

# Effect of nutritionally complete feed with different fatty acid profile on the fatty acid composition of common carp fillet

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## ABSTRACT

Hungarian pond fish production is based on grains, but in the last few years, new ideas and efforts have appeared to intensify carp production technology. The basic objective was to change grain-based feeding to nutritionally complete feeds, which ensure rapid growth and more efficient feed conversion rates. This study aimed to utilise empty ponds during the summer period for carp production. Thus, there is no need for fish producers to catch fish in large ponds at the operating water level to satisfy smaller market demands appearing during the summer.

The other aim was to compare the meat quality of fish raised on traditional and nutritionally complete feed until market size in the last year of production. Fatty acid profile and the levels of saturated, monounsaturated, and polyunsaturated fatty acids in fish fillets were specified, and their ratios were analysed.

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The result showed that nutritionally complete feed with different fatty acid composition affects the fatty acid composition of carp fillet during the rearing period. Quality of the fillet of carp fed with higher unsaturated fatty acid content became more favourable to the consumers due to health promoting effect of poly-unsaturated fatty acids.

## KEYWORDS

common carp, pond feeding, fatty acid profile, lipid peroxidation processes

## 1. INTRODUCTION

Common carp (*Cyprinus carpio* L.) is an important fish of freshwater aquaculture (Roy et al., 2020). In some countries of Europe, carp make up more than 80% of the total fish production (Anton-Pardo et al., 2014). Traditional carp production is principally based on natural food of the pond, and fish growth is also supported by grain feeding. Extruded feeds have better digestion rates compared to cereals, which generates lower feed conversion rates, resulting in a smaller load of organic matter on pond ecosystems (Hardy and Barrows, 2000). Extruded feeds are also applied in carp production; in certain countries are widely used as they provide higher growth rates (Ćirić et al., 2015) and are also able to improve the quality of meat (Trbović et al., 2013).

Meat quality and fatty acid composition of produced fish depend on several factors, the most important endogenous condition of which is the microclimate of animal habitats (Khan and Mir, 2012). Concerning exogenous conditions, as water temperature increases, general metabolism accelerates, promoting protein transformation (Khan and Mir, 2012). Also the number of days exposed to sunlight or snow also affects the natural yields of ponds (Shearer, 1994). Together with geographical location, feeding technology plays an important role in fish's saturated (SFA) and unsaturated fatty acid (UFA) composition (Trbović et al., 2013).

## 2. MATERIALS AND METHODS

### 2.1. Experimental site

The experiment was conducted in small wintering ponds with two-summer-old Nagyatád mirror carp obtained from Czikkhalas Halastavai Ltd (Table 1). There were no parallel ponds during the experiment.

Table 1. Data on experimental stockings

Groups	Pond size		Quantity (individuals)	Total biomass (kg)	Average weight (kg/individual)	Stocking (individual/ha)
	$m^2$	$m^3$				
Contr.	441	900	64	13	0.2	1,440
Exp.	851	1,400	850	170	0.2	10,000



## 2.2. Treatments

Fish were fed thrice daily, the control group with grain (wheat, corn) at 3% per body weight, while the experimental group with nutritionally complete feed of 2.5%. The experimental period was 112 days.

Composition and calculated nutrient content of the experimental feed are given in Table 2.

Analysed fatty acid content of the control and experimental feeds are given in Table 3.

Analysed fat content and fatty acid profile of the control and experimental feeds are given in Table 4.

## 2.3. Measurements

At the start of the experiment in May, three randomly selected animals were sampled as absolute control. The second sampling was done 60 days later in July, the third in September, 112 days

Table 2. Composition and calculated nutrient content of the experimental feed

Feed component			Nutrient content (dry matter)		
Rapeseed meal	10	%	Crude protein	35	%
Soybean meal	22	%	Crude fat	9	%
Fish meal	8	%	N-free extract	37	%
Haemoglobin	2.5	%	Crude fibre	4.6	%
Fish oil	4	%	Crude ash	6.9	%
Wheat	22	%	Digestible energy	14.9	MJ kg <sup>-1</sup>
DDGS	20	%			
Wheat bran	8	%			
Monocalcium phosphate	2	%			
Mineral premix	0.45	%			
Vitamin A	2,500	IE kg <sup>-1</sup>			
Vitamin D	3,500	IE kg <sup>-1</sup>			
Vitamin E	150	mg kg <sup>-1</sup>			

Table 3. Fatty acid components of used feeds (mg/100 g feed)

SFA mg/100 g		MUFA mg/100 g		PUFA mg/100 g				
Contr.	Exp.	Contr.	Exp.	Contr.	Exp.			
C14:0	0	403	C14:1	3	40	C18:2n6c	1,391	1,529
C16:0	328	1,519	C16:1	3	352	C18:3n3c	52	346
C17:0	3	27	C18:1n9c	661	2,217	C20:2	0	181
C18:0	48	240	C18:1n7c	0	315	C20:4n6	0	47
C20:0	11	28	C20:1n9	12	303	C20:5n3 (EPA)	0	461
C22:0	4	41	C22:1	0	395	C22:5n3	0	69
C24:0	4	0				C22:6n3 (DHA)	0	699
Total SFA	399	2,258	Total MUFA	679	3,622	Total PUFA	1,443	3,332



Table 4. Comparison of used feeds based on fat content and fatty acid composition

	Contr.	Exp.
Total amount of SFAs (as % of total amount of fatty acids)	15.7	23.3
Total amount of MUFAs (as % of total amount of fatty acids)	26.9	37.4
Total amount of PUFAs (as % of total amount of fatty acids)	57.2	34.4
Total amount of UFAs (as % of total amount of fatty acids)	84.1	74.8
Fat content (g/100 g wet weight)	2.63	10.1

after the beginning of the feeding experiment. At each sampling, 5 randomly selected fish were taken from the control and 10 from the experimental group.

After slaughtering, whole fillets without skin were sampled and stored at +4°C until laboratory analyses.

The total fat content of the control and experimental feeds and fillets was measured according MSZ ISO (2002). The fatty acid content of feed and fillets was determined according to MSZ (1987) and MSZ ISO (1992).

Content of conjugated dienes (CD) as initial phase markers of lipid peroxidation processes was determined following AOAC (1984), analysis method no. 28.054. In addition, amounts of malondialdehyde (MDA), a meta-stable end product of lipid peroxidation processes, was determined based on the procedure of Menoyo et al. (2003).

## 2.4. Statistical analysis

The statistical program PAST 4.06b (Hammer et al., 2001) was used to prepare statistical analyses of the experiment. Data were checked for normality (Kolmogorov–Smirnov test) and homogeneity of variance (Levene’s test) before being analysed. Student’s two-sample *t*-test was used to compare the means of the two groups fed with control and experimental feeds at specific sampling times. A one-way analysis of variance (ANOVA) followed by Tukey–Kramer’s post-hoc test was used to investigate the time-effect of a specific treatment.

## 3. RESULTS AND DISCUSSION

No significant differences were observed in the total fat content of fillets between the two feeding protocols within the same sampling periods, though fillets of carps fed with experimental feed contained more fat ( $8.83 \pm 1.89\%$ ) than the control ( $7.10 \pm 2.08\%$ ) (Table 5).

When the effect of time on the total fat content of fillets was investigated, a steady increase was observed in both groups. However, in the experimental group, this resulted in a significantly higher concentration at autumn sampling as compared to both the basic value (spring harvest) and summer harvest, while this increase was statistically much less significant in the control group.

The concentration of CDs and MDA were affected by the feed and the sampling time. Significant increases were found in both initial phase (CD) and terminal phase (MDA) markers of lipid peroxidation processes at summer harvest in the experimental group. However, the initial phase marker showed a slight but not significant elevation; MDA concentration was significantly lower than in the control group.



Table 5. Fat, conjugated dienes, and malondialdehyde contents of the fillets raised on control ( $n = 5$ ) and experimental ( $n = 10$ ) feeds and harvested at the beginning, on the 60th, and on the 112th days

Characteristics	Groups	Spring harvest (day 0)	Summer harvest (day 60)	Autumn harvest (day 112)
Fat (g/100 g wet weight)	Contr.	1.26 ± 0.81a	4.36 ± 2.18ab	7.10 ± 2.08bc
	Exp.	–	3.77 ± 1.29ab	8.83 ± 1.89c
Conjugated dienes ( $A_{233}^{1g}$ )	Contr.	0.123 ± 0.006	0.414 ± 0.180	0.876 ± 1.189
	Exp.	–	0.935 ± 0.543*	1.080 ± 0.708
MDA (mg kg <sup>-1</sup> wet weight)	Contr.	0.83 ± 0.32b	0.75 ± 0.27b	0.71 ± 0.20b
	Exp.	–	1.33 ± 0.194c**	0.34 ± 0.03a*

a, b, c: different letters indicate significant ( $P < 0.05$ ) differences between samplings.

\*, \*\*: asterisks indicate significant (\*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ) differences between groups within the same samplings.

Saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) contents of fillets from the two investigated groups harvested at the beginning, on the 60th, and on the 112th days of the experiment are indicated in Table 6. Only fatty acids with a concentration higher than 1 mg/100 g are reported here.

After 60 and 112 days, an increased concentration of myristic acid (C14:0) was observed in the two groups. The consumption of experimental feed resulted in a higher increase of myristic acid than the control; thus, a significantly higher concentration was measured in this group on day 112.

Compared to the initial values, the total concentration of SFAs was significantly higher in both groups on days 60 and 112. However, the different fat composition of feeds resulted in no detectable variations in the SFA content of the fillets.

Among MUFAs, the amounts of palmitoleic acid (C16:1), oleic acid (C18:1n-9), and vaccenic acid (C18:1n-7) increased in fish; significant progress was observed by day 112 in both groups. Regarding the MUFAs, no significant differences were found between the groups, though the experimental feed contained higher levels of palmitoleic and oleic acids.

The concentration of gadoleic acid (C20:1n-9) increased with time. The experimental feed led to a higher increase than the control, which resulted in a significant difference between the groups on day 112.

In the current experiment, the predominant fatty acid in both the control and experimental groups was monounsaturated oleic acid (C18:1n-9), followed by saturated palmitic acid (C16:0). These results are in line with the findings of Trenovszki et al. (2011), who investigated the fatty acid profiles of common carp fillets raised with grain-based diet. Másilko et al. (2015) also observed that in turning from an extensive to semi-intensive feeding system, the above-mentioned fatty acids were the two major components of carp fillet.

In the present study, the concentration of erucic acid did not change in the control group, but after 60 days it showed a 10-times increase in the experimental group fed a diet containing rapeseed. Interestingly, this fatty acid disappeared from both groups by the end of the experimental period.

Polyunsaturated fatty acid (PUFA) composition of fillets from the control and the experimental groups at the beginning, on the 60th, and on the 112nd days of the experiment are shown in Table 7.



Table 6. Saturated and monounsaturated fatty acid composition (mg/100 g of wet weight) of fillets from both investigated groups of fish harvested at the beginning, on the 60th and the 112th days of the experiment (control  $n = 5$ , experimental  $n = 10$ )

		Spring harvest (day 0)	Summer harvest (day 60)	Autumn harvest (day 112)
SFA				
C14:0	Contr.	16.1 ± 13.6a	46.0 ± 24.2ab	71.4 ± 24.3b
	Exp.	–	96.3 ± 32.4b**	234 ± 51.3c***
C16:0	Contr.	213 ± 143a	743 ± 350ab	1,234 ± 334b
	Exp.	–	602 ± 204a	1,584 ± 368b
C18:0	Contr.	77.6 ± 48.2a	235 ± 119ab	335 ± 83.4b
	Exp.	–	133 ± 50.4a	274 ± 63.1b
C22:0	Contr.	11.4 ± 4.57b	22.8 ± 4.62b	51.4 ± 11.4c
	Exp.	–	7.00 ± 1.83b	0.0 ± 0.0a***
Total SFA	Contr.	325 ± 212a	1,058 ± 507ab	1,692 ± 444b
	Exp.	–	854 ± 287ab	2,117 ± 480b
MUFA				
C16:1	Contr.	61.0 ± 44.9a	287 ± 148ab	542 ± 197b
	Exp.	–	192 ± 65.1a	526 ± 135b
C18:1n9	Contr.	438 ± 252a	1,809 ± 1,008ab	3,356 ± 1,032b
	Exp.	–	1,202 ± 470a	2,859 ± 642b
C18:1n7	Contr.	26.2 ± 26.5a	97.6 ± 48.7a	197 ± 131a
	Exp.	–	86.8 ± 23.1a	216 ± 53.2b
C20:1n9	Contr.	32.2 ± 28.7a	87.0 ± 43.9a	173 ± 54.9b
	Exp.	–	125 ± 40.0a	364 ± 76.4c***
C22:1n9	Contr.	4.4 ± 4.0b	3.0 ± 1.41b	0.0 ± 0.0a
	Exp.	–	47.0 ± 40.0c**	0.0 ± 0.0a
Total MUFA	Contr.	569 ± 359a	2,300 ± 1,241ab	4,268 ± 1,380b
	Exp.	–	1,669 ± 625a	3,965 ± 898c

a, b, c: indicate significant ( $P < 0.05$ ) differences between samplings.

\*\*, \*\*\*: asterisks indicate significant (\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ) differences between groups within the same samplings.

PUFAs in the fillets underwent significant changes during the different sampling periods.

Similar to the findings of [Trenovszki et al. \(2011\)](#) and [Másiłko et al. \(2015\)](#), the most represented PUFA in fillets of both groups was linoleic acid (C18:2n-6) in this study as well. It gradually increased in both groups and showed significant differences compared to the first sampling. Experimental feed resulted in twice as much linoleic acid at the end of the experiment than measured at the first sampling. Significant changes were also detected between the control and the experimental group. However, the linoleic acid content of the diets did not differ to that much extend.

At summer harvest, the amount of  $\alpha$ -linolenic acid (C18:3n-3) showed a remarkable increase in the control group, which then dropped back to half of the former value by day 112. However, in the experimental group, the amount of  $\alpha$ -linolenic acid showed a gradual increase during the whole experiment, resulting a much higher value at the end of the experiment than the control. The  $\alpha$ -linolenic acid content of the feeds was also significantly higher in the experimental feed.



At the end of the trial, the amount of arachidonic acid (C20:4n-6) was significantly lower in the experimental group than in control. Arachidonic acid was present in the experimental feed in a rather low quantity.

The US Food and Drug Administration (FDA) officially confirmed that eicosapentaenoic acid (EPA) (C20:5n-3) and docosahexaenoic acid (DHA) (C22:6n-6) could reduce the risk of hypertension and coronary heart diseases (FDA, 2019). At the end of the experimental period,

Table 7. Polyunsaturated fatty acid composition (mg/100 g of wet weight), total amount of n-3 and n-6 fatty acids (mg/100 g wet weight) and the n-3:n-6 ratio of fillets in both investigated groups of fish harvested at the beginning, on the 60th, and on the 112th days of the experiment (control  $n = 5$ , experimental  $n = 10$ )

		Spring harvest (day 0)	Summer harvest (day 60)	Autumn harvest (day 112)
C18:2n6	Contr.	107 ± 58.3a	397 ± 185ab	599 ± 197b
	Exp.	–	498 ± 156b	1,088 ± 236c**
C18:3n6	Contr.	24.4 ± 19.2b	22.0 ± 12.0b	3.60 ± 8.05ab
	Exp.	–	17.8 ± 12.8b	3.40 ± 5.52a
C18:3n3	Contr.	0.99 ± 0.62a	82.0 ± 74.4ab	41.2 ± 28.5ab
	Exp.	–	86.9 ± 39.6b	202 ± 44.4c***
C18:4n3	Contr.	0.0 ± 0.0a	0.0 ± 0.0a	19.8 ± 12.7b
	Exp.	–	0.0 ± 0.0a	77.0 ± 27.0c***
C20:2n6	Contr.	12.0 ± 11.0ab	16.8 ± 10.7b	0.0 ± 0.0a
	Exp.	–	20.6 ± 15.1b	0.0 ± 0.0a
C20:3n6	Contr.	5.47 ± 2.79a	12.8 ± 6.42ab	18.4 ± 6.99b
	Exp.	–	25.9 ± 21.3ab	21.0 ± 5.37ab
C20:3n3	Contr.	0.0 ± 0.0a	5.6 ± 3.85b	0.0 ± 0.0b
	Exp.	–	9.2 ± 4.10b	0.0 ± 0.0a
C20:4n6	Contr.	24.7 ± 13.6abc	32.0 ± 22.5abc	48.2 ± 15.7c
	Exp.	–	15.3 ± 3.47a	25.1 ± 6.72b*
C20:5n3	Contr.	22.3 ± 18.9ab	39.6 ± 43.7ab	13.4 ± 11.0a
	Exp.	–	79.4 ± 27.8c*	199 ± 38.5d***
C22:2	Contr.	5.57 ± 5.20ab	9.0 ± 7.31b	0.0 ± 0.0a
	Exp.	–	14.3 ± 13.6b	47.9 ± 13.2c***
C22:5n3	Contr.	9.85 ± 6.98	13.0 ± 12.3	7.60 ± 8.96
	Exp.	–	31.8 ± 20.1	61.3 ± 8.96***
C22:6n3	Contr.	54.4ab±50.6	26.6 ± 20.2a	19.2 ± 20.1a
	Exp.	–	144 ± 69.5b***	418 ± 77.4c***
Total PUFA	Contr.	271 ± 184a	678 ± 335ab	770 ± 280ab
	Exp.	–	966 ± 304b	2,142 ± 430c***
n-3	Contr.	87.67 ± 76.5a	167 ± 152a	101 ± 67.9a
	Exp.	–	351 ± 131b*	957 ± 182c***
n-6	Contr.	162 ± 93a	464 ± 224ab	669 ± 222b
	Exp.	–	557 ± 163b	1,138 ± 243 c**
n-3:n-6	Contr.	0.52a±0.21	0.47 ± 0.41a	0.15 ± 0.07a
	Exp.	–	0.62 ± 0.09a	0.85 ± 0.06b***

a, b, c: indicate significant ( $P < 0.05$ ) differences between samplings.

\*, \*\*, \*\*\*: asterisks indicate significant (\*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ) differences between groups within the same samplings.



the amounts of EPA, docosadienoic acid (C22:2), docosapentaenoic acid (C22:5n-3), and DHA were significantly higher in the fillets of the experimental group than in the control. DHA was present at an extremely high concentration in the experimental group. The experimental feed contained salient amounts of EPA and DHA.

The total amount of n-3 and n-6 fatty acids and their ratio in the fillets of the two investigated groups at spring, summer, and autumn harvests are indicated in Table 7. Fish meat is one of the vital sources of n-3 fatty acids in human nutrition, which play an important role in the prevention and treatment of several diseases. For example, they reduce the level of triglycerides and cholesterol in serum (Steffens, 1997). No increase was observed in the amount of n-3 fatty acids in fillets of the control group during the 112-day-long experimental period, while the experimental feed resulted in a significantly ( $P < 0.05$ ) higher amount of n-3 fatty acids when compared to the basic value measured at spring harvest. At summer fishing, its amount was only 2.1 times higher ( $P < 0.05$ ), while at autumn harvest already 9.4 times higher amount of n-3 fatty acids was measured than in the control group ( $P < 0.001$ ).

The total amount of n-6 fatty acids increased in both groups, resulting in significantly higher concentrations at the autumn harvest than basic values. However, the ratio of increase was much lower ( $P < 0.01$ ) in the control group (4.1 times higher than the original value) than in the experimental one (7.0 times higher n-6 fatty acid concentration when compared to the original value).

The ratio of accumulation of n-3 and n-6 fatty acids in the fillets showed different patterns in the groups. In the control group, it decreased by 72% at autumn harvest compared to the basic value, while in the experimental group, a 63.5% increase was observed within the same period. This resulted in a significant difference ( $P < 0.001$ ) between the two groups at the end of the experimental period regarding the ratio of n-3 and n-6 fatty acids.

Fluctuations in the ratio of n-3 and n-6 fatty acids in carp meat appear due to different farming factors (Steffens, 1997).

## 4. CONCLUSIONS

Some quality parameters of carp fillet were clearly influenced by the feeding with a complete feed. However, while there was a remarkable difference in the fat content of the feeds, at the end of the 112-days study, no difference was found in the fat content of fillets in case of feeding traditional or nutritionally complete feed. The same was true for saturated fatty acids of fish fillets, where no statistically verifiable difference was found. However, the MUFA content of fish meat was significantly lower in the experimental group. In contrast, the PUFA content, which is more valuable for human nutrition, was significantly (2.78 times) higher than the control group.

The ratio of n-3 and n-6 fatty acids was also significantly higher in the experimental group at the end of the experiment.

The present study demonstrated that fish meat of almost the same quality could be produced in small storage ponds, empty in summer, using a complete feed.

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