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Article

Influence of chronic intake of small doses of mercury on some biochemical parameters of lipid and protein metabolism in goldfish *Carassius auratus* (L., 1758)

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Abstract. The effect of mercury, supplied with food for three months, was studied on some biochemical parameters (concentration of total protein, total cholesterol, and high-density lipoproteins) of the blood serum of goldfish *Carassius auratus* (L., 1758). The content of mercury in the muscles of the fish that consumed the food with a high content of mercury ("HM") increased by 5.8, 10.4, and 11.7 times, with a low content ("LM"), by 1.4, 3.2, and 3.2 times after 1, 2, and 3 months, respectively ($p < 0.05$). Accumulation of mercury resulted in increasing of all the studied parameters; the total cholesterol concentration increased to the maximum in the fish of the experimental group by the end of the experiment (by 3.1 times). At the same time, there was a significant positive relationship between the biochemical parameters of blood and the mercury content in the fish muscles. The obtained results indicated significant changes in the lipid and protein metabolism of fish under the influence of mercury, their intensity depended on the amount of mercury in the feed and the exposure time.

Keywords: fish, heavy metals, blood serum, total protein, total cholesterol, high-density lipoproteins

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Научная статья

Влияние хронического поступления малых доз ртути на некоторые биохимические показатели липидного и белкового обмена у серебряного карася *Carassius auratus* (L., 1758)

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Аннотация. Изучено влияние ртути, поступавшей с кормом в течение трех месяцев, на некоторые биохимические показатели сыворотки крови серебряного карася *Carassius auratus* (L., 1758): концентрацию общего белка, общего холестерина и липопротеинов высокой плотности. Содержание ртути в мышцах рыб группы, потреблявшей корм с повышенным содержанием ртути («ВР»), возрастало в 5.8, 10.4 и 11.7 раза, с пониженным содержанием («НР») – в 1.4, 3.2 и 3.2 раза через 1, 2 и 3 месяца соответственно ($p < 0.05$). Накопление ртути приводило к возрастанию всех изученных показателей; максимально увеличивался уровень общего холестерина у рыб опытной группы к концу эксперимента (в 3.1 раза). При этом показана достоверная положительная связь биохимических показателей крови с содержанием ртути в мышцах рыб. Полученные результаты свидетельствуют о возникновении изменений в липидном и белковом метаболизме рыб под воздействием ртути, степень выраженности которых зависит от количества ртути в корме и длительности эксперимента.

Ключевые слова: рыбы, тяжелые металлы, сыворотка крови, общий белок, общий холестерин, липопротеины высокой плотности

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Introduction

Studies of the effect of toxicants of various nature on the biota of terrestrial and aquatic ecosystems are carried out at various levels of wildlife organization, including biochemical and molecular ones (Nemova, 2005). Heavy metals are highly toxic to most living organisms, so they are considered the most dangerous pollutants in the aquatic environment, since these compounds do not naturally decompose and accumulate in bottom sediments and aquatic organisms. Among the group of heavy metals, mercury occupies a special place due to its high toxicity, variety of forms, and increased ability to transfer via biological pathways in the environment. Mercury vapor from both natural and anthropogenic sources is globally distributed in the atmosphere. As precipitation reaches land and water surfaces, mercury enters the oceans and fresh continental water bodies and may migrate over long distances (Clarkson, 2002; Fitzgerald et al., 1998).

In aquatic ecosystems, sulphate-reducing bacteria in bottom sediments convert inorganic mercury into the more hazardous organic form, methylmercury (Clarkson, 2002; Gilmour et al., 1992; Schafer et al., 2010; Zhu et al., 2018; etc.), the concentration of which in the edible tissues of fish may reach 50–98% of the total amount of mercury compounds (Bloom, 1992; Carrasco et al., 2011). The ratio of concentrations of methylmercury and inorganic mercury compounds varies greatly in different organs of fish. In particular, the muscle tissue may contain up to 80–100% methylmercury of the total mercury content, while its content in the liver may be significantly lower due to increased share of inorganic compounds of this element (Amlund et al., 2007; Batchelar et al., 2013; Olsvik et al., 2021).

Mercury enters the body of fish via gills, digestive system, and skin (to a lesser extent). The metal penetrates through the epithelium into the bloodstream and binds to plasma proteins, then it is transported with the blood flow to all tissues, where it enters the cells via cell membrane (Erickson et al., 2008). Mercury compounds cause stunted fish growth, endocrine disruption, reduced spawning success, immune suppression; they damage liver and kidneys (Klaper et al., 2008; Morcillo et al., 2017), cardiovascular system (Monteiro et al., 2013), nervous system (Berntssen et al., 2003), deteriorate hatching, survival and growth of embryos and larvae (Yu et al., 2019). Molecular mechanisms of negative effects of mercury include several regulatory pathways affecting energy metabolism, oxidative stress, apoptosis, immune response, and lipid metabolism (Morcillo et al., 2017; Nemova, 2005; Olsvik et al., 2021; Wang et al., 2011; Yadetie et al., 2013; etc.). Mercury disrupts intracellular Ca^{2+} metabolism, causing an increase in the calcium concentration in the cell cytosol (Ceccatelli et al., 2010). Due to its ability to bind to thiol groups of proteins, methylmercury may disrupt the conformation of structural and regulatory proteins and inactivate enzyme systems and cell membrane permeability (Ceccatelli et al., 2010; Farina et al., 2011; Nemova, 2005).

Fish consumed by humans is the main, if not the only, source of methylmercury entering in the human body (Toxicological effects of methylmercury, 2000). The main adverse effects of exposure to mercury compounds, including methylmercury, on human health include the risk of cardiovascular disease, neurotoxicity, teratogenicity, nephrotoxicity, and immunotoxicity (Counter, 2002; Farina et al., 2011; Houston, 2011; Ivanova et al., 2021; Ratcliffe et al., 1996; Shuvalova et al., 2021; Sweet and Zelikoff, 2001; Virtanen et al., 2007; etc.). In regions with large water bodies, where a significant part of the population eats fish from them, measures should be taken to develop fish consumption standards (see, for example: Ivanova et al., 2020; Łuczyńska et al., 2017).

The diverse physiological role of tissue and serum proteins, as well as their ability to respond to changes in the intensity and direction of metabolic processes, in particular, anabolism, make it possible to use them as the most important biochemical indicator of the functional state of the body both in normal conditions and under the influence of toxic substances (Chernecky and Berger, 2008). The concentration of total protein in blood serum is an essential integral indicator of protein metabolism, its direction and intensity.

Cholesterol and its transporters (high- and low-density lipoproteins) play an extremely important role in the live organism. It is known that mercury intoxication in humans, in addition to other effects, is accompanied by emergence of cardiovascular diseases: hypertension, coronary heart disease, myocardial infarction, and cardiac arrhythmias, which are due to a decrease in antioxidant protection and increased oxidative stress (Houston, 2011). The content of mercury in the hair of people consuming fish correlates positively with an increased content of mercury in tissues and the level of total cholesterol, triglycerides, and low-density lipoproteins in the blood (Shuvalova et al., 2021; Cho, 2017). High-density lipoproteins (HDL) and their main protein component apolipoprotein A1 (Apo A1) play a protective role in preventing the development of atherosclerosis and related diseases, as they are involved in the reverse transport of cholesterol (Annema and von Eckardstein, 2016; Jomard and Osto, 2019; Verdier et al., 2013; etc.).

In this regard, it seems relevant to assess the biochemical changes that occur in the body of fish in response to chronic exposure to low doses of mercury. The study aims to assess the effects of mercury supplied with food on the content of total water-soluble protein, high-density lipoproteins, and total cholesterol in the blood serum of goldfish.

Materials and methods

The work was carried out in July–October 2021 on goldfish *Carassius auratus* (Linnaeus, 1758), caught in the Barsky pond of the Borok settlement (Nekouzky District, Yaroslavl Oblast, Russia). The age of the fish is 2+...3+, the average weight is 9.8 ± 1.8 g, the average length is 10.6 ± 0.3 cm. Before the experiment started, four groups of fish (16 individuals in each) have been formed, two of which subsequently consumed food with an high content of mercury ("HM") and two, with low one ("LM"). Each group of fish was placed in a 300-L aquarium with constant water flow. Water temperature was set as +20...+22 °C, lighting mode was natural. Feeding the fish with food containing mercury was started the day after they were caught. The experiment continued for three months from the moment the fish were fed for the first time with mercury-containing food.

Goldfish of all groups received food once a day in an amount of 5% of body weight with alternating minced fish and its gelled form; the latter included TetraPondSticks compound feed in addition to minced fish. Minced fish and gelled food were prepared once in sufficient amount for the entire duration of the experiment, frozen at −20 °C in daily portions, and thawed on the day of feeding. Before freezing the food, the mercury content was measured in triplicate in both types. Minced fish for feeding the "HM" group was prepared from the muscles of perch (caught in the Volzhsky Reach of the Rybinsk Reservoir; the mercury content in the finished minced fish was 0.143 mg/kg), for the "LM" group, from pollock muscles (commercial product; mercury content in the finished minced fish 0.019 mg/kg). In the gelled feed, the mercury content was 0.075 and 0.004 mg/kg for the HM and LM groups, respectively. According to the recommendations of the European Commission[1], the content of mercury in fish feed should not exceed a concentration of 0.1 mg/kg. Thus, the content of mercury in the food of the "HM" group may be considered exceeding the recommended value (high), while that of the "LM" group, blow the recommended value (low).

Prior tissue sampling, fish were measured, weighed, and the gonad maturation stage was determined (Sakun and Butskaya, 1968). Blood serum and muscles of goldfish for determining the mercury content of mercury was sampled before the experiment ("zero point") and after 1, 2, and 3 months after the start of feeding. Pieces of white muscles (1–2 g) were cut from both sides of the fish body under the dorsal fin, immediately frozen, and stored at −18 °C. Blood was taken after caudectomy from the tail vessel into Eppendorf-type tubes. The blood was stored at 4 °C for 1–2 hours until a clot was formed naturally. The serum was taken with a dispenser into test tubes and frozen at −18 °C. The total amount of protein in the blood serum was determined by the micro-biuret method (Itzhaki and Gill, 1964). The concentration of total cholesterol (TChol) and high-density lipoprotein (HDL) in blood serum was determined using biochemical kits (Olvex, St. Petersburg, Russia) by the enzymatic colorimetric method (Fishbach and Dunning, 2004).

Mercury content in fish muscle samples was determined on a RA915M atomic absorption spectrometer with a PIRO pyrolytic device (Lumex, St. Petersburg, Russia).

Statistical data processing was performed using the Statistica 6.0 program. The data on the graphs are presented as mean and standard deviation. The data distribution normality was checked by the Shapiro–Wilk test. Since the primary dataset did not have normal distribution, the values of mercury concentration and biochemical parameters of blood serum were logarithmically transformed. These values were then used for the regression analysis to assess the dependence of biochemical parameters on the mercury concentration in fish muscles.

One-way analysis of variance (ANOVA) was applied to determine the effect of accumulated mercury on the indicators of protein and lipid metabolism in fish in regard to the exposure duration. The significance of differences between the "LM" and "HM" groups was calculated using the nonparametric Mann-Whitney test for two independent groups. Differences in all tests used were considered statistically significant at $p < 0.05$.

[1] Commission Directive 2003/100/EC of 31 October 2003. Amending Annex I to Directive 2002/32/ ECof the European Parliament and of the Council on Undesirable Sub-stances in Animal Feed. Official Journal of the European Union l285/33.

Results

During the experiment, the mercury content in the muscles of goldfish increased, it was much more significant in the “HM” group (Fig. 1). The mercury concentration in this group increased 5.8, 10.4, and 11.7 times, respectively, compared to the zero point after 1, 2, and 3 months ($F = 120.5$; $n = 3$; $p = 0.000001$); in the “LM” group, this corresponded to 1.4, 3.2, and 3.2 times ($F = 82.6$; $n = 3$; $p = 0.0000001$). The mercury concentration in fish muscles in the “HM” group was significantly higher comparing to that in the “LM” group throughout the entire experiment: 4 times after one month of the experiment ($p = 0.0002$), 3.3 times after two months ($p = 0.002$), and 3.6 times after three months ($p = 0.003$).

The content of total protein in the blood serum of fish increased significantly comparing with that in beginning of the experiment: in the “HM” group, by 1.9, 2.0, and 2.3 times ($F = 27.0$; $n = 3$; $p = 0.0000001$), in the “LM” group, by 1.9, 2.0, and 2.0 times ($F = 27.0$; $n = 3$; $p = 0.0000001$) after 1, 2, and 3 months, respectively. The total protein content in the “HM” and “LM” groups did not differ during the first two months; an increase in the content of total protein in the blood serum of fish of the “HM” group compared to the “LM” group was noted only after three months ($p = 0.11$) (Fig. 2).

An increase in the protein concentration in the blood serum of both groups of fish could be associated with the consumption of more high-calorie food by goldfish than in their natural habitat, as evidenced by a significant increase of the Fulton’s condition factor during the experiment: $F = 43.4$, $n = 3$, $p = 0.000001$ in the “HM” group; $F = 31.5$, $n = 3$, $p = 0.000001$ in the “LM” group (Table 1). In addition, a significant fat layer around the fish intestine at the end of the experiment was visible with a naked eye. However, there was a significant positive relationship between serum protein concentration and mercury content in fish muscles (regression analysis) in both the “HM” group (Fig. 3**A)** and the “LM” group (Fig. 3**B**).

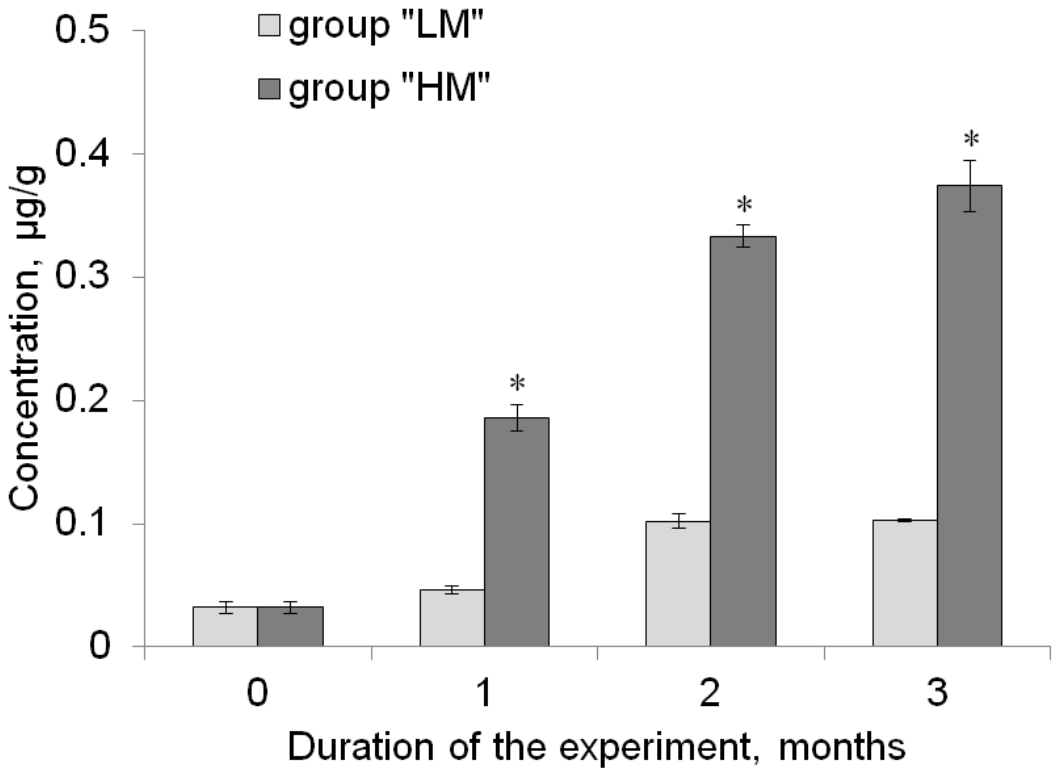

Fig. 1. Dynamics of mercury accumulation in the muscles of goldfish. Here and below: asterisk* indicates significant differences between the “HM” and the “LM” groups (Mann–Whitney test, $p < 0.05$).

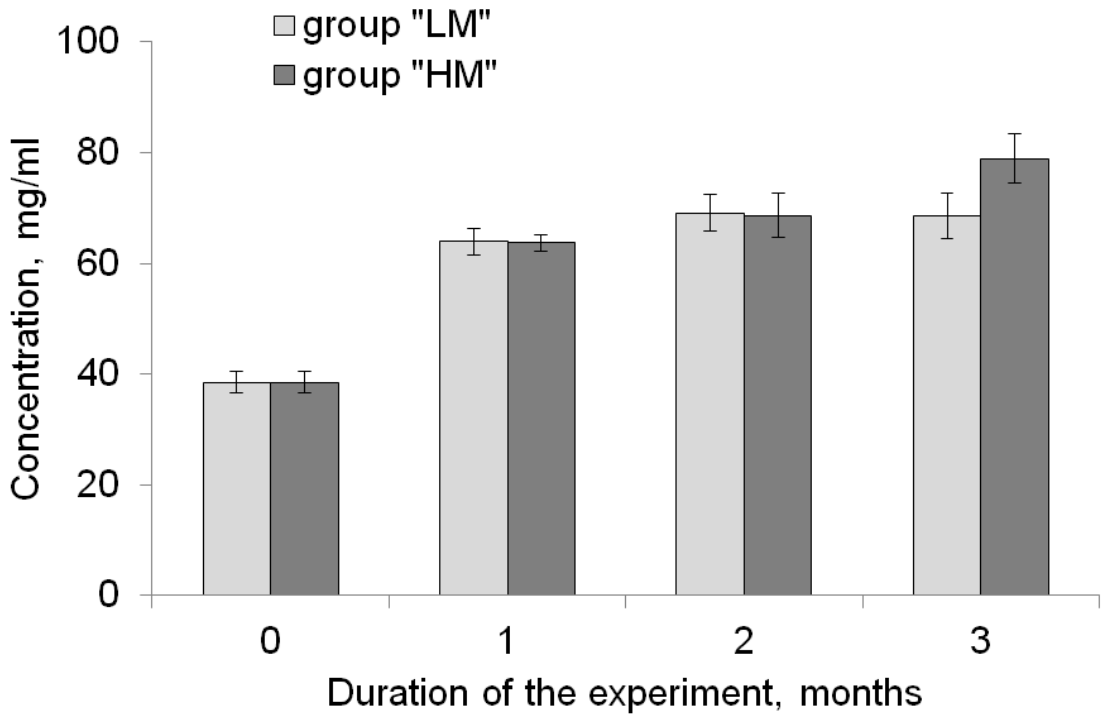

Fig. 2. The concentration of total water-soluble protein in the blood serum of goldfish.

The content of total cholesterol in the blood serum of goldfish of both groups during the experiment increased significantly compared to the initial values: by 1.5, 2.2, and 3.1 times in the “HM” group ($F = 41.3$; $n = 3$; $p = 0.000001$), by 1.1, 1.8 and 1.9 times in the “LM” group ($F = 7.8$; $n = 3$; $p = 0.002$) after 1, 2, and 3 months, respectively (Fig. 4). At the same time, after one and two months of the experiment, the differences between the values of this indicator in the “HM” and “LM” groups were not significant ($p = 0.09$ and $p = 0.33$, respectively); after 3 months, TChol in the “HM” group significantly exceeded that of the “LM” group by 1.7 times ($p = 0.01$).

A significant positive relationship was found between the concentration of total cholesterol in the blood serum and the mercury content in the fish muscles (regression analysis), which was stronger in the “HM” group (Fig. 5).

The dynamics of HDL content in the blood serum during the experiment was even more complex comparing to that of TChol and total protein (Fig. 6). In the “HM” group, HDL decreased by 8% after one month and increased by 2.0 and 1.9 times ($F = 21.4$, $n = 3$, $p = 0.000001$) after two and three months of the experiment, respectively, compared with the values at the beginning of the exposure. In the “LM” group, the HDL concentration decreased by 16% after one month, increased by 1.4 times after two

Table 1. Fulton’s condition factor of the studied fish.

Group	Experiment duration			
	Before exposure	1 month	2 months	3 months
“LM”	2.62 ± 0.07	2.82 ± 0.03	3.42 ± 0.08	3.39 ± 0.06
“HM”	2.73 ± 0.05	2.94 ± 0.04	3.16 ± 0.07	3.63 ± 0.09

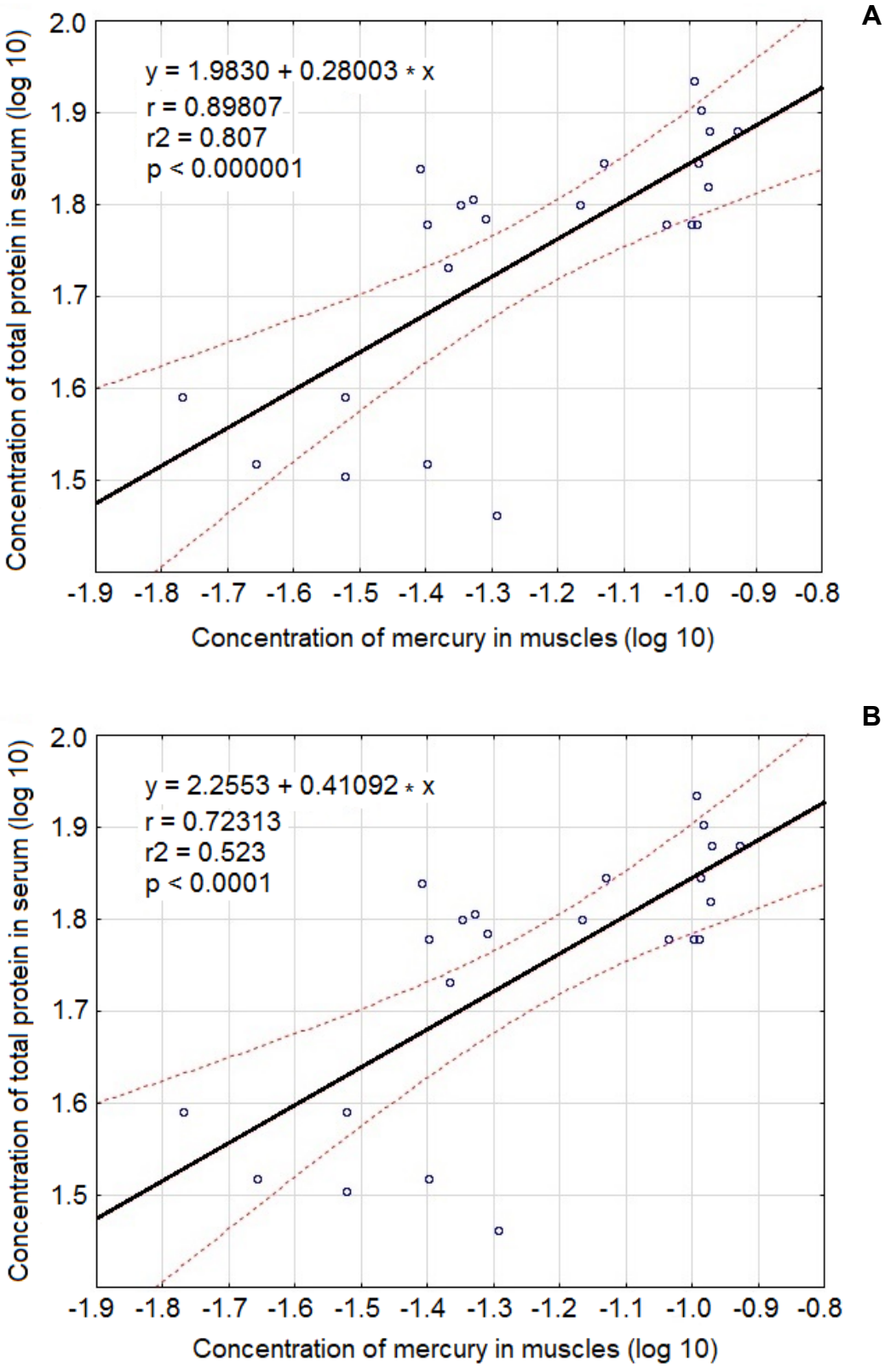

Fig. 3. Dependence of the content of total protein in the blood serum of goldfish on the mercury concentration in muscles in the "HM" group (**A**) and in the "LM" group (**B**) according to regression analysis.

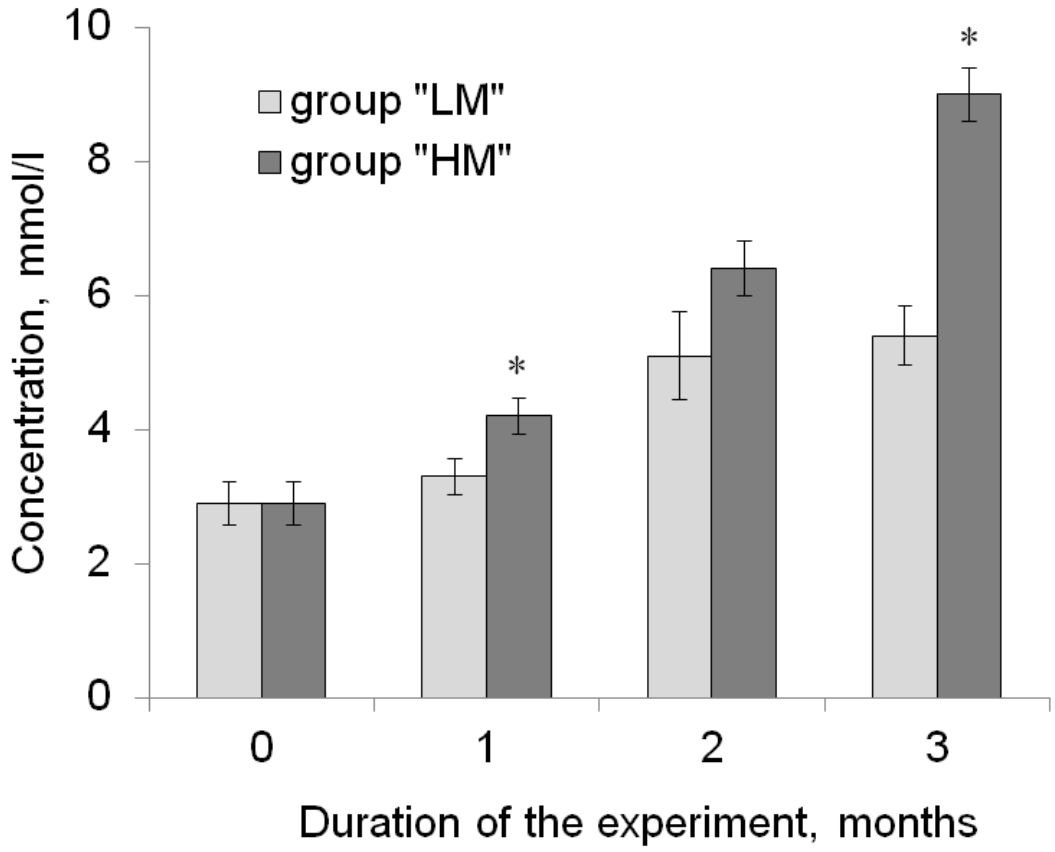

Fig. 4. The concentration of total cholesterol in the blood serum of goldfish.

months, and by 4% only after three months of exposure (F = 6.75, n = 3, p = 0.003). After 3 months, HDL concentration in the “HM” group was significantly comparing to the “LM” group (1.8 times; p = 0.01).

A positive significant relationship between the concentration of HDL in blood serum and the content of mercury in the muscles of goldfish was found, which was significant much stronger in fish belonging to the “HM” group (regression analysis; Fig. 7).

Discussion

High rate of mercury accumulation in the blood serum and muscles of goldfish was observed in our experimental design. In earlier studies, when Atlantic cod *Gadus morhua* (Linnaeus, 1758) was feeding by the food containing methylmercury at a concentration of 0.95 μg/g for three months, this caused an increase in the mercury content in fish muscles up to 0.38 ± 0.04 μg/g wet weight, of which methylmercury accounted for 90–95% (Amlund et al., 2007). In our study, goldfish ate the food containing 6.6 times less mercury content (maximum concentration, the “HM” group), but the mercury concentration in muscle tissue over the same period has reached the same level. Obviously, this can be explained by the influence of a number of factors (feeding regime, rearing conditions, and, possibly, physiological characteristics of the species), which influenced the rate of accumulation of the toxicant in the fish tissues.

According to our data, there were also changes in the protein and lipid metabolism in the fish organism under the influence of mercury contained in the feed. The intensity of these changes depends on the mercury concentration and the duration of exposure. The content of total cholesterol in the blood serum

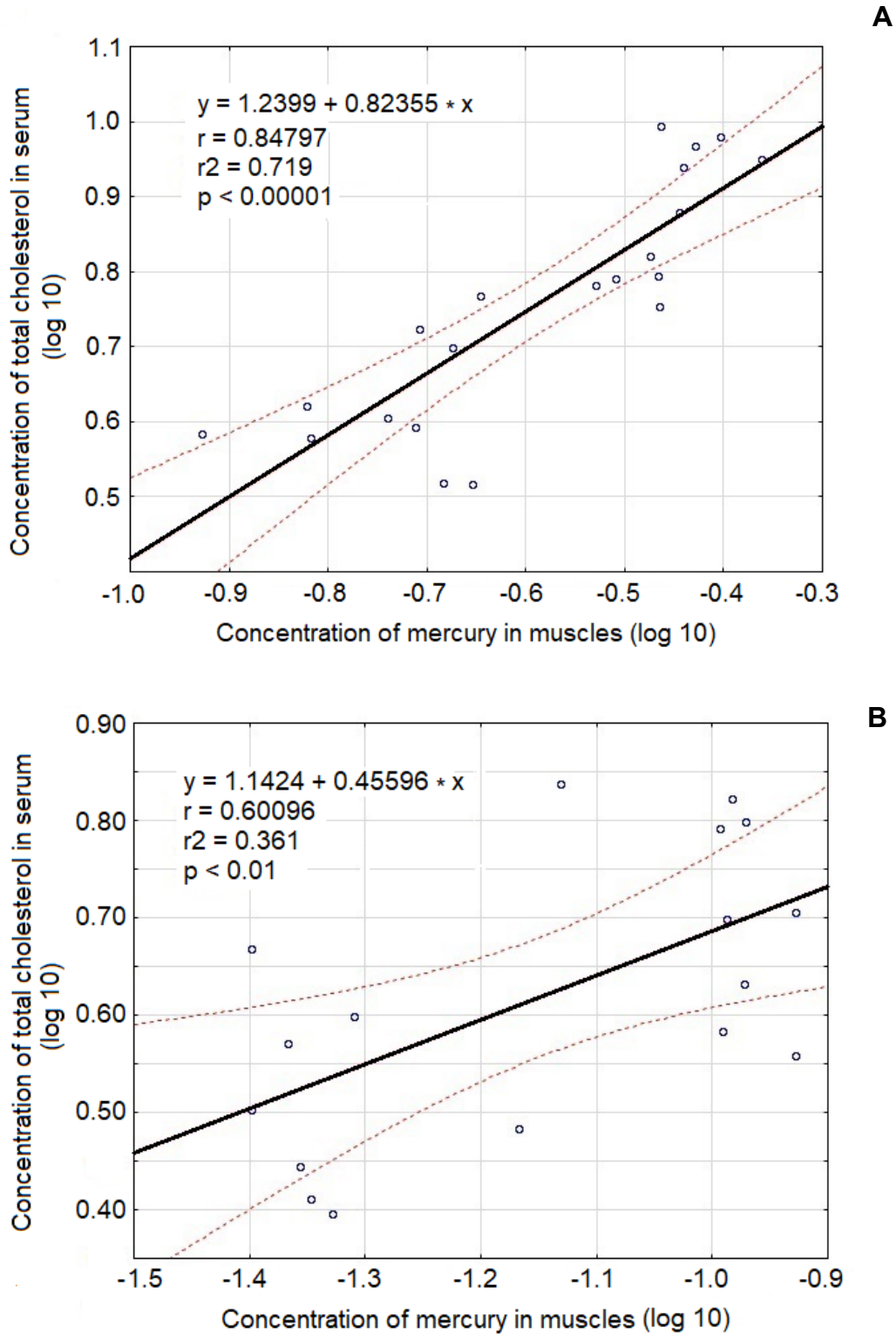

Fig. 5. Dependence of the content of total cholesterol in the blood serum of goldfish on the mercury concentration in the muscles in the “HM” group (**A**) and in the “LM” group (**B**) according to regression analysis.

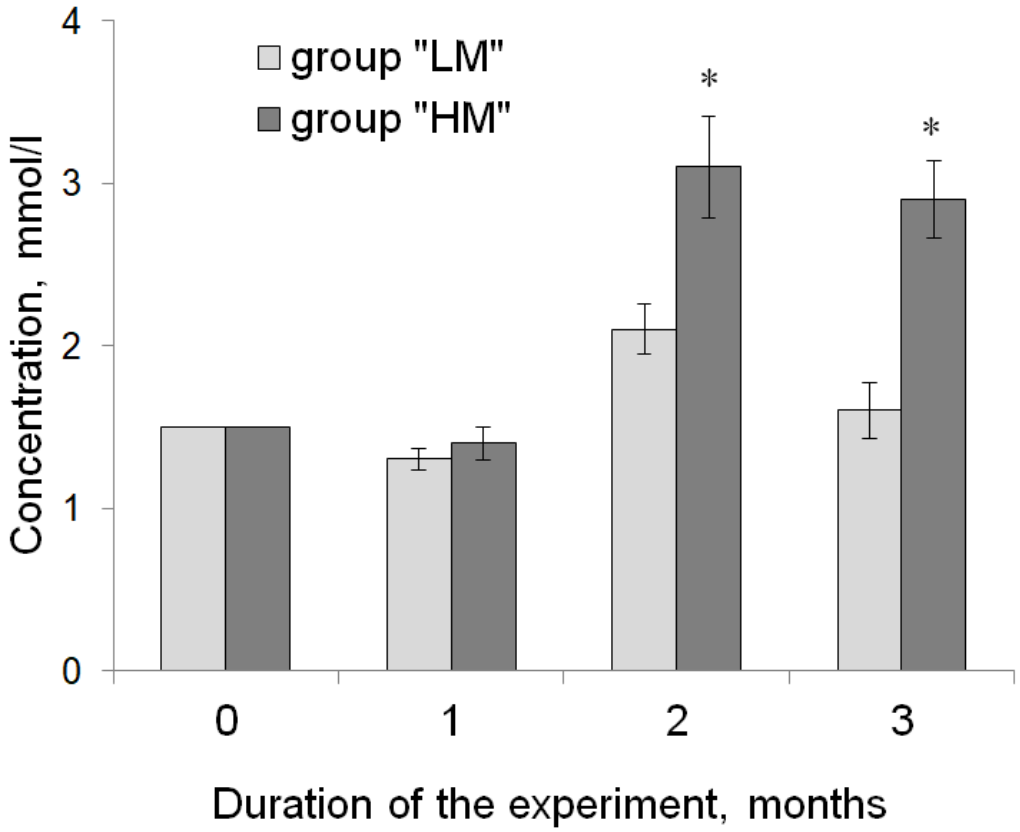

Fig. 6. The concentration of high-density lipoproteins in the blood serum of goldfish.

of goldfish increased the most among the parameters of protein and lipid metabolism studied: three months after the beginning of the experiment, its level increased by 3.1 and 1.9 times comparing with the initial values in the groups that consumed food with a high and low content of mercury, respectively. The content of total water-soluble protein in the blood serum of goldfish increased to a lesser extent: by 2.3 and 2.0 times compared with the initial values in the “HM” and “LM” groups, respectively. Moreover, the strongest and most significant relationship with the concentration of accumulated mercury in the muscles was revealed for this indicator in the “HM” group. Finally, the content of high-density lipoproteins in the blood serum of goldfish increased to the least extent: by 2.0 and 1.4 times in the “HM” and “LM” groups, respectively; the maximum increase was observed after two months of the experiment, and then a decrease of this indicator in both groups. The minor relationship was found between the concentration of accumulated mercury in the muscles and the content of high-density lipoproteins in the blood serum in comparison with other studied parameters of goldfish blood.

According to literature data (Amlund et al., 2007), a diet high in mercury resulted in a ninefold increase of mercury content in fish blood serum after 3 months of exposure. In the blood, mercury circulates as complexes with amino acids and fatty acids; 80–90% of mercury entering the bloodstream binds to erythrocytes (Luzhnnikov, 1994). A negative effect of mercury on the composition of cellular elements of the blood was noted, such as a decrease in the number of erythrocytes and leukocytes, a decrease in the ratio of particular forms of leukocytes and in hemoglobin content (Fletcher and White, 1986; Kuzubova et al., 2000; Patil and Jabde, 1998). We did not find any information in the available literature on the effect of mercury on fish blood serum proteins and lipids in the form of complexes with proteins (lipoproteins). However, it is known that mercury affects negatively a number of other indicators of lipid metabolism in fish; in perch, the level of triacylglycerols in the liver increases with different levels of mercury accumulation in tissues, the concentration of particular fatty acids in muscle lipids and the ratio of some phospholipids in tissues and organs increases, as well as the content of total lipids in

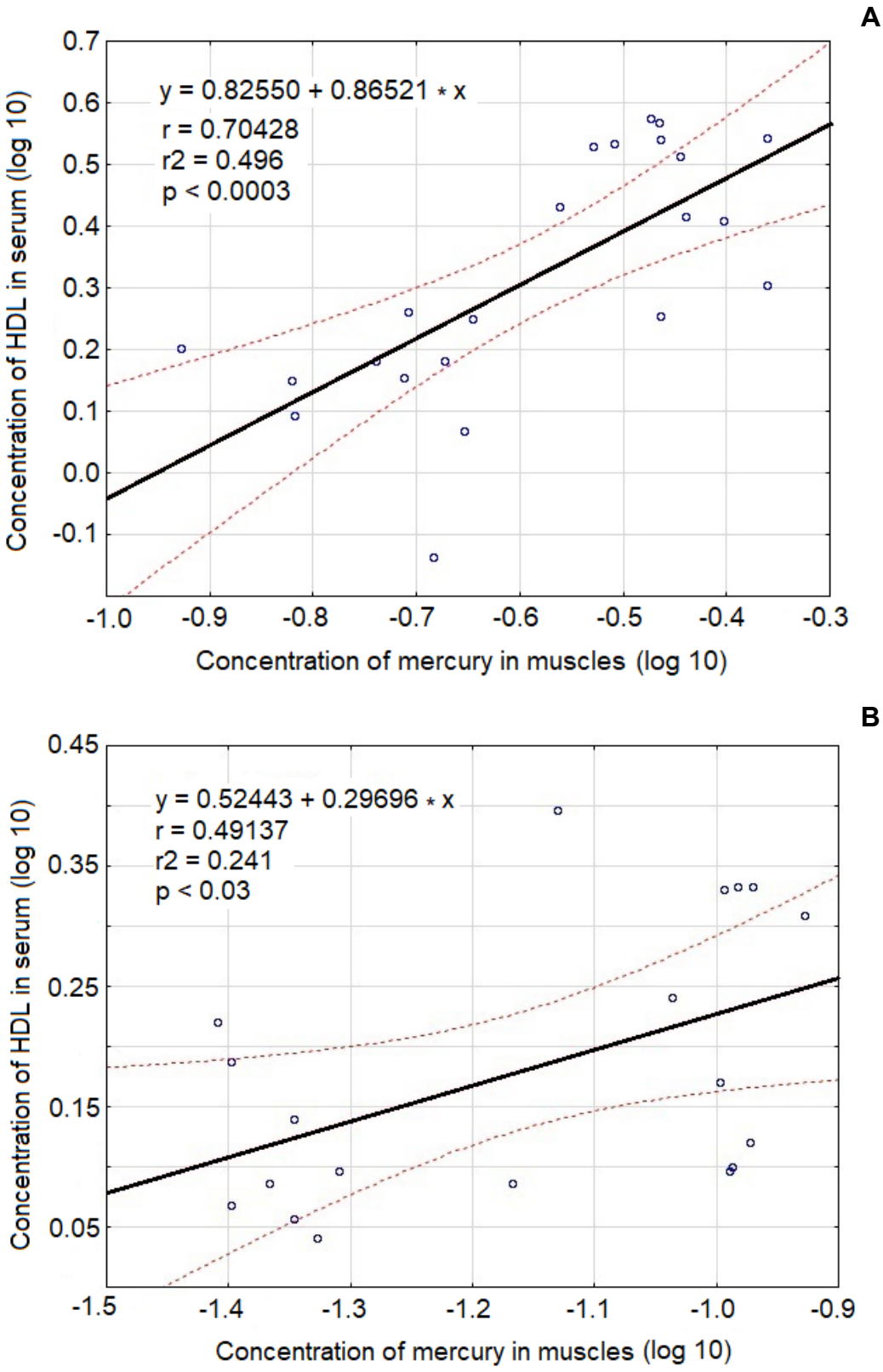

Fig. 7. Dependence of the content of high-density lipoproteins in the blood serum of goldfish on the mercury concentration in the muscles in the “HM” group (**A**) and in the “LM” group (**B**) according to regression analysis.

muscles does (Nemova, 2005; etc). A similar increase in the level of lipids in muscle tissue has been also noted in two catfish species from India (Pal and Ghosh, 2013). The most significant structural and functional changes in the cell membranes of the liver and muscle tissues are associated with the content of docosahexaenoic acid, when a decrease in its concentration may change membrane permeability and transport properties, membrane integrity, activity of membrane-bound enzymes, and receptor expression. In the liver, such a change in the profile of fatty acids entails its malfunctioning (Nemova, 2005). Taken together, these facts indicate the importance of lipid metabolism indicators in assessing the negative impact of mercury on the organisms of aquatic animals.

At first glance, the increase of the total protein concentration in the goldfish blood serum under the influence of mercury in our study contradicts somehow the literature data. In particular, it has been reported earlier that divalent mercury has an affinity for nucleic acids, especially for RNA, being included in the structure of its molecule and thereby negatively affecting DNA synthesis. A decrease in the amount of DNA and RNA in a cell inevitably leads to the suppression of protein synthesis (Kuzubova, 2000). Therefore, it is unlikely that an increase in the content of total blood serum protein is caused by an increase of its biosynthesis in the fish liver. However, the synthesis of certain protective proteins (metallothioneins) is induced rather than inhibited by heavy metals, including mercury (Bebianno et al., 2007; Farina et al., 2011; Morcillo et al., 2017). We assume that decrease of the relative water content in the blood serum is the reason for the increase of the protein concentration due to the redistribution of protein between tissues and blood and thus due to the release of fluid from the vascular space into the tissues. It is known that the distribution of plasma protein between the intra- and extravascular fluids of the fish body depends on the physiological state of the fish body, in particular, on the period of the annual cycle, e.g., spawning or completion of fattening (Andreeva et al., 2015). It is possible that mercury may also affect this process by interacting with sulfhydryl-containing proteins (i.e., carrying SH-domains) that are part of the structure of cell membranes. This causes free-radical and lipid peroxidation of membranes, which leads to a violation of their hydrophobicity and, as a result, higher permeability.

Conclusions

1. It was shown that during the experiment, mercury accumulated in significant amount in the muscles of goldfish, its concentration depended on the amount of mercury in the feed and the exposure duration.

2. Protein metabolism parameters underwent significant changes: total protein in the fish blood serum has increased. Apparently, this was caused by decreasing of the water content in the serum due to the redistribution of protein between tissues and blood and the release of fluid from the vascular space into the tissues.

3. Lipid metabolism has also changed significantly: the level of total cholesterol and high-density lipoproteins in the blood serum of fish increased, the changed of the first parameter were the most significant.

4. The studied biochemical parameters of blood positively correlated with the content of mercury in fish muscles and may be used to assess the negative effect of the toxicant on the fish organism.

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