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THE INFLUENCE OF LINSEED OIL AND FISH OIL DIET SUPPLEMENTS TO THE FATTY ACID SPECTRUM OF COMMON CARP (*Cyprinus carpio* L.) MUSCLE

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Abstract

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Effect of addition 6% of linseed oil (designated L06), 6% and 10% of fish oil (R06 and R10) to feed on the fatty acid spectrum of common carp (*Cyprinus carpio* L.) was investigated. The basic feedmixture which was used as a control variant (K – 34% protein; 9% fat) and the three with oil addition (L06, R06 and R10) were fed to carp fingerling (43.25 g average weight) for 60 days – from 23rd April to 20th June. Before that the fish were fed for 2 month by whey grain and commercial feed for carp fingerling in pond fish-culture (KP feed mixture – 33% protein; 5% fat) at daily feeding rate 1.5% of actually fish mass. This procedure was intended to create feeding conditions closest to those witnessed in market fish farmed in ponds during the vegetation season nevertheless the spectrum of fatty acids present in the fish muscle at the experiment's beginning did not fully correspond to what was observed in carps living in ponds and fed by cereals.

An addition of 6% of linseed oil to the feed lowers the content of the oleic acid and MUFA and, at the same time, it boosts the contents of the α-linoleic acid, n-3 PUFA and the general PUFA in the meat of carp fed on mixtures thus enriched. Additions of 6% and 10% of fish oil to the feed for common carp increases the content of the eicosapentaenoic acid. The 10% addition proved beneficial for also the ratio of n-3/n-6 PUFA. The high content of the docosapentaenoic acid and the general PUFA in the meat of fish as early as the beginning of the experiment resulted in a smaller number of significant changes in the spectrum of fatty acids (particularly the docosahexaenoic acid, PUFA and n-3/n-6 PUFA) found in the fish meat of the L06, R06 and R10 experimental variants.

common carp, fatty acid spectrum, fish oil, linseed oil

A freshwater fish farmed most often in the Czech Republic is the common carp (*Cyprinus carpio* L.). In the recent years the CR production of the carp has stabilized at about 17 thousand tons a year. Europe-wide the carp production has been declining continuously during the last 7 years (by 10% between 2001 and 2007) (source: CR Ministry of Agriculture, MZe, 2008). The producers thus have to seek for new methods capable of increasing the demand, methods aimed to develop more sophisticated carp products.

A contemporary trend towards healthy lifestyle offers new opportunities to find markets for foodstuffs whose content of specific substances prevents the civilization diseases or at least diminishes their incidence. Thanks to its large content of polyunsaturated acids (PUFA), omega n-3, particularly the eicosapentaenoic acid (EPA) and the docosahexaenoic acid (DHA), the meat of fish is one of the foodstuffs proved beneficial to human health. PUFA n-3 has an anti-atherosclerotic effect (Rudel at al., 1998). Lunn and Theobald (2006) reported positive effect of n-3 PUFA also in the treatment of other disea-

ses such as arthritis, nephritis, multiple sclerosis, asthma and skin diseases. To prevent the civilization diseases in humans, WHO (2003) recommended (with special emphasis on the ischemic disease of heart, diabetes and cardiovascular disorders) that the share of fat in food be reduced to 15% to 30% of the overall daily energy intake. Out of this amount of fat the saturated fatty acids should represent less than 10%; the trans-fatty acids less than 1% and the quantity of PUFA should keep between 6% and 10%. The remainder of the daily energy intake from fats should be taken from MUFA, the monounsaturated fatty acids. The quantities of PUFA n-3 and n-6 should range from 1% to 2%, or 5% to 8%, respectively, of the entire amount of fat ingested.

The composition of fatty acids encountered in the fish muscle is affected primarily by the composition of fatty acids found in what the fish feed on (Henderson and Tocher, 1987; Sargent et al., 2002). In the Czech Republic the carp is produced almost exclusively through fish-farming in ponds where the fish receive additional feed in the form of cereals. However, fish kept on high-sacharide diet tend to develop a larger content of the oleic acid (C18:1 n-9) in the muscle (Farkas et al., 1978, Schwarz et al., 1988). Csengeri and Farkas (1993) and Steffens and Wirth (2007) compared the spectrum of fatty acids encountered in carp farmed in ponds with different intensities of farming. They reported a decrease of the n-3 PUFA content in the muscle of common carp receiving additional cereal feed as compared to that found in carp living exclusively on natural feed. Vácha et al. (2007) disclosed an insignificant effect of overwintering on the content of PUFA in muscle. Takeuchi et al. (1978), Runge et al. (1987), Steffens et al. (1995) and Hadjinikolova (2004) investigated a targeted change in the spectrum of fatty acids in the meat of common carp induced by keeping the fish on diet with additions of different kinds of oils and fats, mainly with reliance on increased doses of PUFA and n-3 PUFA.

The spectrum of fatty acids encountered in the fish meat is affected by also other factors. Fajmonová et al. (2003) investigated the effect that the carp sex and the speed of the fish growth in ponds have on the chemical composition of the meat and the spectrum of fatty acids found in the meat. As proved in the investigation, the higher the speed of growing, the greater the amount of fat and dry matter in the carp meat. The same trend was encountered in the volume of MUFA, while the volume of PUFA and the n-3 to n-6 ratio were diminishing. The authors also reported an insignificant effect of the sex on the chemical composition and the spectrum of FA in the carp meat. In common carp the composition of fatty acids in the fat is not substantially influenced by its sex or the kind of hybrid (Buchtová et al., 2007). Fauconneau et al. (1995) describe twice or three times greater activity of enzymes synthesizing the fatty acids in cyprinoids bred in water 2 °C to 10 °C than in fish living in water 20 °C to 30 °C. Farkas et al. (1980) proved that the common carp can develop increased levels of fatty acid saturation in a few hours if exposed to colder environment, while Geri et al. (1995) reported a lower level of n-3/n-6 PUFA in carp bred in natural conditions than in fish of the same category bred in intensive aquaculture of warmed-up water. The effect that the water bloom attributable to the blue-green algae present in the breeding environment may have on the contents of fatty acids in carp muscle is specified in publication by Mareš et al. (2009).

MATERIALS AND METHODOLOGY

In spring 2007 (23 April to 20 June) an experimental recirculation facility on the premises of the Department of Fishery & Hydrobiology was used to conduct a feeding test aimed to investigate the effect that additions of linseed oil and fish oil have on the range of fatty acids in the meat of common carp. Four mixtures of feed were prepared from a basic feeding mixture made according to a uniform recipe (34% of protein and 9% of fat) modified by additions of 6% linseed oil (designated L06) and 6% and 10% of fish oil (R06 and R10, respectively). The reference variant (K) relied on the basic formula of feed, without any oil additions. All the variants were passed through three repeated tests. The formulations, chemical compositions and the spectrum of fatty acids that the experimental feed mixtures contained are specified in table I and table II.

The experiment was carried out in glass tanks large enough to accommodate 60 liters of water, connected to a recirculation circuit and a system of aeration. The flowrate through the tanks was set at 1.5 $l.s^{-1}$. The time schedule of lighting was adjusted so that 14 hours of light were followed by 10 hours of darkness. With the experiment in progress, the physical and chemical parameters of the environment in the tanks were as follows:

- O_2 saturation: 97.6–64.9%
- pH: 7.41–7.86
- temperature: 21.2–23.7 °C
- $N\text{-}NH_4^+$: 0.26–0.73 $mg.l^{-1}$
- $N\text{-}NO_2^-$: 0.04–0.22 $mg.l^{-1}$
- $P\text{-}PO_4^{3+}$: 0.03–0.17 $mg.l^{-1}$

Each tank was populated with 29 carp fingerlings (*Cyprinus carpio* L.) whose average weight was 43.25 g a piece. The fish, production crossbreeds of the Po-L × ROP lines, was bred at the *Rybníkářství Pohořelice*, a. s. (a pond-management company). For 60 days, after having been placed to the testing tanks and before the test was actually launched, the fish was being offered wheat grain upgraded by an addition of KP feed mixture (33% NL, 5% T), a preparation designed to improve the natural feed of fingerlings kept in ponds. The overall quantity of the feed thus daily added to each tank represented 1.5% of the aggregate weight of its fish population. This procedure was intended to create conditions, feeding and others, closest to those witnessed in market fish farmed in ponds during the vegetation season. Just

I: *Componental and biochemical composition of tested feeds (% of wet matter)*

Component	K	L 06	R 06	R 10
Fish meal	16	16	16	16
Rapeseed meal	18	18	18	18
Soybean meal	12	12	12	12
VITEX	10	10	10	10
Whey	3	3	3	3
Wheat pollard	4	4	4	4
Wheatmeal	30	30	30	30
Lecitin	5	5	5	5
AMINOVITAN	2	2	2	2
Linseed oil supplement	-	6	-	-
Fish oil supplement	-	-	6	10
Dry matter	91.6	91.7	93.0	92.6
Crude fat	9.1	17.8	17.3	19.0
Crude protein	33.8	31.7	32.1	29.5
BE [kJ.g^{-1}]*	18.69	20.5	20.65	20.8

* by Steffens (1989)

II: *Fatty acid spectrum of tested feeds and oils (% of total FA)*

	K	L 06	R 06	R 10	Fish oil	Linseed oil
C14:0	1.59	0.70	4.60	5.24	8.06	0.08
C16:0	19.24	11.50	19.52	19.51	20.19	5.07
C16:1 n7	2.33	1.07	4.83	5.56	7.88	0.09
C18:0	3.65	4.42	3.82	3.82	4.04	5.10
C18:1 n9c	19.38	22.26	14.38	13.72	9.70	24.71
C18:1 n7	2.86	1.90	2.97	3.17	3.34	1.08
C18:2 n6c	37.75	27.37	21.46	17.16	1.62	16.99
C18:3 n6	0.07	0.02	0.14	0.17	0.25	0.00
C18:3 n3	3.94	26.47	3.12	2.31	1.04	46.56
C18:4 n3	0.49	0.22	2.04	2.45	3.91	0.01
C20:1	1.27	0.65	1.44	1.48	1.64	0.19
C20:4 n6	0.25	0.12	0.68	0.81	1.20	0.00
C20:4 n3	0.13	0.06	0.49	0.59	0.92	0.00
C20:5 n3	2.90	1.30	9.84	11.67	18.18	0.02
C22:4 n6	0.00	0.01	0.05	0.07	0.11	0.00
C22:5 n6	0.20	0.15	0.20	0.19	0.17	0.09
C22:5 n3	0.57	0.25	1.54	1.80	2.68	0.00
C22:6 n3	3.38	1.53	8.88	10.28	15.07	0.01
SFA	24.48	16.61	27.95	28.59	32.29	10.25
MUFA	25.84	25.89	23.64	23.94	22.56	26.06
PUFA	49.68	57.50	48.41	47.47	45.15	63.69
Total n-6	38.28	27.67	22.51	18.38	3.35	17.08
Total n-3	11.40	29.83	25.90	29.09	41.80	46.60
n-3/n-6	0.30	1.08	1.15	1.58	12.50	2.73

SFA *– C14:0; C16:0; C18:0* ***MUFA*** *– C16:1 n7; C18:1 n9c; C18:1 n7; C20:1* ***PUFA*** *– C18:2 n6c; C18:3 n6; C18:3 n3; C18:4 n3; C20:4 n6; C20:4 n3; C20:5 n3; C22:4 n6; C22:5 n6; C22:5 n3; C22:6 n3 Total* ***n-6*** *– C18:2 n6c; C18:3 n6; C20:4 n6; C22:4 n6; C22:5 n6* ***Total n-3*** *– C18:3 n3; C18:4 n3; C20:4 n3; C20:5 n3; C22:5 n3; C22:6 n3*

before the experiment commenced and in 10-day intervals after the actual weight of the fish population was monitored and the daily doses of feed were adjusted accordingly.

The feed dose always represented 2.5% of weight of the tank populations and the fish was given the feed in 3 daily batches (at 8:00 a.m.; 1:00 p.m. and 6:00 p.m.). On the day when the fish was weighed, no feed was provided.

On the day when the experiment was launched, 6 fingerlings were taken away to analyze the biochemical composition of their muscle and the spectrum of fatty acids found in it. The muscle thus sampled was a vertical strip cut in front of the dorsal fin, always from the left fillet, free from ribs and skin. On the day when the experiment was terminated the same analysis as that carried out at the experiment's outset was performed on 6 fish out of each variant and on the feeds used in the experiment.

Lipids for FA analyse were extracted using a methanol-chloroform solution according to Folch et al. (1957). The spectrum of fatty acids was analyzed by the method of capillary gas chromatography using HP 4890, a chromatograph by Hewlett-Packard, USA, and DB-23, a capillary (*open-tube*) column sized 60 m × 0.25 mm × 0.25 μm). The column outlet was connected to a Flame-Ionizing Detector (FID). The standard used was Supelco 37 Component FAME Mix (Supleco, USA, purchased from Labicom, CR). The temperature program chosen was as follows: $T_1 = 100\,°C$, $t_1 = 3$ min, 10 °C/min, $T_2 = 170\,°C$, $t_2 = 0$ min, 4 °C/min, $T_3 = 230\,°C$, $t_3 = 8$ min, 5 °C/min, $T_4 = 250\,°C$, $t_4 = 15$ min. The injector temperature was 270 °C; the FID temperature was 280 °C. Nitrogen was used for the carrier gas.

The dry matter obtained from the samples was measured gravimetrically at 105 °C until the weight stopped diminishing. The content of nitrogenous substances was examined by the Kjehldal method while the total fats were examined by the Soxhlet method through diethyether extraction of the samples for 10 hours.

The statistic significance of results was assessed by the multiple comparison procedure according to Scheffe, using the UNISTAT 5.1 program. In the result table the statistically significant differences ($P > 0.05$) observed between the measured values are identified by lowercase superscript (X^a) and the highly significant differences ($P > 0.01$) appear as the uppercase superscript (X^A).

RESULTS AND DISCUSSION

Throughout the experiment the fish meat did not exhibit significant changes in the contents of dry matter. The values measured ranged from 21.4% to 25.5%, the initial values included. Feeding diets higher in proteins (29.5% to 33.8%) increased the protein content in the meat of all the fish variants – var. L06 ($P > 0.05$), var. K, R06 and R10 ($P > 0.01$).

At the experiment's beginning the content of fat in the fish meat was higher (6.8%) than what was observed in fish living in natural environment and eating solely the natural feed (Steffens and Wirth, 2007; Vácha et al., 2007). Fat content of 7% in the wet tissue corresponds to the level found in carps living in ponds and partially fed by added cereals as described by Kmínková et al. (2001) and Fajmonová et al. (2003). Steffens and Wirth (2007) reported the fat content of only 3.4% in the meat of carp bred in pond and fed by added cereals. In contrast, Vácha et al. (2007) found the fat content in the meat of fish bred under comparable conditions to be 9.7% to 13.3%, in dependence on the cereals employed. Low level of fat in the "K" feed mixture used in the experiment translated itself into a reduced content ($P > 0.05$) of fat in the meat of this fish variant. The contents of fat encountered in the meat of other fish variants were lower than what was measured at the test beginning, but the figures lacked statistical significance. The spectrum of fatty acids present in the fish muscle at the experiment's beginning did not fully correspond to what was observed in carps living in ponds and fed by cereals as described by Csengeri and Farkas (1993), Steffens and Wirth (2007), Kmínková et al. (2001), Fajmonová et al. (2003), Buchtová et al. (2007), Steffens and Wirth (2007) and Vácha et al. (2007).

Model of nutritional conditions typical of a production pond (cereals + additional feed mixture) returned lower values of the FA spectrum of fish meat at the experiment's beginning (Table III) for the palmitic-oleic acid (C16:1 n-7) and the oleic acid (C18:1 n-9) as compared to the values reported in literature – Kmínková et al. (2001), Fajmonová et al. (2003) and Buchtová et al. (2007). Conversely, higher values were reached for the linoleic acid (C18:2 n-6), the eicosapentaenoic acid (C20:5 n-3) and the docosahexaenoic acid (C22:6 n-3) as compared to what was published by Csengeri and Farkas (1993), Kmínková et al. (2001), Fajmonová et al. (2003), Buchtová et al. (2007) and Steffens and Wirth (2007). The occurrence of general saturated fatty acids (SFA) in the meat of fish subjected to the experiment corresponds to values reported by Kmínková et al. (2001), Buchtová et al. (2007) and Vácha et al. (2007). Early in the experiment the MUFA values in the fish meat were found lower, while the PUFA, n-3 PUFA, n-6 PUFA and n-3/n-6 PUFA were observed higher than those published for fish from production ponds by Csengeri and Farkas (1993), Kmínková et al. (2001), Fajmonová et al. (2003), Buchtová et al. (2007), Steffens and Wirth (2007) and Vácha et al. (2007).

When fed on the reference feed, the fish of this variant developed almost no change in the spectrum of fatty acids. Only the C20:1 group of FA exhibited reduction ($P > 0.05$) as related to the initial value, attributable to the feed provided.

The figures observed for the α-linoleic acid (C18:3 n-3) were, contrarily, greater. The increase of the α-linoleic acid in the fish meat of the L06 variant reached 5.7 times the value measured at the experiment's beginning (the percentage rose from 1.42% to 8.13%). A similar trend of growing contents of the α-linoleic acid in the salmon meat was reported

III: *Fatty acid spectrum (% of total FA) and biochemical composition of carps fillet at the beginning and after 60 days of experiment*

FA	Start	K	L 06	R 06	R 10
C14:0	2.18 ± 0.32^{ABCab}	1.89 ± 0.28^{ABa}	1.46 ± 0.36^{Aa}	2.66 ± 0.44^{BCb}	2.83 ± 0.96^{Cb}
C16:0	18.79 ± 0.29^{ABab}	19.74 ± 0.64^{Aa}	17.09 ± 1.19^{Bb}	19.16 ± 1.19^{ABa}	18.98 ± 2.49^{ABa}
C16:1 n7	5.75 ± 0.71^{a}	4.72 ± 0.85^{ab}	3.60 ± 0.88^{Ab}	4.72 ± 0.65^{ab}	5.59 ± 1.12^{Ba}
C18:0	4.49 ± 0.21	4.87 ± 0.31	5.20 ± 0.92	4.77 ± 0.73	4.52 ± 0.44
C18:1 n9c	29.58 ± 3.22^{Aa}	26.04 ± 2.38^{ABab}	25.52 ± 2.41^{ABab}	22.48 ± 1.76^{Bb}	23.45 ± 3.95^{ABb}
C18:1 n7	3.64 ± 0.14^{A}	3.72 ± 0.12^{A}	3.09 ± 0.16^{B}	3.57 ± 0.14^{A}	3.76 ± 0.49^{A}
C18:2 n6c	14.21 ± 2.17	17.10 ± 0.59	17.89 ± 2.16	16.57 ± 2.06	14.78 ± 1.66
C18:3 n6	0.15 ± 0.01	0.15 ± 0.05	0.20 ± 0.10	0.14 ± 0.03	0.15 ± 0.03
C18:3 n3	1.42 ± 0.21^{A}	1.71 ± 0.12^{A}	8.13 ± 1.93^{B}	2.01 ± 0.28^{A}	1.91 ± 0.47^{A}
C18:4 n3	0.57 ± 0.10^{AB}	0.39 ± 0.07^{A}	0.40 ± 0.08^{A}	0.81 ± 0.17^{B}	0.87 ± 0.27^{B}
C20:1	3.53 ± 0.41^{Aa}	2.74 ± 0.28^{ABb}	2.36 ± 0.25^{Bb}	2.53 ± 0.24^{Bb}	2.41 ± 0.30^{Bb}
C20:4 n6	2.01 ± 0.27	1.90 ± 0.35	1.50 ± 0.58	1.50 ± 0.46	1.49 ± 0.55
C20:4 n3	0.42 ± 0.06^{ABabc}	0.30 ± 0.04^{Aa}	0.40 ± 0.03^{ABab}	0.49 ± 0.07^{Bbc}	0.53 ± 0.17^{Bc}
C20:5 n3	3.64 ± 0.48^{A}	3.63 ± 0.45^{A}	3.43 ± 0.93^{A}	5.91 ± 0.47^{B}	6.25 ± 2.08^{B}
C22:4 n6	0.02 ± 0.02	0.03 ± 0.03	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.02
C22:5 n6	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.01
C22:5 n3	1.21 ± 0.05^{AB}	0.94 ± 0.14^{A}	0.92 ± 0.17^{A}	1.33 ± 0.17^{B}	1.33 ± 0.48^{B}
C22:6 n3	8.39 ± 0.18	10.13 ± 2.24	8.76 ± 2.66	11.35 ± 2.58	11.12 ± 4.27
SFA	25.47 ± 0.19^{ab}	26.50 ± 0.84^{a}	23.75 ± 1.75^{b}	26.59 ± 1.52^{a}	26.33 ± 3.73^{a}
MUFA	42.49 ± 3.32^{a}	37.21 ± 3.25^{ab}	34.57 ± 3.47^{b}	33.29 ± 2.53^{b}	35.22 ± 5.80^{ab}
PUFA	32.04 ± 3.30^{Aa}	36.30 ± 3.30^{ABab}	41.68 ± 2.75^{Bc}	40.12 ± 1.18^{Bbc}	38.46 ± 9.43^{ABabc}
Total n-6	16.40 ± 2.30	19.19 ± 0.70	19.63 ± 1.65^{a}	18.22 ± 1.62	16.45 ± 2.09^{b}
Total n-3	15.64 ± 1.01^{a}	17.11 ± 2.74^{ab}	22.05 ± 3.02^{b}	21.90 ± 2.51^{ab}	22.01 ± 1.84^{b}
n-3/n-6	0.97 ± 0.08^{a}	0.89 ± 0.12^{a}	1.14 ± 0.21^{ab}	1.22 ± 0.24^{ab}	1.34 ± 0.15^{b}
Composition of carps fillet (% of wet matter)					
Dry matter	23.2	21.4	246	25.5	25.4
Crude fat	6.8^{a}	3.9^{b}	4.6^{ab}	4.8^{ab}	5.2^{ab}
Protein	15.4^{Aa}	19.0^{Bb}	18.6^{ABb}	19.1^{Bb}	18.9^{Bb}

SFA *– C14:0; C16:0; C18:0* ***MUFA*** *– C16:1 n7; C18:1 n9c; C18:1 n7; C20:1* ***PUFA*** *– C18:2 n6c; C18:3 n6; C18:3 n3; C18:4 n3; C20:4 n6; C20:4 n3; C20:5 n3; C22:4 n6; C22:5 n6; C22:5 n3; C22:6 n3* ***Total n-6*** *– C18:2 n6c; C18:3 n6; C20:4 n6; C22:4 n6; C22:5 n6 Total* ***n-3*** *– C18:3 n3; C18:4 n3; C20:4 n3; C20:5 n3; C22:5 n3; C22:6n3*

by Tocher et al. (2000). Turchini et al. (2007) found the value of 34.2% in trench. These authors, however, used a higher addition of the linseed oil (about 10%) and the time of feeding was longer (12 and 19 weeks) than in our experiment. The above-mentioned facts considered, we can conclude that even a higher level of the α-linoleic acid in the fish meet could be reached by (1) increasing the amount of oil in the feed mixtures, or by (2) longer time of feeding the fish on a mixture containing a lower amount of the linseed oil. Experimenting with non-carp fish, Tidwell et al. (2007) reached 22.6% of the α-linoleic acid in largemouth bass, while Mourente et al. (2005) achieved in sea bass the percentage of 8.4% when feeding the fish on a diet upgraded with 5% and 10% of linseed oil for 12 weeks and 34 weeks, respectively.

When compared with the initial values, the overall profile of fatty acids in the L06 fish variant demonstrated significant reduction in the level of MUFA ($P > 0.05$) and higher contents of PUFA ($P > 0.01$) and n-3 PUFA ($P > 0.05$). The reduction observed in the content of the oleic acid ($P > 0.01$) and the increase found in the content of the α-linoleic acid ($P > 0.01$) and PUFA ($P > 0.05$) in the L06 variant of fish can be attributed to the addition of linseed oil as evidenced by the significant difference between these fatty acids and the values encountered in the "K" reference fish variant fed on a mixture without the oil addition.

Having comparatively assessed the effect that the addition of the linseed oil and fish oil had on the spectrum of FA in the meat of fish, we can conclude that the L06 fish variant showed a slight decrease in the content of SFA ($P > 0.05$), particularly as regards the myristic acid (C14:0) ($P > 0.01$) and the palmitic acid (C16:0) ($P > 0.05$), as compared with the R06 and R10 variants. Similar results of the SFA comparisons were reported for carp by Runge et al. (1987) and for sea bass by Mourente et al. (2005). Lower values ($P > 0.01$) than those found in the fish variants fed on the fish oil were achieved also with

the vaccen acid (C18:1 n7). In fact the same as achieved with the palmito-oleic acid (P>0.01), but only in comparison with the R10 variant. Difference comparable with what was reported by Mourente et al. (2005) and Runge et al. (1987) was observed in these variants (P > 0.05) as regards the content of n-6 PUFA, where the L06 variant reached a higher value. Despite the lower content of n-6 PUFA, the R10 variant, when compared with the L06 variant, did not demonstrate a significant difference in the value of n-3/n-6 PUFA. The meat of fish of the R06 and R10 variants contained more moroctic acid (C18:4 n-3) (P > 0.01), eicosatetraenoic acid (C20:4 n-3) (P > 0.05), eicosapentaenoic acid (P > 0.01), and docosapentaenoic acid (C22:5 n-3) (P > 0.01) that what was found in the L06 fish variant. This finding has also been confirmed by the results published by Runge et al. (1987), Tocher et al. (2000), Mourente et al. (2005) and Tidwell et al. (2007), all of whom tested the effect that the fish oil and the linseed oil had on the spectrum of FA in fish.

The spectrum of FA in meat of the R06 and R10 carp variants exhibited no significant differences attributable to the different quantities of the added oil. These variants only demonstrated differences when compared with the initial values and with the reference variant values. Neither the 6% nor the 10% addition of fish oil resulted in a change in values of any SFA compared with the initial values. When compared with the reference group, however, both the variants showed a significant increase (R06 – P > 0.05; R10 – P > 0.01) in the myristic acid (C14:0). This observation agrees with the results reported by Runge et al. (1987), Mourente (2005) and Tidwell et al. (2007) who found a higher content of the myristic acid in fish fed on feed with fish oil. Hadjinikolova (2004), conversely, reported a lower content of the myristic acid in the meat of carp fed on mixtures with the fish oil added than was found in the reference variant, despite the fact that feed mixture upgraded by the fish oil contained more of this FA. As regards MUFA, the feed mixture upgraded by the fish oil caused a difference only in the FA (C20:1) group. The R06 and R10 fish variants demonstrated a reduction in the contents of these FAs with a great level of significance (P > 0.01). Beneficial effect of the fish oil was proven by a significant (P > 0.05) lowering of the value also in the "K" variant. Considering the other authors, only Takeuchi et al. (1978) published data on the content of FA C20:1 in carp, but the value reported ranged between 6% and 13.8% in dependence on the type of fish oil used. The fish fed on mixtures of the R06 and R10 variants demonstrated significant (P > 0.01) increase in content of the moroctic acid, eicosatetraenoic acid and docosapentaenoic acid, but only when compared to the values of the reference group of carp. Significant difference (P > 0.01) from the reference variant as well as from the initial values was found in the content of the eicosapentaenoic acid, namely for the R06 and R10 variants. These results correspond to what was published by Runge et al. (1987), Steffens et al. (1995), Tocher et al. (2000) a Tidwell et al. (2007). The feed mixture of R06 decreased the proportion of MUFA (P > 0.05) and increased the proportion of PUFA (P > 0.01) in the meat of these carps as compared to the initial values. Just as is the case with the increase (P > 0.05) in the content of n-3 PUFA in the meat of the R10 fish variant, the effect that the fish oil could have on these changes is not supported by any proof possibly leaning on a significant difference between the observed values and the reference variant. The beneficial effect of the fish oil addition to the feed mixtures has been proven in the n-3/n-6 PUFA parameter, where a significant (P > 0.05) growth was observed in the R10 variant, as compared to both the initial value and the reference variant. The same effect has been achieved by Takeuchi et al. (1978), Runge et al. (1987) and Steffens et al. (1995) who also added fish oil to mixtures used to feed carp. These authors, however, reported beneficial effect of the fish oil addition on also the increase of the content of the docosahexaenoic acid; moreover, the fish meat in their experiments has achieved higher levels of the n-3/n-6 parameter. This minor and insignificant rise in the values observed in our experiment was caused by the high content of the docosahexaenoic acid in the meat of carp as early as at the experiment's beginning. In this case the 60-day simulation of the nutritious conditions existing in the pond environment proved to be insufficient to lower the contents of the FAs down to the values reported by some of the authors, namely by Csengeri a Farkas (1993), Kmínková et al. (2001), Fajmonová et al. (2003), Buchtová et al. (2007), Steffens a Wirth (2007) and Vácha et al. (2007).

CONCLUSION

1. An addition of 6% of linseed oil to the feed lowers the content of the oleic acid and MUFA and, at the same time, it boosts the contents of the α-linoleic acid, n-3 PUFA and the general PUFA in the meat of carp fed on mixtures thus enriched.
2. Additions of 6% and 10% of fish oil to the feed for common carp increases the content of the eicosapentaenoic acid. The 10% addition proved beneficial for also the ratio of n-3/n-6 PUFA.
3. The high content of the docosapentaenoic acid and the general PUFA in the meat of fish as early as the beginning of the experiment resulted in a smaller number of significant changes in the spectrum of fatty acids (particularly the docosahexaenoic acid, PUFA and n-3/n-6 PUFA) found in the fish meat of the L06, R06 and R10 experimental variants.

SUMMARY

A contemporary trend towards healthy lifestyle caused increased interest about foodstutts with positive influence to human health. Pollyunsaturated fatty acids omega n-3 (n-3 PUFA) which are highly conteined in fish meal, can diminish an incidences or participated to treatment of some civilization diseases. In the Czech Republic a carp is produced with using of system where a fish receive additional feed in the form of cereals. This system could has a negative influence to the fatty acid spectrum of carps fillets. If the cereals are feeded in high quantity, that can caused undesired effects of inceasing the value of monounsaturated oleic acid (it has a negative inluence to organoleptic quality of carps meat) and decreasing the n-3 PUFA value of carp muscle. Becouse of this there is requirement from carp producers to finding a way how to restore the fatty acid spectrum of these fish to the values of carps from ponds with naturaly feed or gross it up. The interferencing of FA spectrum of fish meat by oils addition is already approved in service conditions with using intensive fish-farming. This technology wasn't still tested in semi-intensive fish-farming pond conditions. The influence of feed with oils addition to the FA spectrum of carp isn`t still described as good as to others intensive-farmed fish (esp. trout, salmon, tilapia).

The experimental recirculation facility on the premise of the Department of Fishery & Hydrobiology in MZLU Brno was used to conduct a 60 days long fiding test. There were tested 4 carpfeeds prepared from a basic feed mixture formula (34% protein; 9% fat). This one was used like a reference variant (designated K). The other variants were modified by additions of 6% linseed oil (L06) and 6% and 10% of fish oil (R06 and R10). All of feed variant were passed trought three repetitions. Each of 12 pieces of glass tank (60l capacity; 1,5l.s^{-1} flowrate) was populated with 29 carp fingerling (*Cyprinus carpio* L. – production crossbreds of Po-L × ROP lines) whose average weight was 43.25 g. The fish were fed for 60 days with wheat and comercial pondfeed for carp fingerling (33% protein; 5% fat) at daily feeding rate 1.5% of actually fish mass in the tank. This procedure was intedned to create feeding conditions analogous with fish from semi-intensive or intensive pond fish farming. After this period the fish were adapt to experimental feeds and during the feedtest were fed with those diets at daily feeding rate 2.5% of fish mass in each tank. In 10-day intervals during the course of experiment the fish were weighted and the feeding rate was corected. On the day when the experiment was started and on its end 6 fingerlings were taken away to analyze biochemical composition (dry matter, protein, fat) and fatty acid spectrum of their muscle. The muscle thus sampled was a vertical strip cut in dorsal fin, always from the left fillet, free from ribs and skin.

On the start of experiment the palmitic-oleic acid, the oleic acid and the MUFA values were found lower, by contrast the linoleic acid, the eicosapentaenic acid, the docosahexaenic acid, the n-3 and n-6 PUFA and the total PUFA values were found higher that was observed in carps from pond conditions and described in scientific jurnals.

When fed on the reference feed, the fish of this variant developed minimal change in the fatty acid spectrum (the content of the group FA 20:1 decreased as related to the initial tissue).

In contrast to the initial values, the content of the palmitic-oleic acid, the oleic acid, the 20:1 FA group and the MUFA were significantly reduced in the muscle of the fish from L06 variant. The PUFA value increase significantly as related to the initial and control tisuue too. The addition of linseed oil caused significant increasing of the α-linoleic acid content (5.7 times the value measured at the beginning of the experiment).

No significant differences were founded in the spectrum and profile of fatty acid if the muscles of the fish from R06 and R10 variant were compared. These variants only demonstrated differences when compared with initial and control values. Neither the 6% nor the 10% addition of fish oil resulted in a change in values of any SFA in contrast to the initial values. The significant increasing were observed in the moroctic acid, the eicosatetraenic acid and the docosapentaenic acid values in compare both of variant with fish oil and the control tissue. Significant difference from the reference variant as well as from the initial values was found in the content of the eicosapentaenoic acid, for the R06 and R10 variant too. The feed mixture of R06 decreased the content of MUFA and increased the content of PUFA in the meat of carps this variant as compared to the initial values. The n-3 PUFA value of fish from R10 variant increased significantly in compare with control variant value. The n-3/n-6 parameter of these fish increased in compare with control variant and initial tisuue. It means, that increasing the n-3/n-6 PUFA value was caused by adding 10% of fish oil. The change of this parameter wasn't observed in the others variants.

SOUHRN

Vliv přídavku lněného a rybího oleje do krmiva na spektrum mastných kyselin svaloviny kapra obecného (*Cyprinus carpio* L.)

Moderní trend zdravého životního stylu mnoha lidí způsobuje zvýšený zájem o potraviny s pozitivním vlivem na lidské zdraví. Polynenasycené mastné kyseliny řady n-3 (PUFA n-3), vysoce zastoupené v rybím mase, mohou omezit symptomy některých civilizačních chorob či se podílet svým účinkem na jejich léčbě. V podmínkách ČR se kapři chovají s využitím přikrmování obilovinami, které mohou negativně ovlivnit spektrum mastných kyselin. Pokud jsou rybám překládány ve vysokých dávkách, dochází k nežádoucímu jevu zvýšení zastoupení mononenasycené kyseliny olejové (C18:1 n-9), která výrazně negativně ovlivňuje i organoleptické vlastnosti masa, a naopak poklesu obsahu n-3 PUFA oproti rybám, jež se živí výhradně přirozenou potravou. Proto je snaha cíleně ovlivnit spektrum mastných kyselin masa kaprů (zvýšením zastoupení PUFA, zvláště pak řady n-3) využitím dotace krmiv oleji, vítána producenty v ČR i Evropě, kteří hledají nové způsoby jak zvýšit poptávku po kaprovi, jehož produkce v posledních letech klesá. Ovlivnění spektra mastných kyselin masa ryb pomocí různých olejů je již provozně vyzkoušeno v intenzivních chovech. V podmínkách polointenzivního chovu kapra v rybniční akvakultuře nebyla tato technologie dosud ověřena. Též vliv přídavku olejů do krmiva na spektrum mastných kyselin kapra obecného není popsán v takové míře jako u jiných hospodářsky cenných druhů ryb.

Experiment byl uskutečněn na recirkulačním zařízení Oddělení rybářství a hydrobiologie MZLU v Brně po dobu šedesáti dní. Byly testovány čtyři krmné směsi vycházející z jednotné základní receptury (34% protein; 9% tuk) – varianta kontrolní (označená K). Další varianty byly připraveny přidáním 6% lněného oleje (L06), dále pak 6% a 10% rybího oleje (R06 a R10) k základní směsi. Experimentální diety byly testovány ve třech opakováních. Do 60l nádrží s průtokem 1,5l.s^{-1} bylo nasazeno 29 kusů kapřího plůdku (kříženci linií Po-L × ROP) o průměrné hmotnosti 43,25 g. Po aklimatizaci na prostředí nádrží byla rybám po dobu 60 dní předkládána pšenice s krmnou směsí KP (33% NL; 5% T) v množství 1,5% hmotnosti obsádky denně. Tento systém měl za účel navodit u ryb kondiční a výživný stav obdobný jako u ryb z polointenzivního až intenzivního rybničního chovu. Po této době byly ryby převedeny na experimentální krmné směsi a po startu vlastního experimentu jim byla tato krmiva předkládána v množství 2,5% hmotnosti obsádky denně. Na počátku a v 10denních intervalech v průběhu pokusu byly ryby v rámci kontrolních odběrů vzorků váženy a byla jim korigována denní krmná dávka. V den začátku, v kontrolních dnech a na konci pokusu bylo odebíráno 6 kusů plůdku z každé varianty na stanovení biochemického složení (sušina, protein, tuk) a spektra mastných kyselin svaloviny. Ta byla odebírána ve formě vertikálního pruhu masa z levé filety bez žeber a kůže.

U ryb na počátku experimentu bylo zjištěno nižší zastoupení kyseliny palmito-olejové, olejové a MUFA, naopak vyšší zastoupení kyseliny linolové, eicosapentaenové, docosahexaenové, n-3, n-6 i celkových PUFA oproti hodnotám kaprů z rybničních podmínek známým z vědeckých publikací. Předkládání kontrolní varianty krmiva vyvolalo u ryb minimální změnu spektra mastných kyselin masa (snížení zastoupeni skupiny MK 20:1 oproti vstupní hodnotě). Zkrmování směsi L06 způsobilo průkazné snížení obsahu kyseliny palmito-olejové, olejové a skupiny MK 20:1. Přídavek lněného oleje způsobil průkazné zvýšení zastoupení kyseliny α-linolenové (5,7x v porovnání se vstupní hodnotou). V profilu mastných kyselin došlo u ryb této varianty k žádoucímu snížení obsahu MUFA (v srovnání se vstupem) a zvýšení PUFA (oproti počáteční hodnotě i kontrolní variantě). Při vzájemném srovnání krmiv s různým obsahem rybího oleje nebyl nalezen průkazný rozdíl ve spektru ani v profilu mastných kyselin masa ryb těchto variant. Rozdíly jsou patrné až v porovnávání hodnotami vstupu a kontrolní varianty. U ryb obou variant s rybím olejem nedošlo oproti počátečním hodnotám k změnám v zastoupení některé z SFA. Průkazné zvýšení zastoupení v porovnání obou variant s kontrolou bylo zjištěno u kyseliny moroktové, eicosatetraenové a docosapentaenové. Obsah kyseliny eicosapentaenové se u variant s rybím olejem průkazně zvýšil oproti kontrolní variantě i oproti vstupním hodnotám. U ryb varianty R06 bylo oproti počátku zjištěno snížení MUFA a zvýšení celkových PUFA. Zvýšení zastoupení n-3 PUFA bylo zjištěno u varianty R10. Hodnota n-3/n-6 PUFA v mase ryb se průkazně zvýšila vlivem přídavku 10% rybího oleje. U dalších variant nedošlo k průkazným změnám tohoto parametru.

kapr obecný, spektrum mastných kyselin, rybí olej, lněný olej

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REFERENCES

BUCHTOVÁ, H.·SVOBODOVÁ, Z., KŘÍŽEK, M., VÁCHA, F., ·KOCOUR, M., VELÍŠEK, J., 2007: Fatty acid composition of flesh of three-year-old experimental scaly crossbreds of common carp (*Cyprinus carpio*, Linnaeus 1758). *Acta Vet. Brno*, 76: 73–81. ISSN 0001-7213

CSENGERI, I., FARKAS, T., 1993: Effect of essential fatty acid deficient diets on the carcass acids and membrane viscosity in the common carp. In: *Proceeding of EIFAC Workshop on Methodology for Determination of Nutrient Requirements in Fish.* Eichenau, Abstract, p. 62

FAJMONOVÁ, E.,·ZELENKA, J.,·KOMPRDA, T., KLADROBA, D., ŠARMANOVÁ, I., 2003: Effect of sex, growth intensity and heat treatment on fatty acid composionof common carp (*Cyprinus carpio*) fillets. *Czech Journal of Animal Sciences*, 48, 2: 85–92. ISSN 1212-1819

FARKAS, T., CSENGERI, I., MAJOROS, F., OLAH, J., 1978: Metabolism of fatty acids in fish, II. Biosynthesis of fatty acids in relations to diet in the carp, *Cyprinus carpio* Linnaeus 1758. *Aquaculture*, 14: 57–65. ISSN 0044-8486

FARKAS, T., CSENGERI, I., MAJOROS, F., OLAH, J., 1980: Metabolism of fatty acids in fish, III. Combined effect of environmental temperature and diet on formation and deposition of fatty acids in the carp, Cyprinus carpio Linnaeus 1758. *Aquaculture*, 20: 29–40. ISSN 0044-8486

FAUCONNEAU, B., ALAMI-DURANTE, H., LAROCHE, M., MARCEL, J., VALLOT, D., 1995: Growth and meat quality relations in carp. *Aquaculture*, 129: 265–297. ISSN 0044-8486

FOLCH, J., LEES, M., SLOANE-STANLEY, G. H., 1957: A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226: 497–509. ISSN 0021-9258

GERI, G., POLI, B. M., GUALTIERI, M., LUPI, P., PARISI, G., 1995: Body trakte and chemical composition of Musile in common carp (*Cyprinus carpio* L.) as influenced by age and rearingenviroment. *Aquaculture*, 129: 329–333. ISSN 0044-8486

HADJINIKOLOVA, L., 2004: The influence of nutrative lipid sources on the growth and chemical and fatty acid composition of carp (*Cyprinus carpio* L.). *Arch. of Polish Fish.*, 12, 2: 111–119

HENDERSON, R. J., TOCHER, D. R., 1987: The lipid composition and biochemistry of freshwater fish. *Prog. Lipid Res.*, 26: 281–347. ISSN: 0163–7827

KMÍNKOVÁ, M., WINTEROVÁ, R., KUČERA, J., 2001: Fatty acids in lipids of carp (*Cyprinus carpio* L.) tissues. *Czech Journalof Food Sciences*, 19, 5: 177–181. ISSN 1212-1800.

LUNN, J., THEOBALD, H. E., 2006: The health effects of dietary unsaturated fatty acids. *Nutrition Bulletin*, 31: 178–224. ISSN 0141-9684

MAREŠ, J., PALÍKOVÁ, M., KOPP, R., NAVRÁTIL, S., PIKULA, J., 2009: Changes in the nutritional parameters of muscles of the common carp (*Cyprinus carpio*) and the silver carp (Hypophthalmichthys molitrix) following environmental exposure to cyanobacterial water bloom. *Aquaculture Research*, 1–9. ISSN 1355-557X

MOURENTE, G., GOOD, J. E., BELL, J. G., 2005: Partial substitution of fish oil with rapeseed, linseed and olive oils in diets for European sea bass (*Dicentrarchus labrax* L.): effects on flesh fatty acid composition, plasma prostaglandins E_2 and $F_{2\alpha}$, imune function and effectiveness of a fish oil finishing diet. *Aquaculture nutrition*, 11: 25–40. ISSN 1353-5773

MZE ČR: *Ryby – situační a výhledová zpráva.* MZe ČR Praha. 2008, 41 s.. ISBN 978-80-7084-699-5

RUDEL, L. L., PARKS, J. S., HEDRICK, C. C., THOMAS, M., and WILLIFORD, K., 1998: Lipoprotein and cholesterol metabolism in diet-induced coronary artery atherosclerosis in primates. Role of cholesterol and fatty acids. *Prog. Lipid Res.*, 37: 353–370. ISSN 0163-7827

RUNGE, G., STEINHART, H., SCHWARZ, F. J., KIRCHGESNER, M., 1987: Influence of different fats with wariing addition of α-tokoferyl acatate on the fatty acid composion of carp (*Cyprinus carpio* L.). *Fat Sci. Technol.*, 89: 389–393. ISSN 0931-5985

SARGENT, J. R., TOCHER, D. R., BELL, J. G., 2002: The lipids. In: HALVER, J. E.: *Fish nutrition.* 3rd edition, Academic Press, San Diego, 182–246. ISBN 0-12-319652-3

SCHWARZ, F. J., KIRCHGESSNER, M., STEINHART, H., RUNGE, G.,1988: Influence of different fat with varying additions of α-tokcoferyl acetate on growth and body composition of carp (*Cyprinus carpio* L.). *Aquaculture*, 69: 57–67

STEFFENS, W., 1989: Principles of fish nutrition. Ellis Horwood Lim., Chichester, 384 p.

STEFFENS, W., 1997: Effect of variation in Essentials fatty acid in fish feeds on nutritive value of freshwater fish for humans. *Aquaculture*, 151: 97–119. ISSN 0044-8486

STEFFENS, W., WIRTH, M., 2007: Influence of nutrition on the lipid quality of pond fish: common carp (*Cyprinus carpio.*) and tench (*Tinca tinca*). *Aquaculture Int.*, 15: 313–319. ISSN 0967-6120

STEFFENS, W., WIRTH, M., RENNERT, B., 1995: Effect of adding various oils to thr diet on growth, feed conversion and chemical composion of carp (*Cyprinus carpio*). *Arch. animal nutrition*, 47: 381–389. ISSN 0003-942X

TAKEUCHI, T., WATANABE, T., OGINO, C.: Use of hydrogenated fish oil and beef tallow as s dietary energy source for carp and rainbowtrout. *Bull. of Jap. Soc. Sci. Fish.*,1978, 44: 875–881

TIDWELL, J. H., COYLE, S., BRIGHT, L. A., 2007: Effects of different types of dietary lipids on growth and fatty acid composition of largemouth bass. *North Amer. Jour. Of Aquaculture*, 69: 257–264. ISSN 1522-2055

TOCHER, D. R., BELL, J. G, DICK, J. R., HENDERSON, R. J., MCGHEE, F., MICHELL, D., MORRIS, P. C., 2000: Polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr-smolt transformation and the effects of die-

tary linseed and rapeseed oils. *Fish Physiol. And Biochem.*, 23: 59–73. ISSN 0920-1742

TURCHINI, G. M., MORETTI, V. M., MENTASTI, T., ORBAN, E., VALFE, F., 2007: Effects of dietary lipid source on fillet chemical composition, flavour volatile compounds and sensory characteristics in the freshwater tench (*Tinca tinca* L.) *Food chemistry*, 102: 1144–1155. ISSN 0308-8146

VÁCHA, F., VEJSADA, P., HUDA, J., HARTVICH, P., 2007: Influence of supplemental ceresů feeding on the content and structure of fatty acids during long-lasting storage of common carp (*Cyprinus carpio* L.). *Aquaculture Int.*, 15: 321–329. ISSN 0967-6120

WHO: Diet, nutrition and the prevention of chronic diseases: report of a joint WHO/FAO expert consultation, Geneva, 28 Jan.–1 Feb. 2002. (WHO technical report series), 2003, 916 p. ISSN 0512-3054

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