MULTIVARIATE ANALYSIS OF FATTY ACID PROFILES OF CARP MEAT DURING SEMI – INTENSIVE FARMING

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MULTIVARIJANTNA ANALIZA SASTAVA MASNIH KISELINA MESA ŠARANA U TOKU POLUINTENZIVNOG GAJENJA

Apstrakt

Poređenje masnokiselinskog sastava mesa šarana pomoću multivarijantnih metoda, kao što su analiza glavnih komponenti (PCA) i linearna diskriminaciona analiza (LDA), omogućava razdvajanje riba prema načinu ishrane i bolje razumevanje promena u sastavu masnih kiselina tokom uzgoja. U periodu od aprila do oktobra, sa šaranskog ribnjaka u kojem je riba bila prihranjivana ekstrudiranom hranom, ispitano je dvadeset osam šarana. Mase riba su se značajno povećale između juna i septembra (P < 0,001) i septembra i oktobra (P < 0,001), zbog intenzivnog prihranjivanja šarana tokom letnjeg perioda, a sadržaj ukupnih lipida se značajno povećao između septembra i oktobra (P < 0.001). PCA je pokazala da je postojala visoka pozitivna korelacija između mase šarana, sadržaja lipida i oleinske kiseline (r > 0,6; P < 0,0001). Zbog veće dostupnosti prirodne hrane, u aprilu i junu, došlo je do povećanja sadržaja n-3 polinezasićenih masnih kiselina u mesu šarana, što je doprinelo boljem kvalitetu ribe. Prihrana šarana sa ekstrudiranom hranom uticala je na povećanje sadržaja n-6 polinezasićenih masnih kiselina, koje su u septembru bile značajno veće u odnosu na juni (P < 0,01), ali i na smanjenje nutritivno važnih n-3 polinezasićenih masnih kiselina (P < 0,01). Odnos n-3/n-6 je bio najveći u junu (0,30), a najmanji u oktobru (0,16). LDA analizom postignuto je razdvajanje šarana prema periodu uzorkovanja, što je u korelaciji sa vrstom unete hrane u ovim periodima. Najveća sličnost u masnokiselinskom sastavu ustanovljena je između šarana u septembru i oktobru, kao posledica smanjenja količine dostupne prirodne hrane i većeg unosa ekstrudirane hrane. PCA i LDA su pokazale da je došlo do značajnih promena u sastavu masnih kiselina šarana tokom uzgoja ribe. Rezultati koji su dobijeni u ovom

radu o uticaju ishrane na sastav masnih kiselina i sadržaj lipida u mesu šarana doprineće poboljšanju načina ishrane i konsekventno kvalitetu mesa gajenog šarana.

Ključne reči: masne kiseline, šaran, analiza glavnih komponenti, linearna diskriminaciona analiza

Keywords: fatty acids, common carp, principal component analysis, linear discrimination analysis

INTRODUCTION

Fresh water fish belonging to the Cyprinid family is economically important to human nutrition in Serbia. The geo-hydrological conditions in the country are very convenient for carp (*Cyprinus carpio*) breeding which is one of the most cultivated fish species due to rapid growth and easy cultivation.

Fatty acid composition of farmed fish differs from the fatty acid composition of the fish from open waters because of the diets. Other factors, such as the size or age of the fish, reproductive status, geographic location, season, and temperature may influence the fatty acid composition of the fish muscle (Alasalvar et al., 2002, Rasoarahona et al., 2004, Jensen et al., 2007).

Comparison of the fatty acid composition by using multivariate data analysis indicates that the fatty acid composition of the muscle tissue of the fish fed different foods differs and that it is similar to the fatty acids of the food (Barrado et al., 2003). The use of multivariate methods, such as principal component analysis (PCA) and linear discrimination analysis (LDA) affords a better understanding of the fatty acid composition of the carp meat according to the fish diet and summarizes the statistical correlation among fatty acids.

The aim of this study was to determine and compare the fatty acid profiles of carp meat during growth, reared in semi-intensive farming conditions and fed extruded feed as supplementary diet.

MATERIALS AND METHODS

Fish samples

Twenty-eight fish were collected in April, June, September and October 2009 from the fish farm ''Ečka''AD, with a semi-intensive carp breeding system. Fish of one-year age was submitted to trial from spring to autumn. Except naturally occurring food, fish was additionally fed extruded feed consisting of maize, soybean meal and fish meal (23.81 % proteins, 6.97 % lipids), according to the breeding season and to the fish farm productivity. Feed provided to the fish was as follows: in April 0.1 % to 0.3 %, in May 0.3 % to 1 %, in June 1 % to 2 %, in July and August 3 %, in September 2 % to 3 %, with respect to fish biomass and depending on the water temperature, its saturation with oxygen and on the amount of accessible natural food. The weight of each fish was determined upon arrival to the laboratory. Before analysis, the skin, heads, tails, fins, and intestines were removed and fish was filleted. The obtained fillets were homogenized in a laboratory blender.

Fatty acid analysis by GC

Lipids for the fatty acid determination were extracted with hexane/isopropanol mixture by accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA), (Spirić et al., 2010). Total lipids were further converted to fatty acid methyl esters (FAMEs) by trimethylsulfonium hydroxide (EN ISO 5509:2000). FAMEs were determined by gas-liquid chromatography (GLC, Shimadzu 2010) equipped with flame ionization detector and capillary HP-88 column (Spirić et al., 2010). Results were expressed as the mass of the fatty acid (g) in 100 g of fatty acids.

Statistical analysis

Analysis of variance (ANOVA) with Tukey - Kramer test was used to analyze the data at P = 0.05 level. Principal component analysis (PCA) and linear discrimination analysis (LDA) was performed using JMP 8.0.1 software (SAS Institute Inc. NC, USA).

RESULTS AND DISCUSSION

The temperature of water in the farm, the average carp weight and lipid content during rearing are given in Table 1. A significant increase in weight of the fish between June and September ($P < 0.001$), and September and October ($P < 0.001$) was established due to the intensive feeding during summer when carp consumed large quantities of supplementary feed. The favourable environmental conditions in the aquatic environment contributed to the increase of fish biomass as well. The obtained data indicate that from April to September total lipids slightly increased with the size of fish, but a significant increase occurred between September and October ($P < 0.001$). The amount of feed and consequently the intake of energy influenced the increase in the content of lipids in fish muscle. However, the quality of feed is the most important (Haard, 1992; Rasmussen, 2001).

Table1. Water temperature, carp weight and total lipids during rearing

	April (n=6)	June (n=7)	September (n=7)	October (n=8)
Water temperature, °C	14	22	20	6
Fish weight, g	598±162^{C}	874±142^{C}	1439±173^{B}	1984±322^{A}
Total lipids, %	2.25±0.71^{B}	2.37±0.29^{B}	3.02±1.03^{B}	4.72±0.71^{A}

n - number of samples; $^{A, B, C}$ - Values in the same row followed by the same letters do not differ significantly (P>0.05)

Data for the content SFA, MUFA and PUFA, n-3 and n-6 PUFA and n-3/n-6 ratio in carp muscle are presented in Table 2.

Table 2. Fatty acid compositions of carp fed extruded feed (% of total fatty acids) during growth

Fatty acids	April (n=6)	June (n=7)	September (n=7)	October (n=8)
SFA	28.68±5.03^{A}	28.97±1.25^{A}	24.87±1.01^{B}	23.66±0.80^{B}
MUFA	38.77±2.57^{B}	40.52±2.48^{AB}	41.64±2.60^{AB}	42.43±2.93^{A}
PUFA	32.52±4.33^{A}	30.49±3.12^{A}	31.53±1.92^{A}	32.56±2.37^{A}
n-6	24.71±4.88^{AB}	22.86±3.24^{B}	26.96±1.75^{A}	27.99±1.91^{A}
n-3	5.37±1.06^{B}	6.59±0.89^{A}	4.57±0.59^{B}	4.57±0.66^{B}
n-3/n-6	0.23±0.07^{B}	0.30±0.07^{A}	0.17±0.02^{BC}	0.16±0.02^{C}

n - number of samples; $^{A, B, C}$ - Values in the same row followed by the same letters do not differ significantly (P>0.05)

From the presented data, it might be seen that the levels of MUFA significantly increased during fish grow while the levels of SFA decreased. The share of total PUFA did not change significantly ($P > 0.05$).

ANOVA test indicate that the content of n-6 PUFA increased significantly between June and September ($P < 0.01$), what is associated with increased feed intake during summer period, while the levels of n-3 PUFA decreased ($P < 0.01$). The increase in n-6 PUFA led to a reduction of the n-3/n-6 ratio, and, thus, in the reduction of the quality of fish. Henderson and Tocher (1987) reported n-3/n-6 values of 0.5 - 3.8 for freshwater fish. The n-3/n-6 ratio in this study was the highest in June (0.30) and the lowest in October (0.16), what indicates that carp feed was rich in n-6 and poor in n-3 PUFA.

Changes in the fatty acid profile in carp during rearing can be better visualized by PCA. Considering groups of FA and the most important FA, such as oleic, 18:1n-9; linoleic, 18:2n-6; linolenic acid, 18:3n-3; EPA, 20:5n-3; DPA, 22:5n-3 and DHA, 22:6n-3 (which are not presented in the Table 2), PCA clearly differentiate carp according to the investigated period of sampling (Figure 1 and 2). PCA of the fatty acid profiles, taking carp weight and lipid content as variables, resulted in two principal components model describing 74.6 % of the total data variability.

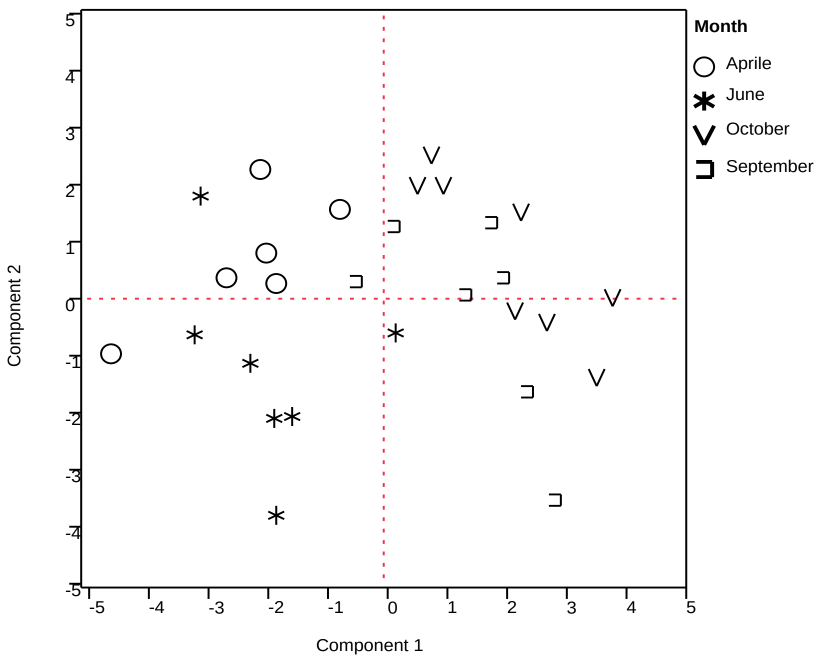

Figure 1. Principal component analysis score plot of the fatty acid profile of carp fed extruded feed

The score plot of the first two principal components (Figure 1) indicates grouping of carps during growth according to months of sampling.

Oleic acid mostly contributed to the variability on the positive part of the PC1. High positive correlation of oleic acid with carp weight and total lipids ($r > 0.6$; $P < 0.0001$) indicates that with the increase of carp weight the total lipids and the content of oleic acid increased as well. Linoleic acid that contributes to the positive part of the PC2 enables to distinguish carp in September and October with higher amounts of this FA.

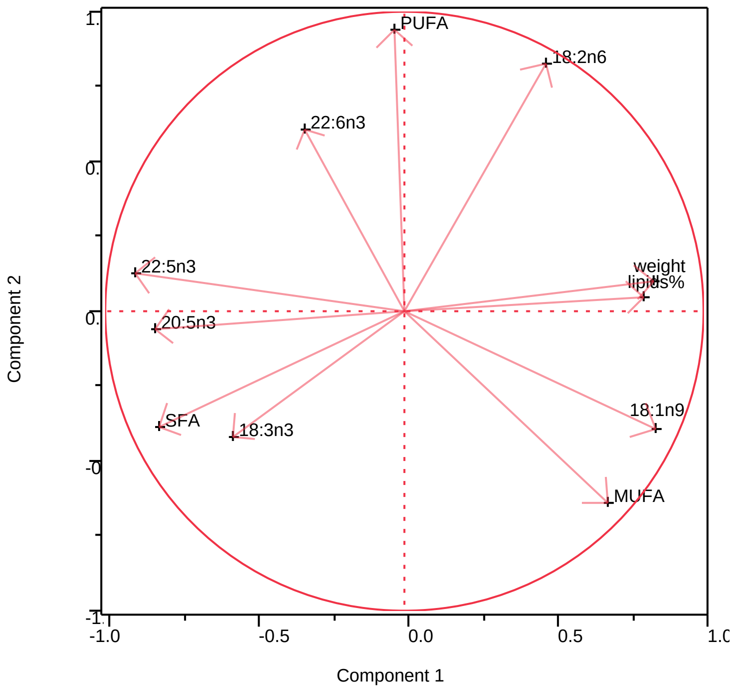

Figure 2. Principal component analysis loading plot of the fatty acid profile of carp fed extruded feed

On the negative part of the PC1, DPA, EPA and linolenic acid correspond to carp which contained higher quantities of these fatty acids in April and June. Carp can adjust the fatty acid metabolism to the prevailing temperature in such a way that increase in the temperature give rise to the creation of saturated fatty acids (Tocher, 2003), what probably is the reason why SFA were presented in higher quantities in June when temperature was the highest. The presence of linolenic acid and long-chain PUFA (EPA, DPA) in carps in April and June could be due to the higher intake of natural food. The natural food, represented by zooplankton and benthos, is a source rich in linolenic acid and EPA (Domaizon et al., 2000; Bogut et al., 2007; Živic et al., 2011), but poor in DHA. Bell et al. (1994) reported that DHA in freshwater invertebrates was present in small amounts. The availability of natural food in April and June probably caused the increase in the content of n-3 fatty acids in carp, what consequently resulted in a better fish quality (Table 2).

By the LDA, the separation between carp samples might be improved. LDA, as shown in Figure 3, clearly differentiates carps in four groups. The results of the classification are very satisfactory and allow 96 % of fish to be correctly grouped. Out of 28, 27 carps were classified according to the months of sampling.

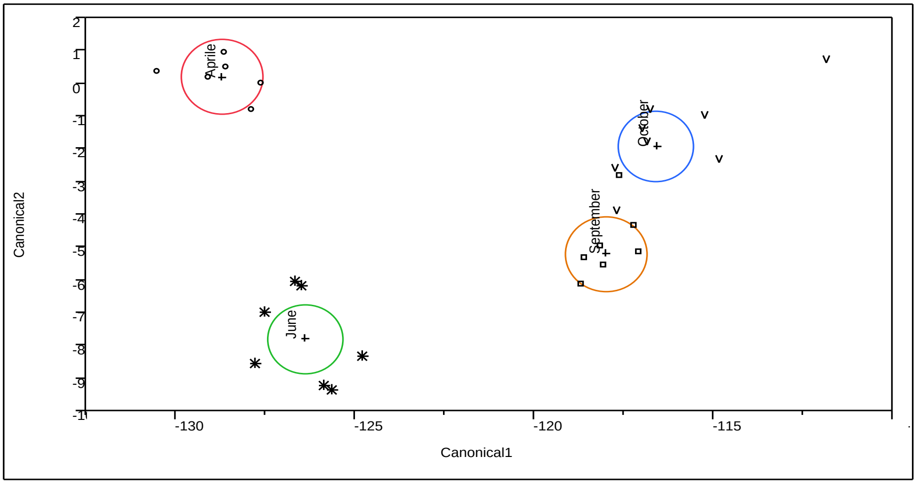

Figure 3. Canonical plot of the fatty acid profiles of carp fed extruded feed

The shortest distance between the points on the canonical plot in Figure 3 represents the smallest differences in the FA profiles of the samples. Fish in April and June were very distant one from the other, and far from September and October, what correlates to the type of ingested food in this period. The greatest similarity was observed between carps in September and October because of the high ingestion of supplementary feed.

CONCLUSION

Based on PCA and LDA it might be concluded that there were significant changes in the fatty acid composition of carp during growth. The presence of natural food in the carp farm influenced the fatty acids composition of carp in different months of sampling. However, the feeding of carp with extruded feed influenced the increase in quantities of MUFA and n-6 PUFA and the decrease in quantities of nutritionally important n-3 PUFA. The highest n-3/n-6 ratio was obtained in June (0.30) and the lowest in October (0.16), what indicates that the applied extruded feed was rich in n-6 and poor in n-3 PUFA. Analysis of the fatty acid composition in combination with multivariate analysis is a useful tool for differentiation of carp during rearing according to the available food in the farm and supplementary feed as well. Data on the effect of diet on the lipid content and fatty acid composition of carp would contribute to improving nutrition and consequently the quality of carp meat.

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