorhynchus mykiss (n=300) were randomly divided into five groups. The studies were performed in triplicate. Tests were carried out for 63 days. The standard 10 m3 cages have been used for this purpose. Density of fish stock, feeding rations and other parts of their growing biotechnology were 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 calculated. Decadal average temperature in cages ranged from 22.5–22.9 °C, dissolved oxygen content was between 7.91–8.54 mg/l.

Sampling of fish meat was performed in compliance with GOST 23481 [27]. 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 [28] and GOST R 51604 [29]. 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 histopathological purposes with hematoxylin and eosin (H&E), periodic-acid Schiff (PAS) and Alcian blue (pH 2.5) [30] and examined microscopically (Leica DM4000 B LED).

Determination of chemical composition of fish meat

Before analyses the fish fillets were minced with skin. Crude protein was determined by the Kieldahl procedure [31]. The moisture was determined by oven drying at 105°C until they reached a constant weight. Total fat was extracted with petroleum ether using the Soxhlet system. Ash was determined using a muffle furnace by heating at 550°C for 8h.

Amino acid content was determined by ion exchange chromatography in an amino acid analyser (AAA-881 "Czechia" amino acid analyser). Prior to hydrolysis, samples were defatted with petroleum ether at room temperature. Acid hydrolysis (6 N HCl) was performed at 110°C for 2h for all amino acids except methionine and a sulphur-containing amino acid. Methionine was measured after oxidation with a performic acid followed by acid hydrolysis [31]. The Beckman Standard STD amino acid solution was used for calibration. The amounts of each amino acid were given as g per 100 g sample. All analyses were conducted with two fold repetition. The amino acid score was calculated according to FAO/WHO [32].

Fatty acids analysis of fish meat

The fatty acid profile of trout samples was determined as fatty acid methyl esters (FAMEs). Prior to analysis, the head, tail, fins, and viscera of the fish were removed. The edible tissue was filleted with the skin left on and homogenized. Fish samples were prepared using direct

saponification with KOH/methanol followed by a derivatization with (trimethylsilyl) diazomethane by the method of Aldai et al. [33].

FA composition was analyzed by GC Agilent with an autosampler (Agilent HP 6890 N, USA) equipped with a flame ionization detector and a Supelco-SP-2330 fused silica capillary column (30 m, 0.25 mm i.d., 0.20 mm film thickness of polyethylene glycol) (Bellefonte, PA). The oven temperature was 140 °C, held at 5 min, raised to 200°C at a rate of 4°C/min and to 220°C at a rate of 1°C/min, while the injector and the detector temperature were set at 220°C and 280°C, respectively. The sample size was 1 µl and the carrier gas was controlled at 16 psi. The split used was 1:100. The individual FAMEs (fatty acid methyl esters) were identified according to similar peak retention times using standard mixture Supelco 37 Component FAME Mix.

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 the results of lipid, protein, fat and ash concentrations in the muscle tissue of the fish in the control and experimental groups. The results of amino acid content in the meat are presented in Table 2. The fatty acid content in the fish is shown in Table 3.

Histological analysis

Histological analysis of the digestive system is considered to be a

Parameter Units of measurement	Control group (n =20)	Experimental group (n=20)			
		1%	2%	3%	4%
Proteins g/100g	16.00 ± 0.17^{a}	16.7 ± 0.32^{a}	17.08 ± 0.54^{a}	17.67 ± 0.28^{a}	18.73 ± 0.36^{a}
Lipids g/100g	5.30 ± 0.11^{b}	5.40 ± 0.14^{a}	5.35 ± 0.11^{a}	5.45 ± 0.10^{a}	5.5 ± 0.21^{a}
Moisture g/100g	73.40 ± 1.15^{a}	73.97 ± 1.21^{b}	73.99 ± 1.14^{b}	73.85 ± 1.64^{b}	74.05 ± 1.34^{b}
Ash g/100g	1.43 ± 0.01^{c}	1.43 ± 0.87^{a}	1.44 ± 0.24^{a}	1.45 ± 0.52^{a}	1.45 ± 0.41^{a}
Digestibility (%)	98.57	98.67	98.77	98.57	97.65
Energy value kcal /100g	112.00 ± 2.61^{c}	115.60 ± 3.67^{c}	113.35 ± 4.14^{c}	114.45 ± 3.62^{c}	115.28 ± 4.25^{c}
Mean values of 20 samples ± standard deviation. The letters in the same line show the differences in the results of statistical analysis.					

Table 1: Chemical profile of the muscle tissue of rainbow trout (Trial groups: control and experimental).

Amino acids	Recommended daily intake (g/70kg/body weight)	Control group	Experimental group			
			1% zeolite	2% zeolite	3% zeolite	4% zeolite
Threonine	0.46	0,72 ± 0.03^{b}	0,87 ± 0.05^{b}	0,86 ± 0.04^{b}	0,89 ± 0.04^{b}	0,95 ± 0.06^{b}
Valine	0.80	0,89 ± 0.07^{b}	0,95 ± 0.07^{b}	0,95 ± 0.08^{b}	0,99 ± 0.06^{b}	1,09 ± 0.09^{b}
Isoleucine	1.1	0,80 ± 0.12^{b}	0,68 ± 0.09^{b}	0,71 ± 0.07^{b}	0,80 ± 0.10^{b}	0,78 ± 0.08^{b}
Leucine	0.67	1,69 ± 0.21^{c}	1,79 ± 0.20^{c}	1,87 ± 0.18^{c}	1,92 ± 0.22^{c}	2,01 ± 0.18^{c}
Tyrosine	0.85	0,54 ± 0.12^{ab}	0,47 ± 0.10^{a}	0,57 ± 0.12^{ab}	0,54 ± 0.15^{ab}	0,61 ± 0.13^{ab}
Phenylalanine		0,89 ± 0.05^{b}	0,91 ± 0.07^{b}	0,99 ± 0.08^{b}	0,94 ± 0.04^{b}	1,01 ± 0.07^{b}
Cysteine	0.85	0,25 ± 0.02^{a}	0,27 ± 0.01^{a}	0,29 ± 0.02^{a}	0,31 ± 0.02^{a}	0,33 ± 0.03^{a}
Methionine		0,45 ± 0.04^{a}	0,55 ± 0.06^{a}	0,52 ± 0.07^{a}	0,51 ± 0.04^{a}	0,55 ± 0.07^{a}
Tryptophan	0.20	0,27 ± 0.03^{a}	0,18 ± 0.01^{a}	0,21 ± 0.02^{a}	0,27 ± 0.03^{a}	0,21 ± 0.02^{a}
Lysine	0.66	1,20 ± 0.21^{c}	1,40 ± 0.22^{c}	1,40 ± 0.18^{c}	1,20 ± 0.28^{c}	1,40 ± 0.19^{c}
Σ of exogenous amino acids EAA	**5.59**	**7,70**	**8,07**	**8,37**	**8,37**	**8,94**
Aspartic acid	-	1,05 ± 0.21^{bc}	1,15 ± 0.17^{bc}	1,25 ± 0.23^{bc}	1,25 ± 0.31^{bc}	1,15 ± 0.28^{bc}
Histidine	-	0,56 ± 0.17^{ab}	0,58 ± 0.14^{ab}	0,65 ± 0.12^{ab}	0,66 ± 0.17^{ab}	0,65 ± 0.11^{ab}
Arginine	-	1,46 ± 0.32^{c}	1,50 ± 0.38^{c}	1,5 ± 0.44^{c}	1,46 ± 0.28^{c}	1,5 ± 0.24^{c}
Serine	-	0,55 ± 0.07^{a}	0,45 ± 0.08^{a}	0,45 ± 0.07^{a}	0,55 ± 0.05^{a}	0,45 ± 0.04^{a}
Glutamic acid	-	3,05 ± 0.23^{c}	3,27 ± 0.18^{c}	3,28 ± 0.19^{c}	3,25 ± 0.19^{c}	3,28 ± 0.21^{c}
Proline	-	0,57 ± 0.04^{a}	0,46 ± 0.04^{a}	0,46 ± 0.04^{a}	0,57 ± 0.05^{a}	0,64 ± 0.06^{a}
Glycine	-	0,82 ± 0.08^{b}	0,60 ± 0.09^{ab}	0,60 ± 0.10^{ab}	0,86 ± 0.11^{b}	0,76 ± 0.09^{b}
Alanine	-	0,94 ± 0.09^{b}	1.00 ± 0.08^{b}	1,11 ± 0.12^{b}	0,94 ± 0.10^{b}	1,26 ± 0.13^{b}
Σ NEAA	**-**	**9**	**9,01**	**9,3**	**9,54**	**9,69**
Σ EAA/Σ NEAA	**-**	0,86 ± 0.06^{b}	0,89 ± 0.07^{b}	0,90 ± 0.06^{b}	0,88 ± 0.07^{b}	0,92 ± 0.08^{b}
The letters in the same line show the differences in the results of statistical analysis.						

Table 2: The amino acids compositions of rainbow trout (control and experimental groups, g/100 g muscle).

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 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 [34]. 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. 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

Fatty acid composition, g /100 g	Control group (n =20)	Experimental group			
		1%	2%	3%	4%
C 12:0 (Lauric acid)	0,04 ± 0.03^{a}	0,04 ± 0.02^{a}	0,05 ± 0.02^{a}	0,05 ± 0.02^{a}	0,04 ± 0.02^{a}
C 13:0 (Tridecanoic acid)	0,02 ± 0.02^{a}	0,01 ± 0.04^{a}	0,03 ± 0.02^{a}	0,02 ± 0.02	0,01 ± 0.023^{a}
C 14:0 (Miristic acid)	3,27 ± 0.06^{a}	3,85 ± 0.07^{a}	3,76 ± 0.03^{a}	3,72 ± 0.03^{a}	3,45 ± 0.02^{a}
C 15:0 (Pentadecanoic acid)	0,37 ± 0.03^{a}	0,37 ± 0.05^{a}	0,37 ± 0.04^{a}	0,36 ± 0.05^{a}	0,36 ± 0.03^{a}
C 16:0 (Palmitic acid)	15,99 ± 0.05^{a}	16,16 ± 0.08^{a}	16,05 ± 0.07^{a}	16,22 ± 0.10^{a}	15,34 ± 0.08^{a}
C 17:0 (Heptadecanoic acid)	0,68 ± 0.02^{a}	0,58 ± 0.03^{a}	0,53 ± 0.05^{a}	0,52 ± 0.02^{a}	0,48 ± 0.03^{a}
C 18:0 (Stearic acid)	3,42 ± 0.13^{a}	3,25 ± 0.02^{a}	3,44 ± 0.04^{a}	3,15 ± 0.04^{a}	3,25 ± 0.05^{a}
C 20:0 (Arachidic acid)	0,22 ± 0.03^{a}	0,21 ± 0.02^{a}	0,16 ± 0.02^{a}	0,23 ± 0.02^{a}	0,21 ± 0.02^{a}
C 22:0 (Behenic acid)	1,54 ± 0.14^{a}	1,26 ± 0.05^{a}	1,32 ± 0.05^{a}	1,48 ± 0.05^{a}	1,53 ± 0.04^{a}
C 23:0 (Tricosanoic acid)	0,04 ± 0.02^{a}	0,04 ± 0.06^{a}	0,03 ± 0.03^{a}	0,04 ± 0.02^{a}	0,04 ± 0.02^{a}
C 24:0 (Lignoceric acid)	1,35 ± 0.02^{a}	1,40 ± 0.03^{a}	1,44 ± 0.04^{a}	1,25 ± 0.05^{a}	1,54 ± 0.03^{a}
Total SFAs	**26,94**	**27,17**	**27,18**	**27,04**	**26,25**
Monounsaturated Fatty Acids					
C 14:1 (Myristoleic acid)	0,23 ± 0.06^{a}	0,23 ± 0.04^{a}	0,24 ± 0.03^{a}	0,23 ± 0.03^{a}	0,21 ± 0.02^{a}
C 16:1 (Palmitoleic acid)	5,30 ± 0.02^{a}	5,29 ± 0.08^{a}	5,25 ± 0.05^{a}	5,36 ± 0.04^{a}	5,95 ± 0.02^{a}
C 17:1 (cis 10 –heptadecenoic acid)	0,26 ± 0.06^{a}	0,23 ± 0.02^{a}	0,21 ± 0.02^{a}	0,23 ± 0.02^{a}	0,35 ± 0.03^{a}
C 18:1 n9 (Oleic acid)	20,98 ± 0.09^{ab}	20,27 ± 0.11^{ab}	21,72 ± 0.10^{ab}	21,52 ± 0.07^{ab}	20,08 ± 0.08^{ab}
C 20:1 (cis -11- eicosenoic acid)	2,43 ± 0.02^{a}	2,41 ± 0.05^{a}	2,35 ± 0.04^{a}	2,41 ± 0.03^{a}	2,41 ± 0.02^{a}
C 24:1 (Nervonic acid)	1,46 ± 0.02^{a}	1,61 ± 0.06^{a}	1,52 ± 0.06^{a}	1,53 ± 0.02^{a}	1,48 ± 0.02^{a}
Total MUFAs	**30,66**	**30,04**	**31,29**	**31,28**	**30,48**
Polyunsaturated Fatty Acids					
C 18:2 n6 (Linoleic acid)	11,06 ± 0.02^{a}	11,11 ± 0.02^{a}	11,24 ± 0.03^{a}	11,35 ± 0.02^{a}	11,45 ± 0.03^{a}
C 18:3 n6 (γ-linolenic acid)	0,29 ± 0.03^{b}	0,42 ± 0.02^{b}	0,35 ± 0.02^{b}	0,28 ± 0.02^{b}	0,26 ± 0.02^{b}
C 18:3 n3 (Linolenic acid)	1,86 ± 0.02^{a}	1,99 ± 0.05^{a}	1,89 ± 0.04^{a}	1,94 ± 0.03^{a}	1,86 ± 0.04^{a}
C 20:2 (cis-11,14-eicosadienoic acid)	0,58 ± 0.02^{b}	0,50 ± 0.03^{b}	0,45 ± 0.04^{b}	0,55 ± 0.04^{b}	0,65 ± 0.04^{b}
C 20:3 n6 (cis-8,11,14-eicosatrienoic acid)	0,24 ± 0.07^{a}	0,23 ± 0.07^{a}	0,24 ± 0.07^{a}	0,21 ± 0.06^{a}	0,23 ± 0.07^{a}
C 20:3 n3 (cis-11,14,17-eicosatrienoic acid)	0,58 ± 0.02^{b}	0,58 ± 0.02^{b}	0,51 ± 0.02^{b}	0,56 ± 0.02^{b}	0,58 ± 0.02^{b}
C 20:4 n6 (Arachidonic acid)	0,87 ± 0.02^{a}	0,69 ± 0.07^{a}	0,79 ± 0.07^{a}	0,71 ± 0.06^{a}	0,87 ± 0.07^{a}
C 20:5 n3 (cis-5,8,11,14,17-eicosapentaenoic acid) EPA	3,86 ± 0.02^{a}	4,05 ± 0.03^{a}	4,11 ± 0.03^{a}	4,12 ± 0.03^{a}	4,21 ± 0.03^{a}
C 22:2 (cis 13,16 –docosadienoic acid)	1,05 ± 0.06^{b}	1,05 ± 0.02^{b}	1,02 ± 0.02^{b}	1,02 ± 0.02^{b}	1,06 ± 0.02^{b}
C 22:6 n3 (cis-4,7,10,13,16,19-docosahexaenoic acid) DHA	15,05 ± 0.02^{a}	16,09 ± 0.02^{a}	15,05 ± 0.02^{a}	15,35 ± 0.02^{a}	15,34 ± 0.02^{a}
Total PUFAs	**35,44**	**36,71**	**35,65**	**36,09**	**36,51**
PUFAs/SFAs	1,32 ± 0.02^{a}	1,35 ± 0.02^{a}	1,31 ± 0.05^{a}	1,33 ± 0.05^{a}	1,39 ± 0.03^{a}
Ó n6	12,46 ± 0.07^{a}	12,45 ± 0.06^{a}	12,62 ± 0.03^{a}	12,55 ± 0.07^{a}	12,81 ± 0.08^{a}
Ó n3	21,35 ± 0.05^{b}	22,71 ± 0.04^{b}	21,56 ± 0.05^{b}	21,97 ± 0.05^{b}	21,99 ± 0.03^{b}
n6/n3	0,58 ± 0.02^{a}	0,55 ± 0.02^{a}	0,59 ± 0.05^{a}	0,57 ± 0.05^{a}	0,58 ± 0.03^{a}
DHA/EPA	3,90 ± 0.08^{a}	3,97 ± 0.08^{a}	3,66 ± 0.07^{a}	3,73 ± 0.05^{a}	3,64 ± 0.06^{a}
Unidentified	6,96 ± 0.02^{a}	6,08 ± 0.02^{a}	5,88 ± 0.03^{a}	5,59 ± 0.02^{a}	6,76 ± 0.04^{a}
The letters in the same line show the differences in the results of statistical analysis.					

Table 3: The fatty acids compositions of rainbow trout (control and experimental groups, % of total fatty acid).

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 (Figure 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 endomysium.

Figure 2: Transversely striated muscle tissue.

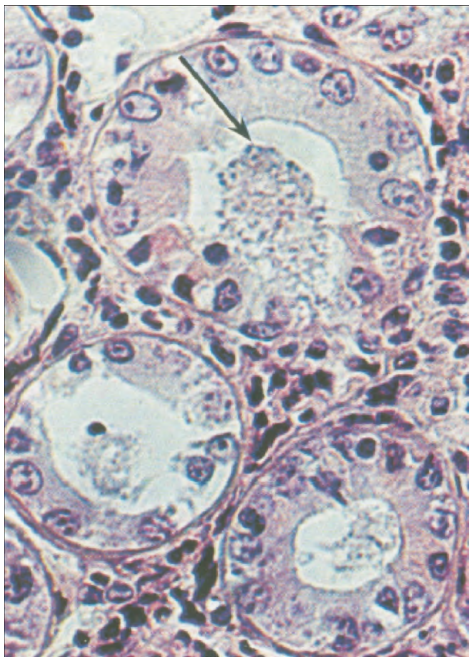

Figure 3: Renal tubules of the kidney of a rainbow trout (the arrow indicates the accumulation of eosinophil protein mass in the lumen of the tubules after hematoxylin and eosin (H&E) staining).

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 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 [35]. Histological investigation (Figure 3) shows that in the kidney of the fish fed with 4% zeolite 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 [36]. Also, Gonzalez et al. [37] 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. [38] 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 [39]: 76.23, 18.57, 3.71 and 1.47%, for moisture, protein, fat and ash, respectively.

Study of amino acid content

The quality of the fish protein evaluated on the basis of the amino acids contained in it. The most important amino acids are exogenous amino acids, which are indispensible for the human body. The amino acid content (g/100 g amino acid) in the rainbow trout from the control and experimental groups has been analyzed, and the results are shown in Table 2. In this study, some amino acids exhibited a significant difference of content between the groups. Differences in the types and amounts of amino acids in fish tissues have been attributed to the location, size of fish, age, food, reproductive status and season [40-43].

The most exogenous amino acids were found in the rainbow trout fed with 4% zeolite feed – 8.94 g of these amino acids were found in 100 g of the muscle tissue. The daily demand for the amino acids required for an adult human weighing 70 kg is equal to 5.59 g [44]. The results of the studies indicated that 100 g of the fish muscle tissue orom each experimental group covered the ingredient's daily demand of an adult human.

A glutamic acid (containing 3.25-3.28%) was present in the highest amount among the amino acids measured in all the experimental groups. Similar results for the glutamic acid was reported in the rainbow trout (O. mykiss), an Atlantic salmon (Salmo salar), a channel catfish (Ictalurus punctatus) [45], Oreochromis niloticus, Tilapia zilli, Sarotherodon galileaus, Clarias anguillaris, Clarias gariepinus and Heterobranchus longifilis [46], and a Beluga Sturgeon (Huso huso) [47]. Glutamine from muscles serves as an important carrier of ammonia (nitrogen) to the immune system [48].

The most important amino acids in terms of nutritional value that cannot be synthesized by the human body and must be supplied in diet are: lysine, methionine, and cysteine. Histidine is another valuable amino acid for the human body and belongs to the group of relatively exogenous amino acids that are produced in a human body. However under certain conditions, e.g. a rapid growth or a disease, its amount is insufficient, and must be supplied in diet.

A particularly advantageous increase of the content was noted for lysine, methionine, and cysteine in the group of the fish fed with the addition of zeolite. The highest content was noted for the group of the fish fed with the addition of 4% zeolite.

In this study, the content of the essential amino acids in the rainbow trout fed with RGM-2M enriched with zeolite was higher (8.07–8.94%) than the content in the control group (7.7%) (Table 2). The non-essential amino acid content was also higher in the rainbow trout from the experimental groups (9.01–9.69%) than in the trout from the control group (9.00%) (Table 2).

These content levels were the evidence that the rainbow trout fed with RGM-2M/zeolite were a very good source of the amino acids. It was significant to note that the rainbow trout contained a broad variety of the amino acids and their isomers, as well as a particularly high proportion of EAA. There were higher levels of the amino acids in the experimental fish than in the control group.

The most significant increases in the experimental groups in comparison to the control group were observed for the leucine and the lysine. The significant decreases were observed for the tryptophan and the glycine.

The proportion of those essential amino acids to the non-essential amino acids was greater for the experimental group (0.89–0.92) than the control group (0.85). In the study, this proportion was greater than it had been stated in other works: it was 0.78 for Huso huso [47], 0.77 for a sea bream (Pagrus major), 0.77 for a mackerel (Scomber japonicus), 0.71 for a mullet (Mugil cephalus), 0.69 for a sardine (Sardina melonosticta), 0.74 for a herring (Clupea pallasi), and 0.75 for a chum salmon (Oncorhynchus keta). Dezhabad et al. [49] had reported that the proportion of the essential amino acids to the non-essential amino acids for the three species: Rutilus frisii, Hypophthalmicthys molitri and Oncorhynchus mykiss, ranged from 1.03 to 1.19.

Study of the fatty acid content

It is known that the fish meat does not contain significant amounts of the lipids. However, the study of its fatty acid content is of significant interest, not from the perspective of determining its biological value, but as the indicator which points at cell abnormalities in biochemical processes. It was found that the zeolite increased mainly the level of polyunsaturated fatty acids in the fish meat.

The biochemical composition may be affected by the species of the fish, environmental factors, size, age, and diet [41-43,50-52]. The fish can be a source of essential fatty acids [53]. In this study, FA contents (% of total FAs) in the rainbow trout fed with RGM-2M with zeolite from the experimental and control groups were given in Table 3.

After analysing of the fatty acids it was found that there were higher and lower levels of the fatty acids in the experimental fish than in the control group.

The FA contents of the fish in the control and experimental groups ranged from 26.25% to 27.37% of the saturated fatty acids (SFAs), 30.04–31.29% of monounsaturated acids (MUFAs) and 35.44–36.71% of PUFAs.

The lipids in the fatty muscle tissue of the trout fed with 2 and 3% zeolite feed contained the most saturated fatty acids. The majority of monoene acids were contained in the lipids of the muscle tissue of the fish fed with feed with the addition of 2 and 3% zeolite. The level of SFAs was comparable, and MUFAs was significantly lower than the level observed by Łuczyńska [54]. Most n-6 polyene fatty acids were noted in the muscle tissue of the fish fed with the 1 % zeolite feed. However this content was higher in all the experimental groups in comparison to the control group. Among them, those present in the highest content in the experimental group of the fish were C18:1n9, an oleic acid (OLA, 20,08–21,72 %), C16:0, a palmitic acid (PAA, 15.34–16.22%), DHA (15.05–16.09%), C18:2 a linoleic acid (LIA 11.06–11.45%), a palmitoleic acid (PLA 5.30–5.69%), C16:1 EPA (3.86–4.21%), a stearic acid (STA 3.15–3.44%), and C14:0, and a myristic acid (MYA, 3.27–3.85%).

Epidemiological studies showed that an n-3 fatty acid intake is inversely related to cancer, cardiovascular diseases [55], psychiatric disorders [56], asthma [57], bone mineral density [58] and type 2 diabetes [59]. Because of this fact, the polyunsaturated fatty acids (PUFAs) should be separated into the n-3 and n-6 fatty acids. Although the n-3 and n-6 PUFA levels in the two experimental groups (1% and 3% zeolite) were higher than in the control group, the difference was statistically significant.

The muscle tissue of the trout fed with 4% zeolite feed was characterized as the richest source of EPA. The linoleic acid was dominant in the group of n-6 fatty acids, and DHA and EPA were dominant in the n-3 group. Other researchers have made similar observations [54,60,61].

The proportions of FAs-n3 (21.35%; 21.56–22.71% control and experimental groups) were generally higher than those of FAs-n6

(12.46%; 12.45–12.81%). The UK Department of Health recommends an ideal n6/n3 ratio of 4.0 at maximum [62]. Values higher than the maximum value are harmful to health and may promote cardiovascular diseases [63]. In this study, the n6/n3 ratio was found to be 0.55–0.59 in all the experimental groups.

The recommended minimum value of the PUFAs/SFAs ratio is 0.45 [62], which is lower than the values of 1.32 and 1.31–1.39 from the control group and the experimental groups treated with RGM-2M and additives. DHA/EPA ratio ranged from 0.72 to 6.89 in some fresh water fish species [64] and it was equal to 1.56 in the rainbow trout [65].

In this study, the ratio of DHA/EPA in the rainbow trout fed with RGM-2M enriched in 1% zeolite was found to be 3.97 and was greater than in the control group (3.90). In other groups, this ratio was lower and amounted to 3.64–3.73.

On the basis of the conducted analysis no single correlation could be found between the content of individual fatty acids and the percentage of zeolite's addition to the feed. Undoubtedly, the addition of the zeolite had an influence on the profile of fatty acids in lipids in the muscle tissue of rainbow trout, and it also increased the content of n-3 and n-6 polyene fatty acids advantageously.

Conclusion

The nutritive value of the fish and its physiological role as a source of bioactive substances for humans is dependent on the proportions of proteins, fats and mineral substances, the study of the chemical composition of the fish meat is an important part of the veterinary-sanitary examination The addition of various amounts of the additive to the fish feed does not only improve the aesthetics of the outer appearance of fish products but also increases their storage life, as well as the content of vitamins, mineral elements, and food materials.

The results of this study confirm that the zeolites have a positive effect on the chemical composition and physical and biochemical features of the meat. A negative effect of clinoptilolite hasn't been determined. From a practical point of view, the rainbow trout (*O. mykiss*) fed on RGM-2M with up to 4% zeolite contains a higher amount of essential amino acids desirable in a daily diet. This study shows the increase in the level of the polyunsaturated fatty acids. Moreover, using the zeolites as the feed additive for the fish can be a significant part of a comprehensive program to control the fish meat quality.

The introduction of the natural zeolites from the Chankanay deposit into the diet of the fish in the amount of 1–4 % weight of the diet does not cause pathological changes in the liver, muscles and other organs of the fish in the experimental group. Therefore, it has no negative effects on the proteolytic enzyme systems of the fish or on breeding. Our studies show that zeolites are a valuable mineral feed additive of natural origin that promotes the production of the fish meat both qualitatively and quantitatively.

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