

## LIVER CONTENT OF FATTY ACIDS AND REPRODUCTIVE INDICES OF CARP IN RELATION TO LEVEL OF DIETARY VITAMIN A

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**Abstract.** We investigated the effect of increased amounts of vitamin A in the diet of spawning carp on the content of fatty acids of the total lipids in the liver, as well as the reproductive ability of the fish. The experiment was conducted in the prespawning period in three groups of carp. The control group were offered the standard granulated mixed fodder. The test groups were additionally administered retinyl acetate added to the diet. It was established that in both females and males liver of the test groups, which received higher amounts of vitamin A, the content of fatty acids of total lipids increased. In the liver of the test groups carp, the increases were due to saturated fatty acids with an even and odd number of carbon atoms in the chain, monounsaturated fatty acid of families n-7 and n-9, and polyunsaturated fatty acids of the n-3 and n-6 families. The test group females, which were given vitamin A in quantities of 2500 and 5000 IE · kg<sup>-1</sup> feed, showed a significant and dose-dependent increase in both working and relative fecundity. The males had also an increased volume of milt. The supplementation also significantly and dose-dependently increased the percentage of larvae per spawn.

**Key words:** carp, vitamin A, fatty acids

### INTRODUCTION

Metabolic processes in the organism and the reproductive ability of pond fish, including carps, largely depend on meeting their needs for vitamins [Grytsyniak 2007, Smolyaninov 2012] especially vitamin A [Harrison 2005]. Given above vitamin affects the visual, antioxidant and immune functions of the body of fish.

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Also vitamin A has effects on different parts of the metabolic processes in the body of pond fish [Zhel'tov 2003].

The content of vitamin A in the blood, organs and tissues of pond fish, including carps, varies greatly depending on its content in the diet [Clagett-Dame and Knutson 2011]. The deficiency of vitamin A in the diet leads to inhibition of metabolic processes in the body and reproductive capacity of carps [Vorobyov 2008].

Fatty acids in the organism of fish are a source of a number of biologically active substances (prostaglandins, thromboxane and leukotrienes), which have a significant impact on the reproductive system [Popyk 2011]. However, so far the impact remains unknown endogenous and exogenous vitamin A content of fatty acids of total lipids in the liver and the reproductive ability of females and carp males.

Based on the above, the aim of the work was to investigate the effect of increased amounts of vitamin A in the diet on the content of fatty acids of total lipids in the liver and the reproductive ability of females and carp males.

## MATERIAL AND METHODS

The experiment was conducted at the Experimental Station ponds Lviv Institute of Fisheries NAAS. Lviv Research Station IRH NAANU was organized on March 6th in 1958 on the base of fisheries "Horodok" (Velykyi Lubin, Lviv region). All interventions and slaughter of fish were conducted in compliance with the European Convention for the Protection of vertebrate animals used for experimental and scientific purposes and the decision of the First National (Ukrainian) Congress on Bioethics. There were formed three groups of Lyubin scaly carps (*Cyprinus carpio* L.) age of six (each group has ten females and males). Each group of carp was kept in ponds with independent water supply. In ponds periodically it was determined the number and biomass of natural food – benthos.

Carps of each group were receiving standard granular mixed fodder K 111-2 from 50% -s protein per 4% of body weight every day at 8:00 during one month. The first group of carps was the control group and was receiving mixed fodder given above with sunflower oil in the amount of 3%. The second and third groups of carps were the research groups and additionally received as a part of the above-mentioned mixed fodder retynil acetate (production of "Technologist" Uman). The latter was applied to the mixed fodder in the above amount of oil. And carps of the first and the second research groups received mixed fodder with 2500 and 5000 IE · kg<sup>-1</sup> of vitamin A.

At the end of the experiment fish was caught from ponds by trawl method. From caught females and males of each group were obtained roe and milk by

hormone-induced method. It was being determined the absolute and relative fertility of females from each group. It was being defined the number of milk obtained from males of each group. At the same time it was being determined the output of the fertilized roe from females of each group. The fertilized roe was in the Weiss apparatus in the laboratory conditions.

After the decapitation of four females and males from each group, liver samples were collected for laboratory analysis. The samples were assayed for the content of fatty acids in the total lipids, according to Ravis and Fedoruk [2010].

The resulting data were processed by the variational statistics method using Student's *t*-test. We calculated arithmetic means (M), errors of means ( $\pm m$ ) and the probability of the difference between the averages (P). The changes were considered significant at  $P \leq 0.05$ . For calculations it was used a standard statistical package of computer software packages Origin 6.0 and Excel (Microsoft, USA).

## RESULTS AND DISCUSSION

It was established that in the liver of females and carp males of research groups, which in the prespawning period as a part of a standard granular fodder received additional amounts of vitamin A, compared with the liver of females and carp males of the control group that received fodder without additives, dose-dependent increases the content of fatty acids, total lipids (Tables 1 and 2).

From the above tables it is shown that the content of fatty acids of total lipids in the liver of females and carp males of the experimental group, compared with females and males of the control group, increases the expense of saturated, monounsaturated and polyunsaturated fatty acids. Moreover, the content of saturated fatty acids in the liver of carp of the research group increases due to fatty acids with an even (in females of the first and the second experimental groups according to 3.92 and 3.92 to 3.60 to 3.60  $\text{g} \cdot \text{kg}^{-1}$  wet weight in control, and males – 3.85 and 3.87 vs. 3.61) and odd (females of the first and the second experimental groups according to 0.08 and 0.08 to 0.06, and males – 0.07 and 0.07 against 0.05) the number of carbon atoms in the chain monounsaturated fatty acids – fatty acids n-7 families (females of the first and the second experimental groups respectively to 0.34 and 0.35 to 0.30, and males – 0.33 and 0.34 vs. 0.27) and n-9 (females of the first and the second experimental groups respectively to 7.97 and 8.06 to 7.88, and males – 7.80 and 7.85 against 7.71) and polyunsaturated fatty acids – fatty acids n-3 families (females of the first and the second experimental groups according to 7.56 and 7.67 to 6.66, and males – 6.72 and 6.81 against 6.03) and n-6 (females of the first and the second experimental groups according to 7.09 and 7.19 to 6.31, and males – 6.67 and 6.77 to 5.88  $\text{g} \cdot \text{kg}^{-1}$  wet weight in control). However, it should be noted that in the liver of carp females of the research

Table 1. The content of fatty acids of total lipids in the liver of female spawning carp,  $g \cdot kg^{-1}$  wet weight,  $M \pm m$ ,  $n = 4$

Tabela 1. Zawartość kwasów tłuszczowych całkowitych lipidów w wątrobie samic karpia – tarlaków,  $g \cdot kg^{-1}$  świeżej masy,  $M \pm m$ ,  $n = 4$

Fatty acids, codes Kwasy tłuszczowe, kody	Groups of fish – Grupy ryb		
	Control – Kontrolna	I	II
Caprylic, 8:0 – Kaprylowy, 8:0	0.01 ± 0.002	0.03 ± 0.002***	0.03 ± 0.002***
Capric, 10:0 – Kaprynowy, 10:0	0.05 ± 0.002	0.07 ± 0.002***	0.07 ± 0.002***
Lauric, 12:0 – Laurynowy, 12:0	0.08 ± 0.002	0.10 ± 0.002***	0.10 ± 0.002***
Myristic, 14:0 – Mirystynowy, 14:0	0.11 ± 0.002	0.13 ± 0.002***	0.13 ± 0.002***
Pentadecanoic, 15:0 – Pentadekanowy, 15:0	0.06 ± 0.002	0.08 ± 0.002***	0.08 ± 0.002***
Palmitic, 16:0 – Palmitynowy, 16:0	1.65 ± 0.063	1.91 ± 0.036*	1.93 ± 0.031***
Palmitoleic, 16:1 – Palmitoleinowy, 16:1	0.30 ± 0.008	0.34 ± 0.006***	0.35 ± 0.007***
Stearic, 18:0 – Stearynowy, 18:0	1.60 ± 0.043	1.57 ± 0.040	1.56 ± 0.036
Oleic, 18:1 – Oleinowy, 18:1	7.85 ± 0.171	7.93 ± 0.166*	8.01 ± 0.167*
Linoleic, 18:2 – Linolowy, 18:2	4.01 ± 0.108	4.47 ± 0.066*	4.51 ± 0.056***
Linolenic, 18:3 – Linolenowy, 18:3	1.97 ± 0.084	2.30 ± 0.046*	2.33 ± 0.040***
Arachidic, 20:0 – Arachidowy, 20:0	0.04 ± 0.002	0.03 ± 0.002	0.02 ± 0.004
Eicosanoic, 20:1 – Eikozainowy, 20:1	0.03 ± 0.002	0.04 ± 0.002*	0.05 ± 0.004*
Eicosadienoic, 20:2 – Eikozadienowy, 20:2	0.07 ± 0.002	0.09 ± 0.002***	0.09 ± 0.002*
Docosadienoic, 20:3 – Dokozadienowy, 20:3	0.48 ± 0.147	0.54 ± 0.011*	0.55 ± 0.009***
Arachidonic, 20:4 – Arachidonowy, 20:4	0.75 ± 0.022	0.86 ± 0.016***	0.88 ± 0.018***
Eicosapentaenoic, 20:5 – Eikozapentaenowy, 20:5	0.80 ± 0.026	0.90 ± 0.029*	0.94 ± 0.020***
Docosadienoic, 22:2 – Dokozadienowy, 22:2	0.33 ± 0.008	0.37 ± 0.006***	0.38 ± 0.008***
Docosatrienoic, 22:3 – Dokozatrienowy, 22:3	0.38 ± 0.012	0.44 ± 0.008***	0.45 ± 0.008***
Docosatetraenoic, 22:4 – Dokozatetraenowy, 22:4	0.67 ± 0.018	0.76 ± 0.019*	0.78 ± 0.018***
Docosapentaenoic, 22:5 – Dokozapentaenowy, 22:5	1.45 ± 0.053	1.63 ± 0.017*	1.64 ± 0.017*
Docosahexaenoic, 22:6 – Dokozaheksaenowy, 22:6	2.06 ± 0.073	2.29 ± 0.021*	2.31 ± 0.019*
Total fatty acids, incl.: Kwasy tłuszczowe ogółem, w tym:	24.75	26.88	27.19
Saturated – Nasycone	3.60	3.92	3.92
Monounsaturated – Jednonienasycone	8.18	8.31	8.41
Polyunsaturated – Wielonienasycone	12.97	14.65	14.86
n-3/n-6	1.05	1.06	1.06

\*  $P \leq 0.02-0.05$ ; \*\*  $P \leq 0.01$ ; \*\*\*  $P \leq 0.001$

groups, which in the pre-spawning period as part of a standard granular fodder received additional amounts of vitamin A, compared with the liver of carp females of the control group that received feed without additives, more rapidly grows the content of polyunsaturated fatty acids n-3 family, and males – family n-6 (Tables 1, 2). The above, apparently it is associated with gender differences in the me-

Table 2. The content of fatty acids of total lipids in the liver of male spawning carp, g · kg<sup>-1</sup> wet weight, M ±m, n = 4Tabela 2. Zawartość kwasów tłuszczowych całkowitych lipidów w wątrobie samców karpia – tarlaków, g · kg<sup>-1</sup> świeżej masy, M ±m, n = 4

Fatty acids, codes Kwasy tłuszczowe, kody	Groups of fish – Grupy ryb		
	Control – Kontrolna	I	II
Caprylic, 8:0 – Kaprylowy, 8:0	0.02 ±0.002	0.04 ±0.002***	0.04 ±0.002***
Capric, 10:0 – Kaprynowy, 10:0	0.04 ±0.002	0.06 ±0.002***	0.06 ±0.002***
Lauric, 12:0 – Laurynowy, 12:0	0.07 ±0.002	0.09 ±0.002***	0.10 ±0.004***
Myristic, 14:0 – Mirystynowy, 14:0	0.11 ±0.002	0.13 ±0.002***	0.14 ±0.004***
Pentadecanoic, 15:0 – Pentadekanowy, 15:0	0.05 ±0.002	0.07 ±0.002***	0.07 ±0.002***
Palmitic, 16:0 – Palmitynowy, 16:0	1.58 ±0.057	1.79 ±0.027*	1.82 ±0.029***
Palmitoleic, 16:1 – Palmitoleinowy, 16:1	0.27 ±0.008	0.33 ±0.009***	0.34 ±0.007***
Stearic, 18:0 – Stearynowy, 18:0	1.74 ±0.059	1.70 ±0.057	1.67 ±0.058
Oleic, 18:1 – Oleinowy, 18:1	7.68 ±0.155	7.76 ±0.159*	7.80 ±0.156*
Linoleic, 18:2 – Linolowy, 18:2	3.93 ±0.127	4.42 ±0.056*	4.45 ±0.054***
Linolenic, 18:3 – Linolenowy, 18:3	1.77 ±0.051	1.99 ±0.031*	2.02 ±0.024***
Arachidic, 20:0 – Arachidowy, 20:0	0.05 ±0.002	0.04 ±0.005	0.04 ±0.004
Eicosanoic, 20:1 – Eikozainowy, 20:1	0.03 ±0.002	0.04 ±0.002*	0.05 ±0.005*
Eicosadienoic, 20:2 – Eikozadienowy, 20:2	0.06 ±0.002	0.08 ±0.002***	0.08 ±0.002***
Docosadienoic, 20:3 – Dokoziadenowy, 20:3	0.41 ±0.013	0.47 ±0.006***	0.48 ±0.006***
Arachidonic, 20:4 – Arachidonowy, 20:4	0.66 ±0.018	0.69 ±0.007*	0.71 ±0.008*
Eicosapentaenoic, 20:5 – Eikozapentaenowy, 20:5	0.71 ±0.025	0.80 ±0.010*	0.81 ±0.010***
Docosadienoic, 22:2 – Dokoziadenowy, 22:2	0.29 ±0.010	0.33 ±0.004***	0.35 ±0.004***
Docosatrienoic, 22:3 – Dokoziatrienowy, 22:3	0.34 ±0.013	0.40 ±0.004***	0.41 ±0.004***
Docosatetraenoic, 22:4 – Dokoziatetraenowego, 22:4	0.59 ±0.018	0.68 ±0.015*	0.70 ±0.012***
Docosapentaenoic, 22:5 – Dokoziapentaenowy, 22:5	1.30 ±0.042	1.44 ±0.011*	1.46 ±0.010*
Docosahexaenoic, 22:6 – Dokoziheksaenowy, 22:6	1.91 ±0.045	2.09 ±0.021*	2.11 ±0.193***
Total fatty acids, incl.: – Kwasy tłuszczowe ogółem, w tym:	23.61	25.44	25.71
Saturated – Nasycone	3.66	3.92	3.94
Monounsaturated – Jednonienasycone	7.98	8.13	8.19
Polyunsaturated – Wielonienasycone	11.97	13.39	13.58
n-3/n-6	1.02	1.00	1.00

\* P ≤ 0.02–0.05; \*\* P ≤ 0.01; \*\*\* P ≤ 0.001

tabolism of polyunsaturated fatty acids in the organism of carp [Vorobyov 2008, Popyk 2011].

The growth of contents polyunsaturated fatty acids of families n-3 and n-6 in liver of females and carp males for more vitamin A in their diet, apparently is associated with a decrease in lipid peroxidation [Smolyaninov 2010]. However, the increased number of protected vitamin A family of polyunsaturated fatty acids n-3

Table 3. Reproduction indices of spawning carp by level of vitamin A added to feed,  $M \pm m$ ,  $n = 10$

Tabela 3. Wskaźniki reprodukcyjne karpia tarłowego w zależności od dodatku witaminy A w paszy,  $M \pm m$ ,  $n = 10$

Indices Wskaźniki	Groups of fish – Grupy ryb		
	Control – Kontrolna	I	II
Working fertility in females carps, thousand eggs Płodność absolutna samic karpia, tys. jaj	669.0 $\pm$ 10.01	709.4 $\pm$ 6.23**	719.5 $\pm$ 6.97**
Relative fecundity in females carps, thousand eggs Płodność względna samic karpia, tys. jaj	92.4 $\pm$ 2.82	115.9 $\pm$ 4.93**	119.8 $\pm$ 4.52**
The volume of milt carps, ml Objętość spermy, ml	24.6 $\pm$ 0.66	28.4 $\pm$ 0.57**	29.4 $\pm$ 0.68***
Larvae from roe, % Larw z ikry, %	70.1 $\pm$ 0.47	72.9 $\pm$ 0.32**	73.4 $\pm$ 0.27***

and n-6 not only promotes greater synthesis in the organism of females and carp males biologically active substances (prostaglandins, thromboxane and leykotrey-eniv). We see that the increased number of fatty acids, mentioned above, helps to transform available in fish cholesterol in estrogen and androgens.

We found that the increase of the content of polyunsaturated fatty acids n-3 families and n-6 in the liver leads to improvement of the reproductive capacity of females and carp males. Table 3 shows that the female carp of the research groups, which in the prespawning period as part of standard granulated fodder were fed extra amounts of vitamin A, compared with carp females of the control group which were fed a standard granular fodder without supplements significantly and dose-dependent increases working and relative fertility, in the male carp – the volume of milk. This increases significantly and dose-dependent the larvae out of eggs.

## CONCLUSIONS

In the liver of carp females and males of the first and the second research groups, which in the prespawning period as part of a standard granular fodder received vitamin A in quantities of 2500 and 5000 IE  $\cdot$  kg<sup>-1</sup> of mixed fodder increases the content of fatty acids of total lipids. The content of fatty acids of total lipids in the liver of carp of research groups increases due to saturated fatty acids with an even and odd number of carbon atoms in the chain, monounsaturated fatty acid families n-7 and n-9 and polyunsaturated fatty acids and n-3 families and n-6.

In the liver of carp females, which in prespawning period as part of standard granulated mixed fodder were fed the additional vitamin A in quantities of 2500

and 5000 IE · kg<sup>-1</sup> fodder, more intensively increases the content of polyunsaturated fatty acids n-3 family, and carp males – n-6 family.

In the female carp, which in prespawning period as part of standard granulated mixed fodder were fed the additional vitamin A in quantities of 2500 and 5000 IE · kg<sup>-1</sup> fodder, dose-dependent increases significantly and relative fecundity and working in carp males – volume of milk. This increases significantly and dose-dependent the larvae out of eggs.

Prospects for the further research. In future it is planned to investigate the impact of increased amounts of vitamin A in the diet on the fatty acid composition of triacylglycerols skeletal muscles of carps females and males.

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## ZAWARTOŚĆ KWASÓW TŁUSZCZOWYCH W WĄTROBIE ORAZ WSKAŹNIKI ROZRODCZE KARPIA W STOSUNKU DO POZIOMU W DIECIE WITAMINY A

**Streszczenie.** Badano wpływ zwiększonej ilości witaminy A w diecie karpia na zawartość kwasów tłuszczowych w lipidach ogólnych w wątrobie, jak również na zdolności reprodukcyjne u ryb. Eksperyment przeprowadzono w okresie przedtarłowym w trzech grupach karpia. W grupie kontrolnej, karpie otrzymywały standardową granulowaną mieszankę paszową. Grupy eksperymentalne dodatkowo otrzymywały octan retinylu dodawany do diety. Stwierdzono zarówno u samic jak i u samców, że wątroby w grupach eksperymentalnych, które otrzymały wyższą ilość witaminy A, zawartość kwasów tłuszczowych była zwiększona. Spowodowane to było przez wzrost zawartości nasyconych kwasów tłuszczowych o parzystej i nieparzystej liczbie atomów węgla w łańcuchu, kwasów jednonienasyconych, kwasów tłuszczowych z rodziny n-7 i n-9 oraz wielonienasyconych kwasów tłuszczowych n-3 i n-6. Samice, którym podawano witaminę A w ilości 2500 i 5000 IE · kg<sup>-1</sup> paszy, wykazywały znaczące i zależne od dawki zwiększenie płodności, zarówno całkowitej jak i względnej. Samce produkowały większą objętość spermy. Suplementacja istotnie i zależnie od dawki zwiększyła procent larw z ikry.

**Słowa kluczowe:** karp, witamina A, kwasy tłuszczowe

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