

FATTY ACIDS METABOLISM IN SKELETAL MUSCLES AND GROWTH RATE IN COMMON CARP (*CYPRINUS CARPIO* L.) AFTER FEEDING DIET WITH VARIED COPPER AND ZINC CONCENTRATIONS

Yosyp Rivis¹, Nataliya Yanovych²✉

¹Institute of Agriculture of Carpathian Region, Lviv, Ukraine

²Lviv National University of Veterinary Medicine and Biotechnology named after S. Gzhyskyi, Lviv, Ukraine

ABSTRACT

Increasing zinc and copper concentration in diet is accompanied with increasing of their content in common carp (*Cyprinus carpio* L.) tissues, namely in skeletal muscles. Correspondingly, concentration of anionic forms of fatty acids in the skeletal muscles is also increasing. Total content of non-esterified fatty acids tends to increase in the skeletal muscles of carp at copper and zinc concentration in mixed fodder 8 and 100 mg/kg respectively, while at 16 and 200 mg/kg of copper and zinc respectively, it is decreasing. Increasing of copper and zinc concentration in the diet of carp is accompanied by increasing of level of fatty acids of total lipids in their skeletal muscles. Simultaneously, in total lipids of the skeletal muscles of carp, ratio of polyunsaturated fatty acids of ω -3 family to ω -6 family is increasing. Wherein in total lipids of the skeletal muscles of carp, efficiency of linolic and linolenic acids transformation to their more long-chain and more unsaturated derivatives is growing; concurrently intensity of transformation of myristinic, palmitinic, stearinic and arachinic acids of total lipids to their corresponding mono-unsaturated derivatives is increasing. During the period of the feeding trial, carp of the treatment groups had higher weight gains compared to control.

Key words: carp, skeletal muscles, zinc, copper, fatty acids, metabolism

INTRODUCTION

Metabolic processes in freshwater fishes, including carp, are greatly affected by trace elements. Deficiency of trace elements may lead to disorders of vitally important functions, delay of growth and development, anemia, oxidation stress etc. At the same time, the excessive content of trace elements in the water and diet may cause biochemical, structural and functional pathologies [Yanovych and Yanovych 2014]. As a result of industrial and agricultural production, fishponds can accumulate increased concentrations of heavy metals [Sandor et al. 2001, Jing Li et al. 2015], those, depending on their properties, valence and content, can affect metabolic processes in the freshwater fishes [Clearwater et al. 2002, Rajamanickam and Murhuswamy 2008, Štrbac et al. 2015], mainly as a constituents of numerous enzymes [Yanovych and Yanovych 2014]. For instance, zinc is a part of the antioxidant enzyme superoxide dismutase; also, it regulates the activity of Δ^3 -, Δ^4 -, Δ^5 - and Δ^6 -desaturases [Huang et al.

1982, Reed et al. 2014, Yanovych and Yanovych 2014]. Copper influences the activity of antioxidant enzymes superoxide dismutase and, indirectly, catalase; besides, it affects the activity of Δ^9 -desaturase [Wahle and Davies 1975]. Abovementioned desaturases are involved in fatty acids metabolism in fishes. Moreover, divalent heavy metals, such as copper and zinc, are able to form salts with fatty acids, which are hardly dissolved and have low metabolic activity [Lewis 2007]. Regardless numerous investigations of lipids and fatty acids metabolism in fish, its tissue specificity under influence of copper and zinc, as well as influence of copper and zinc on growth of freshwater fish are not clarified yet.

The aim of our research was estimation of concentration of non-esterified fatty acids, anionic forms of fatty acids and fatty acids of total lipids in skeletal muscles of carp, and carp growth intensity under different copper and zinc content in diet.

✉yandeni77@gmail.com

MATERIAL AND METHODS

Experiment was conducted on three groups (4 fish each) of two-year-old common carp with average body weight 332 g. During 45 days carp were kept in ponds 0.04 square hectares each. Carp of control group received standard granulated mixed fodder (K 111–3) without copper and zinc addition. Carp of 1st and 2nd experimental groups received the same mixed fodder with addition of sulfates of copper and zinc. The concentration of copper and zinc in the feed for 1st group was 8 and 100 mg per kg, respectively, and for 2nd group it was 16 and 200 mg per kg, respectively. Salts of copper and zinc were added to the fodder at its granulation. Natural mixed fodder and mixed fodder with copper and zinc addition were fed to carp daily at 8 a.m. in the rate of 6% of their body weight.

Finally, the carp of control and treatment groups were weighted, and samples of skeletal muscles were taken for laboratory test. The samples were measured for the concentration of copper, zinc, non-esterified fatty acids, anionic forms of fatty acids and fatty acids of total lipids.

Concentration of copper and zinc in the muscles was determined by spectrophotometry [Price 1972]. Content of non-esterified fatty acids, anionic forms of fatty acids and fatty acids of total lipids was determined by gas chromatographic method [Rivis and Fedoruk 2010].

Separation of methyl ethers of fatty acids was conducted with chromatograph Chrom-5 (Laboratorni pristroje, the Czech Republic). Stainless steel column with the length 3700 mm and inner diameter 3 mm was filled with Chromaton-N-AW, with size of parts 60–80 mesh, sialinized with HMDS (hexamethyldisilazane), covered with polyethylene glycol adipate (stationary liquid phase) in 10% concentration.

Identification of peaks on chromatogram was conducted with the method of “carbonic numbers” calculation [Ackman 1969], with the usage of chemical pure, standard solutions of methyl ethers of fatty acids. Calculation of separate fatty acids concentration was conducted with the formula, which includes correction coefficients for each of them [Rivis and Fedoruk 2010].

Results of investigations were processed mathematically. Mean value (M), standard error ($\pm m$) and probability of differences between two average magnitudes (p) were calculated. Difference between two average magnitudes was considered probable at $P \leq 0.05$. For calculations the program Origin 6.0, Excel (Microsoft, USA) was used.

RESULTS AND DISCUSSION

It was revealed, that zinc concentration in the skeletal muscles of carp was 15.80 ± 0.52 mg/kg ($P \leq 0.05$) in 1st experimental group and 16.23 ± 0.50 mg/kg ($P \leq 0.05$) in 2nd experimental group comparing to 13.50 ± 0.55

mg/kg of natural weight in control group. At the same time, copper concentration in the skeletal muscles was 0.85 ± 0.038 mg/kg ($P \leq 0.05$) in 1st experimental group and 0.90 ± 0.037 mg/kg ($P \leq 0.05$) in 2nd experimental group in comparing to 0.67 ± 0.038 mg/kg of natural weight in control group.

Increasing zinc and copper concentration in carp skeletal muscles results in changes of content of non-esterified fatty acids, anionic forms of fatty acids and fatty acids of total lipids in them (Tables 1, 2 and 3). In particular, in the skeletal muscles of carp of 1st experimental group, in comparison with fishes of control group, the tendency to increasing of total content of non-esterified fatty acids with high metabolic activity was observed (Table 1). The presented table shows, that this tendency occurs due both to monounsaturated and polyunsaturated fatty acids.

Tendency of increasing total content of non-esterified fatty acids in the skeletal muscles of carp of 1st experimental group is caused by higher concentration of monounsaturated fatty acids of ω -7 and ω -9 family, and polyunsaturated fatty acids of ω -3 and ω -6 family in their composition. Herewith, the ratio of non-esterified polyunsaturated fatty acids of ω -3 family to ω -6 family did not change (Table 1). Simultaneously, in the skeletal muscles of carp of 1st experimental group, intensity of transformation of non-esterified form of linoleic acid to its more unsaturated derivatives with longer chains is increasing.

In the skeletal muscles of carp of 1st experimental group, total content of non-esterified polyunsaturated fatty acids with even and odd numbers of carbon atoms in chain is decreasing. Herewith, in carp skeletal muscles, the intensity of desaturation of non-esterified forms of myristic, palmitic, stearic and arachidic acids to their monounsaturated derivatives – myristo-oleic, palmito-oleic, oleic and eicosenic acids is increasing.

In the skeletal muscles of carp of 2nd experimental group, in compare to carp of control group, total content of non-esterified fatty acids with high metabolic activity is decreasing (Table 1). The presented table shows, that this decreasing is occurs due to saturated, monounsaturated and polyunsaturated fatty acids.

Decreasing total content of non-esterified fatty acids in the skeletal muscles of carp of 2nd experimental group is occurs due to lower concentration of saturated fatty acids with even and odd numbers of carbon atoms in the chain (1.37 against 1.60), monounsaturated fatty acids of ω -7 and ω -9 family and polyunsaturated fatty acids of ω -3 and ω -6 family in their composition. Herewith, the ratio of non-esterified polyunsaturated fatty acids of ω -3 family to ω -6 family did not change (Table 1). However, the efficiency of conversion of non-esterified linoleic and linolenic acids to their more unsaturated derivatives with longer chain did not change, either. Herewith, in carp

Table 1. Non-esterified fatty acids content in skeletal muscles of carp at different concentration of copper and zinc in mixed fodder mg/kg of natural weight ($M \pm m$, $n = 4$)

Tabela 1. Zawartość kwasów tłuszczowych nie zestryfikowanych w mięśniach szkieletowych karpi w różnym stężeniu miedzi i cynku w mieszaninie paszowej w mg/kg masy mokrej ($M \pm m$, $n = 4$)

Non-esterified fatty acids and their abbreviation Nienasycone kwasy tłuszczowe i ich oznaczenie	Control group Grupa kontrolna	1st Experimental group 1. grupa doświadczalna	2nd Experimental group 2. grupa doświadczalna
Capric acid, 10:0 – Kwas kaprylowy, 10:0	0.70 ±0.058	0.63 ±0.088	0.47 ±0.033*
Lauric acid, 12:0 – Kwas laurynowy, 12:0	1.20 ±0.058	1.00 ±0.058	0.97 ±0.033*
Myristic acid, 14:0 – Kwas mirystynowy, 14:0	7.80 ±0.173	7.47 ±0.176	7.20 ±0.115*
Pentadecanoic acid, 15:0 – Kwas pentadekanowy, 15:0	1.60 ±0.058	1.50 ±0.058	1.37 ±0.033*
Palmitic acid, 16:0 – Kwas palmitynowy, 16:0	146.50 ±4.451	138.70 ±5.292	129.27 ±4.586
Palmitoleic acid, 16:1 – Kwas palmitoleinowy, 16:1	13.60 ±0.346	14.03 ±0.376	12.43 ±0.203*
Stearic acid, 18:0 – Kwas stearynowy, 18:0	44.40 ±1.710	41.47 ±1.683	39.30 ±0.586*
Oleic acid, 18:1 – Kwas oleinowy, 18:1	806.70 ±22.351	840.03 ±19.904	741.70 ±7.998
Linoleic acid, 18:2 – Kwas linolowy, 18:2	251.87 ±7.538	263.70 ±9.500	220.97 ±7.403*
Linolenic acid, 18:3 – Kwas linolenowy, 18:3	162.80 ±4.277	173.13 ±4.518	142.60 ±5.460*
Arachidic acid, 20:0 – Kwas arachidowy, 20:0	90.57 ±2.206	85.70 ±2.170	82.40 ±1.677*
Eicosenic acid, 20:1 – Kwas eikosenowy, 20:1	134.50 ±3.496	141.40 ±3.951	126.20 ±3.580
Eicosadienoic acid, 20:2 – Kwas eikozadienowy, 20:2	90.60 ±2.658	95.63 ±3.106	79.33 ±2.738*
Eicosatrienoic acid, 20:3 – Kwas eikozatrienowy, 20:3	48.37 ±1.855	52.33 ±1.923	39.53 ±2.085*
Eicosatetraenoic (arachidonic) acid, 20:4 Kwas eikozatetraenowy (arachidonowy), 20:4	66.17 ±2.092	71.33 ±2.571	58.07 ±1.622*
Eicosapentaenoic acid, 20:5 – Kwas eikozapentaenowy, 20:5	99.57 ±2.541	101.57 ±4.341	82.43 ±2.572**
Docosadienoic acid, 22:2 – Kwas dokozadienowy, 22:2	19.50 ±0.723	20.70 ±0.681	17.37 ±0.376
Docosatrienoic acid, 22:3 – Kwas dokozatrienowy, 22:3	38.43 ±1.680	42.77 ±1.848	32.13 ±1.502*
Docosatetraenoic acid, 22:4 – Kwas dokozatetraenowy, 22:4	39.93 ±2.021	44.10 ±1.692	35.70 ±1.762
Docosapentaenoic acid, 22:5 – Kwas dokozapentaenowy, 22:5	83.07 ±2.677	89.10 ±3.308	77.53 ±1.752
Docosahexaenoic acid, 22:6 – Kwas dokozahexaenowy, 22:6	143.43 ±6.953	152.63 ±7.214	125.80 ±2.540
Total content of fatty acids – Całkowita zawartość kwasów tłuszczowych	2291.31	2378.92	2052.77
Including saturated – W tym nasycone	292.77	276.47	260.98
Monounsaturated – Jednonienasycone	954.80	995.46	880.33
Polyunsaturated – Wielonienasycone	1043.74	1106.99	911.46
ω -3/ ω -6	1.02	1.02	1.02

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P < 0.001$.

skeletal muscles, the intensity of desaturation of non-esterified forms of myristic, palmitic, stearic and arachidic acids to their monounsaturated derivatives – myristoleic, palmitoleic, oleic and eicosenic acids did not change.

These monounsaturated derivatives can incorporate in lipids of cell membranes, thus increasing their penetration for heavy metals. Thus, unaltered intensity of desaturation of non-esterified forms of myristic, palmitic, stearic and arachidic acids in the skeletal muscles of carp after increasing copper and zinc concentration in their diet may indicate existence of protection mechanisms against heavy metals deposition in their body.

Long-chain fatty acids (with 18 and more carbon atoms in the chain) with high metabolic activity are able to bind trace elements with the formation of soaps of fatty acids [Lewis 2007], which are poorly soluble, and have low biological importance [Gurr et al. 2002]. Results of

the experiment show that after increasing zinc and copper levels in diet, in the skeletal muscles of carp of both experimental groups, comparing to carp of control group, total content of anionic fatty acids has increased (Table 2). The table shows that the increase of total concentration of anionic fatty acids in skeletal muscles of carp is observed due to saturated, monounsaturated and polyunsaturated fatty acids.

An increase of total concentration of anionic fatty acids in the skeletal muscles of carp of both experimental groups occurs due to higher content of saturated fatty acids with odd and even number of carbon atoms in the chain, monounsaturated fatty acids of the ω -7 and ω -9 families, and polyunsaturated acids of the ω -3 and ω -6 families in their composition. Herewith, the ratio of anionic polyunsaturated acids of the ω -3 family to ω -6 family did not change (Table 2).

Table 2. Anionic forms of fatty acids content in skeletal muscles of carp at different concentration of copper and zinc in mixed fodder, mg/kg of natural weight ($M \pm m$, $n = 4$)

Tabela 2. Anionowe formy zawartości kwasów tłuszczowych w mięśniach szkieletowych karpia w różnych stężeniach miedzi i cynku w mieszanej paszy, mg/kg masy mokrej ($M \pm m$, $n = 4$)

Anionic forms of fatty acids and their abbreviation Anionowe formy kwasów tłuszczowych i ich oznaczenie	Control group Grupa kontrolna	1st Experimental group 1. grupa doświadczalna	2nd Experimental group 2. grupa doświadczalna
Capric acid, 10:0 – Kwas kaprylowy, 10:0	0.37 ±0.033	0.47 ±0.033	0.57 ±0.033*
Lauric acid, 12:0 – Kwas laurynowy, 12:0	0.80 ±0.058	0.90 ±0.058	1.03 ±0.033*
Myristic acid, 14:0 – Kwas mirystynowy, 14:0	6.60 ±0.115	6.83 ±0.145	7.03 ±0.088*
Pentadecanoic acid, 15:0 – Kwas pentadekanowy, 15:0	1.20 ±0.058	1.30 ±0.058	1.47 ±0.067*
Palmitic acid, 16:0 – Kwas palmitynowy, 16:0	123.57 ±5.112	130.07 ±5.170	140.77 ±4.503
Palmitoleic acid, 16:1 – Kwas palmitoleinowy, 16:1	10.40 ±0.693	11.17 ±0.639	12.47 ±0.260*
Stearic acid, 18:0 – Kwas stearynowy, 18:0	36.47 ±1.444	38.77 ±1.707	42.93 ±1.444*
Oleic acid, 18:1 – Kwas oleinowy, 18:1	628.13 ±10.024	639.80 ±10.543	663.10 ±7.697
Linoleic acid, 18:2 – Kwas linolowy, 18:2	216.87 ±7.552	231.23 ±5.523	242.00 ±4.562*
Linolenic acid, 18:3 – Kwas linolenowy, 18:3	120.07 ±5.603	127.93 ±5.124	138.73 ±3.811
Arachidic acid, 20:0 – Kwas arachidowy, 20:0	76.60 ±2.139	81.57 ±1.713	85.43 ±2.396
Eicosenic acid, 20:1 – Kwas eikosenowy, 20:1	116.83 ±4.215	125.20 ±4.796	133.87 ±4.045*
Eicosadienoic acid, 20:2 – Kwas eikozadienowy, 20:2	72.33 ±2.230	77.43 ±2.558	81.80 ±1.950*
Eicosatrienoic acid, 20:3 – Kwas eikozatrienowy, 20:3	36.73 ±1.906	40.07 ±1.790	43.67 ±1.387*
Eicosatetraenoic (arachidonic) acid, 20:4 Kwas eikozatetraenowy (arachidonowy), 20:4	42.50 ±1.704	46.07 ±1.617	48.27 ±1.241
Eicosapentaenoic acid, 20:5 – Kwas eikozapentaenowy, 20:5	80.67 ±2.512	86.80 ±2.960	90.67 ±2.140*
Docosadienoic acid, 22:2 – Kwas dokozadienowy, 22:2	14.37 ±0.924	15.47 ±0.996	17.13 ±0.467
Docosatrienoic acid, 22:3 – Kwas dokozatrienowy, 22:3	26.20 ±1.097	28.83 ±1.241	30.83 ±1.071*
Docosatetraenoic acid, 22:4 – Kwas dokozatetraenowy, 22:4	24.40 ±1.012	27.23 ±0.984	28.60 ±0.866*
Docosapentaenoic acid, 22:5 – Kwas dokozapentaenowy, 22:5	62.37 ±1.855	66.73 ±1.638	68.77 ±1.472
Docosahexaenoic acid, 22:6 – Kwas dokozahexaenowy, 22:6	116.97 ±4.853	128.80 ±3.503	131.87 ±2.483
Total content of fatty acids – Całkowita zawartość kwasów tłuszczowych	1814.45	1912.67	2011.01
Including saturated – W tym nasycone	245.61	259.91	279.23
Monounsaturated – Jednonienasycone	755.36	776.17	809.44
Polyunsaturated – Wielonienasycone	813.48	876.59	922.34
ω -3/ ω -6	1.00	1.00	1.00

For explanations see Table 1.
Objaśnienia podano w tabeli 1.

The changes of non-etherified and anionic fatty acids content in the skeletal muscles of carp influenced on concentration of fatty acids of total lipids in them. It was revealed, that after increasing of copper and zinc concentration in diet of carp of experimental groups, in their skeletal muscles concentration of fatty acids of total lipids was higher, than in the skeletal muscles of carp of control group (Table 3). Table 3 shows, that increasing of concentration of fatty acids of total lipids in the skeletal muscles of carp of experimental groups occurs due to monounsaturated and polyunsaturated fatty acids, namely due to monounsaturated fatty acids of ω -7 and ω -9 family and polyunsaturated fatty acids of ω -3 and ω -6 family (Table 3). Intensity of transformation of linoleic and linolenic acids to their more unsaturated derivatives with longer chains is increasing in consequence to zinc influence. It is known, that zinc activates Δ^3 -, Δ^4 -, Δ^5 -

and Δ^6 -desaturases [Huang et al. 1982, Reed et al. 2014], which contribute to formation of more unsaturated fatty acids from corresponding monounsaturated and polyunsaturated fatty acids.

Increasing of content of fatty acids of total lipids in the skeletal muscles of carp of experimental groups occurs in the background of decreasing of content of saturated fatty acids with even number of carbon atoms in the chain in their composition. Wherein, in their skeletal muscles, the intensity of desaturation of myristic, palmitic, stearic and arachidic acids of total lipids to corresponding monounsaturated derivatives – myristo-oleic, palmito-oleic, oleic and eicosenic acids is increasing due to copper influence, since copper activated Δ^9 -desaturase enzyme [Wahle and Davies 1975], which contributes in formation of monounsaturated fatty acids from corresponding saturated forms [Gurr et al. 2002].

Table 3. Fatty acids of total lipids content in skeletal muscles of carp at different concentration of copper and zinc in mixed fodder, g/kg of natural weight ($M \pm m$, $n = 4$)

Tabela 3. Kwasy tłuszczowe w całkowitej zawartości lipidów w mięśniach szkieletowych karpi w różnych stężeniach miedzi i cynku w mieszankach paszowych, g/kg of natural weight ($M \pm m$, $n = 4$)

Fatty acids of total lipids and their abbreviation Kwasy tłuszczowe lipidów ogólnych i ich oznaczenie	Control group Grupa kontrolna	1st Experimental group 1. grupa doświadczalna	2nd Experimental group 2. grupa doświadczalna
Capric acid, 10:0 – Kwas kaprylowy, 10:0	0.01 ±0.000	0.01 ±0.000	0.01 ±0.000
Lauric acid, 12:0 – Kwas laurynowy, 12:0	0.02 ±0.000	0.01 ±0.003*	0.01 ±0.003*
Myristic acid, 14:0 – Kwas mirystynowy, 14:0	0.04 ±0.003	0.03 ±0.003	0.03 ±0.003
Pentadecanoic acid, 15:0 – Kwas pentadekanowy, 15:0	0.01 ±0.000	0.01 ±0.000	0.01 ±0.000
Palmitic acid, 16:0 – Kwas palmitynowy, 16:0	0.88 ±0.032	0.82 ±0.030	0.80 ±0.033
Palmitoleic acid, 16:1 – Kwas palmitoleinowy, 16:1	0.06 ±0.003	0.07 ±0.003	0.07 ±0.003
Stearic acid, 18:0 – Kwas stearynowy, 18:0	0.23 ±0.011	0.20 ±0.011	0.19 ±0.011
Oleic acid, 18:1 – Kwas oleinowy, 18:1	4.48 ±0.202	5.23 ±0.191	5.11 ±0.173
Linoleic acid, 18:2 – Kwas linolowy, 18:2	1.37 ±0.055	1.30 ±0.058	1.27 ±0.058
Linolenic acid, 18:3 – Kwas linolenowy, 18:3	0.72 ±0.026	0.67 ±0.026	0.64 ±0.023
Arachidic acid, 20:0 – Kwas arachidowy, 20:0	0.81 ±0.029	0.76 ±0.026	0.72 ±0.023
Eicosenic acid, 20:1 – Kwas eikosenowy, 20:1	0.93 ±0.035	1.10 ±0.046*	1.04 ±0.043
Eicosadienoic acid, 20:2 – Kwas eikozadienowy, 20:2	0.51 ±0.017	0.60 ±0.026*	0.58 ±0.029
Eicosatrienoic acid, 20:3 – Kwas eikozatrienowy, 20:3	0.27 ±0.020	0.36 ±0.020*	0.33 ±0.021
Eicosatetraenoic (arachidonic) acid, 20:4 Kwas eikozatetraenowy (arachidonowy), 20:4	0.32 ±0.017	0.41 ±0.023*	0.38 ±0.023
Eicosapentaenoic acid, 20:5 – Kwas eikozapentaenowy, 20:5	0.55 ±0.026	0.67 ±0.026*	0.63 ±0.029
Docosadienoic acid, 22:2 – Kwas dokozadienowy, 22:2	0.09 ±0.006	0.12 ±0.006*	0.10 ±0.006
Docosatrienoic acid, 22:3 – Kwas dokozatrienowy, 22:3	0.11 ±0.006	0.14 ±0.006*	0.13 ±0.006
Docosatetraenoic acid, 22:4 – Kwas dokozatetraenowy, 22:4	0.21 ±0.011	0.26 ±0.014*	0.26 ±0.014*
Docosapentaenoic acid, 22:5 – Kwas dokozapentaenowy, 22:5	0.42 ±0.020	0.52 ±0.026*	0.50 ±0.023
Docosahexaenoic acid, 22:6 – Kwas dokozahexaenowy, 22:6	0.85 ±0.037	1.02 ±0.049	0.99 ±0.040
Total content of fatty acids – Całkowita zawartość kwasów tłuszczowych	12.90	14.33	13.82
Including saturated – W tym nasycone	2.00	1.84	1.77
Monounsaturated – Jednonienasycone	5.48	6.42	6.24
Polyunsaturated – Wielonienasycone	5.42	6.07	5.81
ω -3/ ω -6	0.96	0.99	0.98

For explanations see Table 1.

Objaśnienia podano w tabeli 1.

Table 4. Average body weight of two-year old carp at different concentration of copper and zinc in mixed fodder, g ($M \pm m$, $n = 4$)

Tabela 4. Średnia masa ciała karpi dwuletnich w różnych stężeniach miedzi i cynku w mieszankach paszowych, g ($M \pm m$, $n = 4$)

Control group Grupa kontrolna	1st Experimental group 1. grupa doświadczalna	2nd Experimental group 2. grupa doświadczalna
At the beginning of experiment – Na początku doświadczenia		
332.2 ±1.77	332.5 ±1.51	332.3 ±1.92
At the end of experiment (45 days) – Na zakończenie doświadczenia		
548.0 ±2.94	645.0 ±2.94***	604.8 ±3.53***

For explanations see Table 1.

Objaśnienia podano w tabeli 1.

Significant increase in the contents of products of myristic, palmitic, stearic and arachidic acids desaturation, and more unsaturated derivatives of linoleic and linolenic acids with longer chains leads to increased permeability

of cell membranes for different metabolites and xenobiotics. These membranes have a higher permeability compared to cell membranes with a higher content of saturated fatty acids [Anderson and Ma 2009].

Changes in the content of non-etherified fatty acids, anionic fatty acids and fatty acids of total lipids in the skeletal muscles of carp are accompanied by changes in body weight (Table 4). At the end of the experiment, body weight of carp of the control group increased 1.65-fold, and body weight of carp of 1st and 2nd experimental groups – 1.94- and 1.82-fold, respectively. The results of our experiments show that copper and zinc in concentration 8 and 100 mg/kg of feed, respectively, significantly influenced the growth of carp.

CONCLUSIONS

1. Increased copper and zinc content in the feed results in an increase in the concentration of these elements in the skeletal muscles of carp ($P \leq 0.05$).
2. Increased copper and zinc content in the diet is accompanied by a higher content of anionic fatty acids in carp's skeletal muscles due to saturated, monounsaturated and polyunsaturated fatty acids ($P \leq 0.05$).
3. Total content of non-etherified fatty acids in the muscles in the 1st experimental group tended to increase ($P \leq 0.1$), while in the 2nd experimental group it showed a decreasing trend ($P \leq 0.05$). Changes in non-etherified fatty acids content in the skeletal muscles of carp of 1st experimental group are observed due to monounsaturated and polyunsaturated fatty acids, while in the 2nd experimental group – due to saturated, monounsaturated and polyunsaturated fatty acids.
4. The levels of fatty acids of total lipids in the skeletal muscles of carp of the 2nd and, especially, 1st experimental groups has increased, due to monounsaturated and polyunsaturated fatty acids ($P \leq 0.05$ – 0.01).
5. In total lipids of the skeletal muscles of carp of the 1st and 2nd experimental groups, ratio of polyunsaturated fatty acids of ω -3 family to ω -6 family has increased ($P \leq 0.01$ – 0.001). Also, in total lipids of the skeletal muscles of carp, the intensity of linoleic and linolenic acids transformation to their more saturated derivatives with longer chains has increased ($P \leq 0.01$ – 0.001). At the same time, the intensity desaturation of myristic, palmitic, stearic and arachic acids to corresponding monounsaturated derivatives in total lipids of the skeletal muscles of carp has increased too ($P \leq 0.01$ – 0.001).
6. During the 45-day experiment, the carp of control group gained their weight 1.65-fold, while carp of 1st and 2nd experimental groups – 1.94- and 1.82-fold, respectively ($P \leq 0.001$).

REFERENCES

- Ackman, R.G. (1969). Gas-liquid chromatography of fatty acids and esters. In: Lowenstein J.M. (ed.) *Methods in Enzymology*. Academic, New York, 329–381.
- Anderson, B.M., Ma, D. (2009). Are all n-3 polyunsaturated fatty acids created equal? *Lipids Health Dis.*, 8(1), 33.
- Clearwater, S.J., Farag, A.M., Meyer, J.S. (2002). Bioavailability and toxicity of dietborne copper and zinc to fish. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.*, 132(3), 269–313.
- Gurr, M.I., Harwood, J.L., Frayn, K.N. (2002). *Lipid biochemistry. An Introduction*. 5-th ed. Blackwell Sci., 337 p.
- Huang, Y.S., Cunnane, S.C., Horrobin, D.F., Davignon, J. (1982). Most biological effects of zinc deficiency corrected by gamma-linolenic acid (18:3 omega 6) but not by linoleic acid (18:2 omega 6). *Atherosclerosis*, 41, 193–207.
- Jing, L., Haixin, Y., Yaning L. (2015). Meta-Analysis of the Copper, Zinc, and Cadmium Absorption Capacities of Aquatic Plants in Heavy Metal-Polluted Water. *Int. J. Environ. Res. Publ. Health*, 12(12), 14958–14973.
- Lewis, R.J., Sr. (2007). *Hawley's condensed chemical dictionary*, 15th ed. New York; Hoboken: Wiley, 1379 p.
- Price, W.J. (1972). *Analytical Atomic Absorption Spectrometry*. Heyden and Son Ltd., London. 239 p.
- Rajamanickam, V., Muthuswamy, N. (2008). Effect of heavy metals induced toxicity on metabolic biomarkers in common carp (*Cyprinus Carpio* L.). *Mj. Int. J. Sci. Technol.*, 2(01), 192–200.
- Reed, S., Xia, Q., Ran-Ressler, R., Brenna, J.-T., Glahn, R.P., Tako, E. (2014). Dietary zinc deficiency affects blood linoleic acid: dihomogamma-linolenic acid (LA:DGLA) ratio; a sensitive physiological marker of zinc status in vivo (*Gallus gallus*). *Nutrients*, 6(3), 1164–1180.
- Rivis, J.F., Fedoruk, R.S. (2010). Quantitative and qualitative chromatographical methods of some lipids and fatty acids determination in biological material. *Lviv, Spolom*, 110 p. [In Ukrainian].
- Sandor, Z., Csengeri, I., Oncsik, M.B., Alexis, M.N., Zubcova, E. (2001). Trace metal levels in freshwater fish, sediment and water. *Environ. Sci. Pollut. Res. Int.*, 8(4), 265–268.
- Štrbac, S., Kašanin-Grubin, M., Jovančićević, B., Simonović, P. (2015). Bioaccumulation of Heavy Metals and Microelements in Silver Bream (*Brama brama* L.), Northern Pike (*Esox lucius* L.), Sterlet (*Acipenser ruthenus* L.), and Common Carp (*Cyprinus carpio* L.) From Tisza River, Serbia. *J. Toxicol. Environ. Health A.*, 78(11), 663–665.
- Wahle, K.W.J., Davies, N.T. (1975). Effect of dietary copper deficiency in the rat on fatty acid composition of adipose tissue and desaturase activity of liver microsomes. *Br. J. Nutr.*, 34, 105–112.
- Yanovych, N.E., Yanovych, D.O. (2014). Trace elements role in pond fishes vital functions. *Scientific messenger of LNUVM and BT named after S.Z. Gzhytskyi*, 16(2), 345–372.

METABOLIZM KWASÓW TŁUSZCZOWYCH W MIĘŚNIACH SZKIELETOWYCH I TEMPO WZROSTU KARPIA (*CYPRINUS CARPIO* L.) W EFEKCIE SKARMIANIA PASZĄ O RÓŻNEJ ZAWARTOŚCI MIEDZI I CYNKU

STRESZCZENIE

Zwiększenie stężenia cynku i miedzi w diecie prowadzi do wzrostu ich zawartości w tkankach karpia (*Cyprinus carpio* L.), między innymi w mięśniach szkieletowych. Odpowiednio wzrasta również stężenie anionowych form kwasów tłuszczowych w mięśniach szkieletowych. Całkowita zawartość nieestryfikowanych kwasów tłuszczowych zwiększa się w mięśniach karpia przy stężeniu miedzi i cynku w mieszankach paszowych na poziomie odpowiednio 8 i 100 mg/kg, zaś maleje przy stężeniu miedzi i cynku odpowiednio 16 i 200 mg/kg. Zwiększeniu stężenia miedzi i cynku w diecie karpia towarzyszy wzrost poziomu kwasów tłuszczowych w lipidach całkowitych w mięśniach szkieletowych. Jednocześnie, w całkowitych lipidach mięśni szkieletowych karpi wzrasta stosunek kwasów ω -3 do ω -6. W lipidach mięśni szkieletowych karpi rośnie szybkość transformacji kwasów linolowych i linolenowych do ich bardziej długołańcuchowych i bardziej nienasyconych pochodnych. Jednocześnie wzrasta intensywność przemiany kwasu mirystynowego, palmitinowego, stearynowego i arachinowego do odpowiadających im jednonienasyconych pochodnych. W trakcie doświadczenia karpie grup doświadczalnych wykazały większe przyrosty masy ciała w porównaniu do ryb z grupy kontrolnej.

Słowa kluczowe: karp, mięśnie szkieletowe, cynk, miedź, kwasy tłuszczowe, metabolizm

