Slov Vet Res 2016; 53 (1): 19-28
UDC 613.2:664.33:547.915:597.551.214

Original Scientific Article

COMMON CARP RESPONSE TO THE DIFFERENT CONCENTRATION OF LINSEED OIL IN DIET

Boris Župan[1], Dragana Ljubojević[2]*, Miloš Pelić[2], Miroslav Ćirković[2], Vesna Đorđević[3], Ivan Bogut[4]

[1]Agricultural Extension Service, Savska 41, 10000 Zagreb, Croatia, [2]Scientific Veterinary Institute „Novi Sad", Rumenački put 20, 21000 Novi Sad; [3]Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Serbia, [4]Faculty of Agriculture, University of Osijek, Kralja Petra Svačića 1d, 31000 Osijek, Croatia

*Corresponding author, E-mail: ljubojevic.ljubojevicd.dragana@gmail.com

Summary: Common carp (*Cyprinus carpio* L.) fingerlings were fed five diets in triplicate containing 0, 2, 3, 4 and 5% of linseed oil for 75 days to determine the effect of the different concentration of linseed oil on growth parameters, as well as on the proximate and fatty acid composition of the fish flesh. The fish had the average initial weight of 200 g and were stocked in 15 cages. The fish which were fed with the diets supplemented with 3, 4 and 5% linseed oil had significantly better growth parameters compared to the fish fed with the diets supplemented with 0 or 2% linseed oil. The lipid content in muscle increased from 1.25% in the control group without the addition of linseed oil, to 1.46%; 1.56%; 1.94% and 2.37% in the groups of fish fed with the diets supplemented with 2, 3, 4 and 5% linseed oil, respectively. The fatty acid profiles in the muscle tissue reflected the diet concentrations with significant increases ($p < 0.05$) in 18:3n-3 in the fish fed with the diets supplemented with 2, 3, 4 and 5% of linseed oil. Other fatty acids which increased significantly ($p < 0.05$) in the muscle tissue, of fish fed with the diets supplemented with linseed oil, were: eicosatrienoic acid (C20:3n-3), eicosapentaenoic acid (EPA, C20:5n-3), docosapentaenoic acid (DPA, C22:5n-3), docosahexaenoic acid (DHA, C22:6n-3), as well as polyunsaturated fatty acids (PUFA), total n−3 fatty acids, and the n-3/n-6 ratio. The fatty acids which decreased significantly ($p < 0.05$) were monounsaturated fatty acids (MUFA) as well as linoleic acid (LA, C18:2n-6). The fish fed with the diet supplemented with 5% of linseed oil had three times more the n−3 fatty acids (24.02%) than those from the control group (7.5%). The n-3/n6 ratio ranged from 0.59 in fish from the control group to 4.14 in fish fed with the diet supplemented with 5% of linseed oil, mainly due to the difference in content of the alfa-linolenic acid (ALA, C18:3n-3). The inclusion of 5% linseed oil in diets showed the most favourable effects on the content of essential fatty acids in the tissue of carp as well as on other tested parameters.

Key words: *Cyprinus carpio*; cages; chemical composition; fatty acid; growth parameters; nutrition

Introduction

The n-3 highly unsaturated fatty acids (n-3 HUFA) are well known to have numerous beneficial effects on human health (1) and, undoubtedly, fish meat represents the best source of these nutrients in the human diet (2, 3). Moreover, the consumption of fish meat is encouraged due to the high protein content, the presence of essential amino acids, minerals and vitamins (4). Dietary lipids have an important role in aquafeeds as they are the source of energy and fatty acids which are essential for normal growth and development of fish. Furthermore, lipids can spare protein in the diets from being used as the source of energy (5) and additionally they can decrease ammonia production (6). Fish oil (FO) represents the main source for lipid in fish feed, especially for carnivorous fish species. The fish feed industry uses approximately 87% of the total produced FO, from which 66% is specifically used by salmon

Received: 15 February 2015
Accepted for publication: 9 July 2015

species. However, the significance of cyprinid and other warm-water fish species, when it comes to FO consumption, cannot be ignored due to their high share in the total world aquaculture production (7). Merino et al. (8) predicted that the price of FO will rise significantly in the future. An important concern is the possibility of contamination of the FO with persistent organic pollutants (9), which emphasizes the increasing international demand for safe and high quality fish feeds. Because of this, there is a growing need for sustainable alternatives to FO for aquafeeds, representing a considerable challenge for the future development of aquaculture. The replacement of FO with vegetable oils (VO) should be as such, where the catabolism of eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) is minimized while the deposition of linoleic acid (LA, C18:2n-6) has to be avoided (10). Some vegetable oils are considered to be alternatives to FO, such as rapeseed oil, soybean oil and linseed oil (LO), and have been tested as an alternative lipid source in different fresh water cyprinid fish species (11, 12, 13, 14, 15) and particularly in common carp (5, 16) one of the most important fish species worldwide. LO is rich in C18:2n-6 and especially rich in α-linolenic acid (ALA, C18:3n-3) (17), so the addition of this oil could increase the amount of C22:6n-3 and C20:5n-3 in fish meat, regarding the fact that fresh water fish species could synthesize n-3 HUFA from dietary C18:3n-3 (12). Feed with an addition of different content of LO, a vegetable oil rich with n-3 polyunsaturated fatty acids (PUFA) and especially with C18:3n-3, as an alternative lipid source, seems to produce a more similar fatty acid profile to FO than other vegetable oils in some cyprinids, without compromising fish growth or feed utilization (13, 15, 18). Enhancement in fish meat n-3 fatty acids causes a more convenient n-3/n-6 ratio which may improve the health value of cultured fresh water fish, whereas a modern diet contains high levels of n-6 and low levels of n-3 PUFAs (19). High dietary lipid content can also decrease feed consumption and reduce the growth of some fish species (11, 12) and it can lead to an increase in the lipid deposition in the body of fish (20) and consequently affect the nutritive quality of fish meat. Hence, the optimal dietary lipid level and source should to be investigated and carefully evaluated. Therefore, the aim of the present work was to investigate the effects of the different levels of linseed oil in diets on the growth performance, proximate composition and tissue fatty acid composition in common carp, which is one of the most commercially important fish species worldwide.

Materials and methods

Fish and facilities

The cage platform with 15 cages was set up in a 650 m² fishpond at a fish farm in Grabovo (Croatia). Water was added after the fishpond had been disinfected with 160 kg of Ca $(HCO_3)_2$. For the purpose of this trial, triplicate groups of common carp, were distributed in 15 cages. Approximately 60 one-year-old fish, with an initial mean weight of 200 g, were randomly distributed in each cage. All fish were reared under variable natural atmospheric conditions.

Experimental diets and feeding

Five comercial extruded diets were formulated to provide 32% of protein. Experimental diets were further prepared by an addition of 0% (control group – C), 2% (group L2), 3% (group L3), 4% (group L4) or 5% (group L5) of LO (Table 1). The daily feed amount was given in three sessions at 8.00, 13.00, and 17.00 h; by automatic feeders. The feed was provided in an amount of 1–2% with respect to fish biomass and depending on the water temperature, and water saturation with oxygen. The diet samples were collected at the beginning of the trial. Prior to the feeding trial, the fish were fed with commercial extruded feeds for one month. The trial lasted for 75 days, from April until June. Growth performance indicators such as specific growth rate (SGR), feed conversion ratio (FCR), weight gain (WG) and the survival rate (SR) were calculated (Table 3).

The water content in the diets and fish flesh was estimated by drying at 103 ± 2°C until their weight was constant for 24 hours. The level of crude protein (N × 6.25) was assessed with the Kjeldahl method (Manual Book, Kjeltec Auto 1030 Analyzer; Tecator, Höganäs, Sweden), and the total ash was determined after the combustion at 550 ± 25°C overnight. Crude fat from the diets samples and fish flesh was extracted with a Soxhlet extractor.

Lipid extraction and fatty acid analysis

The fatty acid composition analysis of experimental diets and fish flesh was performed as described previously (21). Briefly, the fatty acids in the experimental samples were determined following the extraction of total lipids by means of accelerated solvent extraction (ASE) on Dionex ASE 200. The mixture of n-hexane and isopropanol (60:40, v/v) was used for lipid extraction at 100°C and nitrogen pressure of 10.3 MPa in two static cycles lasting a total of 10 minutes. Fatty acid methyl esters were separated on a polar cyanopropyl aril column HP-88 (column length 100 m, diameter 0.25 mm, film thickness 0.20 µm; Agilent, Santa Clara, USA), in a programmed temperature range, on a capillary gas chromatograph (Shimadzu 2010; Shimadzu, Kyoto, Japan), with a flame ionisation detector. The temperature of the injector was 250°C and the detector temperature was 280°C. The carrier gas was nitrogen, with a flow rate of 1.33 ml min and a split ratio 1:50. The identification of fatty acid methyl esters was based on comparing their retention times with the standard, Supelco 37 Component FAME Mix (Supelco, Bellefonte, USA). The content of each fatty acid was expressed as the percentage of the total fatty acid content.

Statistical analyses

All data is shown as means ± SD (n=3). Statistical analyses were conducted by using a statistical software program Statistica 12 for Windows (Statistica Version 12.0; StatSoft Inc., Tulsa, USA), to determine if variables differed between treatments. Significant effects were further explored using analysis of variance (one-way ANOVA) with repeated measurements, as well as Tukey's post-hoc multiple range test. A significance level of $p < 0.05$ was used.

Results

Diet composition

The oil increased concentrations of linseed oil led to an increasing content of crude fat in the experimental diets, as well as increasing content of dry matter, while protein and ash content remain constant (Table 1). The oil concentration in the diets showed a significant effect on the dietary fatty acid compositions (Table 2). The C diet was free of external lipid addition and depended on the internal lipid contents of the diet ingredients. The C diet contained 21% of saturated fatty

Table 1: Composition and proximate analysis of the extruded formulated diet

Ingredients (%)	Diet for C	Diet for L2	Diet for L3	Diet for L4	Diet for L5
Soybean meal	50	50	50	50	50
Sunflower meal	18	18	18	18	18
Brewery yeast	5	5	5	5	5
Linseed oil	0	2	3	4	5
Wheat flour	10.6	10.6	10.6	10.6	10.6
Corn	12	12	12	12	12
Methionin	0.1	0.1	0.1	0.1	0.1
Lysine L	0.3	0.3	0.3	0.3	0.3
Vitamin mix[1]	2	2	2	2	2
Mineral mix[2]	2	2	2	2	2
Chemical analysis (%)					
Dry matter	89.91	90.15	90.27	90.38	90.5
Crude protein	32.49	32.33	32.25	32.17	32.09
Crude fat	1.56	3.46	4.4	5.36	6.31
Crude ash	4.49	4,46	4.45	4.44	4.43
NFE[3]	61.45	59.74	58.88	58.03	57.17

[1]C-control group; L2-group with addition of 2% of linseed oil; L3- group with addition of 3% of linseed oil; L4-group with addition of 4% of linseed oil; L5-group with addition of 5% of linseed oil; [1]Vitamin mix(mg/kg of diet): vitamin B1, 15; vitamin B2, 10; vitamin B6, 20; vitamin B12, 0,15; vitamin K3, 15; inositol, 250; Ca-pantothenic acid, 80; nicotinic acid, 100; folic acid, 1; vitamin H (biotin), 1; vitamin E, 140; vitamin C, 500; vitamin A, 20 000 IU; vitamin D3, 6 000 IU; choline chloride, 1 800, and cellulose was used as a carrier.
[2]Mineral mix (mg/kg of diet): Cu 20, Fe 40, Mn 30, Se 0.4, Zn 125, and cellulose was used as a carrier
[3]NFE, nitrogen-free extract, g/kg DM = 100 – (CP + CF + CA)

Table 2: Fatty acid composition of control diet (C) and experimental diets supplemented with 2% (L2), 3% (L3), 4% (L4) and 5% (L5) linseed oil (LO)

Fatty acid (%)	Diet for C	Diet for L2	Diet for L3	Diet for L4	Diet for L5
Myristic acid, C14:0	0.45	0.5	0.5	0.5	0.5
Pentadecylic acid, C15:0	0.1	0.1	0.1	0.1	0.1
Palmitic acid, C16:0	15.2	14.2	14.1	14.2	13.6
Palmitoleic acid, C16:1	0.62	0.4	0.4	0.4	0.5
Stearic acid, C18:0	4.67	3.5	3,6	3.5	3.4
Oleic acid, C18:1cis-9	29.5	32.9	31.7	31.7	28.5
Vaccenic acid, C18:1cis-11	1.2	0.5	0.5	0.5	0.6
Linoleic acid, C18:2 n-6	40.4	23.1	19.5	16.9	15.9
γ- linolenic acid, C18:3 n-6	0.15	0.19	0.13	0.15	0.1
α-linolenic acid, C18:3 n-3	5.56	22.3	27.5	30.1	33.9
Arachidic acid,C20:0	0.5	0.3	0.3	0.25	0.4
Eicosenoic acid, C20:1	0.24	0.15	0.25	0.37	0.3
Behenic acid, C20:2 n-6	0.1	0.2	0.2	0.2	0.2
Dihomogammalinolenic acid, C20:3 n-6	0.84	0.76	0.70	0.69	0.6
Eicosatrienoic acid, C20:3 n-3	0.2	0.2	0.2	0.2	0.2
Arachidonic acid, C20:4 n-6	0.2	0.1	0.1	0.1	0.1
Eicosapentaenoic acid, C20:5 n-3	0	0	0	0	0
Docosapentaenoic acid, C22:5 n-3	0	0	0	0	0
Docosahexaenoic acid, C22:6 n-3	0	0	0	0	0
SFA	20.92	18.6	18.6	18.55	18.1
MUFA	31.56	33.95	32.85	32.97	29.9
PUFA	47.45	46.85	48.33	48.34	51
∑ n -6	41.69	24.35	20.63	18.04	16.9
∑ n -3	5.76	22.5	27.7	30.3	34.1
n-3/n-6	0.14	0.92	1.34	1.65	2.02
n-6/n3	7.24	1.08	0.74	0.6	0.5
PUFA/SFA	2.27	2.52	2.6	2.34	2.82
USFA/SFA	3.78	4.35	4.36	4.38	4.47

C-control group; L2-group with addition of 2% of linseed oil; L3- group with addition of 3% of linseed oil; L4-group with addition of 4% of linseed oil; L5-group with addition of 5% of linseed oil; SFA-saturated fatty acids, MUFA-monounsaturated fatty acids; PUFA-polyunsaturated fatty acids of n-3 and n-6 series; USFA-unsaturated fatty acids Proximate composition of the fish and fish feed

Table 3: Growth performance of experimental fish

Variable	C	L2	L3	L4	L5
Initial number of fish	180	180	180	180	180
IBW(g)	201.67±1.53	203.67±2.52	201±1	201±2.65	201±1
FBW (g)	$484.3±4.04^{a}$	$492.33±4.93^{ab}$	$502±2^{ab}$	$508±2^{b}$	$519.67±2.52^{b}$
Final number of fish	141	147	150	147	153
Survival rate (%) SR	$78.33±2.3^{a}$	$81.67±2.1^{a}$	$83.33±1.9^{ab}$	$81.67±2.4^{a}$	$85±2.4^{b}$
WG (gfish^{-1})	$282.67±2.51^{a}$	$288.67±2.42^{ab}$	$301±1.73^{ab}$	$307±3.61^{bc}$	$318.67±3.5^{c}$
DGR (g day^{-1})	$3.77±3.2^{a}$	$3.84±3.1^{a}$	$3.99±3.2^{ab}$	$4.01±2.8^{b}$	$4.2±3.1^{b}$
SGR (%·day^{-1})	$1.17±1.29^{a}$	$1.18±0.03^{ab}$	$1.22±0.01^{ab}$	$1.24±0.02^{b}$	$1.27±0.01^{b}$
FCR (g·g^{-1})	$2.16±0.04^{a}$	$1.93±0.03^{b}$	$1.76±0.01^{c}$	$1,79±0.03^{c}$	$1,59±0.02^{d}$

C-control group; L2-group with addition of 2% of linseed oil; L3- group with addition of 3% of linseed oil; L4-group with addition of 4% of linseed oil; L5-group with addition of 5% of linseed oil; All values are mean ± SD (n = 3). Groups with different letter indexes in the same row statistically significantly different ($P < 0.05$); IBW, initial body weight; FBW, final body weight; SR, survival rate = (Final fish number/initial fish number) X 100; WG, weight gain = [Final body weight (g) - initial body weight (g)] X100 X [initial body weight (g)]$^{-1}$; DGR, Daily growth rate = [final body weight (g) - initial body weight (g)] X time^{-1} of rearing (days); SGR, specific growth rate = 100 X [ln final body weight (g) - ln initial body weight (g)] X time^{-1} of rearing (days); FCR, feed conversion ratio = total food supply (g) X [final fish biomass (g) - initial fish biomass (g)]$^{-1}$.

Table 4: Proximate composition of experimental fish

Parameters (%)	C	L2	L3	L4	L5
Moisture content (%)	79.16±0.07^{a}	78.87±0.16^{ab}	78.61±0.07^{b}	78.53±0.03^{b}	77.84±0.15^{c}
Protein content (%)	17.13±0.08^{a}	17.99±0.14^{b}	18.24±0.02^{b}	18.62±0.03^{c}	18.87±0.1^{c}
Fat content (%)	1.25±0.12^{a}	1.46±0.23^{a}	1.56±0.1^{a}	1.94±0.05^{b}	2.37±0.28^{c}
Ash content (%)	0.99±0.03	0.99±0.03	0.98±0.03	0.96±0.01	1.01±0.05

C-control group; L2-group with addition of 2% of linseed oil; L3- group with addition of 3% of linseed oil; L4-group with addition of 4% of linseed oil; L5-group with addition of 5% of linseed oil; Values are means ± SD (n = 3); Values in the same row with different letter notation statistically significantly differ at $p < 0.05$

Table 5: Fatty acid composition in fillets of experimental fish

Fatty acid (%)	C	L2	L3	L4	L5
C14:0	0.93±0.07	0.92±0.1	0.95±0.02	0.91±0.02	0.94±0.06
C15:0	0.16±0.03^{a}	1.17±0.01^{b}	1.17±0.03^{b}	1.33±0.01^{bc}	1.63±0.01^{c}
C16:0	21.29±3.58	21.25±2.03	21.07±0.16	21.36±0.49	21.12±0.6
C16:1	5.33±0.58^{a}	4.96±0.4^{b}	4.88±0.51^{b}	4.79±0.75^{b}	4.85±0.51^{b}
C17:0	0.27±0.04	0.29±0.03	0.28±0.02	0.28±0.04	0.26±0.01
C18:0	4.67±1.66	4.6±0.83	4.58±0.15	4.52±0.37	4.54±0.36
C18:1cis-9	38.29±2.67^{a}	35.27±1.94^{b}	35.34±2.81^{b}	36.23±0.73^{b}	33.20±0.78^{b}
C18:1cis-11	5.12± 0.17^{a}	1.73± 0.24^{b}	1.65± 2.86^{b}	1.53±0.22^{b}	0.21±0.00^{c}
C18:2 n-6	9.56±0.29^{a}	1.12±0.33^{b}	1.73± 0.08^{c}	1.86±0.05^{c}	1.75±0.19^{c}
C18:3 n-6	0.15±0.02	0.19±0.06	0.13±0.01	0.15±0.04	0.19±0.09
C18:3 n-3	5.42±3.32^{a}	14.93 ±0.44^{b}	15.21±0.72^{c}	15.46±0.05^{c}	17.72±1.19^{d}
C20:0	0.21±0.03	0.27±0.05	0.26±0.01	0.25±0.02	0.20±0.01
C20:1	2.54±0.24	2.38±0.15	2.44±0.25	2.31±0.37	2.31±0.37
C20:2 n-6	0.46±0.05	0.45±0.07	0.49±0.02	0.45 ±0.09	0.44±0.09
C20:3 n-6	0.84±0.16^{a}	0.86±0.14^{a}	0.70±0.03^{b}	0.69±0.07^{b}	0.62±0.06^{b}
C20:3 n-3	0.21±0.04^{a}	0.4±0.07^{b}	0.5±0.01^{b}	0.62±0.07^{bc}	0.73±0.04^{c}
C20:4 n-6	1.70±0.48^{a}	2.57±0.07^{b}	2.65±0.06^{b}	2.76±0.04^{bc}	2.8±0.01^{c}
C20:5 n-3	0.18±0.02^{a}	1.22±0.01^{b}	1.25±0.01^{b}	1.28±0.01^{b}	1.31±0.02^{b}
C22:5 n-3	0.16±0.02^{a}	1.21±0.02^{b}	1.27±0.02^{b}	1.30±0.01^{bc}	1.36±0.03^{c}
C22:6 n-3	1.54±0.26^{a}	2.67±0.1^{b}	2.68±0.02^{b}	2.79±0.02^{c}	2.9±0.07^{d}
SFA	27.52±5.25^{a}	28.5±2.86^{b}	28.31±0.33^{b}	28.65±0.53^{b}	28.69±0.3^{b}
MUFA	51.28±3.42^{a}	44.34±2.49^{b}	44.31±0.4^{b}	44.86±0.96^{b}	40.57±0.69^{c}
PUFA	20.22±3.42^{a}	25.62±0.35^{b}	26.61±0.86^{bc}	27.35±1.02^{c}	29.82±1.26^{d}
∑ n -6	12.71±3.05^{a}	5.19±0.33^{b}	5.7±0.81^{bc}	5.91±0.92^{c}	5.8±1.44^{c}
∑ n -3	7.51±0.4^{a}	20.43±0.34^{b}	20.91±0.07^{b}	21.45±0.12^{c}	24.02±0.18^{d}
n-3/n-6	0.59±0.01^{a}	3.94±0.02^{b}	3.67±0.01^{b}	3.63±0.01^{b}	4.14±0.02^{c}
n-6/n3	1.69±0.02^{a}	0.25±0.03^{b}	0.27±0.05^{b}	0.28±0.02^{b}	0.24±0.03^{b}
PUFA/SFA	0.73±0.3^{a}	0.90±0.22^{b}	0.94±0.26^{b}	0.95±0.32^{bc}	1.04±0.34^{c}
USFA/SFA	3.90±0.38^{a}	2.45±0.26^{b}	2.51±0.21^{b}	2.52±0.34^{b}	2.46±0.38^{b}

C-control group; L2-group with addition of 2% of linseed oil; L3- group with addition of 3% of linseed oil; L4-group with addition of 4% of linseed oil; L5-group with addition of 5% of linseed oil; Values are means ± SD (n = 3); Values in the same row with different letter notation statistically significantly differ at $p < 0.01$. SFAsaturated fatty acids; MUFAmonounsaturated fatty acids; PUFApolyunsaturated fatty acids from the n3 (n3 PUFA) and n6 (n6 PUFA) families.

acids (SFA) of which approximately two thirds (15.2%) was palmitic acid (C16:0) and 31.56 % monounsaturated fatty acids (MUFA), whereas the content of oleic acid (OA, C18:1) was 29.5%. The C diet contained 41.7 % n-6 PUFA, predominantly C18:2n-6 (40.4%) and 5.76 % n-3 PUFA, with 5.56% C18:3n-3. Increasing oil content resulted in a decreased level of SFA, which was 18.1 % in the L5 diet and the content of MUFA was almost the same among the diets. The inclusion of the increased content of LO in the experimental diets resulted in a decreased content of C16:0 and arachidonic acid (AA, C20:4n-6), and in a slightly increased content of eicosanoic acid (C20:1). The n-3/n-6 ratio increased from 0.14 in the C diet to 2.02 in the L5 diet, mainly due to the increasing content of n-3 (especially C18:3n-3) from 5.8% in C diets to 34% in L5 diets. The total content of n-6 PUFA was decreased from 41.7% in the C diet to 16.9 in L5 diet.

Production performance

There were no significant differences in the initial mean weights of the fish (Table 3). Following the 75 days of trial, the mean weight was between 484 g in the C and 520 g in the L5 group. The statistically significant effect of oil concentration was observed in the final body weight, and the highest body weight was observed in the L4 and L5 groups. Moreover, the significant effect of the above mentioned factor was identified in the growth parameters (SGR, DGR and WG). The feed conversion ratio (FCR) was satisfactory for all treatments and ranged from 1.59 to 2.16. One-way ANOVA showed a significant effect of oil concentration on the FCR.

Proximate composition of common carp filets

A significant effect of treatments was observed in the content of crude protein and lipid in the common carp fillets (Table 4). Moisture content was significantly lower in the carp fillets comparing to groups which received diets with higher concentrations of LO which were accompanied with a higher content of fat in muscle tissue. The ash content was the same in all the analysed groups. Oil content showed a significant effect on the proximate composition of the carp fillets, and also resulted in significant changes in the fillets fatty acid compositions (Table 5).

Fatty acid composition of common carp fillets

The proportions of the analysed fatty acid groups were significantly affected by the used diet. The fatty acid compositions of the carp muscle tissue are shown in Table 5. The addition of linseed oil significantly affected the muscle fatty acid composition. However, the significant effect regarding oil concentration in the diets was observed only for a few fatty acids. It was observed that the content of C18:1 decreased slightly, the total MUFA decreased approximately by 14%, the C18:2n-6 decreased to a 7-fold and the total n-6 PUFA 2-fold in the flesh of common carp from groups with an addition of linseed oil compared to the flesh of the carp from C group. On the other hand, the content of saturated fatty acids (SFA) slightly increased, C20:4n-6 increased 1.4-fold, C18:3n-3 3-fold, eicosatrienoic acid (C20:3n-3) from 2 to 3 fold, C20:5n-3 6-fold, docosapentaenoic acid (DPA, C22:5n-3) 6 – fold, C22:6n-3 1.7-fold and n-3/n-6 ratio 6-fold in the flesh of carp from groups L1, L2, L3, L4 and L5 compared to C group. It is interesting that the content of C22:6n-3 was higher in the muscle tissue of the carp fed with all the experimental diets compared to the content of C22:6n-3 in the diets. The L5 group contained the highest levels of SFA (28.69%), while the lowest was measured in the C group (27.52%); the most prominent differences concerned the pentadecylic acid (C15:0), which was almost ten times higher in the L5 than in the C group (1.63% vs 0.16%). The content of MUFA in the fillets of the common carp from the C group was significantly higher than in the other groups (51.28% vs 40.57–44.86%). The contents of C18:2n-6 and C18:3n-3 in the common carp fillets were lower than determined in the diets analysed, and the contents of C20:4n-6, C20:5n-3, C22:5n-3 and C22:6n-3 were higher than the content in the diets. Increased levels of the intermediates eicosadienoic acid (C20:2n-6) and C20:3n-3 in fish that were fed with LO diets were detected. Regarding the C20:4n-6, its content in the fish fillets from the groups fed with an addition of linseed oil was higher (2.57-2.8%) than in those fed with the C diet (1.7%). However, statistically significant differences were noted in the contents of C20:4n-6, C20:5n-3, or C22:6n-3 in the fillets of common carp fed with the diets supplemented with different concentrations of LO ($P < 0.05$; Table 5). The ratio of n-3/n-6 ranged from 4.1 (L5 group) to 0.6 (C group) ($P < 0.05$).

Discussion

Production performance

Several studies have shown that the use of vegetable oils, including LO in diets of cyprinid fish species, has no negative effects on fish growth and growth parameters (13, 15, 18, 22). Growth performance was significantly affected by an addition of increased concentrations of LO in fish diets. It should be noted that the growth rate of carp fed diets supplemented with LO in this study was significantly faster than that of fish fed with diets without the supplementation of LO. The concentration of added oil also showed significant influence on the growth performance. This could indicate that increased fat contents in the diets, which is in the present study LO, has a favorable impact on the growth of common carp. Similar results were reported for catfish (23) and for tench (13). The results of the present research agreed with some reports that by increasing the dietary lipid content, the growth of the fish may improve (24, 25). On the other hand, some authors have reported that a high dietary lipid level (more than 7%) could reduce fish growth (24, 25) which could be due to the low ability to digest and absorb high lipid, the reduction in feed intake and fatty acid imbalance in the diet.

Proximate composition of common carp fillets

The increase of dietary oil levels herein is usually associated with an increase in fish flesh lipid content. A positive correlation between the dietary lipid levels and the total lipid levels in muscle tissue was also observed previously in other species (24, 26). On the contrary, the increase of dietary oil levels led to a decrease in the fish flesh moisture content. A negative correlation between the lipid and moisture contents in the flesh of the carp was also previously shown (27, 28). This is in accordance with previous studies (15, 20) which also showed that the chemical composition of carp flesh was influenced by the experimental diets, especially the content of fat. There were no adverse effects of oil addition on the proximate composition of the common carp fillets, after 75 days of feeding. Our findings are in agreement with previous studies in cyprinid species (goldfish, *Carassius auratus*; common carp, *C. carpio*; grass carp, *Ctenopharyngodon idella*; tench, *Tinca tinca*) in which the addition of VO in feed did not negatively affect the chemical composition of these fish over either short or long-term periods (13, 15, 29, 30).

Fatty acid composition of common carp fillets

The fatty acid composition of muscle lipids of common carp is known to be highly influenced by dietary fatty acids (15, 16) and a linear correlation exists between individual fatty acids in muscle lipid and their concentration in dietary lipid. In the present study, the differences in the muscle tissue fatty acid composition resulted from the different levels of oil in the diets. It seems that common carp also utilize C18:2n-6 and C18:3n-3 as a source of metabolic energy. Lower levels of the above mentioned fatty acids in the muscle tissue, regardless of the dietary treatment, might designate highly active mitochondrial enzymes oxidising fatty acids as previously noted (31). This can further be related to the fact that mitochondrial β-oxidation is of specific significance to muscle tissues (32). Higher levels of C18:2n-6 and C18:3n-3 in muscle tissue of freshwater fish species fed diets with different VO were also previously noted (5, 15, 18). The intermediates C20:2n-6 and C20:3n-3 acid were detected in the carp fillets in the LO groups. Since VO are mainly deprived of these fatty acids and they are an important part of the biosynthetic pathways of n-6 and n-3 HUFA, this result highlights the adjustable attempts to alleviate HUFA deficiencies. When the level of C18:3n-3 or C18:2n-6 in the diets was increased, C20:3n-3 or C20:2n-6 level in muscle was also increased. A similar phenomenon was previously observed in tench (15) and in common carp (5). Previous studies have shown that grass carp (11, 12), tench (15) and common carp (15) can synthesise n-3 HUFA from dietary C18:3n-3. In the present trial, although experimental diets contained no HUFA, including C20:5n-3 and C22:6n-3, the mentioned fatty acids were found in the muscle tissues of common carp fed with experimental diets (both control diet and diets supplemented with LO) for 75 days, preventing essential fatty acids (EFA) deficiency and also suggesting that the common carp was able to synthesise n-3 HUFA from the C18:3n-3 in the diets. Besides that, in the muscle

tissue of groups, the presence of C20:4n-6 formed from C18:2n-6 upholds this theory. In the present experiment, increasing levels of C22:5n-3 and C22:6n-3 in the common carp fillets relative to their respective dietary levels were observed and they induced the bioconversion of C20:5n-3 to C22:6n-3. A similar pattern of selective storage was observed in n-6 fatty acids in which increased retention was noted in C20:4n-6. The changes in muscle fatty acid composition suggest selective utilisation or deposition of individual fatty acids as previously reported for cyprinid omnivorous fish species (5, 13, 14, 15, 18, 22, 30). It should be emphasized that the main consequence of higher C18:3n-3, C20:5n-3, C22:5n-3 and C22:6n-3 and lower C18:2n-6 contents in the fillets of the common carp was the increased value of the n-3/n-6 ratio compared to the levels of this ratio in the diets. However, in the case of the C group, this value was significantly lower than that in the groups fed diets supplemented with LO. The abundance of C18:3n-3 apparently fulfils the essential fatty acids needs of the common carp in this study. This is presumably due to the ability of common carp to selectively retain C22:6n-3 and bioconvert C18:3n-3 to C20:5n-3 and C22:6n-3; C20:5n-3 to C22:6n-3, and C18:2n-6 to C20:4n-6. Results obtained in the present study suggest that common carp have high tolerance to diets that differ significantly in lipid content and fatty acid composition. In the present research, n-6/n-3 ratios of all experimental groups were below 4.0, which is in accordance with the recommendation of Simopoulos (19) for human nutrition. The PUFA/SFA ratio was the highest in Diet L5 (2.82), mostly due to the very high content of C18:3n-3, which was 33.9 % in this sample. The lowest level of PUFA/SFA ratio recommended by WHO and FAO is 0.4 (33) and all experimental diets comply with this recommendation. The mentioned differences are even more obvious in the fatty acid composition of carp meat. All groups fed with the experimental diets supplemented with LO had a better n-6/n-3 ratio than the control group, and the common carp fed with diet L5 showed the best results with a n-6/n-3 ratio of 0.24. In this group, C20:5n-3 content increased about seven times in comparison to the control group (1.31% vs. 0.18 %), while no significant differences were observed between the common carp fed diets supplemented with LO. C22:6n-3 content increased 1.9 times (2.9% vs. 1.54%) and significant differences were observed between groups fed diets with different levels of LO. It can be concluded that the addition of higher concentrations in fish feed (4 or 5%) gave more favorable fatty acid composition of carp meat than the addition of LO in lower concentrations (2 or 3%) and especially more than the control group without the addition of LO. Based on the obtained results, we conclude that LO in the diet in concentrations of 4 or 5% leads to satisfactory production performance, as well as favorable proximate composition of muscles and lipid quality regarding fatty acid composition of common carp. The significance of the present study also relies on the fact that the study was conducted in natural atmospheric conditions, while the majority of the previous studies (11, 12, 13, 14) were conducted in indoor closed tanks; so the results of this study are more applicable to the aquaculture industry.

Acknowledgements

This work was supported by grants from the Ministry of Education, Science, and Technological Development of the Republic of Serbia (project no. TR31011).

Reference

1. Balk EM, Lichtenstein AH, Chung M, Kupelnick B, Chew P, Lau J. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. Atherosclerosis 2006; 189: 19–30.

2. Topic Popovic N, Kozacinski L, Strunjak - Perovic I, et al. Fatty acid and proximate composition of bluefin tuna (*Thunnus thynnus*) muscle with regard to plasma lipids. Aquac Res 2012; 43: 722–9.

3. Sarma D, Akhtar MS, Das P, et al. Nutritional quality in terms of amino acid and fatty acid of five coldwater fish species: implications to human health. Natl Acad Sci Lett 2013; 36: 385–91.

4. Hathwar SC, Rai AK, Modi VK, Narayan B. Characteristics and consumer acceptance of healthier meat and meat product formulations: a review. J Food Sci Technol 2012; 49: 653–64.

5. Ljubojević D, Radosavljević V, Puvača N, et al. Interactive effects of dietary protein level and oil source on proximate composition and fatty acid composition in common carp (*Cyprinus carpio* L.) J Food Compost Anal 2015; 37: 44–50.

6. Mungkung R, Aubin J, Prihadi TH, Slembrouck J, van der Werf HM, Legendre M. Life cycle assessment for environmentally sustainable aquaculture management: a case study of combined aquaculture systems for carp and tilapia. J Clean Prod 2013; 57: 249–56.

7. Tacon AG, Metian M. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. Aquaculture 2008; 285: 146–58.

8. Merino G, Barange M, Blanchard JL, et al. Can marine fisheries and aquaculture meet fish demand from a growing human population in a changing climate? Glob Environ Change 2012; 22: 795–806.

9. Bell JG, Dick JR, Strachan F, Guy DR, Berntssen MH, Sprague M. Complete replacement of fish oil with a blend of vegetable oils affects dioxin, dioxin-like polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in 3 Atlantic salmon (*Salmo salar*) families differing in flesh adiposity. Aquaculture 2012; 324: 118–26.

10. Thanuthong T, Francis DS, Manickam E, Senadheera SD, Cameron - Smith D, Turchini GM. Fish oil replacement in rainbow trout diets and total dietary PUFA content: II. Effects on fatty acid metabolism and in vivo fatty acid bioconversion. Aquaculture 2011; 322: 99–108.

11. Du ZY, Liu YJ, Tian LX, He JG, Cao JM, Liang GY. The influence of feeding rate on growth, feed efficiency and body composition of juvenile grass carp (*Ctenopharyngodon idella*). Aquac Int 2006; 14: 247–57.

12. Du ZY, Clouet P, Huang LM, Degrace P, Zheng WH, He JG. Utilization of different dietary lipid sources at high level in herbivorous grass carp (*Ctenopharyngodon idella*): mechanism related to hepatic fatty acid oxidation. Aquac Nutr 2008; 14: 77–92.

13. Zakęś Z, Jankowska B, Jarmolowicz S, Zmijevski T, Partyka K, Zakęś DK. Effects of different dietary fatty acids profiles on the growthperformance and body composition of juvenile tench (*Tinca tinca* L.). Rev Fish Biol Fisheries 2010; 20: 389–401.

14. Sun S, Ye J, Chen J, Wang Y, Chen L. Effect of dietary fish oil replacement by rapeseed oil on the growth, fatty acid composition and serum non-specific immunity response of fingerling black carp, *Mylopharyngodon piceus*. Aquac Nutr 2011; 17: 441–50.

15. Ljubojević D, Ćirković M, Novakov N, et al. Comparison of meat quality of tench, *Tinca tinca*, reared in extensive and semi-intensive culture systems. J Appl Ichthyol 2014; 30: 50–7.

16. Steffens W, Wirth M. Influence of nutrition on the lipid quality of pond fish: common carp (*Cyprinus carpio*) and tench (*Tinca tinca*). Aquac Int 2007; 15: 313–9.

17. Łukaszewicz M, Szopa J, Krasowska A. Susceptibility of lipids from different flax cultivars to peroxidation and its lowering by added antioxidants. Food Chem 2004; 88: 225–31.

18. Turchini GM, Moretti VM, Mentasti T, Orban E, Valfre F. Effects of dietary lipid source on fillet chemical composition, flavour volatile compounds and sensory characteristics in the freshwater fish tench (*Tinca tinca* L.). Food Chem 2007; 102: 1144–55.

19. Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med 2008; 233: 674–88.

20. Keshavanath P, Manjappa K, Gangadhara B. Evaluation of carbohydrate rich diets through common carp culture in manured tanks. Aquac Nutr 2002; 8: 169–74.

21. Spirić A, Trbović D, Vranić D, Đinović J, Petronijević R, Matekalo - Sverak V. Statistical evaluation of fatty acid profile and cholesterol content in fish (common carp) lipids obtained by different sample preparation procedures. Anal Chim Acta 2010; 672: 66–71.

22. Kukačka V, Chalopkova L, Fialova M, Kopp R, Mareš J. The influence of linseed oil and fish oil supplements to the fatty acid spectrum of common carp (*Cyprinus carpio* L.) muscle. Acta Univ Agric Silvic Mendel Brun 2009; 57 (5): 193–2.

23. Bogut I, Has - Schön EM, Čačić M, Milaković Z, Novoselić D, Brkić S. Linolenic acid supplementation in the diet of European catfish (*Silurus glanis*): effect on growth and fatty acid composition. J Appl Ichthyol 2002; 18: 1–6.

24. Pei Z, Xie S, Lei W, Zhu X, Yang Y. Comparative study on the effect of dietary lipid level on growth and feed utilization for gibel carp (*Carassius auratus* gibelio) and Chinese longsnout catfish (*Leoiocassis longirostris* Günther) Aquac Nutr 2004; 10: 209–16.

25. El-Marakby M. Effect of dietary sources and levels of lipids on growth performance and feed utilization of fry Nile tilapia, *Oreochromis ni-*

loticus (L.) (Teleostei: Perciformes). J Fish Aquat Sci 2006; 1: 117–25.

26. Chou BS, Shiau SY. Optimal dietary lipid level for growth juvenile hybrid tylapia, *Oreochromis niloticus* X *Oreochromis aureus*. Aquaculture 1996; 143: 185–95.

27. Ljubojević D, Ćirković M, Đorđević V, et al. Fat quality of marketable fresh water fish species in the Republic of Serbia. Czech J Food Sci 2013; 31: 445–50.

28. Ljubojević D, Ćirković M, Novakov N, et al. Productivity and meat nutrient in fish: the diet effect. Kafkas Univ Vet Fak Derg 2013; 19: 43–9.

29. Steffens WM, Rennert B. Effects of adding variousoils to the diet on growth, feed conversion and chemicalcomposition of carp (*Cyprinus carpio*). Arch Anim Nutr 1995; 47: 381–9.

30. Pozernick M, Wiegand MD. Use of canola oil in the feed of larval and juvenile goldfish, *Carassius auratus* (L.). Aquac Res 1997; 28: 75–83.

31. Torstensen BE, Lie O, Froyland L. Lipid metabolism and tissue composition in Atlantic salmon (*Salmo salar* L.): effects of capelin oil, palm oil, and oleic acid-enriched sunflower oil as dietary lipid sources. Lipids 2000; 35: 653–64.

32. Nanton DA, Vegusdal A, Rora AMB, Ruyter B, Baeverfjord G, Torstensen BE. Muscle lipid storage pattern, composition, and adipocyte distribution in different parts of Atlantic salmon (*Salmo salar*) fed fish oil and vegetable oil. Aquaculture 2007; 265: 230–43.

33. FAO/WHO. Report of the WHO/FAO Joint Expert Consultation. Diet nutrition and the prevention of chronic diseases. Geneva : World Health Organization, 2003. (Technical Report Series, No. 916)

POVPREČNA ODZIVNOST KRAPOV NA RAZLIČNE KONCENTRACİJE LANENEGA OLJA V HRANİ

B. Župan, D. Ljubojević, M. Pelić, M. Ćirković, V. Đorđević, I. Bogut

Povzetek: Mladice krapa (*Cyprinus carpio* L.) so bile krmljene 75 dni s petimi različnimi krmili v treh ponovitvah, ki so vsebovala 0, 2, 3, 4 ali 5 % lanenega olja, z namenom določanja učinka različnih koncentracij lanenega olja na rastne parametre kakor tudi na sestavo neposredne in maščobno-kislinske sestave mesa rib. Ribe so imele povprečno začetno težo 200 g in so bile nastanjene v 15 kletkah. Ribe, ki so bile krmljene s krmili, dopolnjenimi s 3, 4 ali 5 % lanenega olja, so imele bistveno boljše kazalnike rasti v primerjavi z ribami, ki so prejemale krmila, dopolnjena z 0 ali 2 % lanenega olja. Vsebnost maščob v mišicah se je povečala z 1,25 % v kontrolni skupini brez dodatka lanenega olja na 1,46 %, 1,56 %, 1,94 % in 2,37 % v skupinah rib, krmljenih s krmo, dopolnjeno z 2, 3, 4 oziroma 5 % lanenega olja. Maščobno-kislinski profili mišičnega tkiva so pokazali znatno povečanje ($p < 0,05$) vsebnosti maščobnih kislin 18:3n-3 pri ribah, krmljenih s krmili, ki so vsebovala 2, 3, 4 oz. 5 % lanenega olja. Druge maščobne kisline, ki so se v mišičnem tkivu rib, krmljenih s krmili, dopolnjenimi z lanenim oljem, znatno povečale ($p < 0,05$) so bile: eikozanojska kislina (C20: 3n-3), eikozapentanojska kislina (EPA, C20: 5n-3), dokozanojska kislina (DPA, C22: 5n-3), dokoheksanojska kislina (DHA, C22: 6n-3) kakor tudi polinenasičene maščobne kisline (PUFA), skupne n-3 maščobne kisline, spremenilo pa se je tudi razmerje maščobnih kislin n-3 : n6. Maščobne kisline, katerih vsebnost se je bistveno zmanjšala ($p < 0,05$) so mononenasičene maščobne kisline (MUFA), pa tudi alfa linolne kisline (LA C18: 2n-6). Ribe, ki so bile krmljene s krmili s 5 % lanenega olja, so imele trikrat več n-3 maščobnih kislin (24,02 %) kot ribe iz kontrolne skupine (7,5 %). Razmerje n-3/n6 je bilo ugotovljeno v razponu od 0,59 pri ribah iz kontrolne skupine do 4,14 pri ribah, krmljenih s krmili s 5 % lanenega olja, predvsem zaradi razlike v vsebnosti alfa-linolenske kisline (ALA, C18: 3n-3). Vključitev 5 % lanenega olja v krmi je pokazala najugodnejše učinke na vsebnost esencialnih maščobnih kislin v mesu krapov, pa tudi drugih testiranih parametrov.

Ključne beside: *Cyprinus carpio*; gojenje; kemična sestava mišic; maščobne kisline; parametri rasti; prehrana