The changes in the fatty acid profile of some tissues of female carp after feeding of vitamin and mineral supplement

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Abstract. The problem of vitamin and mineral nutrition of pond fish and its influence on metabolic processes in their organism is a matter of vital importance in the last decades all over the world. The aim of the study was to assess the effect of vitamin and mineral supplement on lipid individual classes and fatty acid composition in the body of female carp in pre-spawning period. For this purpose, lipids were determined after separation by thin layer chromatography and the fatty acid composition by gas chromatography, on 5-7 samples of fish tissues. Vitamin and mineral supplementation of the carp diet leads to the increase of the level of mono- and polyunsaturated fatty acids in the skeletal muscle of female carps from the second experimental group: a significant decrease of palmitic acid to 14.1%, compared to 19.06% in control ($P<0.5$) and a simultaneous increase of oleic acid to 50.81%, compared to 44.84% in control ($P<0.5$) was demonstrated. A higher level of phospholipids was found in the liver and skeletal muscles of carps after introduction of vitamins and mineral supplements. These results suggest the positive changes in n the lipid metabolism in female carp under influence of vitamin and mineral supplements at pre-spawning period, in regard of enhancement of their reproductive function.
Key Words: lipid individual classes, polyunsaturated fatty acids, supplement.

Introduction. Fish is a subject of increased stress under conditions of intensive aquaculture owing to environmental (water quality and hypoxia) and health(parasites and infectious diseases) factors with negative impacts on fish well-being and overall performance, and consequent economic losses (Petitjean et al 2019). Adequate vitamin and mineral supplements are essential to avoid malnutrition and to maintain adequate performance and health. Furthermore, it is becoming evident that diets supplemented with specific nutrients, vitamins or minerals, at levels above the requirements, may improve the health condition and disease resistance (Oliva-Teles 2012). In recent years, the investigation of the influence of vitamins and trace elements on the fish organism has been focused on studying the essential biologically active compounds on the reproductive function and body metabolism of carps. Vitality and reproductive ability of fish depend on ensuring their requirements in these nutrients. This is due to a wide range of biological effects of trace elements in humans and animals on their metabolism (Mette et al 2006; Yanovych & Rivis 2016). At the same time, vitamins deficiency is commonly observed in the commercial aquaculture (Ringø et al 2014). However, in practice, diets are rarely deficient in only one specific micronutrient and usually the clinical signs and histopathological features are not particularly specific (Chanda et al 2015). Detailed signs of vitamin deficiency or excess were reviewed by Halver (2002).

Diet supplementation with certain minerals, at levels above the requirements for normal growth and below those causing toxicity, may enhance the immune function and disease resistance in fish, although such effects are not always evident (Gatlin 2002). Another issue is in the focus of investigators: the role of adequate mineral and vitamin nutrition in proper functioning of reproductive system of female carp, especially at pre-spawn period (Lim et al 2008). In this context, the aim of the study was to investigate the effect of supplementation with different levels of fat-soluble vitamins A, D, E and trace elements, such as selenium, zinc and iodine on the lipid individual classes and fatty acids composition in the body of female carp in pre-spawning period.

Material and Method

Animals and diet. The experiment was conducted in Lviv Research Station of the Institute of Fisheries of NAAS in 3 groups of five years old female carps of the local breed, that were divided, based on the principle of analogy, into control and two experimental groups of 10 individuals each. Fish were kept in a special basin under conditions of Recirculating Aquaculture System (RAS). The water temperature gradually increased from 10 to 20°C. Starting 30 days before the intended spawning, female carp of the control group were fed with granulated feed (fish meal, wheat, rye flour, oil). During that month, female carps of the first experimental group were fed with a similar food and "Tryvit" (Fortis Pharma, Ukraine), that contains 2,500 IU vitamin A, 3,333 IU vitamin D_3 and 1.7 mg vitamin E, and also trace elements: potassium iodide, at a dose of 5 mg kg^{-1} of food, 40 mg kg^{-1} of zinc sulfate and 0.3 mg kg^{-1} of sodium selenite. The diet of female carps of the second experimental group was supplemented with "Tryvit" containing 5,000 IU vitamin A, 6,666 IU vitamin D_3 and 3.3 mg vitamin E and also the following trace elements: potassium iodide, at a dose of 10 mg kg^{-1} of food, 60 mg kg^{-1} of zinc sulfate and 0.5 mg kg^{-1} of sodium selenite.

Collection of samples. The samples of liver and skeletal muscles for biochemical researches were taken after finishing the experiment. From five to seven separate samples of each tissue were taken from ten separate fishes of every group. Each sample was homogenized in cold Tris-HCl buffer (Sigma-Aldrich, USA) (100 mM, pH 7.4) to obtain a 10% (w/v) tissue homogenate.

Lipid extraction and fatty acid analysis. Fatty acid content was determined by total muscle lipid extraction (Folch et al 1957) and was measured using gas chromatography on Hewlett-Packard 7890B (Agilent Technologies, USA) with capillary column sp-2560 (Sigma-Aldrich Supelco, USA). Methylation was conducted with a methanol-sulphuric acid solution (100:1) (Merck, Germany). Fatty acids content was expressed as FA% (fractions) from the total fatty acid content (5 measurements for each parameter).

Statistical analysis. Numerical data have been treated by biometric variations nonparametric analysis using the Microsoft Excel software package of the Microsoft Office Professional XP and the software Statistica 6.0. The significance levels were 0.05, 0.01 and 0.001.

Results. The fatty acid composition in the liver of female carp after adding vitamin and mineral supplementation are listed in the Table 1. The data show an increase of the overall content of monounsaturated acids, decrease of saturated acids and a slight decrease of polyunsaturated fatty acids, especially in the second experimental group. These changes are due to the significant increase of oleic acid by 57% ($P<0.5$) and to the decrease of palmitic acid by 67% ($P<0.01$) in the second experimental group, compared to the control. At the same time, a tendency to growth of the eicosenic and linoleic acids has been established. Almost the same changes have been observed in fatty acids composition in the skeletal muscles of female carp (Table 2). In the second experimental group, a significant decrease of the palmitic acid to 14.1%, compared to 19.06% in control ($P<0.5$), and a simultaneous increase of oleic acid to 50.81% compared to 44.84% in control ($P<0.5$), and also a tendency to decrease of the miristic, palmitoleic and arachidonic acids in fish of both experimental groups were demonstrated. These data suggest the intensification of the processes of elongation and desaturation of fatty acids in skeletal muscles of female carp under the influence of supplementation with vitamins and minerals.

Table 1

Fatty acids composition in the liver of female carp (M±m, %, n=5-7)

Name of acid	*Code of acid*	*Control*	*Experimental 1*	*Experimental 2*
Myristic	14:0	1.05±0.11	1.13±0.06	0.69±0.08
Palmitic	16:0	26.35±1.30	26.11±0.22	17.78±0.14**
Palmitoleic	c-7- 16:1	1.43±0.26	2.15±0.48	0.89±0.44
	c-9- 16:1	4.78±0.36	3.19±0.56	3.78±0.58
Margaric	17:0	0.36±0.04	0.41±0.21	0.43±0.02
Heptadecenoic	17:1	0.43±0.07	0.39±1.16	0.37±0.01
Stearic	18:0	9.33±0.74	11.32±1.14	9.12±0.28
Oleic	t 6 18:1	0.30±0.02	0.39±0.14	0.23±0.02
	cis 9 18:1	31.79±2.46	32.8±0.14	46.98±4.65*
	cis 11 18:1	5.80±0.26	5.29±0.26	5.46±0.25
Linoleic	c-9,12 18:2	6.39±0.58	6,40±0.69	7.24±0.46
Arachidic	20:0	0.03±0.007	0.05±0.009	0.05±0.006
Linolenic	18:3 ω6 γ	0.32±0.04	0.42±0.07	0.34±0.009
	18:3 ω3 ἀ	0.41 ±0.06	0.43±0.21	0.3±0.09
Eicosenoic	c 9 20:1	0.46±0.12	0.41±0.16	0.7±0.04
	c 11 20:1 ω9	2.76±0.6	2.77±0.40	3.24±0.14
	c 13 20:1	0.33±0.02	0.40±0.11	0.37±0.006
Eicosadienoic	20:2	1.55±0.28	1.11±0.39	0.48±0.08*
Behenic	22:0	0.16±0.05	0.23±0.08	0.06±0.02
Eicosatryenoic	20:3	0.63±0.02	0.53±0.14	0.26±0.04**
Arachidonic	20:4	2.48±0.29	1.80±0.35	0.81±0.14**
Eicosapentaenoic	20:5	1.02±0.22	0.91±0,14	0.95±0.05
Docosahexaenoic	22:6	1.84±0.12	1.36±0.60	1.40±0.12
Including: Saturated		37.28	39.25	28.13
Monounsaturated		48.67	48.4	62.66
Polyunsaturated		14.05	12.35	11.14

* – P<0.05; ** – P<0.01;*** – P<0.001.

Table 2

Fatty acids composition in skeletal muscles of female carp (M±m, %, n=5-7)

Name of acid	*Code of acid*	*Control*	*Experimental 1*	*Experimental 2*
Myristic	14:0	1.13±0.13	0.99±0.09	0.76±0.07
Palmitic	16:0	19.06±0.79	18.14±1.74	14.14±0.74*
Palmitoleic	c-7- 16:1	0.70±0.05	0.74±0.16	0.54±0.07
	c-9- 16:1	6.44±0.76	5.64±1.03	4.47±0.20
Margaric	17:0	0.22±0.03	0.22±0.07	0.15±0.02
Heptadecenoic	17:1	0.32±0.05	0.31±0.12	0.25±0.07
Stearic	18:0	7.32±0.08	8.66±1.52	8.16±0.75
Oleic	t 6 18:1	0.35 ±0.02	0.33 ±0.04	0.29±0.006*
	cis 9 18:1	44.84±2.08	43.03 ±2.41	50.81±1.59*
	cis 11 18:1	5.45 ±0.28	6.24 ±0.33	4.59±0.30
Linoleic	c,c-9,12 18:2	7.52±0.88	8.25±1.28	8.96±1.09
Arachidic	20:0	0.31±0.03	0.41±0.06	0.38±0.006
Linolenic	18:3 ω6 γ	0.77±0.19	0.76±0.33	0.70±0.14
	18:3 ω3 ἀ	0.55±0.08	0.53±0.03	0.8±0.08
Eicosenoic	c 9 20:1	2.72±0.36	2.91±0.51	3±0.28
	c 11 20:1 ω9	0.41±0.08	0.43±0.14	0.33±0.07
	c 13 20:1	0.04±0.009	0.05±0.02	0.04±0.006
Eicosadienoic	20:2	0.3±0.06	0.34±0.07	0.35±0.03
Behenic	22:0	0.76±0.21	0.87±0.31	0.67±0.19
Eicosatryenoic	20:3	0.46±0.18	0.37±0.15	0.21±0.05
Arachidonic	20:4	0.33±0.2	0.56±0.17	0.43±0.06
Including: Saturated		27.77	28.07	23.22
Monounsaturated		61.37	59.73	64.75
Polyunsaturated		10.86	12.2	12.03

* – P<0.05; ** – P<0.01;*** – P<0.001.

Table 3 presents the changes of individual lipid classes in the skeletal muscle and liver of female carp under the influence of investigated supplements. The data show that the supplementation of vitamins and minerals leads to a significant increase of phospholipids by 1.5 (P<0.001) and decrease of triacylglycerols by 1.68 (P<0.001) in the skeletal muscles and liver of female carp, compared to control group.

Table 3

Lipid classes in skeletal muscles and liver carp (M±m; %, n=7)

Lipids	*Groups*		
	Control	*Experimental 1*	*Experimental 2*
Skeletal muscles			
Total lipids %	3.5±0.01	4.1±0.02	4.3±0.01
Phospholipids	17.75±0.22	21.05±0.27	27.00±0.13***
Diacylglycerols	12.55±0.31	12.87±0.09	14.75±0.18
Free cholesterol	16.12±0.13	16.72±0.37	17.56±0.23
NEFA	10.28±0.25	10.25±0.39	10.35±0.38
Triacylglycerols	37.87±0.75	34.2±0.47*	22.86±1.31**
Cholesterol ethers	5.00±0.76	5.55±0.35	7.47±0.89*
Liver			
Total lipids %	4.5±0.01	6.3±0.03	6.8±0.07**
Phospholipids	19.90±0.62	22.86±0.17*	23.19±0.13**
Diacylglycerols	13.79±0.30	13.95±0.36	14.78±0.05
Free cholesterol	16.08±0.05	16.50±0.38	16.18±0.30
NEFA	10.17±0.21	11.29±0.03	11.23±0.38
Triacylglycerols	35.11±0.79	29.18±0.57**	27.18±0.76**
Cholesterol ethers	4.93±0.30	6.28±0.36*	7.32±0.46*

* – P<0.05; ** – P<0.01;*** – P<0.001.

Discussion. The aim of the study was to show the effect of vitamin and mineral supplements on the individual classes of lipids and fatty acids composition in some tissues of female carp in the pre-spawning period, and also to evaluate the significance of possible changes in the light of ensuring the reproductive function of female carps.

First of all, we elucidated some changes in fatty acid composition in the liver. The increase of monounsaturated oleic acid and the simultaneous decrease of saturated palmitic acid may lead to the overall increase of unsaturity of cell membranes. Such changes were probably caused by the supplementation with vitamins and tracer elements in higher doses. These changes can be caused by the intensification of the desaturation processes of the palmitic acid, that occur in liver under the influence of diet supplementation of vitamins and minerals. It is known that at least one of the compounds of the supplement, the Zn, plays a significant role in the metabolism of polyunsaturated fatty acids: zinc is a cofactor of Δ-6-desaturase, a limiting enzyme that catalyzes the transformation of linoleic to γ-linolenic acid. It is believed that a lack of zinc acid is a factor in the inhibition of this enzyme (Yary et al 2017).

On the other hand, a serious decrease of polyunsaturated arachidonic and eicosatrienoic acids under the influence of vitamin and mineral supplementation has been determined. It is well known that linoleic and linolenic acids are parent acids of omega-3 and omega-6, providing a synthesis of various series of prostaglandins (Smolyaninov et al 2002). Thus, the decrease of the content of arachidonic and eicosatryenoic acid may be caused by their more intensive utilization in eicosanoids synthesis in the liver, under the influence of mineral supplements.

It is generally recognized that polyunsaturated fatty acids (PUFA) composition might vary among species of fish (Muhamad & Mohamad 2012). Among the PUFA’s are known very important n−3 PUFA’s class acids such as linolenic acid (18:3n−3), eicosapentaenoic acid (EPA) (20:5n−3) and docosahexaenoic acid (DHA) (22:6n−3) which are appreciated for their anti-thrombogenic and anti-atherogenic effects in human (Hrytsyniak et al 2010). On the contrary, among the saturated fatty acids, the lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0), are recognized as health risk factors (Garaffo et al 2011; Sanfilippo et al 2011).

The data presented in Table 2 suggests the intensification of the processes of elongation and desaturation of fatty acids in the skeletal muscles of female carp, under the influence of vitamins and minerals supplementation. Thus, inclusion to the diet of female carp of vitamin and mineral supplementation leads to an increase of the mono- and polyunsaturated acids in the muscle of female carps. The changes in fatty acid composition in skeletal muscles may be treated as almost similar as in liver, being possibly determined by the same factors.

Another topic of our research is the study of changes in separate lipid classes after the action of vitamins and trace elements. The data show that the supplementation with vitamins and minerals increases significantly the level of phospholipids and decreases the triacylglycerols. This means that these dietary additives stimulate the synthesis of structural lipids by using mainly phospholipids, the major structural lipids in tissues, which contain high amounts of polyunsaturated fatty acids. Triacylglycerols are the main energy depots in fish (Etursdottir et al 2008).

It is known that phospholipids serve as structural compounds of cell membranes, while triacylglycerols are a reserve of energy and they are mainly stored in adipose tissues. Thus, the higher the level of fatness, the higher the content of triacylglycerols, as it can be observed (Mráz 2011). Increased phospholipids in the liver and skeletal muscles of carps fed with additional vitamin and mineral supplements may indicate the growth of these tissues. The decrease of triacylglycerols and increase of total lipids in tissues of carp causes the accumulation of fat in their organism and induce the reserve required for the adaptive capacity of the body. It is known that in the early summer and autumn, the intensity of lipid peroxidation in the liver of carp is much lower than in the winter and especially in early spring, when the stocks of energy substrates and biologically active substances in their body decreases (Oleksiuk & Yanovich 2006). The reason for this is to accommodate the water temperature and hypoxic conditions in which the fish are in early spring (Lushchak & Bagnyukova 2007; Orel 2007) and to reduce the enzymatic activity and the antioxidant system's nonenzymatic reactions in their body (Oleksiuk & Yanovich 2006; Rudenko & Vishchur 2016). Such changes can be explained by the results of our previous studies, which show that the addition of 5,000 IU of vitamin A led to decrease of triacylglycerols in the carp blood. The addition of 2,500 IU of vitamin A decreased level of lipid hydroperoxides and thiobarbituric acid reactive substances (TBARS), and increased the activity of superoxide dismutase and glutathione peroxidase, in comparison with control group carps (Popyk & Smolyaninov 2012).

Researchers proved the essential role of fatty acids in the reproductive function. Therefore, one of the objectives of our comprehensive research is also to examine this aspect (Hrytsyniak et al 2010). The various data suggest that a dietary supplementation of both n-3 and n-6 PUFA, is essential to improve the gonadal maturation, breeding performance and spawn recovery in the Catla Female broodstock (Samiran et al 2001). Moreover, our recent results (Masyuk 2018) suggest the subsequent changes in fatty acid metabolism in reproductive organs of female carp in pre-spawn period under the influence of vitamins and trace elements, resulting in beneficial changes of fatty acid composition of their spawn.

Conclusions. The results suggest that vitamin and mineral supplement to carp diet leads to the increase of the level of mono- and polyunsaturated fatty acids in the liver and skeletal muscles of female carps. It has been observed a higher level of phospholipids in the studied tissues of carps after introduction of vitamins and mineral supplements. These results suggest the positive changes in lipid metabolism in female carp under the influence of vitamin and mineral supplementation, at the pre-spawning period, resulting in an enhancement of their reproductive function. Taking into consideration the data obtained in this study and also the results of our previous investigations, we propose for the use in the fishery practice a vitamin and mineral supplement which contains 2,500 IU vitamin A, 3,333 IU vitamin D_3 and 1.7 mg vitamin E, and also the following trace elements: 5 mg kg^{-1} of food of potassium iodide, 40 mg kg^{-1} of zinc sulfate and 0.3 mg kg^{-1} of sodium selenite.

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Conflict of interest. The authors declare no conflict of interest.

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