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THE INFLUENCE OF NUTRATIVE LIPID SOURCES ON THE GROWTH AND CHEMICAL AND FATTY ACID COMPOSITION OF CARP (*CYPRINUS CARPIO* L.)

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Abstract. The purpose of the present study was to determine the influence of animal and plant lipids (cod-liver, soybean and sunflower-seed oil, sunflower-seed and tallow fatty acids, sunflower-seed lecithin) on the growth and chemical and fatty acid composition of carp, *Cyprinus carpio* L. Eight isocaloric diets that were processed into granules and distributed in three experiments were used. The nutritive lipid sources that were a component of the carp diets stimulated growth, protein absorption, and the retention of fats and protein. The fatty acids extracted from the carp in the 2+ age group were either oleic-linoleic-palmitic or oleic-palmitic-linoleic. The individual fatty acid profile was influenced by the composition of the lipid sources.

Key words: CARP (*CYPRINUS CARPIO*), NUTRITION, LIPIDS, GROWTH, BIOCHEMICAL PARAMETERS, FATTY ACID COMPOSITION

INTRODUCTION

Carp, *Cyprinus carpio* L., is a major cultivated fish species in Bulgaria since it has a stable growth rate and it tastes good. One of the factors that most directly influences carp productivity is feed. Increasing the nutritional values of feed rations using different nutritive and stimulating additives leads to improved nutrition efficiency. Research on enriching feeds with various lipid components is of great importance since they are well absorbed by fish and provide irreplaceable essential fatty acids, phospholipids, liposolvent vitamins, sterols, etc. It is believed that when the above substances are introduced to living organisms, lipids perform specific functions that are of much more significance than the non-specific ones related to their caloric equivalent. While assessing the biological role of the lipids, their position as participants in the establishment and functioning of the cellular membrane was also taken into account. Some investigation results showed that the introduction of lipids in the diet stimulates fish somatic growth, protein absorption, and protein and lipid retention

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(Borlogan and Parazo 1991, Steffens 1987). According to Viola and Rapport (1978, 1979), carp absorb free fatty acids more readily from neutral oil.

The study was conducted in order to determine what impact lipids of animal and plant origin present in feed have on growth and the chemical and fatty acids composition of carp.

MATERIAL AND METHODS

EXPERIMENTAL FEEDS

Eight isocaloric diets processed into granules (4 mm) were used for the experiments. They were distributed in three experiments according to the scheme in Table 1.

TABLE 1

Scheme of experiments

Type of lipids	Experiment I-variants				Experiment II- variants			Experiment III- variants			
	1*	2**	3	4	5	6*	7	8	9*	10	11
Cod-liver oil	-	3%	-	-	-	-	-	-	-	-	-
Soy-bean oil	-	-	3%	-	-	-	-	-	-	-	-
Sunflower-seed oil	-	-	-	3%	-	-	-	-	-	-	-
RssFA***	-	-	-	-	3%	-	5%	-	-	7%	4%
Sunflower-seed lecithin	-	-	-	-	-	-	-	-	-	-	3%
DTFA****	-	-	-	-	-	-	-	5%	-	-	-

* - variants 1, 6, and 9 are controls (without a lipid component in their composition)

** - variants 2, 3, 4, 5, 7, 8, 10, 11 are experimental and contain different lipids of varied sources that were added to the feed by surface oiling

***. RSsFA - Refined Sunflower-seed Fatty Acids

****DTFA- distilled tallow fatty acids

The lipid component was included in the feed by surface oiling. Diets 1, 6, and 9 did not have lipid additives and were the control for the experiments: diet 1 - control experiment I; diet 6 - control experiment II; diet 9 - control experiment III. Diets 2, 3 and 4 consisted of 3% cod-liver, soy-bean and sunflower-seed oil, respectively, whereas diet 5 consisted of 3% RSsFA (Refined Sunflower-seed Fatty Acids, a by-product of sunflower-seed oil production, that consisted of 67-68% free fatty acids, mainly oleic and linoleic acid, and over 85% and 30-31% of neutral oil). Diets 7 and 8 consisted of 5% RSsFA and 5% DTFA (distilled tallow fatty acids - a by-product of tallow oil production, that consisted of 90-94% free fatty acids, including isomeric and mainly oleic and linoleic acids (over 70%)), respectively. Diet 10 consisted of 7% RSsFA, and diet 11 consisted of a 7% mixture of 3% sunflower-seed lecithin and 4% RSsFA. Each combination had two-fold repeatability.

The nutritive value of the feeds used in the experiments is presented in Table 2, while the fatty acids composition of the experimental rations is presented in Table 3. The individual fatty acids profile was determined and the content of unsaturated fatty acids from the C₁₈ - C_{18:1} group and C_{18:2} prevailed with a sum that was significantly higher for the variants where sunflower-seed oil and its derivatives – RSsFA and sunflower-seed lecithin, were used.

TABLE 2

General chemical composition of diets used in the experiments

Parameters (%)	Experiment I -variants				Experiment II- variants				Experiment III- variants		
	1	2	3	4	5	6	7	8	9	10	11
Crude protein	25.14	25.26	25.26	25.26	25.06	25.08	25.08	25.08	25.10	25.10	25.10
Crude fats	3.00	6.00	6.00	6.00	6.20	3.60	8.60	8.60	3.00	10.00	10.00
Crude fiber	5.62	6.34	6.34	6.34	6.18	6.40	6.40	6.40	5.56	5.56	5.56
Lysine	1.37	1.27	1.27	1.27	1.25	1.39	1.39	1.39	1.36	1.36	1.36
Methionine	0.58	0.61	0.61	0.61	0.59	0.84	0.84	0.84	0.72	0.72	0.72
Calcium	2.70	2.58	2.58	2.58	2.53	2.03	2.03	2.03	2.66	2.66	2.66
Phosphorus	2.04	2.01	2.01	2.01	2.00	2.03	2.03	2.03	2.04	2.04	2.04
Water	10.70	10.64	10.64	10.64	10.66	11.58	11.58	11.58	11.52	11.52	11.52

TABLE 3

Fatty -acid composition of experimental diets, % of fat (N = 3).
Variants are described in the Material and Methods

		Experiment I -variants				Experiment II- variants				Experiment III- variants		
		1	2	3	4	5	6	7	8	9	10	11
C _{14:0}	mean	1.8	8.5	1.8	1.7	1.3	0.4	0.6	0.8	1.2	1.2	1.8
	SD	0.07	0.13	0.07	0.08	0.11	0.06	0.04	0.03	0.11	0.16	0.10
C _{14:1}	mean	-	0.5	-	0.3	-	-	-	-	-	-	-
	SD	-	0.02	-	0.02	-	-	-	-	-	-	-
C _{16:0}	mean	19.5	11.6	18.4	14.7	14.0	19.8	12.5	10.1	26.7	12.6	12.4
	SD	0.32	0.38	0.18	0.25	0.25	0.17	0.24	0.17	0.19	0.25	0.15
C _{16:1}	mean	4.3	5.4	1.3	2.7	0.7	4.1	4.5	2.5	0.7	0.7	0.7
	SD	0.17	0.14	0.09	0.13	0.06	0.11	0.15	0.11	0.06	0.03	0.03
C _{18:0}	mean	5.4	7.2	9.3	5.5	5.2	5.8	5.3	4.9	3.4	5.6	5.6
	SD	0.14	0.16	0.28	0.19	0.16	0.14	0.12	0.14	0.12	0.12	0.17
C _{18:1}	mean	25.3	29.0	21.2	19.4	20.4	23.2	27.7	28.9	31.4	25.3	26.5
	SD	0.19	0.25	0.25	0.32	0.38	0.19	0.41	0.28	0.19	0.38	0.31
C _{18:2}	mean	41.4	25.1	44.8	52.5	56.7	42.8	47.6	47.6	35.0	52.7	52.0
	SD	0.53	0.12	0.74	0.65	0.56	0.33	0.68	0.32	0.64	0.74	0.44
C _{18:3}	mean	2.3	8.3	3.2	3.2	1.7	3.9	1.8	-	1.2	1.1	-
	SD	0.14	0.17	0.15	0.11	0.12	0.12	0.05	-	0.09	0.08	-
C _{20:0}	mean	-	0.2	-	-	-	-	-	-	0.4	0.4	0.6
	SD	-	0.05	-	-	-	-	-	-	0.03	0.15	0.04
C _{22:0}	mean	-	4.2	-	-	-	-	-	2.04	-	-	-
	SD	-	0.17	-	-	-	-	-	0.14	-	-	-
C _{18 izom.}	mean	-	-	-	-	-	-	-	3.2	-	-	-
	SD	-	-	-	-	-	-	-	0.19	-	-	-
Σ saturated		26.7	31.7	29.5	21.9	20.5	26.0	18.4	17.8	31.7	20.2	20.4
Σ unsaturated		73.3	68.3	70.5	78.1	79.5	74.0	81.6	82.2	68.3	79.8	79.6
Unsaturated /saturated		2.74	2.15	2.38	3.56	3.87	2.85	4.43	4.61	2.15	3.95	3.90

FISH AND NUTRITION

Age group 1+ carp reared at the Fisheries and Aquaculture Institute, Plovdiv Branch, Bulgaria were used in the experiments. Earthen experimental ponds with an area ranging from 0.1 to 0.25 ha were prepared prior to the experiments. They were stocked with 2800 fish specimens ha^{-1} .

The first experiment lasted for 140 days at a water temperature range of 19-27°C. The dissolved oxygen concentration in the water was 4.0-6.0 mg l^{-1} and the pH was 7.0-8.5. The second experiment lasted for 160 days at a water temperature range of 18 - 25°C. The dissolved oxygen concentration in the water was 4.6-6.6 mg l^{-1} and the pH was 6.5 - 8.5. The third experiment lasted for 158 days at a water temperature of 18 - 24°C. The dissolved oxygen concentration in the water was 4.6 - 7.0 mg l^{-1} and the pH was 7.0 - 8.0.

The fish were fed twice daily at 09:00 and 14:00, and the rations were determined based on the monthly allocation of feed.

CHEMICAL ANALYSES AND CALCULATIONS

Samples of skinless meat from ten carp from each variant were collected at the beginning and end of the experiments. After homogenization, the samples were used to determine the following parameters: water (105°C, 24 h); proteins (Kjeldahl and Pdrnas-Wagner distillation of N); lipids (Soxhlet method); ash using standard methods (Kyosev 1978).

Lipids were extracted from feed and fish samples with the Bligh and Dyer method (1959). The fatty acid composition was determined using the gas-chromatography of the fatty acid methyl esters. A gas chromatograph with a flame-ionized detector was used. Component identification was performed with standards produced by Carbo Erba.

The effect achieved by the addition of lipids was registered by calculating the parameters of weight, growth, food utilization, and protein retention for each variant based on data of assimilated food and chemical analysis of fish (Borlogan and Parazo 1991, EIFAG 1989, Viola and Lahav 1991) as follows:

- DGR (Daily Growth Rate) = (final weight-initial weight) days^{-1} (g d^{-1});
- SGR (Specific Growth Rate) = (Ln final weight-Ln initial weight) days^{-1} (%);
- FCR (Feed Conversion Ratio) = Dry food intake/fish weight gain;
- PER (Protein Efficiency Ratio) = Weight gain/protein intake;
- PRE (Protein Retention Efficiency) = Body protein gain/protein intake $\times 100$.

The data was processed statistically with Windows 98 statistical software. The reliability of the difference between the values of two samples was determined by means of the T-test at a degree of probability of $P \leq 0.05$.

RESULTS

The data obtained from the experiments on growth rate, such as growth, DGR, and SGR, are presented in Table 4.

TABLE 4
Influence of dietary lipid additives on carp growth and food conversion. Variants are described in the Material and Methods

Variants	Initial body weight (g)	Final body weight (g)	Weight gain				Food conversion			
			Total (kg d ⁻¹)	Individual (g)		DGR (g d ⁻¹)	SGR (%)	FCR	PER	PRE
				mean	SD					
Experiment I										
1	105	1004	224	899 ^b	28,5	6.42	1.61	3.0	1.32	22.25
2	90	1033	220	943 ^b	30.4	6.74	1.74	3.0	1.32	22.96
3	95	955	207	860 ^b	29.3	6.14	1.65	3.3	1.20	21.07
4	96	1018	200	922 ^b	31.1	6.59	1.69	3.4	1.16	20.09
5	101	1152	257	1051 ^a	35.4	7.51	1.74	2.6	1.54	26.95
Experiment II										
6	94	900	215	806 ^e	27.3	5.04	1.41	3.4	1.17	19.43
7	96	1180	262	1084 ^c	30.4	6.77	1.57	2.7	1.48	26.21
8	96	1030	221	934 ^d	31.2	5.84	1.50	3.3	1.21	21.19
Experiment III										
9	55	934	216	879 ^g	22.3	5.56	1.79	3.1	1.28	21.44
10	55	1080	272	1025 ^f	29.6	5.48	1.88	2.5	1.60	28.28
11	55	1040	256	985 ^f	28.1	6.23	1.86	2.7	1.48	26.93

* - 280 carp specimens on average in every variant from two ponds

** - different superscripts indicate statistically significant differences ($P < 0.05$)

The carp reacted to the various experimental diets in different ways. Higher individual weight and growth were registered in variants 5 (3% RSsFA), 7 and 8 (5% RSsFA and 5% DTFA), and 10 and 11 (7% RssFA and 7% lipid mixture). The fish from control groups 1, 6, and 9 (without additional lipids) had significantly lower individual growth in comparison with experimental variants 5, 7, 8, 10, and 11. The FCR values were lower (15-20%) in comparison to the control portions in variants 5, 7, and 10, in which different quantities of RSsFA were applied. Higher values were also registered for PER and protein retention in these variants.

The chemical composition of the meat of carp from age group 2+ during the experimental period changed in proportion to decreased water and increased protein and lipid contents (Table 5).

TABLE 5
Influence of lipid additives on the chemical composition of carp meat (N = 5) (% of wet weight).
Variants are described in the Material and Methods

Variants	Water		Protein		Lipids	
	mean*	SD	Mean	SD	mean	SD
	Experiment I					
1	78.57 ^a	0.33	16.78 ^b	0.13	3.19 ^c	0.12
2	77.55 ^a	0.24	17.40 ^a	0.14	4.15 ^a	0.10
3	77.53 ^b	0.21	17.56 ^a	0.12	4.02 ^a	0.10
4	77.85 ^{ab}	0.30	17.26 ^a	0.16	4.13 ^a	0.13
5	77.39 ^b	0.20	17.56 ^a	0.14	3.70 ^b	0.12
	Experiment II					
6	78.44 ^c	0.18	16.57 ^d	0.13	2.86 ^e	0.10
7	77.56 ^d	0.26	17.74 ^c	0.18	3.57 ^d	0.16
8	77.82 ^{cd}	0.33	17.63 ^c	0.21	3.23 ^d	0.10
	Experiment III					
9	78.63 ^e	0.25	16.70 ^f	0.18	2.92 ^g	0.12
10	77.37 ^f	0.22	17.75 ^e	0.14	3.81 ^f	0.15
11	77.83 ^{ef}	0.31	18.24 ^e	0.21	3.11 ^g	0.10

*- different superscripts indicate statistically significant differences ($P < 0.05$)

The retention of proteins and lipids in the meat for every variant with lipid additives was significantly higher in comparison to control variants 1, 6, and 9. The absolute values of protein varied from 17.26% to 18.24% and of lipids from 3.11% to 4.15%. The lipid level in carp meat is dependent on the type and quantity of the added lipid.

The fatty acid composition of lipids from age group 2+ carp was comparatively complicated and included representatives of fatty acids from C₁₄ to C₂₂ with an even number of carbon atoms, both saturated and unsaturated (Table 6). The level of the saturated fatty acids was considerably lower than that of the unsaturated. As far as the variants of fatty acids are concerned, the lipids extracted from age group 2+ carp were oleic-linoleic-palmitic. The content of butyric-acid was also influenced to a certain degree by the composition of lipid sources.

DISCUSSION

The results from the present study prove that the introduction of lipids to feeds stimulates carp somatic growth, protein assimilation, and protein and lipid retention, and these results conform with those of other authors (Abrosimov and Bibikov 2003,

TABLE 6

Fatty-acid composition of carp meat (% of fat; N = 3). Variants are described in the Material and Methods

	Experiment I -variants				Experiment II- variants				Experiment III- variants		
	1	2	3	4	5	6	7	8	9	10	11
C _{14:0} mean	2.0	1.1	1.8	2.0	1.7	0.4	0.6	0.8	1.2	1.6	1.8
SD	0.08	0.12	0.12	0.09	0.10	0.06	0.04	0.03	0.11	0.16	0.10
C _{14:1} mean	0.4	0.5	0.4	0.4	1.0	-	-	-	-	-	-
SD	0.03	0.08	0.03	0.05	0.04	-	-	-	-	-	-
C _{14:2} mean	-	-	-	-	-	0.1	0.4	0.4	-	-	-
SD	-	-	-	-	-	0.03	0.06	0.04	-	-	-
C _{16:0} mean	25.2	24.6	27.4	18.6	22.4	25.6	17.5	21.6	22.5	16.0	19.9
SD	0.29	0.27	0.35	0.25	0.33	0.32	0.26	0.37	0.38	0.33	0.37
C _{16:1} mean	5.7	6.7	7.0	2.4	5.9	1.2	5.1	5.6	5.6	7.9	5.6
SD	0.18	0.18	0.14	0.19	0.19	0.10	0.19	0.18	0.09	0.24	0.25
C _{16:3} mean	0.1	0.4	0.2	0.3	0.5	-	-	-	-	-	-
SD	0.02	0.06	0.06	0.03	0.03	-	-	-	-	-	-
C _{18:0} mean	4.5	5.6	4.5	5.5	5.8	4.6	5.6	5.2	2.4	2.8	3.1
SD	0.17	0.17	0.14	0.21	0.11	0.15	0.16	0.22	0.12	0.23	0.15
C _{18:1} mean	38.2	42.8	40.7	43.8	34.6	46.4	41.3	41.0	45.6	42.9	44.9
SD	0.33	0.53	0.38	0.38	0.55	0.63	0.65	0.51	0.33	0.76	0.51
C _{18:2} mean	19.8	14.0	13.8	22.1	21.4	18.4	24.9	20.7	20.4	26.1	23.6
SD	0.25	0.24	0.22	0.31	0.34	0.19	0.35	0.38	0.24	0.27	0.26
C _{18:3} mean	3.1	4.0	3.6	4.3	4.3	0.3	0.3	0.3	0.5	0.6	0.4
SD	0.15	0.12	0.14	0.14	0.13	0.02	0.04	0.06	0.05	0.05	0.06
C _{20:0} mean	-	0.3	0.6	0.6	-	-	-	-	1.3	0.6	1.1
SD	-	0.04	0.08	0.06	-	-	-	-	0.09	0.08	0.14
C _{20:1} mean	-	-	-	-	-	2.2	3.2	3.4	-	-	-
SD	-	-	-	-	-	0.13	0.11	0.12	-	-	-
C _{20:2} mean	-	-	-	-	-	-	0.5	0.4	-	-	-
SD	-	-	-	-	-	-	0.06	0.05	-	-	-
C _{20:3} mean	-	-	-	-	-	-	-	0.4	-	0.5	-
SD	-	-	-	-	-	-	-	0.03	-	0.09	-
C _{20:4} mean	0.5	-	-	-	1.2	-	-	-	-	-	-
SD	0.04	-	-	-	0.09	-	-	-	-	-	-
C _{22:0} mean	0.5	-	-	-	1.2	-	-	-	-	1.2	-
SD	0.06	-	-	-	0.07	-	-	-	-	0.09	-
Σ saturated	32.2	31.6	34.3	26.7	31.1	31.4	24.3	27.8	27.9	22.0	25.5
Σ unsaturated	67.8	68.4	65.7	73.3	68.9	68.6	75.7	72.2	72.1	78.0	74.5
unsaturated /saturated	2.10	2.16	1.91	2.75	2.22	2.18	3.11	2.59	2.59	3.44	2.92

Borlogan and Parazo 1991, Steffens 1987). Lower productivity and protein retention in control variants 1, 6, and 9 were registered. Regardless of the overall positive influence of lipids, the difference in the values of the studied parameters (growth, FCR, protein efficiency, and retention) indicates the specific response of carp to the lipids of various origin that were used. The most favorable were the values of the variants that used different quantities of RSsFA. In light of carp active lipid metabolism, the preceding fact relates to the greater absorption by the carp of the free fatty acids in neutral oil, which

complies with other studies (Viola and Rappaport 1978, 1979). The same can account for lower values when using 3% cod-liver, soy-bean, and sunflower-seed oil.

The level of lipids in carp meat and their modification depends both on the quantity and type of lipids used. An increase in dietary lipids does not always correspond to an increase in the general lipid level in carp. The investigations indicate that this process can be controlled to a certain extent by selecting a suitable lipid source and a quantity consistent with the level of protein in the feed respective of protein in relation to energy. This observation was made in the author's previous studies and by other authors (Viola and Rappaport 1979, Viola et al.1988).

A comparative analysis of protein accumulation in carp meat has shown that in all cases the lipid additions produce a protein-saving effect at differing values, which also complies with other studies (Abrosimov and Bibikov 2003). For example, the protein accumulation in carp meat from variants 5 and 11 was 4.60 and 9.22% higher than in the controls.

With regard to the fatty acid composition of carp meat lipids, the spectrum of unsaturated fatty acids was extended to the greatest degree in variants 5, 7, 8, and 10. The synthesis of fatty acids with 20 carbon atoms containing from 1 to 4 double bonds was favored. The highest relative share of essential fatty acids (35 - 39%) occurred when RSsFA was included in the diets. Both the quantity and the type of lipids used impacted the composition of carp muscle lipids. This was also confirmed by the results of the variants where, along with the typical modifications of the reduction of saturated fatty acids as a whole (16:0 above all) and the increase of unsaturated fatty acids of C₁₈ group, certain changes were noted that are attributable to the nature of the lipid additive.

The results of the study indicate that the introduction of nutritive lipid sources in carp diets stimulates carp growth, protein absorption, and the retention of proteins and lipids in carp meat.

The selection of an appropriate lipid component and quantity consistent with the level of protein in the diet allows for the total quantity of lipids in carp to be properly monitored. As far as various fatty acids are concerned, lipids that have been extracted from the meat of age group 2+ carp are oleic-linoleic-palmitic or oleic- palmitic-linoleic.

Their individual fatty acid profile was influenced by the composition of the lipid sources. The tendency identified is one of the more favorable impact of RSsFA on the studied parameters that characterize the development (growth, DGR, SGR), food conversion (FCR and PER), protein retention (PRE), chemical, and fatty acid composition of carp.

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STRESZCZENIE

WPLYW LIPIDÓW RÓŻNEGO POCHODZENIA NA SKŁAD CHEMICZNY I ZAWARTOŚĆ KWASÓW TŁUSZCZOWYCH W CIELE KARPIA (*CYPRINUS CARPIO* L.)

Celem niniejszej pracy było określenie wpływu tłuszczów roślinnych i zwierzęcych (tran z wątroby dorsza, olej sojowy i słonecznikowy, rafinowany olej słonecznikowy i talowy, lecytyna uzyskana z ziaren słonecznika) na wzrost, skład chemiczny mięsa i zawartość kwasów tłuszczowych w ciele karpia (*Cyprinus carpio* L.). W trzech doświadczeniach użyto ośmiu izokalorycznych pasz granulowanych. Tłuszcze użyte w doświadczeniach, pochodzące z różnych źródeł, stymulowały wzrost, absorpcję białek oraz magazynowanie białek i tłuszczów. Kwasy tłuszczowe wyestrahowane z mięśni dwuletnich karpia podzielono na dwie grupy: kwasy typu oleinowo-linolowo-palmitynowy oraz oleinowo-palmitynowo-linolowy. Profile kwasów tłuszczowych wyestrahowanych z indywidualnych osobników były ściśle związane ze źródłem tłuszczu, który wykorzystano w komponowaniu paszy.