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THE INFLUENCE OF SUPPLEMENT FEED PREPARATION ON THE FATTY ACID COMPOSITION OF CARP AND CHIRONOMIDAE LARVAE IN A SEMI-INTENSIVE PRODUCTION SYSTEM

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Abstract - In order to examine how extruded and pelleted feed affects the fatty acid composition of carp meat and Chironomidae larvae, two-month-old carp specimens were set in two fishponds (L1 and L2). The fatty acid composition of extruded and pelleted feed is characterized by a significantly higher content of ω-3 fatty acids and higher ω-3 to ω-6 fatty acids ratio (ω-3/ω-6) in extruded (11.34±0.12% and 0.315±0.005, respectively) compared to pelleted feed (7.72±0.08%, 0.180±0.001, respectively). The fatty acid composition of carp meat is characterized by higher ω-3 fatty acid content and ω-3/ω-6 in carp fed with extruded feed (L1, 6.98±0.53% and 0.295±0.022, respectively) compared to carp fed with extruded feed (L2, 5.46±0.07% and 0.232±0.009, respectively). Chironomidae larvae from the fishpond L2 had significantly higher ω-3 fatty acid content (8.22±0.89%), and therefore higher ω-3/ω-6 (0.81±0.09) in comparison to Chironomidae from the L1 fishpond where these parameters were 4.48±0.06% and 0.21±0.01, respectively.

Key words: Fatty acids, pelleted feed, extruded feed, carp, Chironomidae larvae

Abbreviations: PUFA: polyunsaturated fatty acids; MUFA: monounsaturated fatty acid; SFA: saturated fatty acids; ALA: Alpha-linolenic acid; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid; DPA: Docosapentaenoic acid; FAME: fatty acid methyl esters; CA: Correspondence analysis CA

INTRODUCTION

Carp is one of the most cultivated fish species in the world and the most important species in Serbian aquaculture (Marković, 2010)). The most common carp farming system in Serbia is the semi-intensive production system, based on the combination of natural (bottom fauna and zooplankton) and supplement feed. The grains were the dominant type of carp feed until 2005. In order to increase production per fishpond unit area, but also to improve the quality of the carp meat, in recent years fish are provided with concentrated feed (pelleted and extruded feed). This way of carp feeding is spreading from Serbia to other neighboring countries: Bulgaria, Romania, Bosnia and Herzegovina (Vandeputte et al., 2008, Marković et al., 2009; Marković, 2010).

Despite containing biologically important proteins, fish meat is one of the main sources of essential

fatty acids in human diet. Many factors affecting the lipid and fatty acid composition in fish tissues, such as environmental (Farkas, 1984; Viola et al., 1988; Vacha and Tvrzicka, 1995; Alasalvar et al., 2002; Rasmussen et al., 2006; Kandemir et al., Polat, 2007; Guler et al., 2008) and genetic predisposition (Bieniarz et al., 2001; Fajmonová et al., 2003; Buchtove et al., 2007, 2010; Łuczyńska et al., 2012). Examination of the fatty acid composition in carp meat has shown that different methods of breeding and feeding has caused significant variations in the proportion of n-3 and n-6 polyunsaturated fatty acids.

There are studies indicating that the fatty acid composition of fish feed influences the composition of fatty acids in fish meat (Farkas 1978; Fauconneau et al., 1991, 1995; Steffens and Wirth, 2007). Feed rich in n-3 polyunsaturated fatty acids significantly increase the n-3/n-6 PUFA ratio under the same growing conditions (Grisdale-Helland et al., 2002; Person-Le Ruyet et al., 2004; Skalli et al., 2006).

Essential fatty acids are important for biocenosis stability in aquatic ecosystems because aquatic organisms are the primary source of available essential fatty acids (Arts et al., 2000) that are implemented through the food chains in multiple trophic levels. As regards carp, the main natural source of essential fatty acids is Chironomidae larvae, especially *Chironomus plumosus* as the dominant species in carp ponds (Bogut et al., 2007; Živić et al., 2011).

Very few studies have dealt with the effects of supplement feed preparation. These studies indicate that food technology influences different meat quality parameters in trout (Hilton et al., 1981; Cappell-Vens, 1984), sea bream (Deguara, 1997), *Nile tilapia* and striped mullet *Mugil cephalus* (Ammar et al., 2008). Even fewer studies have dealt with the influence of concentrated treatment methods on fatty acid composition, and there are none on carp. According to the literature, the only similar survey was carried out on sea bass meat (*Dicentrarchus labrax* Linnaeus, 1758) in Turkey (Aslan et al., 2009). In this survey, an extruded feed diet resulted in an increase of the n-3/n-6 fatty acid ratio in relation to fish fed with pelleted feed by about 11%, due to a slight decrease of n-6 and an increase in n-3 fatty acid content, while other significant effects have not been observed (Aslan et al., 2009).

Having in mind that carp is very common in human nutrition and different types of technology can be used for carp growing based on a variety of feed, as well as various methods for concentrated mixture processing being used for carp diet, it is important to perceive the influence of feed treatment on the fatty acid content in carp meat and its importance as a natural food for Chironomidae larvae. The main objective of this study was to determine the effect of pelleted and extruded feed on fatty acid content in carp meat and Chironomidae larvae.

MATERIALS AND METHODS

Sampling and design

In order to examine how supplement (extruded and pelleted) feed affects the fatty acid composition of carp meat and Chironomidae larvae (natural carp feed), an experiment was carried out in two fishponds of the same area (650 m^2 in bottom base). The ponds lie side-by-side within an experimental fish farm of the Center for Fishery and Applied Hydrobiology, Faculty of Agriculture, University of Belgrade. One thousand two hundred carp individuals were placed in both ponds (L1 and L2). Specimens are genetically identical, aged two months with an individual weight of 5.69±0.02g in pond L1 and 5.71±0.02g in pond L2. In addition to natural feed, the carp was fed with extruded (pond L1) and pelleted (pond L2) feed in the course of three months (July-October, 2010). Two categories of fish feed (extruded and pelleted) were produced by using the same components (28.0% soybean, 14.0% fish meal, 8.0% yeast, 10.0% wheat, 20.3% corn and 16% soybean meal), with two different feed production technologies (extrusion and pelleting). The mass of the daily fishmeal was 3% compared to ichthyomass in the fishpond. Chironomidae larvae were sampled with a modified Ekman-Birge grab, adapted for usage in carp farms, with a grasping area of 87.55 cm^2. In order to analyze the

chemical and fatty acid composition of *Chironomus plumosus* larvae, 374 individuals were collected from pond L1 and 262 individuals from pond L2 (fourth and fifth larval instar). Until the laboratory work began, the samples were kept at -18°C. For the analysis of the fatty acid composition of carp meat, six individual, randomly selected fish were taken from each fishpond, after three months of fish growing. Mean fish weight was 121.4±8.4 g for the fish in pond L1 and 79.8±5.5 g (P=0.002) for the fish in pond L2. Samples were preserved at -18˚C until the day they were examined in the laboratory.

Fatty acid analysis by capillary gas chromatography

Fish samples were thawed overnight at +4°C before analysis. Analysis was carried out on homogenized fish muscle after removing the skin, bones, head and viscera. Total lipids of fish muscle, feed and chironomids for fatty acid determination were extracted with a mixture of n-hexane and isopropanol (60:40, v/v) by accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA) (Spirić et al., 2010). The solvent was removed until dry under a stream of nitrogen at 50°C. The fat extract was further converted to fatty acid methyl esters (FAMEs) by trimethylsulfonium hydroxide (EN ISO 5509:2000). FAMEs were determined by capillary gas chromatography on GC instrument Shimadzu 2010 (Kyoto, Japan) equipped with split/splitless injector, flame ionization detector and cyanopropyl HP-88 column, length 100 m, i.d. 0.25 mm, film thickness 0.20 μm (J&W Scientific, CA). Injector and detector temperature were 250°C and 280°C, respectively. As carrier gas, nitrogen was used at a flow rate of 1.33 mL/min. The injected volume was 1 μL and injector split ratio was set at 1:50. The column temperature was programmed from the initial 125°C to 230°C. Total analysis time was 50.5 min. The chromatographic peaks in the samples were identified by comparing the relative retention times of FAME peaks with peaks in a Supelco 37 Component FAME mix standard (Supelco, Bellefonte, USA). Results were expressed as mass of fatty acid (g) in 100 g of total fatty acids. Fatty acid content and total lipid analysis of carp meat were made in the Institute of Meat Hygiene and Technology in Belgrade (Serbia).

Statistical methods

Correspondence analysis (CA) was used to analyze the relationship between fatty acids in extruded and pelleted feed, carp fed with one of the two types of feed and Chironomidae that inhabited the two types of ponds (with different treatment). Correspondence analysis was performed using the Brodgar program (Highland Statistics Ltd, UK). To obtain differences between mean values of the two samples, the t-test was used in case of normal distribution of data or the Mann Whitney Rank Sum Test if this wasn't the case, both with statistical importance of $P<0.05$. Both tests were realized by the Sigma Start ver. 2 program. In this study, Simpson's index was used (Simpson, 1949) to determine the degree of uniformity in the quantitative composition of the main groups of fatty acids. It was determined by BioDiversity Professional software McAleece (1997)).

RESULTS AND DISCUSSION

Correspondence analysis showed a clear distinction between fish, Chironomidae and supplement feed in relation to fatty acid composition. These differences are a consequence of saturated fatty acid (SFA) dominance in Chironomidae, monounsaturated fatty acid (MUFA) dominance in carp and polyunsaturated fatty acid (PUFA) dominance in feed (Fig. 1).

There were several significant differences from this general trend. Thus, 18:1 *cis* 11 was the most dominant in Chironomidae, regardless of diet type and alpha linolenic acid in Chironomidae from the L2 pond (Table 1 and Fig. 1). In addition, n-6 acids, C20:2 n-6 and C20:3 n-6 were the most common in fish meat, regardless of the diet type, while C22:6 n-3 (docosahexaenoic acid, DHA) was the most common in the carp fed with extruded feed. PUFA domination in supplement feed was strongly influenced by the higher content of linoleic acid, which constituted 98.5% of total n-6 acids in the pelleted, and 97.9% in the extruded feeds. Similary, in the case of MUFA, oleic acid was the most dominant in fish meat (70%, regardless diet type). The striking

Table 1. Fatty acid composition in % of the pelleted and extruded feed and Chironomidae and carp meat from ponds L1 and L2 where extruded and pelleted feed was used as supplemental feed respectively.

Fatty acids	Pelleted feed	Extruded feed	Chironomidae L1	Chironomidae L2	Fish L1	Fish L2
C14:0	1.225±0.005[a]	1.77±0	2.335±0.065	4.79±0.07	1.208±0.027[a,e]	1.138±0.021[e]
C15:0	0.125±0.005	0.145±0.005	1.665±0.055[c]	1.615±0.015[c]	0.242±0.009[e]	0.252±0.019[e]
C16:0	14.28±0.02	13.88±0.07	26.15±0.29	32.27±0.17	18.25±0.32[e]	18.70±0.35[e]
C16:1	1.525±0.035	2.04±0.02	6.86±0.27[c]	4.20±0.01	6.17±0.41[c]	7.33±0.07
C17:0	0.175±0.005	0.175±0.005	2.365±0.025	2.08±0.18	0.340±0.018[e]	0.338±0.015e
C18:0	4.44±0.02[a]	4.645±0.015[b]	10.75±0.23[c]	10.82±0.32[c]	4.59±0.20[a,b,e]	4.315±0.077[a,e]
C18:1cis-9	23.05±0.03	23.285±0.045	12.63±0.06	15.955±0.205	30.640±0.876[e]	32.12±0.58[e]
C18:1 cis-11	2.5±0.02	2.765±0.045	7.015±0.165[c]	6.995±0.185[c]	4.142±0.093[e]	4.05±0.11[e]
C18:2n-6	41.80±0.21	35.715±0.085	21.37±0.66c	9.775±0.075	21.788±0.270[c,e]	21.88±0.69[c,e]
C20:0	0.285±0.005[a]	0.28±0.02[a]	2.09±0.02	1.905±0.095	0.168±0.012	0.125±0.003
C18:3n-6	n.d.	0.035±0.035[a,b]	n.d.	n.d.	0.155±0.00957	0.275±0.029
C18:3n-3	3.555±0.025	3.70±0.01	3.205±0.085	7.78±0.93	2.408±0.077[e]	2.55±0.061[e]
C20:1	1.45±0.03	2.14±0.02	1.6±0.04	0.88±0.17	2.745±0.031	2.530±0.065
C20:2n-6	0.205±0.005	0.295±0.025	n.d.	0.27±0.04	0.708±0.0309	0.518±0.032
C20:3n-6	0.435±0.025[a]	0.435±0.035[a]	n.d.	0.22±0,01	1.010±0.057	0.785±0.031
C20:3n-3	1.325±0.015	2.56±0.24	n.d.	n.d.	0.795±0.028	0.570±0.017
C22:1+C20:4	0.34±0.06[a]	0.52±0.10[a,b]	0.695±0.055[b,c]	0.24±0[a]	0.885±0.1282[b,c,e]	0.590±0.014[b,e]
C20:5n-3	1.135±0.045	1.775±0.015	1.27±0.02	0.445±0.045	0.805±0.083	0.55±0.02
C22:5n-3	0.54±0.22[a]	1.18±0.05	n.d.	n.d	0.458±0.039[a]	0.342±0.0063[a]
C22:6n-3	1.175±0.055	2.13±0.05[b]	n.d.	n.d.	2.510±0.460[b,e]	1.448±0.076[e]
C24:0	0.125±0.015[a]	0.12±0.01[a]	n.d.	n.d.	n.d.	n.d.
C24:1	0.11±0	0.18±0.01	n.d.	n.d.	n.d.	n.d.
C22:0	0.225±0.005[a]	0.215±0.005[a]	n.d.	n.d.	n.d.	n.d.
SFA	20.88±0.06	21.24±0.11	45.36±0.14	53.47±0.45	24.79±0.53[e]	24.87±0.41[e]
MUFA	28.64±0.02[a]	30.41±0.01	28.11±0.42[a,c]	28.04±0.56[a,c]	43.692±1.25[e]	45.78±0.48[e]
PUFA	50.16±0.11	47.83±0.01	25.85±0.60	18.38±0.89	30.64±0.63[e]	28.91±0.66[e]
N-6	42.44±0.19	36.48±0.11	21.37±0.66	10.16±0.01	23.66±0.27[e]	23.46±0.66[e]
N-3	7.725±0.075[a]	11.345±0.115	4.475±0.065	8.22±0.89[a,d]	6.975±0.534[a,d]	5.455±0.068
N-3/N-6	0.18±0	0.315±0.005[b]	0.21±0.01	0.81±0.09	0.295±0.022[b]	0.2325±0.00854

*Data in the same row sharing the same letter show no statistically significant difference (P>0.05).

dominance of oleic acid could be explained by a significant proportion of grains in the supplement feed (30%), and thus higher carbohydrate content in the pelleted and extruded feeds. A similar effect was observed previously (Vacha et al., 2007, Kiminkova et al., 2001; Buchtova et al., 2007). Experiments that included Acetyl CoA, with radioactive mark, have shown that this occurrence is not caused by selective accumulation of oleic acid, but by activating of its *de novo* synthesis caused by a diet abundant in carbohydrates and low in lipids, especially in linoleic acid whose presence blocks enzymes for *de novo* synthesis of oleic acid (Farkas et al., 1978; Csengeri, 1996; Henderson, 1996).

Palmitic acid was predominant among the saturated fatty acids in *Chironomus plumosus* larvae, but to a much lesser extent (57.7% in larvae from L1 and 60% in larvae from L2). In fact, if Simpson's diversity index is taken as a measure of fatty acid uniformity, it is obvious that the fatty acid composition of SFA and MUFA was the most uniform in Chironomidae larvae (Fig. 2). According to the results of the Simpson's index, n-6 acids are characterized by the least uniform composition in all groups (Fig. 2) due to a pronounced dominance of linoleic acid (Table 1). This dominance is most apparent in Chironomidae from the L1 pond where linoleic acid was the only n-6 fatty acid. Additionally,

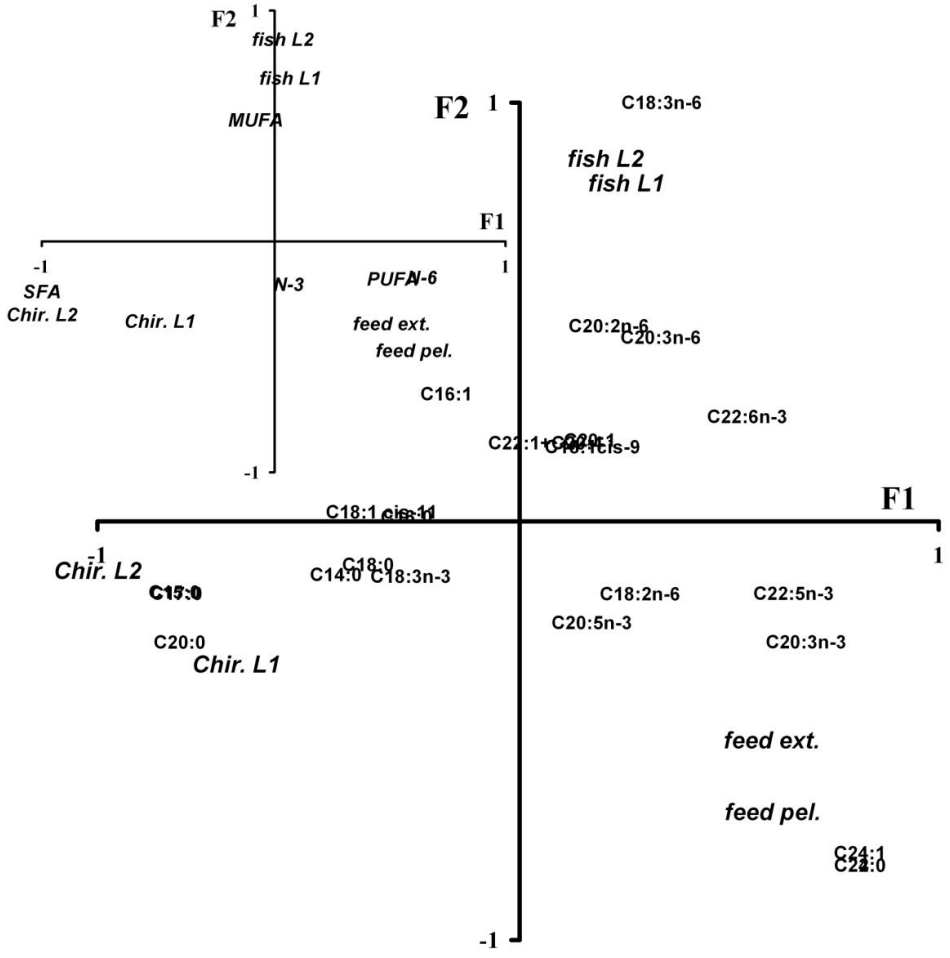

Fig. 1. Correspondence analysis biplot showing interrelationship between detected fatty acids and samples they were determined from. Insert: Correspondence analysis biplot showing interrelationship between main groups of fatty acids and samples they were determined from.

n-3 acids were characterized by the most balanced composition, with the exception of Chironomidae, particularly in the L2 pond (Fig. 2) due to distinct dominance of ALA and total absence of DHA (Table 1). The absence of DHA or its very low proportion (<0.2%) seems to be typical for Chironomidae larvae, as has been observed in a number of studies (Sushchik et al., 2003; Makhutova et al., 2011; Ghioni et al., 1996; Bell et al., 1994). However, there are some exceptions (Bogut et al., 2007) where eicosapentaenoic acid (EPA) is the primary (dominant) polyunsaturated fatty acid in Chironomidae, which is confirmed by our results.

According to the results shown in Table 1, differences among the groups were most pronounced at the n-3 fatty acid level. The content of monounsaturated and unsaturated fatty acids was similar in both feed types (P>0.05), and differences in PUFA, in addition to the linoleic acid content, was primarily reflected in the content of omega-3 fatty acids. Thus, it is statistically significantly higher (P<0.001) for extruded (11.34±0.12%) than for pelleted (7.72±0.08%) feed. This gave rise to significant differences in the ratio of omega-3/omega-6 fatty acids, which was significantly higher (P<0.001) for extruded (0.315±0.005%) than for pelleted (0.180±0.001%) feed. These results

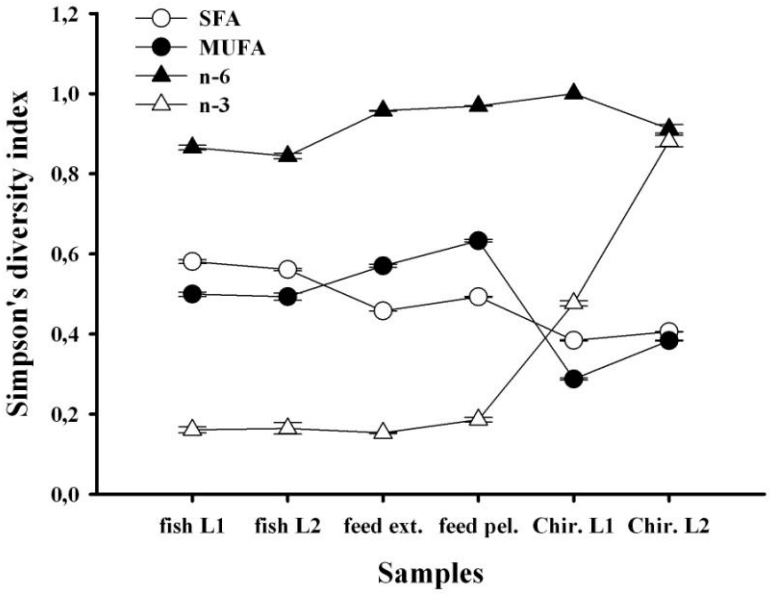

Fig. 2. Values of Simpson's index for main groups of fatty acids from examined samples.

are in correlation with published data (Aslan et al., 2009). Although different, both of these relationships are relatively low as a consequence of soybean over fishmeal domination in both types of feed. Besides, the differences in the content of omega-3 fatty acids were due to the increased content of higher omega 3 fatty acids (20:5 n-3 EPA, 22:5 n-3 DPA, 22:6 n-3 DHA) in extruded feed, while the content of linoleic acid is approximately equal.

These differences in n-3 PUFA content in fish feed were fully reflected in the profile of n-3 PUFA content in the carp meat. Omega 3 fatty acids were more prevalent (P=0.030) in carp fed with extruded feed (6.98±0.53%) compared to those fed with pelleted feed (5.46±0.07%). This is a consequence (as in the case of supplement feed) of the increased content of higher omega 3 acids (EPA, DPA, DHA) in the carp meat fed with extruded feed, while the ALA content was the same, regardless of feed type. In accordance with the higher content of omega-3 fatty acids, their relationship with the omega-6 acids was significantly higher (P=0.039) in the carp meat fed with extruded (0.295±0.022) than in those fed with pelleted feed (0.232±0.009). The composition of both feed types was identical, but observed differences are due to the different methods of feed production technologies (extrusion and pelleting).

In the case of *Chironomus plumosus* larvae, the situation was the opposite. Chironomidae from L2, with the pelleted feed treatment, had a significantly higher content of omega 3 fatty acids (8.22±0.89%, P=0.002) and higher ratio of omega 3 to omega 6 (0.81±0.09, P<0.001, Table 1) compared to Chironomidae from L1 with the extruded feed treatment (4.48±0.06% and 0.21±0.01, Table 1). In addition, differences in the content of omega 3 fatty acids were the result of the far higher ALA content in Chironomidae from the pond with pelleted feed treatment. CA analysis could identify such large differences in n-3 and n-6 fatty acids content (Table 1) in Chironomidae larvae, depending on the feed treatment in the ponds. These differences are unobservable in the fatty acid composition of carp even in the supplement feed, especially along the F1 axis, which explains most of the variation (71.3%). Unambiguous evidence for this claim is provided by correspondence analysis when PUFA is only taken into account (Fig. 3). In this case, both types of fish feed are relatively

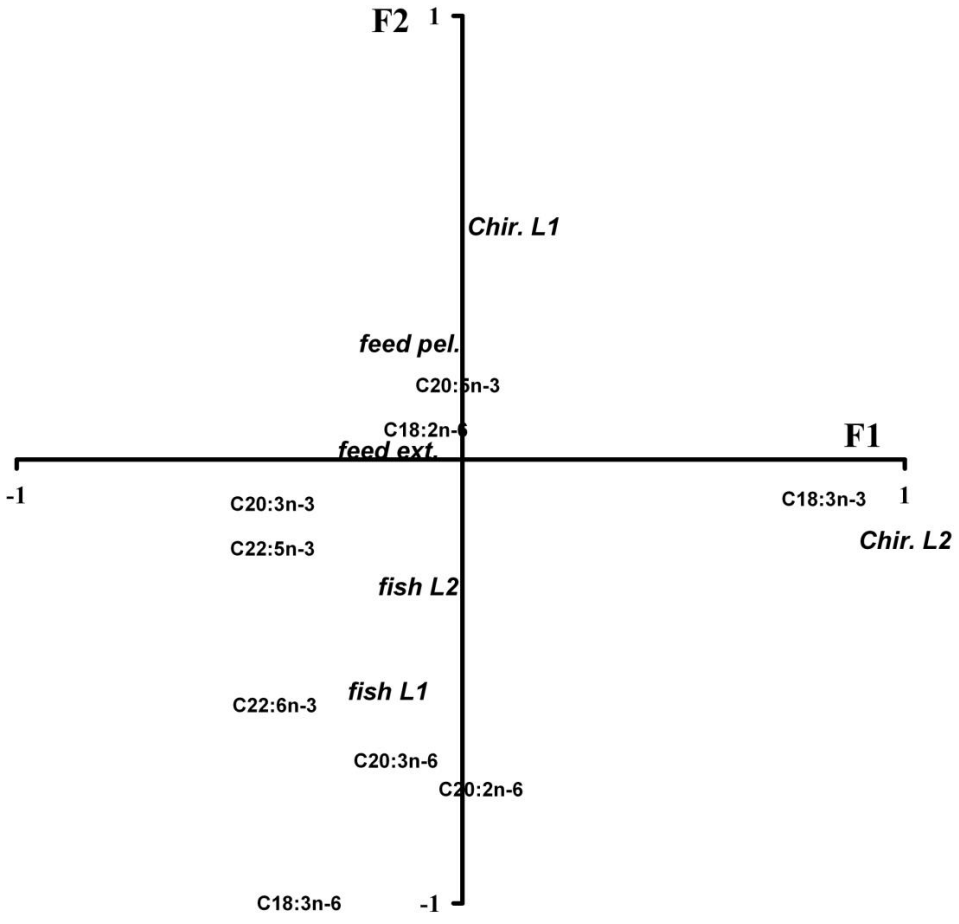

Fig. 3. Correspondence analysis biplot showing interrelationship between detected polyunsaturated fatty acids (PUFA) and samples they were determined from.

closely grouped, especially along the F1 axis, while Chironomidae from the ponds with pelleted and extruded feed treatment are drastically more separated along both axes in relation to PUFA content. The main cause of this separation is clearly ALA, showing the highest correlation with the F1 axis. In fact, due to the high ALA content, the PUFA content of Chironomidae from L2 is clearly separated not only in relation to Chironomidae from L1, but in relation to both feed types and fish with both feed treatments.

On the basis of our results, it can be concluded that supplement feed has a greater impact than natural feed when it comes to fatty acid composition. Having in mind the relatively small amount of Chironomidae in the studied ponds, as well as the fact that in the presence of supplement feed carp rather chooses extra, easily accessible food than organisms of the benthos (Marković, 2010), this result was to be expected. However, it was expected far less that the type of supplement feed would have such a significant impact on the fatty acid composition in Chironomidae larvae. Since Chironomidae are represented only by predominantly detritivorous genus *Chironomus*, the cause of different feed type effect on Chironomidae in relation to fish, can be found in the fact that when carp are fed with pelleted feed, wastage is considerably higher compared to when its fed with extruded feed. Specifically, pelleted feed decomposes into small particles in contact with water and thus

becomes available to detritivores like Chironomidae larvae, while extruded feed stays compact in the water much longer until it is eaten by carp, thereby not being available to Chironomidae larvae (Marković, 2010).

CONCLUSIONS

In order to examine how supplement (extruded and pelleted) feed affects the fatty acid composition of carp meat and Chironomidae larvae (natural carp feed), an experiment was carried out (from July to October, 2010) in two fishponds (L1 and L2) of the Centre for Fishery and Applied Hydrobiology, Faculty of Agriculture, University of Belgrade. As the composition of both feed types was identical, differences in omega 3 PUFA (11.34±0.12% for extruded and 7.72±0.08% for pelleted feed) and the omega 3/ omega 6 ratio (0.315±0.005 for extruded feed and 0.180±0.001 for pelleted feed) were probably due to the technology of food processing. The specific heat treatment used for extruded feed makes its lipids more accessible to fish than those used for pelleted feed.

The experiment showed that supplement feed had a great impact on fatty acid composition in carp meat, even though its natural food (mostly Chironomidae larvae) was available to the carp.

Fatty acid composition of carp fed with extruded and pelleted feed was nearly identical, with the exception of the higher content of omega 3 fatty acids (p=0.03) in carp fed with extruded feed (6.98±0.53%) compared to the carp fed with pelleted feed (5.46±0.07%) and thereby the higher ratio of omega 3 and omega 6 fatty acids in carp fed with extruded feed (p=0.039).

Chironomus larvae from the L2 pond had a significantly higher content of omega 3 fatty acids (8.22±0.89%, p=0.002) and thus a higher ratio of omega 3 to omega 6 fatty acids (0.81±0.09, p<0.001) compared to Chironomidae from L1 (4.48±0.06% and 0.21±0.01).

The difference in the prevalence of omega 3 fatty could be due to the greater availability of pelleted feed compared to extruded feed in Chironomidae diet.

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REFERENCES

Alasalvar, C., Taylor, K.D.A., Zubcov, E., Shahidi, F. and *M. Alexis* (2002). Differentiation of cultured and wild sea bass (*Dicentrarchus labrax*): total lipid content, fatty acid and trace mineral composition. *Food Chem.* **79**, 145-150.

Ammar, A.A., Abd-Elgawad, A.S. and *A.A. Salam* (2008). Effect of extruded and non-extruded fish pellet on growth performance and total production of Nile tilapia and grey mullet fingerlings reared in a polyculture system in earthen ponds. 8th International Symposium on Tilapia in Aquaculture, Cairo, Egypt, pp. 1199-209.

Arts, M. T., Ackman, R. G. and *B. J. Holub* (2001). Essential fatty acids in aquatic ecosystems: a crucial link between diet and human health and evolution. *Can. J. Fish. Aquat. Sci.* 58, 122-137.

Aslan, S.S., Çoban, B., Tekinay, A., Gezgin, T. and *K.C. Guven* (2009). Effects of pellet and extruded feed on fatty acid composition of European sea bass (*Dicentrarchus labrax*) using HRGC-MS. *Fresen. Environ. Bull.* **18**, 112-116.

Bell, G., Ghioni, C. and *J. Sargent* (1994). Fatty acid compositions of 10 freshwater invertebrates which are natural food organisms of Atlantic salmon parr (*Salmo salar*): a comparison with commercial diets. *Aquaculture*. **128,** 301-313.

Bieniarz, K., Koldras, M., Kaminski, J. and *T. Mejza* (2001). Fatty acids, fat and cholesterol in some lines of carp (*Cyprinus carpio*) in Poland. *Arch. Pol. Fish.* **9**, 5-24.

Bogut, I., Has-Schön, E., Adámek, Z., Rajković, V. and *D.Galović (*2007). *Chironomus plumosus* larvae - a suitable nutrient for freshwater farmed fish. *Agriculture*. 13, 1-5.

Buchtova, H., Svobodova, Z., Križek, M., Vacha, F., Kocour, M. and *J.Velišek* (2007). Fatty acid composition in intramuscular lipids of experimental scaly crossbreds in 3-year-old common carp *(Cyprinus carpio L.). Acta Vet Brno*. **76**, S73-S81.

Buchtová, H., Svobodová, Z., Kocour, M. and *J. Velíšek* (2010). Chemical composition of fillets of mirror crossbreds common carp (*Cyprinus carpio* L.). *Acta Vet Brno.* **79**, 551-557.

Cappell-Vens, B. (1984). The effects of extrusion and pelleting of feed for trout on the digestibility of protein, amino acids and energy and on feed conversion. *Aquacult. Eng.* **3**, 71-89.

Csengeri, I. (1996) Dietary effects on fatty acid metabolism of common carp. *Arch. of Anim. Nut.* **49**, 73-92.

Deguara, S. (1997). Evaluation of different pressed and extruded fish meal based diets on the growth of gilthead sea bream, *Sparus aurata* L. *Cah. Opt. Mediterr.* **22**, 123-139.

Fajmonová, E., Zelenka, J., Komprda, T., Kladroba D. and *I. Šarmanová* (2003). Effect of sex, growth intensity and heat treatment on fatty acid composition of common carp (*Cyprinus carpio*) fillets. *Czech J. Anim. Sci.* **48**, 85-92.

Farkas, T. (1984). Adaption of fatty acids composition to temperature-a study on carp (*Cyprinus carpio L.*) liver slices. *Comp. Biochem. Physiol.* **79B,** 531-535.

Farkas, T., Csengeri, I., Majoros, F. and *J. Oláh* (1978). Metabolism of fatty acids in Fish. II. Biosynthesis of fatty acids in relation to diet in the carp, *Cyprinus carpio* Linnaeus 1758. *Aquaculture*. **14**, 57-65.

Fauconneau, B., Corraze, G., Lebail, P.Y. and *J.M. Vernier* (1991). Lipid storage in fish: cellular, metabolic and hormonal control. *Inra. Prod. Anim.* **3**, 369-381.

Fauconneau, B., Alami-Durante, H., Laroche, M., Marcel, J. and *D. Vallot* (1995). Growth and meat quality relations in carp. *Aquaculture.* **129**, 265–297.

Hilton, J.W., Cho, C.Y. and *S.J. Slinger* (1981). Effect of extrusion processing and steam pelleting diets on pellet durability, pellet water absorption and the physiological responses of rainbow trout (*Salmo gairdneri* R.). *Aquaculture.* **25,** 185-1 94.

Ghioni, C., Bell, J.G. and *J.R. Sargent* (1996). Polyunsaturated fatty acids in neutral lipids and phospholipids of some freshwater insects. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **114,** 161-170.

Grisdale-Helland, B., Ruyter, B., Rosenlund, G., Obach, A., Helland, S. J., Sandberg, M. G., Standal, H. and *C. Rosjo* (2002). Influence of high contents of dietary soybean oil on growth, feed utilization, tissue fatty acid composition, heart histology and standard oxygen consumption of Atlantic salmon (*Salmo salar*) raised at two temperatures. *Aquaculture*. **207**, 311-329.

Guler, G.O., Kiztanir, B., Aktumsek, A., Citil, O.B. and *H. Ozparlak* (2008). Determination of the seasonal changes on total fatty acid composition and ω3/ω6 ratios of carp (*Cyprinus carpio* L.) muscle lipids in Beysehir Lake (Turkey). *Food Chem.* **108**, 689-694.

Henderson, R.J. (1996). Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids. *Arch. of Anim. Nut.* **49**, 5-22.

Kandemir, S. and *N. Polat* (2007). Seasonal variation of total lipid and total fatty acid in muscle and liver of rainbow trout (*Oncorhynchus mykiss* W., 1792) reared in Derbent Dam Lake. *Turkish J. Fisheries Aquatic Sci.* **7**, 27-31.

Kiminkova, M., Winterova R. and *J. Kučera* (2001). Fatty acids in lipids of carp (*Cyprinus carpio*) tissues. *Czech J. Food Sci.* **19**, 177-181.

Łuczyńska, J., Beata Paszczyk, B., Borejszo, Z. and *Ł. Tarkowski* (2012). Fatty acid profi le of muscles of freshwater fish from Olsztyn Markets. *Pol. J. Food Nutr. Sci.* **62**, 51-55.

Makhutova, N.O., Sushchik, N.N., Gladyshev, I.M., Ageev, A.A., Pryanichnikova, G.E. and *S.G. Kalachova* (2011). Is the fatty acid composition of freshwater zoobenthic invertebrates controlled by phylogenetic or trophic factors? *Lipids*. **46**, 709-721.

Marković, Z. (2010). *Šaran, Gajenje u ribnjacima i kaveznim sistemima.* Prof. dr Zoran Markovic, Beograd, 1-152.

Marković, Z., Poleksić, V., Dulić, Z. and *M. Stanković* (2009). Carp production intensification in traditional semiintensive culture systems by application of extruded feed and selected fish fry. Aquaculture Europe 09, Trondheim, Norway, pp. 498-499.

McAleece, N. (1997). *BioDiversity. Professional Beta.* The Natural History Museum. London, 1-125.

Person-Le Ruyet, J., Skalli, A., Dulau, B., Le Bayon, N., Le Delliou, H. and *J.H. Robin* (2004). Does dietary n-3 highly unsaturated fatty acids level influence the European sea bass (*Dicentrarchus labrax*) capacity to adapt to a high temperature? *Aquaculture.* **242**, 571-588.

Rasmussen, R.S., Morrissey, M.T. and *S. Carroll* (2006). Effect of seasonality, location, size on lipid content in North Pacific Troll-Caught Albacore Tuna (*Thunnus alalunga*). *J. Aquat. Food Prod. Technol.* **15**, 73-86.

Runge, G., Steinhart, H., Schwarz, F.J. and *M. KirchgeBner* (1987). Influence of different fats with varying addition of ru-tocopheryl acetate on the fatty acid composition of carp *(Cyprinus carpio* L.). *Fat. Sci. Technol.* **89**, 389-393.

Simpson, E.H. (1949). Measurement of diversity. *Nature.* **16**, 1-688.

Skalli, A., Robin, J.H., Le Bayon, N., Le Delliou, H. and *J. Person-Le Ruyet* (2006). Impact of essential fatty acid deficiency and temperature on tissues' fatty acid composition of European sea bass (*Dicentrarchus labrax*). *Aquaculture*. **255**, 223-232.

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

Steffens, W. and *M. Wirth* (2007). Influence of nutrition on the lipid quality of pond fi sh: common carp (*Cyprinus carpio*) and tench (*Tinca tinca*). *Aquacult. Int***. 15**, 313-319.

Steffens, W. (1997). Effects of variation in essential fatty acids in fish feeds on nutritive value of freshwater fish for humans. *Aquaculture*. 151, 97-119.

Sushchik, N.N., Gladyshev, M.I., Moskvichova, A.V., Makhutova O.N. and *G.S.Kalachova* (2003). Comparison of fatty acid composition in major lipid classes of the dominant benthic invertebrates of the Yenisei River. *Comp. Biochem. Physiol. B. Biochem. Mol. Biol.* **134**, 111-122.

Sýkora, M. and M. Valenta (1978). Lipidy rybničních ryb čeledi Cyprinidae. *Živoč. Výr*. **23**, 811-824.

Vacha, F. and *E. Tvrzicka* (1995). Content of polyunsaturated fatty acids and cholesterol in muscle tissue of tench (*Tinca tinca*), common carp (*Cyprinus carpio*) and hybrid of bighead carp (*Aristichthys nobilis*) with silver carp (*Hypophthalmichthys molitrix*). *Pol. Archs Hydrobiol.* **42**, 151-157.

Vacha F., Vejsada P., Huda J. and *P. Hartvich* (2007). Influence of supplemental cereal feeding on the content and structure of fatty acids during long-lasting storage of common carp (*Cyprinus carpio* L.). *Aquacult. Int.* **15**, 321-329.

Vandeputte, M., Kocour, M., Mauger, S., Rodina, M., Launay, A., Gela, D., Dupont-Nivet, M., Hulak, M. and *O. Linhard* (2008). Genetic variation for growth at one and two summers of age in the common carp (*Cyprinus carpio* L.): Heritability estimates and response to selection. *Aquaculture*. **277**, 7-13.

Viola, S., Mokady, S., Behar, O. and *U. Cogan* (1988). Effects of polyunsaturated fatty acids in feeds of tilapia and carp. 1. Body composition and fatty acids profiles at different environmental temperatures. *Aquaculture*. **75**, 27-137.

Živić, I., Trbović, D., Živić, M., Bjelanović, K., Stanković, M., Vukojević, D. and *Z. Marković* (2011). *Chironomus plumosus* (Diptera, Insecta) larvae as a source of essential fatty acids in feed of carp fry. Conference proceedings 5th International conference Aquaculture and Fishery, Belgrade Serbia, pp. 497-503.