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FATTY ACID PROFILE IN MUSCLES OF CARP (*CYPRINUS CARPIO* L.) RAISED IN A SEMI-INTENSIVE PRODUCTION SYSTEM FED WITH GRAINS, PELLETED AND EXTRUDED FEED

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Abstract - The effects of grains, pelleted and extruded feed on the fatty acid content in carp meat has not been examined yet. In this work, we present evidence that the high carbohydrate content in all three types of feed causes oleic acid to predominate in all meat samples. A higher PUFA content in the meat of fish fed with granulated feed was detected. The extruded feed diet led to 69% greater n-3, and 53% lower n-6 fatty acid contents. Their ratio is thus 2.64-fold higher than in meat of carp fed with pelleted feed. A higher content of n-3 fatty acids in fish fed with extruded feed was the consequence of higher DHA (1.6 times) and EPA (3.3 times) contents. The detected differences could be the consequence of the thermal treatment of extruded feed that makes the proteins, carbohydrates and lipids more accessible to fish than in a pelleted feed.

Key words: Common carp, fatty acids, extruded feed, pelleted feed

Abbreviations: PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acid; ALA, Alpha-linolenic acid; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid; LA, Linoleic acid; FAME , fatty acid methyl esters; PER, Protein Efficiency Ratio; ANPU Apparent Net Protein Utilization; ANLU, Apparent Net Lipid Utilization.

INTRODUCTION

After the silver carp (*Hypophthalmichthys molitrix*, Valenciennes 1844) and the grass carp (*Ctenopharyngodon idellus*, Valenciennes 1844), the common carp (*Cyprinus carpio*, Linnaeus 1758) is the most abundant cyprinid fish species in the world's aquaculture (Takeuchi et al., 2002; FAO, 2009). The quality of its meat is very important in human nutrition. The quality of meat in farmed fish is largely defined by the quality of their nutrition. Farmed salmonid fish are grown only in intensive systems under strictly defined conditions. The fish are fed with rich processed energetic feeds that conform to standard quality (Cho, 1992). Common carp is grown in a semi-intensive aquaculture (Tacon, 1993). Semi-intensive production is primarily based on a grain diet (corn, wheat, barley). In recent years, primarily during periods of depression of natural feed (from June to September), pelleted and extruded concentrated feeds are increasingly used in some countries in southeastern Europe (Serbia, Bosnia-Herzegovina, Bulgaria, Romania) (Marković et al., 2009). The different quality of supplemental feeds leads to differences in the quality of meat in farmed carp.

Besides containing biologically valuable proteins, minerals and vitamins, fish meat is one of the

basic sources of n-3 fatty acids in human nutrition. The n-3 fatty acids reduce the level of triglycerides and cholesterol in serum (Sidhu, 2003; Steffens, 1997). It was shown that n-3 fatty acids are important in the prevention and treatment of a multitude of diseases: they reduce the risk of coronary heart disease (Yaqoob, 2004; Wang et al., 2006), hypertension (Berry and Hirsch, 1986), and have a positive role in the prevention and treatment of inflammatory (Calder, 2001) and autoimmune diseases (Zamaria, 2004), malignant diseases (Terry et al., 2004) and diabetes (Nettleton and Katz, 2005). As a result, the FDA (U.S. Food and Drug Administration) officially confirmed in 2004 that EPA and DHA can reduce the risk of coronary heart diseases, and recommends that consumers use food that contains these important compounds in order to improve health (2004).

If the presence of n-3 fatty acids in carp meat is analyzed along with the ratio to n-6 fatty acids, there is considerable variability due to different farming factors (Steffens, 1997). Therefore, depending on the method of carp farming, the n-3 to n-6 ratio can range from 0.1 (Runge et al., 1987) to as much as 3.02 (Sýkora and Valenta, 1978). There have been studies on the effects of feed mixture (Steffens, 1997), gender (Fajmonová et al., 2003), age (Geri et al., 1995) and farming temperature (Viola et al., 1988) on fatty acid composition, however, the effects of different methods of feed processing have not been analyzed, despite there being so many. When it comes to trout (Hilton et al., 1981) and gilt-head bream (Deguara, 1997), it has been shown that the use of feeds produced by different processing methods greatly influences various quality parameters of the meat of these fish. As regards sea bass, a significant effect of two types of feed on the n-3/n-6 fatty acid relationship has been shown (Aslan et al., 2009).

Taking into consideration the importance of carp in human nutrition, as well as the variety of feed treatments in the dominant semi-intensive breeding system, it is very important to understand the influence of the method of feed treatment on the fatty acid content in carp meat. The main goal of this paper was to establish the effect of certain feeds (non-treated grains, pelleted and extruded feeds) on the fatty acid content in carp meat.

MATERIALS AND METHODS

The experiment was performed in three 650-m² ponds positioned side by side in an experimental fish farm of the Center for Fishery and Applied Hydrobiology, University of Belgrade, Faculty of Agriculture, Serbia.

Experiment design

Four hundred carp from the commercial fish farm aged 11 months and weighing 150 +/- 18 g were placed in each pond. Initial filling and later refilling was done with the same quantity of water. After three weeks of adaptation to the new conditions in the ponds, the carps were fed for six months (May-October 2009) with different feeds: grains, pelleted feed and extruded feed. The grain feed was comprised of wheat, corn and barley at a 1:1:1 ratio. The other two types of feed contained the same components (Table 1), but the pellets were obtained by different treatments: one was derived by pelleting and the other by an extrusion process.

Table 1. Extruded and pelleted feed formulation.

Components	%
Soybean	28.00
Fish flour	5.00
Yeast	6.00
Wheat	22.00
Corn	30.00
Soybean meal	6.40
Chalk	0.80
Lysine	0.20
Premix for fish 1%	1.00
Monocalcium phosphate	0.50
Salt	0.10

Table 2. Chemical composition of experimental diets.

	Grains	Extruded feed	Pelletted feed
Proteins	11.3±0.8	28.5±0.9	26.5±0.8
Lipids	3.3±0.2	7.8±0.1	8.0±0.1
Ash	1.9±0.3	4.7±0.2	4.7±0.3
Fibers	7.4±0.6	3.5±0.1	3.3±0.4
Moisture	9.8±0.7	9.4±0.3	12.0±0.5
Accessible carbohydrates	66.2±2.0	46.1±1.3	45.5±1.7

A daily fish portion during the experiment was 3% relative to the ichtiomass of the pond. The ichtiomass was determined by measuring samples of fish (50 each) every 15 days, and calculated according to the total number of fish in an experimental pond. The fish mass was determined by measuring on a CASBEE scale (Model MW 120) to the nearest 0.001 g.

Three fish were taken as samples for analyzing the fatty acid content in their meat before being placed in the ponds to represent control samples. Three fish from each pond were taken after six months of feeding. Samples were preserved at -18˚C until examination in the laboratory. The fish were thawed for 1 h at room temperature. The head, tail, skin and entrails were removed to obtain homogenized fillets. Determination of the fatty acid content and total lipid analysis of carp meat was performed in the Institute of Meat Hygiene and Technology in Belgrade (Serbia).

Chemical analysis

The percentage of moisture in the meat was measured gravimetrically by thermal drying to a constant weight in an oven at 110°C for 24 h. The crude protein content was determined by Kjeldahl analyses with a Tecator Kjeltec Autoanalyzer. Crude fat was determined by acid hydrolysis using a Soxtec System 1047 hydrolyzing unit (Tecator application note 92/87), followed by exhaustive Soxhlet extraction using petroleum ether (40-60°C; boiling point) on a Soxtec System HT6 (Tecator application note 67/83). The ash content was determined by drying the samples in porcelain crucibles in a muffle furnace at 600°C overnight. Extraction of crude fiber was conducted using a Tecator 1020 extraction apparatus at 550°C for 2 h according to the method described in the Tecator application note 01/78. All methods were based on those described in the AOAC. All analyses of chemical composition of fish feed were performed in the laboratories of the Institute of Veterinary Medicine of Serbia. The chemical composition of each type of feed is given in Table 2. It can be seen that there is a significant difference between the pelleted and extruded feed in moisture only ($p=0.001$), while both extruded and pelleted feeds have higher protein, lipid and ash contents and a lower number of fibers and accessible carbohydrate when compared to grains ($p<0.001$).

Total lipids for fatty acid determination were extracted from fish muscle tissues by accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA). A homogenate of the sample mixed with diatomaceous earth was extracted with a mixture of n-hexane and isopropanol (60:40 v/v) in a 33 ml extraction cell at 100°C and nitrogen pressure of 10.3 MPa. The extracts were collected, and the solvent was removed under a stream of nitrogen in a Dionex Solvent Evaporator 500 at 50°C until dryness. The fat extract was further used for fatty acid determination.

Fatty acid methyl esters (FAME) were prepared by transesterification, using trimethylsulfonium hydroxide according to EN ISO 5509:2000 procedure. The GC instrument Shimadzu 2010 (Kyoto, Japan)

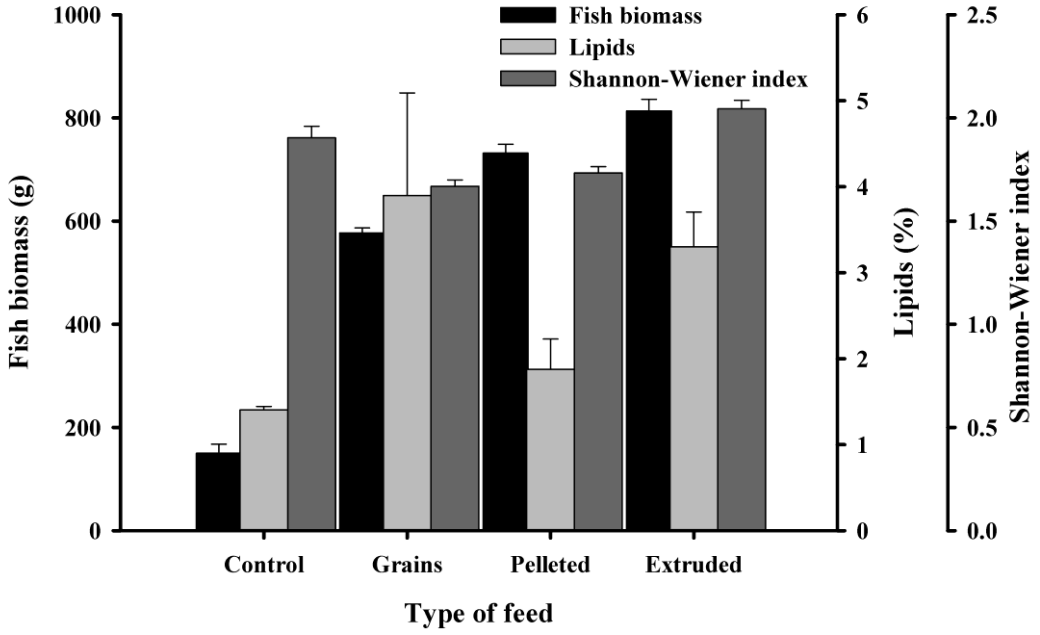

Fig. 1. Biomass values (black columns) of total lipids (light gray columns) and Shannon-Weaver diversity index of fatty acids (dark gray columns) in carp fillets at the beginning of the experiment (control) and after six months feeding with grains, pelleted and extruded feed.

used for FAME determination was equipped with a split/splitless injector, fused silica cyanopropyl HP-88 column (length 100 m, i.d. 0.25 mm, film thickness 0.20 μm, J&W Scientific, USA) and a flame ionization detector. The column temperature was programmed. Injector temperature was 250°C and detector temperature was 280°C. The carrier gas was nitrogen at a flow rate of 1.33 ml/min and injector split ratio 1:50. The injected volume was 1 μl and the total analysis time 50.5 min. The chromatographic peaks in the samples were identified by comparing the relative retention times of FAME peaks with the peaks obtained in a Supelco 37 Component FAME mix standard (Supelco, Bellefonte, USA).

Statistical methods

The Shannon-Weaver index was used (Shannon and Weaver, 1949) to determine the diversity of fatty acids in the samples, which was determined using BioDiversity Professional software McAleece (1997). The Student t test was used for comparison of samples and Pierson's product-moment correlation for determination of correlation between them, both with statistical importance of $P<0.05$. Both tests were realized by the Sigma Start ver. 2 program.

Principal component analysis (PCA) correlation matrix was used to describe the interactive relation between the four types of feed and certain fatty acids from the differently fed carps. The data were normalized using the following equation:

$$y_i = \frac{x_i - \overline{X}_i}{SD_i}$$

where y*i* represents the transformed value of variable *i*, xi the original value of variable *i,* X_i the mean value of variable *i* for all sampling dates and SD*i* the standard deviation of variable *i*. The PCA was determined by the ADE-4 program (Thioulouse et al., 1997).

RESULTS

Analysis of the influence of feed on carp mass at the end of a growth period of six months showed that fish

Table 3. Fatty acid composition of carp fillets fed with grains, extruded and pelleted feed and fatty acid composition of used food.

	Beginning of the experiment	Fatty acid – meat			Fatty acid – (feed)		
		Grains	Pelleted feed	Extruded feed	Grains	Pelleted feed	Extruded feed
PUFA	15.26±1.93	12.38±0.68	34.90±0.35	27.21±1.42	59.2±2.50	56.09±2.10	59.72±1.62
n-6	12.39±1.40	10.65±0.67	30.64±0.15	19.98±1.23	57.12±2.11	50.22±2.02	53.42±1.41
n-3	2.87±0.54	1.72±0.22	4.27±0.26	7.23±1.21	2.08±0.11	5.87±0.23	6.30±0.27
n-3/n-6	0.23±0.01	0.16±0.02	0.140±0.006	0.37±0.07	0.036±0.005	0.105±0.005	0.12±0.004
MUFA	53.16±1.79	60.37±0.80	43.24±0.94	46.31±1.42	21.83±1.02	25.75±1.15	23.75±1.34
SFA	31.57±1.35	26.99±1.29	21.85±0.71	26.47±0.45	18.97±0.80	17.51±0.92	15.93±0.83
C22:6 n-3	0.50±0.18	0.22±0.02	0.76±0.19	2.16±0.79	ND	0.46±0.04	0.47±0.03
C22:5 n-3	0.11±0.11	0.08±0.04	0.18±0.02	0.65±0.16	ND	ND	ND
C22:1+C20:4	1.25±0.26	0.97±0.41	0.67±0.11	0.98±0.21	ND	0.25±0.03	0.22±0.03
C20:5 n-3	0.24±0.06	0.20±0.04	0.230±0.006	0.76±0.15	ND	0.36±0.01	0.32±0.02
C20:3 n-6	0.92±0.08	0.74±0.10	0.99±0.07	1.09±0.29	ND	0.18±0.04	0.18±0.01
C20:3 n-3	0.71±0.06	0.51±0.27	0.34±0.10	1.49±0.13	ND	ND	ND
C20:2	0.66±0.07	0.29±0.02	0.55±0.07	0.68±0.08	ND	0.22±0.02	0.21±0.01
C18:3 n-6	0.04±0.04	0.23±0.05	0.39±0.12	0.16±0.02	ND	ND	ND
C18:3 n-3	1.32±0.25	0.72±0.17	2.76±0.08	2.17±0.08	2.08±0.11	5.05±0.21	5.51±0.26
C18:2 n-6	9.53±0.95	6.76±1.73	28.04±0.07	17.07±0.95	57.12±2.11	50.04±2.00	53.24±1.41
C20:1	2.49±0.06	2.07±0.06	1.46±0.11	2.97±0.07	ND	ND	ND
C18:1 cis-9	39.01±2.23	48.02±0.77	37.67±1.44	34.86±1.55	21.74±1.02	25.50±1.13	23.57±1.32
C18:1 cis-11	4.06±0.15	3.56±0.24	0.99±0.57	3.55±0.15	ND	ND	ND
C16:1	7.60±0.28	6.73±0.35	3.13±0.18	4.93±0.06	0.09±0.01	0.25±0.03	0.18±0.04
C20:0	ND	ND	ND	ND	0.37±0.07	0.32±0.02	0.30±0.02
C18:0	7.26±0.35	4.99±0.54	4.54±0.16	4.70±0.09	1.38±0.11	3.90±0.15	3.60±0.17
C17:0	0.55±0.06	0.23±0.03	0.167±0.009	0.413±0.007	ND	ND	ND
C16:0	22.21±0.93	20.65±1.08	16.36±0.70	19.36±0.40	17.05±0.6	13.26±0.62	12.00±0.71
C15:0	0.40±0.03	0.25±0.04	0.137±0.003	0.38±0.03	ND	ND	ND
C14:0	1.14±0.03	0.87±0.05	0.64±0.02	1.62±0.08	0.17±0.02	0.03±0.004	0.03±0.004

fed with extruded feed had an average mass of 813±22 g. This was significantly greater than that of fish that were fed with pelleted feed (732±17 g; p=0.045) and fish fed with grains (577±10 g, p<0.001). The significantly lower mass of fish fed with grains (p=0.001), even in comparison to fish fed with pelleted feed (Fig. 1) was expected, since grains have a lower protein content than either pelleted or extruded feeds. Compared to the control group (one-year-old carp spawn at the beginning of the experiment; 1.40±0.04%), the lipid content was significantly increased in fish fed with grains (3.90±1.19%; p=0.45) and in fish fed with extruded feed (3.30±0.40%; p=0.40). However, this was not the case in fish that were fed with pelleted feed (1.88±0.35%; p=0.252) in which the lipid content was significantly lower (p=0.045) than in fish fed with extruded feed, but not of those fed with grains (p=0.18) (Fig.1).

Table 3 shows the fatty acid content in the fish meat from the control group and from fish fed with the three types of feed. PCA analysis revealed the ef-

Table 4. Correlation matrix of fatty acid composition of fish meat at the beginning of the experiment and after the experiment

	Control	Grains	Pelleted feed	Extruded feed
Control	1.000			
Grains	0.983*	1.000		
Pelleted feed	0.886*	0.861*	1.000	
Extruded feed	0.967*	0.946*	0.966*	1.000

* Statistical importance of correlation coefficient (Pierson`s correlation; P<0.001)

fects of the different feeds on the total fatty acid composition of fish meat (Fig. 2, insert). Only two axes (95% (F1) and 4.3% (F2)), which carry the total inertia, remained in the PCA analysis. The PCA results clearly show that the fatty acid content in the meat of the fish fed with grains was the most similar to the content determined in control fish. The largest difference compared to the control was in fish fed with the pelleted feed. The fatty acid composition of fish fed with the extruded feed is similar to the control group and to the group fed with the pelleted feed.

To establish the statistical significance of these results, the fatty acid compositions of all samples were compared using Pierson's correlation. All tested cases were characterized by a high degree of statistical importance ($p<0.001$), as well as a high degree of correlation (correlation coefficient $r>0.8$). These results confirmed the conclusions from the PCA data (Table 4). Thus, the statistically highest degree of correlation was between the control group and the fish fed with grains. On the other hand, the lowest coefficient of correlation was between fish fed with the pelleted feed and the control group, and the fish fed with grains. The fatty acid composition of fish fed with the extruded feed had the lowest coefficient of correlation compared to fish fed with grains, while correlation with the two other groups was slightly higher.

The content of oleic acid was highest (C 18:1, $p<0.001$) in comparison to all detected fatty acids in all examined fish. Beside this shared characteristic, a number of specifities characterize the fatty acid composition of carp spawn fed with different types of feed. These are clearly shown in the combined PCA graph (Fig. 2) which illustrates the mutual relations of the detected fatty acids and their relationships to the types of feed control.

The PCA graph shows that the meat of fish fed with pelleted and extruded feed is outstanding for its PUFA content, of fish fed with grains for its oleic acid content, and the meat of control fish for its saturated fatty acid and MUFA contents. This is also supported by the results presented in Table 3 that shows that the meat of fish fed with grains has the highest oleic acid content ($p<0.05$). In addition, oleic acid predominates in the fatty acid profile (C 18:2; $p<0.001$). The fatty acid composition of fish meat from the beginning of the experiment differs in its high content of saturated fatty acid (SFA) compared to the other groups ($p<0.05$). Palmitic (C 16:0), stearic (C 18:0) and myristic (C 14:0) acids are the predominant saturated fatty acids. Palmitic acid predominates in all three groups of fish at the end of the experiment as well as in the control ($p<0.001$). Although its presence in the control sample was significantly greater than in fish fed with the pelleted ($p=0.007$) and extruded ($p=0.048$) feeds, this is not the case with fish that were fed with grains. The concentration of stearic acid was significantly higher in the control group compared to the other three samples. Unlike these two fatty acids, the content of myristic acid is significantly higher ($p<0.006$) in the meat of fish that were fed with the extruded feed.

Fish fed with pelleted and extruded feeds had significantly higher contents of PUFA than the fish that were fed with grains and the control sample ($p<0.05$). However, the presence of some PUFA in these two groups was very different. In fish that were fed with

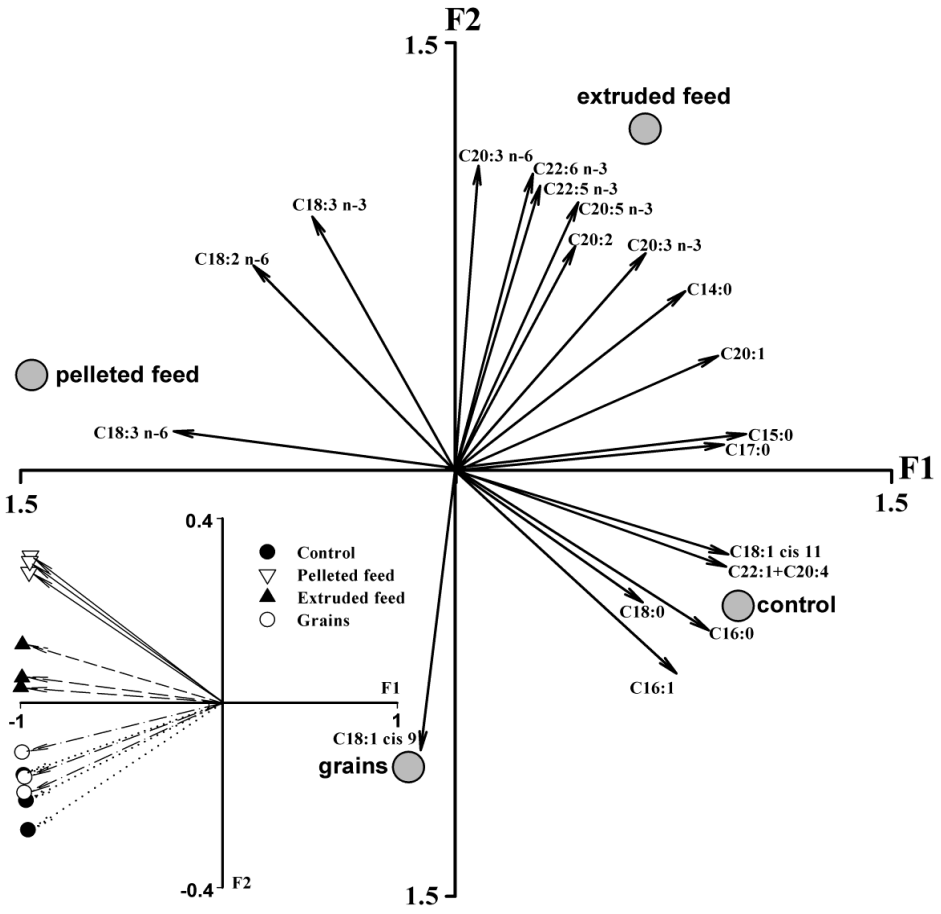

Fig. 2. Combined PCA graphic shows mutual relations of detected fatty acids (arrows), and their relation to examined types of feed and control sample (gray circles). Insert: the PCA analysis of feed types depending on fish meat fatty acid composition.

the pelleted feed there was a significantly higher content of linoleic acid compared to the other three experimental groups ($p<0.001$). The fatty acid content of the meat of fish fed with the extruded feed was characterized by a high proportion of n-3 fatty acids (7.23%), which is significantly higher than in the other three groups ($p<0.05$). This is the consequence of the much greater content of higher n-3 fatty acid. Thus, there was a greater content of docosahexaenoic acid (C 22:6; DHA 2.16%), eicosapentaenoic acid (C 20:5; EPA 0.76%) and eicosatrienoic acid (C 20:3; 1.49) in fish fed with the extruded feed compared to the other three groups. As it is characterized by the highest proportion of n-3 acids, carp meat also has the largest n-3/n-6 fatty acid ratio.

Besides the relationships between the fatty acid contents, PCA analysis shows that the fish that was fed with the extruded feed had the most balanced fatty acid composition, since the greatest number of fatty acids is also found in this type of feed.

Unlike the fatty acid profile of carp meat, in the feed that the fish were fed with linoleic acid predominates. This fatty acid is predominates in all three feeds as compared to carp meat, and makes up more than half of all of the fatty acids in the feed (50.04-57.12%). Apart from linoleic acid, there is a high percentage of oleic and palmitic acids in fish feed. These fatty acids accounted for 88% and 95.9% of all fatty acids detected in granulated and grain feeds,

respectively. There was a notably lower diversity of fatty acids in the feeds compared to the fish muscle at the end of the experiment (in all groups: SFA, MUFA and especially of PUFA fatty acids). The quantification of this observation is possible using the Shannon-Weaver diversity index. The value of this index is conditioned by two components: the number of detected fatty acids, which is identical for all four groups, and the comparableness of their numbers, which the PCA analysis shows as very different. The Shannon-Weaver index values (Fig. 1) decline as follows: extruded feed (2.04±0.04), control (1.90±0.06), pelleted feed (1.73±0.03) and grains (1.67±0.03). This confirms that the fatty acid composition of the meat obtained from fish that were fed with the extruded feed is the most balanced.

DISCUSSION

Investigation of the fatty acid composition in the muscle of cultivated carp revealed that the high content of grains in all three types of feed caused oleic acid, a monounsaturated fatty acid (MUFA), to predominate. This is a significant deviation from general opinion that the fatty acid composition of the feed determines the fatty acid composition of fish meat (Steffens, 1997). The linoleic acid content significantly surpassed the oleic acid content in grain and granulated feeds. The predominance of oleic acid in the meat of carp fed with the feed with a high content of grain was previously observed (Vacha et al., 2007; Kiminkova et al., 2001; Buchtova et al., 2007). Experiments that included radioactively labeled acetyl CoA have shown that this occurrence is not caused by a selective accumulation of oleic acid, but that it is the result its *de novo* synthesis caused by feeding with feed rich in carbohydrates and low in lipids, especially linoleic acid whose presence blocks the enzymes for *de novo* synthesis of oleic acid (Farkas et al., 1978; Csengeri, 1996; Henderson, 1996). Thus, it is possible to explain the predominance of oleic acid in carp meat in this experiment, even more so because it statistically prevails in the meat of fish fed with grains only, which has the highest carbohydrate content and the lowest linoleic acid content. Such an explanation confirms that the granulated feed has a higher level of oleic acid and a lower level of linoleic acid as compared to grains, while the content of these fatty acids in the meat of carp fed with pelleted and extruded feeds is reversed.

Although the fatty acid compositions of grains and granulated feed are very similar, at least with regards to the prevailing fatty acid content, there are still clear and significant differences, above all in the prevalence of n-3 fatty acids and the diversity of fatty acids as a whole; this is significantly higher in the granulated feed due to presence of soybean, fish flour and dry yeast. Therefore, some differences in fatty acid composition in carp meat can be expected. Thus, the meat of fish fed with pelleted feed has a significantly higher level of n-6 and less n-3, while the oleic acid content does not differ from that of fish fed with the extruded feed (Table 3). An equal amount of oleic acid was expected because of the equal content of this fatty acid in the feed, but also of carbohydrates, which can stimulate the *de novo* synthesis of this acid. However, the reasons for the significantly higher differences in the n-3 and n-6 fatty acid contents are not so clear. Very little research dealing with the influence of feed preparation on the fatty acid content has been done, and none on carp meat. According to the literature, the only similar experiment examined sea-bass meat (*Dicentrarchus labrax*, Linnaeus, 1758) in Turkey (Aslan et al., 2009). Herein we observed that feeding fish with the extruded feed led to an increase in the n-3/n-6 fatty acid ratio by about 11% as compared to fish fed with pelleted feed. This was caused by the slight decrease in n-6 content and increase in n-3 fatty acid content. Other significant effects were not observed.

Our results reveal a greater difference in the n-3 and n-6 fatty acid content. The main difference is in the PUFA content. In carp fed with the extruded feed the n-3 content was 69% higher whereas the content of n-6 was 53% lower, so that their ratio was 2.64-fold higher compared to that observed in the meat of carp fed with the pelleted feed. The higher content of n-3 fatty acids in fish fed with the extruded feed was not caused by the higher content of ALA, as its presence is lower in the meat of fish fed with the

pelleted feed; it was caused by significantly higher DHA (1.6-fold) and EPA (3.3-fold) contents, whose presence was significantly higher than in the feed itself. This means that an accumulation of DHA and EPA in carp meat occurs, in contrast to ALA, which exhibits an opposite trend. A comparison of results obtained on sea bass is hard to achieve. On one hand there is a significant difference in feed composition and its fatty acid content, and on the other, the fatty acid synthesis pathways in these species are very different. In the case of sea bass, fish flour and fish oil predominate in both types of feed, so that the lipid and protein content is much higher than carbohydrates. In addition, the n-3/n-6 ratio is greater than 1, and ALA is not predominant in n-3, but DHA and EPA are (Aslan et al., 2009). In the carp feed, grains and soy predominate with ALA among the n-3 fatty acids. In addition, carp, being a freshwater fish, has the ability to synthesize long-chain n-3 and n-6 fatty acids from ALA, i.e. LA, while sea bass, as a typical sea fish, does not (Henderson, 1996).

Bearing in mind that the compositions of both types of concentrated feed are nearly identical and that the fish were genetically uniform and grown under the same conditions, the reasons for the observed differences should be sought in the specific feed treatments. Although the influence of the extruding processing of feed on the fatty acid content of fish has not been intensively researched, its effects in comparison to other breeding and quality parameters have been investigated (Hilton et al., 1981; Deguara, 1997; Vergara et al., 1999). These studies have shown that the gelatinization of starch due to the higher temperature applied during the process of feed extrusion makes it much more accessible to fish (Hilton et al., 1981; Hilton and Slinger, 1983). This is also the case with proteins and lipids, as the PER (Protein Efficiency Ratio), ANPU (Apparent Net Protein Utilization) and ANLU (Apparent Net Lipid Utilization) are higher in fish fed with extruded than with pelleted feed (Deguara, 1997; Vergara et al., 1999). We observed that in carp meat, as in gilthead bream meat (Deguara. 1997), the lipid content was higher in the fish that were fed with the extruded feed than with the pelleted feed despite the contents being practically identical. This could be the consequence of the greater efficiency in ANLU. As the EPA and DHA content in both types of feed is lower than 0.5%, it may be that the efficiency of lipid utilization from feed enabled these poorly represented types of fatty acids to be taken up more efficiently from the extruded than from the pelleted feed where the EPA content in the fish meat was 57% lower than in the feed. In contrast, in the meat of fish fed with the extruded feed, the presence of DHA and EPA is far higher than in the feed, which points either to its accumulation in the muscles or its *de novo* synthesis. Henderson (1996) revealed the ability of carp for *de novo* synthesis of EPA and DHA from ALA. This, together with the lower presence of ALA in fish fed with the extruded feed as compared to fish that were fed with the pelleted feed indicates possible *de novo* synthesis of EPA and DHA. This is a potential mechanism for increasing their amounts, especially if we take into account the difference in carbohydrate structure between these two types of feed and their significance to the regulation of fatty acid biosynthesis (Farkas et al., 1978; Csengeri, 1996; Henderson, 1996).

The values of the n-3/n-6 ratios are lower than was obtained in other experiments carried out on carp. Steffens and Wirth (2007) found n-3/n-6 values from 0.8 to 2.4, depending on whether the fish were provided with supplementary feed or not. In other experiments that were carried out in carp raised under a semi-intensive breeding system, these values were even higher (Mráz and Pickova, 2009). However, when making these comparisons one should be cautious considering that the levels of lipids and fatty acids vary depending on which part of the fish samples are taken (Katikou et al., 2001). In addition, different strains of carp have different levels and ratios of fatty acids in their meat (Mráz and Pickova, 2009). Similar results for the n-3/n-6 ratio were provided by Mráz et al. (2012), where this level was around 0.25; however, in the same paper a higher percentage of PUFA in comparison to all the groups examined in our experiment was also shown. Fatty acids can also originate from natural foods. Organisms such as zooplankton (Domaizon et al., 200), phytoplank-

ton (Sushchik et al. 2004) and zoobenthos (Arts et al., 2001) have high levels of PUFA, especially in the group of zoobenthos, *Chironomus plumosus*, which is predominant in carp fish-farms (Bogut et al., 2007; Živić et al., 2001, 2013). In the ponds where our experiment was carried out, the biomass of the plankton and benthos was relatively low and there were no differences between the ponds (Dulić et al., 2010). Therefore, we assume that natural food equally influenced the examined groups.

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