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Dynamics of fatty acid composition in the muscles of crucian carp and pike under the influence of elevated concentrations of cobalt ions

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In today's conditions of growing anthropogenic pressure on aquatic ecosystems, the problem of heavy metal accumulation, including cobalt ions, is becoming particularly relevant. Cobalt ions can enter aquatic environments as a result of mining activities, metallurgy, and the production of batteries, dyes, catalysts, and magnetic materials. It is collectively may lead to local exceedances of permissible concentration limits in freshwater ecosystems. Despite the fact that cobalt is a biogenic element in low concentrations, its excess has a toxic effect on hydrobionts, in particular affecting lipid homeostasis. One of the sensitive indicators of metabolic disorders in fish is a change in the fatty acid composition of tissues, which can be used as a bioindicator of toxic pressure and the functional state of the organism. The paper analyses changes in the fatty acid composition of the muscle tissue of crucian carp (*Carassius gibelio* Bloch) and pike (*Esox lucius* L.) under the influence of cobalt ions at concentrations of 0.1 mg/dm³ and 0.25 mg/dm³ over a period of 14 days. These concentrations correspond to 2 and 5 maximum permissible concentrations. Total lipids were extracted and transesterified to obtain fatty acid methyl esters, which were then analyzed by gas chromatography for the quantitative determination of individual fatty acids. In crucian carp, the influence of cobalt ions led to significant changes in the composition of essential fatty acids. In particular, the fractions of saturated myristic acid (14:0) and long-chain monounsaturated eicosenoic acid (20:1) increased proportionally to the concentration of the investigated metal, while the levels of stearic acid (18:0) and oleic acid (18:1) decreased. A more systematic effect was observed in pike muscles: the content of many saturated fatty acid fractions decreased compared to the control group, while changes in the proportions of key polyunsaturated fatty acids likely indicate impaired desaturase activity. In the pike organism, a more systemic effect of cobalt ions was observed, which consisted in a tendency to decrease the amount of most saturated fatty acids. Some nutritional indices for assessing fatty acids were also analyzed.

Key words: fishes, fatty acids, defense system, physiological response, regulation, adaptive reactions, biomonitoring, toxic pollution, cobalt

Introduction

Heavy metal contamination of aquatic ecosystems is recognized as one of the most significant environmental concerns in contemporary ecological research. The growth of industrial activity, wastewater discharge and the accumulation of man-made waste lead to increased concentrations of these elements. This poses a significant threat to the environment due to the high potential of metals for bioaccumulation and biomagnification in living organisms [15, 24].

Fish are constantly exposed to heavy metals in the aquatic environment, which explains their widespread use as model organisms in ecotoxicological studies [22, 40]. The accumulation and toxicity of heavy metals in fish organisms are multidirectional and often lead to physiological and biochemical changes, such as excessive formation of reactive oxygen species (ROS), enzyme dysfunction, and imbalance of redox reactions. This usually leads to structural damage to lipids, especially unsaturated fatty acids [32]. This may frequently result in the degradation of fatty acid molecules, which are essential for the structural and functional integrity of fish biological membranes and significantly contribute to their nutritional value in the human diet [12, 27].

Fish is distinguished by its valuable nutritional composition, primarily due to the presence of high-quality higher fatty carboxylic acids fatty acids, among which long-chain omega-3 (ω−3) fatty acids are of particular importance [36, 46]. The lipid composition of fish muscle tissue differs from that of mammals in terms of the number of double bonds. [27]. Omega-6 (ω−6) and omega-3 (ω−3) are important metabolic precursors of long-chain polyunsaturated fatty acids (C 20–24), such as arachidonic acid (20:4), eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6), which are mainly found in fish with a high fat content [2, 21, 45].

Changes in the composition of fatty acids can indicate not only the quality of fish products, but also the level of toxicant contamination of freshwater environments [27]. Lipids and individual fatty acids are responsible for the permeability, fluidity and integrity of biological cell membranes [3, 39]. They are precursors of bioactive and signaling molecules responsible for regulating biochemical reactions in cells [7]. Eicosapentaenoic acid is a precursor of eicosanoids, which have a wide range of physiological effects and play a role in immune and inflammatory responses, nervous system functions, and reproduction [37]. Eicosanoids also help fish adapt to environmental stressors [27].

The presence of pollutants such as heavy metals can significantly affect the fatty acid profile in fish tissues due to changes in the relative amounts of individual fatty acids [14, 41, 43]. Changes in lipid metabolism and fatty acid profiles were used to better understand how metals affect aquatic organisms in food chains, as well as the integrative biochemical response to the impact of pollutants and their accumulation in aquatic organisms [31]. This makes them promising biomarkers for assessing the impact of pollutants. Most previous studies have focused exclusively on metal accumulation or on analyzing the fatty acid composition in fish [25, 29, 35, 50].

Considering that the lipid content in fish muscle tissue is influenced by species-specific characteristics, the ecological status of the water body, and the presence of toxic agents, it is important to investigate alterations in the fatty acid composition of muscle lipids caused by metal ions in general, with particular attention to cobalt. Cobalt is a technically important trace element with wide applications across various industries and technologies, including its use in high-temperature alloys for jet engines and in improving the efficiency of magnetic materials and rechargeable batteries. This metal is rarely present in freshwater environments at elevated levels, except in cases where mining, manufacturing, or other anthropogenic activities cause local increases in cobalt concentrations [44]. Although cobalt is an important element contained in vitamin B_{12}, only trace amounts are needed for life, and an increase in its content can lead to toxic effects on aquatic biota [5].

Materials and Methods

Experimental studies were conducted on two-year-old crucian carp (*Carassius gibelio* Bloch) and pike (*Esox lucius* L.), with average body weights of 200–220 g and 150–170 g, respectively. The effects of two elevated concentrations of cobalt ions, 0.1 and 0.25 mg/dm³, corresponding to 2 and 5 times the maximum permissible concentrations, were investigated. Metal in the form of $CoCl_2 \cdot 6H_2O$ was added to the water in 200-litre aquariums containing test groups of fish (five individuals in each). To limit the influence of fish exometabolites on their physiology, the water in each aquarium was replaced every two days. To ensure the full development and maximal functional expression of compensatory-adaptive responses to the metal, fish were acclimatized over a 14-day period. According to the author [34], this period is sufficient for the formation of adaptive responses in the bodies of exothermic animals. The control group consisted of fish tissue samples taken from aquariums without added cobalt chloride. The fish were not fed during the research. The experiments were conducted in accordance with the rules of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes [17] and the principles of animal research ethics [12].

To determinate the content of lipids and fatty acids, the muscles were ground in cold glass homogenizers, followed by extraction of total lipids from the tissue with a chloroform-methanol mixture in a 2:1 ratio using the Folch method [20]. At the same time, 20 parts of the extracting mixture were added to one mass part of the tissue and left for 12 hours for extraction. Non-lipid impurities were removed from the extract by washing with a 1 % KCl solution. Total lipids were obtained after distillation of the extract mixture [26]. The dried samples were dissolved in 1 ml of hexane and used for further analysis of fatty acids.

Fatty acid methyl esters (FAME) were obtained from total lipids by acid-catalyzed transesterification [11]. 100 mg of extracted lipid was dissolved in 1.5 ml of dichloromethane and 3 ml of methanol-sulphuric acid solution (200:3 by volume) and shaken vigorously. The reaction mixture was heated at 100 °C for approximately 1 hour and then cooled to room temperature. Then, 3 ml of hexane and 1 ml of distilled water were added to the mixture. After mixing and layering, the entire upper phase was collected. One gram of anhydrous sodium sulphate was added to the collected sample and then left for 24 hours to allow separation. After filtration, the sample was evaporated until its mass became constant under a stream of nitrogen. Heptane was added to the sample to achieve a final concentration of 50 mg/dm^3.

FAME analysis was performed using a gas chromatograph (*Agilent6890*, California, USA) equipped with a BPX70 fused silica capillary column (length 50 m; inner diameter 0.22 mm; film thickness 0.25 μm) and a flame ionization detector (FID). Nitrogen was used as the carrier gas, and the split ratio was set at 1:100.

The temperatures for the injection port and detector were set at 270 °C and 300 °C, respectively. The mass spectrum data were processed using *STAR-GC3800* software. Fatty acids were identified by comparing their retention times with a mixture of standards (*NU-CHEK PREP, Inc.*). Each fatty acid was quantified (%) by calculating its peak and area relative to the total peak area.

The results of the studies were statistically processed using Student's t-test and R software to determine the significant difference between the experimental and control groups.

Research Results and Discussion

Fish tissues are characterized by a significant variety of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) [23]. As a result of experimental research, we identified 13 fatty acids in the muscle lipids of crucian carp and pike, including five SFA, three MUFA and five PUFA (table).

In the muscles of crucian carp, under the action of both concentrations of cobalt ions among SFA, some increase in the content of myristic acid (14:0) by 35.6 % and 33.1 % and a decrease in stearic acid (18:0) by 34.9 % and 33.3 % relative to the control values were found. This probably indicates that the elongation stages (which normally convert C14:0 to C18:0) were disrupted, leading to the accumulation of shorter SFA. Heavy metals such as cobalt can affect the activity of enzymes that synthesize lipids. In particular, it has been shown that cobalt exposure alters the activity of desaturase and elongase in fish [10, 18]. Therefore, cobalt ions can influence the balance between the synthesis of saturated and unsaturated fatty acids.

Analysis of SFA in pike muscles indicated some decrease in lauric acid (12:0) content by 14.2 % and arachidic acid (20:0) content by 25.4 % under the influence of 0.1 mg/dm^3 cobalt ions, while under the influence of 0.25 mg/dm^3 of metal, the proportions of all saturated fatty acids decreased: lauric (12:0) by 21.3 %, myristic (14:0) by 10.4 %, palmitic (16:0) by 5 %, stearic (18:0) by 4.2 %, and arachidic (20:0) by 20.8 %. Under the action of maximum cobalt ion concentration, the total content of all detected SFAs decreased by 5.4 % compared to the control group. Various fish species have different lipid metabolisms and therefore react differently to toxins. In particular, due to biomagnification, predatory fish can accumulate larger amounts of heavy metal ions compared to species at lower trophic levels [24]. Under the influence of cobalt ions, pike showed a significant decrease in the amount of virtually all measured fatty acids, which indicates their intensive mobilization or breakdown. In fish, cobalt ions contribute to the generation of active forms of oxygen, among which the hydroxyl radical (·OH) plays a leading role, capable of initiating peroxide oxidation of unsaturated fatty acids [24, 32].

Differences in the content of MUFA were found in fish muscles: in crucian carp, their total amount in the control group was 37.6 %, and in pike — 13.5 %. As a result of exposing fish to cobalt ions at a concentration of 0.25 mg/dm^3, a decrease in the content of essential important monounsaturated fatty acids in fish muscles was observed oleic acid (18:1) by 20.1 % in crucian carp and palmitoleic acid (16:1) by 9.1 % in pike. At the same time, an increase in the concentration of eicosenoic acid (20:1) was found in both species of fish studied — by 46.2 % under the action of 0.25 mg/dm^3 of cobalt ions in crucian carp, and by 19.1 % and 23.6 % under the influence of both concentrations of metal in pike. An increase in the proportion of eicosenoic acid (20:1) can alter the fluidity of cell membranes (making them more rigid), which potentially affects the transport of substances, the activity of membrane enzymes and receptor function. This corresponds to the phenomenon of cellular adaptation to stressful conditions — homeoviscous adaptation, when a change in lipid composition ensures the preservation of the physicochemical properties of membranes under the influence of toxicants [16, 49]. The oleic acid (18:1) content in crucian carp muscles was the highest, and its decrease under the action of 0.25 mg/dm^3 cobalt ions led to a decrease in the total amount of MUFA in this group of fish. In addition, the decrease in oleic acid (18:1) levels is likely to be the result of its active use in the process of lipid restructuring and cell membrane restoration. Similar metabolic reactions are observed in stress response models, where short MUFA are converted or directed into PUFA synthesis to maintain the physicochemical stability of membranes [47]. Fish under stress often remodel membrane lipids to maintain cell function [19].

Unlike saturated and monounsaturated fatty acids, the content of PUFA in crucian carp muscles increased by 23.2 % under the influence of 0.25 mg/dm^3 cobalt ions. The highest concentrations of the studied metal caused the biggest changes in the content of docosahexaenoic acid (DHA) (22:6), which is an omega-3 fatty acid. Thus, its percentage increased by 29.2 % and 78.8 % under the action of 0.1 and 0.25 mg/dm^3 of cobalt ions. Elevated DHA levels may help stabilize cell membranes under the influence of heavy metals or modulate anti-inflammatory signals. This effect is likely a compensatory adaptation: increased DHA helps maintain fluidity and integrity of membranes under the influence of toxic metal [18, 28, 41].

Table. The content of fatty acids in fish muscles under the influence of cobalt ions, % (M±m, n=5)

Fatty acids	Crucian carp			Pike		
	Control	0.1 mg/dm³	0.25 mg/dm³	Control	0.1 mg/dm³	0.25 mg/dm³
C 12:0	0,15±0,01	0,14±0,01	0,17±0,01	0,49±0,01	0,42±0,02*	0,38±0,02*
C 14:0	2,34±0,09	3,18±0,23*	3,12 ± 0,18*	0,55±0,02	0,56±0,02	0,49±0,01*
C 16:0	16,27±0,63	17,33±1,56	16,11±0,72	16,87±0,20	16,62±0,36	16,02±0,24*
C 18:0	8,09±0,63	5,27±0,52*	5,40±0,53*	4,92±0,07	4,93±0,15	4,71±0,04*
C 20:0	0,20±0,02	0,22±0,02	0,20±0,02	0,047±0,002	0,035±0,003*	0,037±0,002*
∑ SFA	27,05±1,10	26,14±1,45	24,99±0,35	22,87±0,15	22,56±0,42	21,65±0,29*
C 16:1	9,65±0,66	11,02±1,05	8,58±0,65	2,77±0,08	2,63±0,11	2,52±0,04*
C 18:1	25,00±1,36	23,28±1,86	19,97±0,66*	10,20±0,35	10,49±0,34	10,47±0,49
C 20:1	2,93±0,24	3,08±0,19	4,28±0,26*	0,51±0,02	0,60±0,02*	0,63±0,03*
∑ MUFA	37,58±1,43	37,38±0,89	32,83±0,82*	13,48±0,41	13,72±0,38	13,62±0,53
C 18:2	7,41±0,50	7,42±0,58	7,14±0,69	5,26 ± 0,15	5,88±0,48	7,07±0,38*
C 18:3	2,84±0,30	2,61±0,29	2,94±0,24	2,27±0,07	2,23±0,18	2,39±0,07
C 20:4	4,81±0,34	4,40±0,36	5,26±0,48	8,47±0,11	9,12±0,20*	9,11±0,21*
C 20:5	3,32±0,32	2,69±0,21	2,96±0,30	5,43 ± 0,14	4,76±0,16*	4,39±0,10*
C 22:6	7,86±0,55	10,16±0,33*	14,02±0,93*	32,17±0,92	31,79 ± 1,13	31,66±1,35
∑ PUFA	26,23 0,76	27,29±1,17	32,31±0,74*	53,60 ± 0,99	53,78±1,50	54,62±1,30
Other	9,14±0,27	9,20±0,13	9,87±0,03	10,05±0,97	9,94±1,18	10,12±1,03

Note. * — difference from control is confident ($P<0,05$); SFA — saturated fatty acids, MUFA — monounsaturated fatty acids, PUFA — polyunsaturated fatty acids

In pike muscles, more systematic changes were found in the amount of other important PUFAs. In particular, this increase in the proportion of linoleic acid (18:2) by 34.5 % under the influence of 0.25 mg/dm³ of cobalt ions, arachidonic acid (20:4) by 7.6 % and 7.5 % under the influence of 0.1 and 0.25 mg/dm³ of cobalt ions, and a decrease in eicosapentaenoic acid (20:5) by 12.4 % and 19.1 % under the influence of both concentrations of the studied metal relative to the control values. Increased concentrations of linoleic (C18:2) and arachidonic (C20:4) fatty acids in tissues may indicate activation of pro-inflammatory pathways: linoleic acid is a precursor of arachidonic acid [48], which in turn is a substrate for the synthesis of pro-inflammatory eicosanoid [1, 8]. The augmented production of these mediators reflects a heightened immune response triggered by toxic or damaging influences. In fish, some highly unsaturated fatty acids with more than three double bonds, such as arachidonic acid (20:4), eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6), are considered essential [38]. These PUFAs are of crucial importance for the maintenance of vital activity and the normal physiological functioning of aquatic organisms. For example, eicosapentaenoic acid (20:5) is an excellent source of energy and a precursor to eicosanoids, which regulate inflammation and immune responses [6, 28].

Important indicators of environmental changes are nutritional indices for assessing fatty acids [9]. In particular, at the maximum concentration of cobalt ions in the muscles of crucian carp, an increase in the PUFA/SFA ratio of 32.5 % was found, indicating a shift in the balance towards higher unsaturation and, probably, a compensatory reaction to maintain membrane fluidity and cell stress signaling: membranes become more fluid and dynamic, oxidative damage is buffered, and the balance of lipid mediators shifts, which contributes to the survival of fish under the influence of toxic substances [10, 18]. The same trend towards an increase in the PUFA/SFA ratio was observed in pike muscles exposed to 0.25 mg/dm³ of cobalt ions. However, a number of other publications indicate the opposite — metal-induced lipid peroxidation breaks the double bonds of PUFAs, leading to a decrease in PUFA content in fish muscles [24, 27, 33]. Part of the adaptive response of the crucian carp organism may also be an increase in the unsaturation index by 22.2 % under the action of 0.25 mg/dm³ of cobalt ions, indicating a high degree of general unsaturation [9].

The increase in Fish Lipid Quality (FLQ) in crucian carp muscles by 15.0 % and 51.9 % at both studied concentrations of cobalt ions is an atypical result, as this metal in high concentrations is usually considered toxic to fish and can cause disturbances in lipid metabolism and overall health, which typically leads to a decrease rather than an increase in this nutritional index [4, 27, 30].

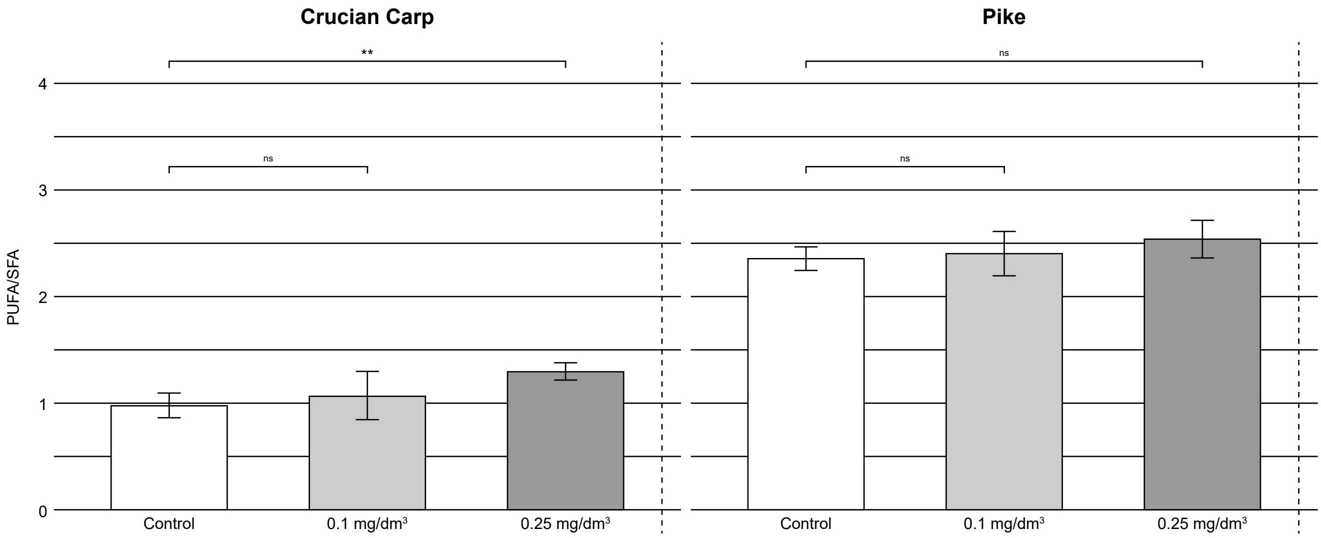

Fig. 1. The ratio of PUFA/SFA in fish muscles under the influence of cobalt ions, % (M±m, n=5)

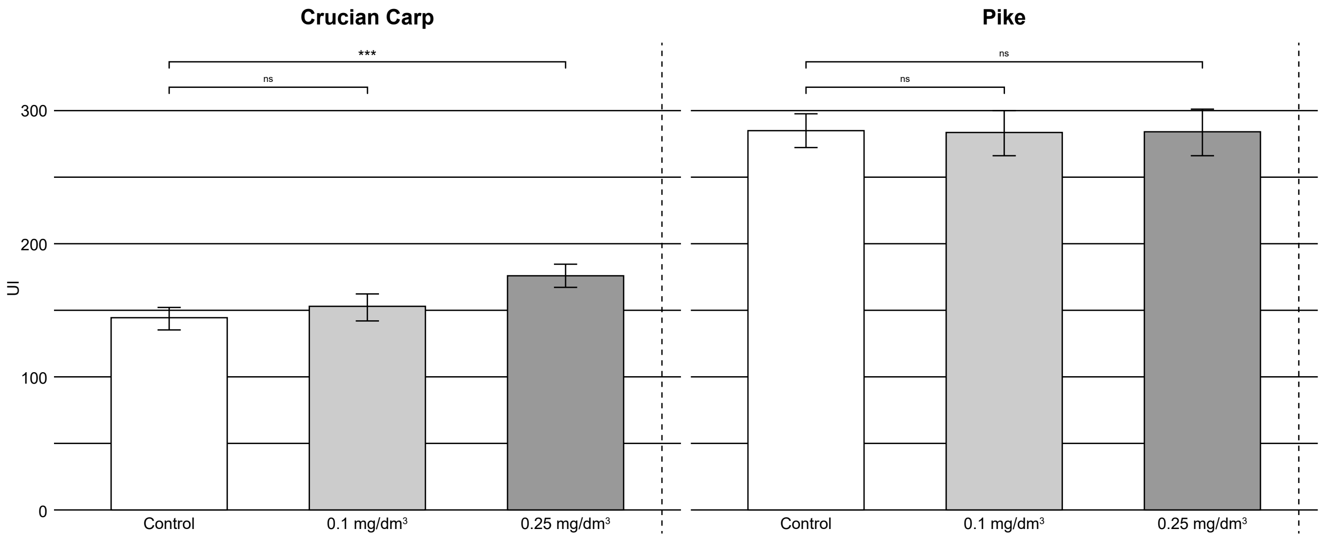

Fig. 2. The unsaturation index (UI) in fish muscles under the influence of cobalt ions, % (M±m, n=5)

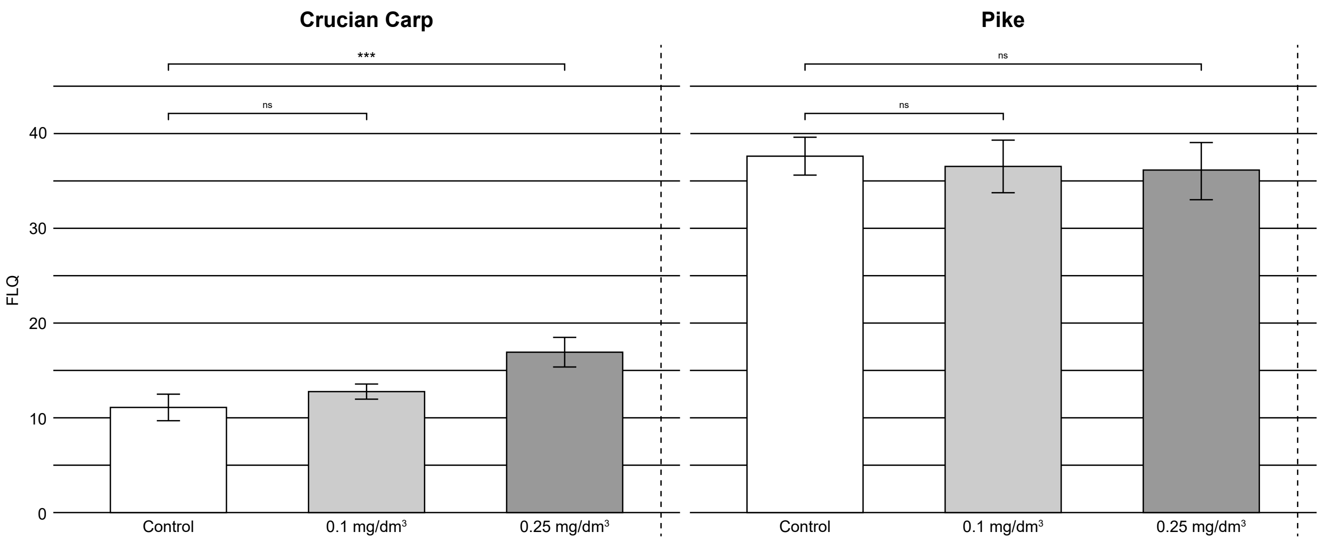

Fig. 3. The Fish Lipid Quality index (FLQ) in fish muscles under the influence of cobalt ions, % (M±m, n=5)

Therefore, it was established that the composition of fatty acids in fish muscles under the influence of elevated concentrations of cobalt ions in water is primarily determined by the concentration of the metal and the type of fish. In crucian carp, the influence of cobalt ions led to an increase in the proportions of myristic (14:0) and eicosenoic (20:1) acids, while the levels of stearic (18:0) and oleic (18:1) acids decreased. A more systemic response was observed in the pike's body: the content of many SFAs decreased compared to the control group, and multidirectional changes in the proportions of key PUFAs likely indicate a disruption in desaturase activity. It should be noted that highly specialized and finely regulated adaptive mechanisms are engaged to preserve the optimal fluidity of cellular membranes, thereby ensuring their structural integrity and functional performance in response to environmental fluctuations in aquatic habitats.

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Динаміка складу жирних кислот у м’язах карася та щуки під впливом підвищених концентрацій іонів кобальту

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У сучасних умовах зростаючого антропогенного навантаження на водні екосистеми проблема накопичення важких металів, зокрема іонів кобальту, стає особливо актуальною. Іони кобальту можуть потрапляти у водне середовище в результаті гірничодобувної діяльності, металургії, виробництва акумуляторів, барвників, каталізаторів та магнітних матеріалів. У сукупності це може призвести до локальних перевищень допустимих концентрацій у прісноводних екосистемах. Незважаючи на те, що кобальт є біогенним елементом у низьких концентраціях, його надлишок має токсичну дію на гідробіонтів, зокрема впливаючи на ліпідний гомеостаз. Одним із чутливих індикаторів порушень обміну речовин у риб є зміна жирнокислотного складу тканин, що може бути використано як біоіндикатор токсичного впливу та функціонального стану організму. У статті аналізуються зміни жирнокислотного складу м’язової тканини карася сріблястого (*Carassius gibelio* Bloch) та щуки звичайної (*Esox lucius* L.) під впливом іонів кобальту в концентраціях 0,1 мг/дм³ та 0,25 мг/дм³ протягом 14 днів. Ці концентрації відповідають 2 та 5 гранично допустимим концентраціям. Загальні ліпіди екстрагували та перетворювали на метилові ефіри жирних кислот, які потім аналізували методом газової хроматографії для кількісного визначення окремих жирних кислот. У карася вплив іонів кобальту призвів до суттєвих змін у складі основних жирних кислот. Зокрема, частки насиченої міристинової кислоти (14:0) та довголанцюгової мононенасиченої ейкозенової кислоти (20:1) збільшувалися пропорційно концентрації досліджуваного металу, тоді як рівні стеаринової кислоти (18:0) та олеїнової кислоти (18:1) знижувалися. В організмі щуки було відмічено більш системний ефект дії іонів кобальту, який полягав у тенденції до зменшення кількості більшості насичених жирних кислот. Також були проаналізовані деякі показники харчування для оцінки жирних кислот харчові індекси для оцінювання жирних кислот.

Ключові слова: риби, жирні кислоти, система захисту, фізіологічна реакція, регуляція, адаптивні реакції, біомоніторинг, токсичне забруднення, кобальт

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