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FATTY ACID PROFILE IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AS INFLUENCED BY DIET

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Abstract: With the aim of reinforcement S&T capacities in aquaculture, studies on the influence of three commercial pelleted diets on fatty acid profile in rainbow trout production were undertaken. Commercial diets for rainbow trout contained significantly different quantities of saturated fatty acids (SFA), ($p<0.05$), ranging from 21.02 to 38.50%. Significant differences between diets ($p<0.05$) were established in the proportions of monounsaturated fatty acids (MUFA), (29.56-45.21%) and polyunsaturated fatty acids (PUFA), (31.95 to 36.43%). The established quantities of EPA (C20:5 n-3) and DHA (C22:6 n-3) in diets were 8.69, 9.11, 11.36% and 3.90, 5.30, 8.18%, respectively. The share of n-3 and n-6 fatty acids (FA) ranged from 17.97 to 26.33% and from 10.10 to 13.98%, respectively. The n-3/n-6 ratio was in the range 1.29-2.61. The major SFA (total SFA: 22.17-35.63%) in fish samples was palmitic acid (C16:0), (15.61-24.65%). Oleic acid (C18:1*cis*-9) was the most abundant from the MUFA family (21.90-31.89%), (total MUFA: 35.00-43.50%). From PUFA family (total PUFA: 29.38-34.33%), linoleic acid (C18:2 n-6), (10.09-10.97%), EPA (2.95-4.26%) and DHA (7.53-10.39%) were present in significant quantities in trout. Quantities of n-3 FA ranged from 17.08 to 21.12% and significant differences ($p<0.05$) were established between trout fed Diet I and Diet III. The proportion of n-6 FA ranged from 12.29 to 13.21% and no significant differences were established ($p>0.05$). The n-3/n-6 ratio ranged from 1.39 to 1.60 and differences were not statistically significant ($p>0.05$). The obtained results indicate that fatty acid profile of fish reflects the fatty acid composition of fish diets, with some variabilities which indicate that the incorporation of fatty acids in fish tissue is under some metabolic effects.

Key words: fatty acids, diet, rainbow trout

Introduction

Uncontrolled and long term exploitation of sea resources as well as knowledge on the favorable impact of n-3 polyunsaturated fatty acids (PUFA) on human health, contributed to considerable investments in aquaculture all over the

world (*Williams, 1998*). Nutritional and health benefits achieved by consumption of fish increased the demand of these products on the market (*Burger and Gochfeld, 2009)*. Proteins of high biological value, low fat content and relatively low cholesterol content, as well as valuable quantities of essential fatty acids makes fish one of the most appreciable food stuffs in human nutrition *(Conor, 2000; Sidhu, 2003)*.

Nutritional value of different fish species from aquaculture might be considered as valuable as nutritional value of fish species from open waters (*Weaver et al., 2008*). However, some fish species from inland aquaculture are more capable than marine fish to desaturate and elongate C_{18} polyunsaturated fatty acids to valuable highly unsaturated fatty acids, EPA (eicosapentaenoic acid) and DHA (docasahexaenoic acid), even when their feed is less rich in these fatty acids (*Tocher et al., 2004*).

It has been proved that nutritional value of fish can vary due to species, diet, location, environmental conditions, age, etc (*Petterson et al., 2009; Sicuro et al., 2010*). In farmed trout, where environmental conditions are more constant throughout the year, development and fish growth as well as fatty acid profile is more affected by food supply (*Almeida et al., 2011*). Many literature data indicate to a strong connection between fish diet and fatty acid profile of fish. (*Caballero et al., 2002; Valente et al., 2007)* Under the same rearing conditions, feed reach in n-3 PUFAs significantly increase the n-3/n-6 ratio in fish (*Skalli and Robin, 2004*).

Rainbow trout is a salmonid fish species originating from the tributaries of the Pacific Ocean in Asia and North America. This fish species has been introduced for food in many countries from almost all continents. Aquaculture of salmonid fish species started to grow exponentially in 1950s, particularly in Europe. In Norway, the ocean cage production of salmon trout to supply export markets started to expand, while inland production of rainbow trout has increased in many European countries to support domestic markets.

Aquaculture as a country economy in Serbia is mainly related to carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) production (*Trbović et al., 2009; Vranić et al., 2011*). Rainbow trout production presents close to 15% of the national aquaculture and has been developed on mountain areas, abundant in cold spring waters. However, rainbow trout production still remains on low technical and technological level due to lack of investment in aquaculture. With the aim of reinforcement S&T capacities in aquaculture, studies on the influence of commercial pelleted diets on fatty acid profile in rainbow trout production were undertaken.

Materials and Methods

The intensive fish farm

Samples of rainbow trout (*Oncorhynchus mykiss*) were collected from an intensive fish farm located 931 m above sea level, on the mountains area of

Zlatibor, Serbia. Water of potable quality is captured directly from the spring and, by gravity force, through channel system, is directed to the farm, with a capacity of 500 l/s. The annual level of production is about 450 t of fish.

Animals and samplings

Rainbow trout was manually fed commercial extruded sinking pelleted diets, according to good aquaculture practice. Based on specifications, Diet I consisted of fish meal (17%), blood meal (13%), fish oil (10%) and other ingredients, like oil seed cakes and soybean, rapeseed, and sunflower meals. Diet II consisted of fish meal (27%), soybean products and other ingredients like chops from soybean extraction, extruded soybean seed, soybean protein concentrate, wheat, rapeseed chops, and oils (rapeseed, fish and palm oil). Diet III consisted of fish meal, soybean protein products, corn, wheat, minerals and vitamins.

Marketable size fish, 30-35 cm length and 250- 300 g weight, were sampled. During each sampling, six fishes were collected along with the appropriate feed (n=6). Fish fillets, obtained after evisceration and previous deprivation of skin, tail, head, fins and bones were homogenized in a laboratory blender (Braun CombiMax 600), separately placed in plastic bags and stored at -25°C until analyzed. A day before analysis samples were defrosted overnight, at +4°C.

Chemicals and standards

Analytical-grade solvents were obtained from Merck (Darmstadt, Germany) and Sigma Aldrich (Germany). Reagent for derivatization of fatty acids (TMSH 0.25 M in methanol) was purchased from Fluka. Fatty acid standards were supplied by Supelco (Bellefonte, USA), (Supelco 37 comp. FAME mix 10 mg mL^{-1} in CH_2Cl_2).

More detailed data on the extraction of lipids by accelerated solvent extraction (ASE) and on fatty acids capillary gas chromatography determination are presented in our previous publications (*Spirić et al., 2009; Spirić et al., 2010*). A brief review of accelerated solvent extraction of lipids and fatty acids determination is presented below.

Accelerated solvent extraction (ASE) of lipids

Total lipids for FA determination were extracted from fish muscle by ASE (ASE 200, Dionex, Sunnyvale, CA). Extraction was performed in 33 mL stainless steel extraction cells filled with diatomaceous earth, at 100ºC, under 10.3 MPa nitrogen pressure, with a mixture of n-hexane and iso-propanol (60:40 v/v), in two static cycles. Solvent was removed under stream of nitrogen (Dionex Solvent evaporator 500), at 50°C.

Determination of fatty acids by GC/FID

After ASE extraction of total lipids and their transesterification by trimethylsulfonium hydroxide (EN ISO 5509:2000), fatty acids have been determined, as methyl esters, by Shimadzu 2010 capillary gas chromatograph equipped with flame ionization detector (GC/FID) and cianopropyl-aryl HP-88 capillary column. Chromatographic peaks in the extracts were identified by comparing relative retention times of FAME peaks with peaks in the Supelco 37 Component FAMEs mix standard. Quantification was performed by using heneicosanoic acid methyl ester as the internal standard. The total share of SFA, MUFA and PUFA consisted of weight percentages of the appropriate individual fatty acids (Pavlovski et al., 2011).

Statistical analysis

For data analysis UnscramblerX statistical software (CamoSoft, Norway) was used. Means within each group were compared with ANOVA and Tukey - Kramer's multiple range tests.

Results and Discussion

Data obtained for fatty acid composition (% of total fatty acids) of rainbow trout diets are presented in Table 1.

The obtained data indicate that the predominant fatty acids in all fish diets were palmitic (C16:0), palmitoleic (C16:1), oleic (C18:1*cis*-9), linoleic (C18:2 n-6), EPA and DHA fatty acid. Commercial diets for trout contained significantly different quantities of SFAs, $p<0.05$, (21.02%, Diet I; 38.50%, Diet II and 30.35%, Diet III). Significant differences between diets ($p<0.05$) were established in the proportions of MUFAs, (45.21 %, Diet I; 29.56%, Diet II and 33.21%, Diet III). PUFAs were present in quantities of 33.73% (Diet I), 31.95% (Diet II) and 36.43% (Diet III). The established quantities of EPA and DHA in the diets were 8.69% (Diet I), 9.11% (Diet II), 11.36% (Diet III) and 3.90% (Diet I), 5.30% (Diet II), 8.18% (Diet III), respectively. Significant differences between all three diets ($p<0.05$) were established in the content of n-3 fatty acids. The highest share of n-3 fatty acids was determined in Diet III, (26.33%), followed by 20.16%, in Diet I and 17.97%, in Diet II. The proportion of n-6 fatty acids in Diet III was 10.10% and this value was significantly lower ($p<0.05$) than in Diet II (13.98%) and Diet I (13.58%). The n-3/n-6 ratios in fish feed were 1.29 (Diet II), 1.49 (Diet I) and 2.61 (Diet III). Fatty acids of n-3 and n-6 PUFA family are considered to be of high nutritional value for fish and their increased levels in diets are expected to be reflected on fatty acid profile of fish, as reported by *Caballero et al. (2002) and Fonseca-Madrigal et al. (2005).*

Table 1. Fatty acid composition (% of total fatty acids) of marketable size trout diets (mean ± standard deviation)

Fatty acids	Diet I	Diet II	Diet III
14:0	4.29 ± 0.01^{B}	4.35 ± 0.05^{B}	6.45 ± 0.02^{A}
15:0	0.36 ± 0.03^{AB}	0.33 ± 0.01^{B}	0.41 ± 0.02^{A}
16:0	13.10 ± 0.04^{C}	28.15 ± 0.20^{A}	19.18 ± 0.02^{B}
16:1	4.76 ± 0.02^{B}	4.79 ± 0.02^{B}	7.33 ± 0.02^{A}
17:0	0.28 ± 0.03^{A}	0.28 ± 0.01^{A}	0.30 ± 0.01^{A}
17:1	0.77 ± 0.02^{B}	0.61 ± 0.03^{C}	1.00 ± 0.01^{A}
18:0	2.64 ± 0.02^{C}	5.00 ± 0.04^{A}	3.84 ± 0.36^{B}
18:1cis-9	34.82 ± 0.05^{A}	19.40 ± 0.16^{B}	18.20 ± 0.13^{C}
18:1cis-11	3.14 ± 0.02^{B}	3.35 ± 0.10^{B}	3.96 ± 0.04^{A}
18:2 n-6	12.21 ± 0.12^{A}	12.02 ± 0.37^{A}	8.06 ± 0.06^{B}
18:3 n-6	0.06 ± 0.01^{C}	0.12 ± 0.01^{B}	0.14 ± 0.01^{A}
18:3 n-3	5.12 ± 0.06^{A}	1.33 ± 0.01^{C}	1.89 ± 0.01^{B}
20:0	0.35 ± 0.01^{B}	0.40 ± 0.01^{A}	0.19 ± 0.01^{C}
20:1	1.46 ± 0.03^{B}	1.14 ± 0.01^{C}	2.38 ± 0.04^{A}
20:2	0.16 ± 0.04^{B}	0.19 ± 0.06^{B}	0.41 ± 0.02^{A}
20:3 n-6	0.54 ± 0.04^{A}	0.42 ± 0.20^{A}	0.47 ± 0.12^{A}
20:3 n-3	1.35 ± 0.01^{B}	1.00 ± 0.05^{B}	2.51 ± 0.20^{A}
22:1+20:4	0.62 ± 0.01^{B}	1.24 ± 0.18^{A}	1.04 ± 0.02^{AB}
20:5 n-3	8.69 ± 0.02^{B}	9.11 ± 0.26^{B}	11.36 ± 0.45^{A}
22:5 n-3	1.11 ± 0.02^{C}	1.24 ± 0.04^{B}	2.39 ± 0.01^{A}
22:6 n-3	3.90 ± 0.02^{C}	5.30 ± 0.25^{B}	8.18 ± 0.41^{A}
24:1	0.27 ± 0.02^{B}	0.29 ± 0.01^{B}	0.35 ± 0.02^{A}
SFA	21.02 ± 0.11^{C}	38.50 ± 0.19^{A}	30.35 ± 0.35^{B}
MUFA	45.21 ± 0.04^{A}	29.56 ± 0.19^{C}	33.21 ± 0.12^{B}
PUFA	33.73 ± 0.18^{B}	31.95 ± 0.01^{C}	36.43 ± 0.46^{A}
n-3	20.16 ± 0.12^{B}	17.97 ± 0.45^{C}	26.33 ± 0.69^{A}
n-6	13.58 ± 0.07^{A}	13.98 ± 0.44^{A}	10.10 ± 0.23^{B}
n-3/n-6	1.49 ± 0.01^{B}	1.29 ± 0.07^{B}	2.61 ± 0.13^{A}

$^{A, B, C}$: values in the same row with different superscript are significantly different ($p<0.05$)

The obtained data indicate that fatty acid composition of fish diets is only to a certain degree reflected on fatty acid profiles of fish, as reported in Table 2.

Table 2. Fatty acid composition of fish lipids (% of total fatty acids) in marketable size trout (mean ± standard deviation)

Fatty acids	Pond I	Pond II	Pond III
14:0	3.33 ± 0.26^{B}	2.82 ± 0.64^{B}	4.63 ± 0.45^{A}
15:0	0.15 ± 0.02^{C}	0.26 ± 0.05^{B}	0.38 ± 0.01^{A}
16:0	15.61 ± 0.83^{C}	20.39 ± 2.62^{B}	24.65 ± 2.89^{A}
16:1	4.83 ± 0.27^{B}	3.72 ± 1.16^{B}	5.94 ± 0.60^{A}
17:0	0.20 ± 0.02^{B}	0.22 ± 0.03^{B}	0.30 ± 0.02^{A}
17:1	0.53 ± 0.05^{AB}	0.35 ± 0.16^{B}	0.60 ± 0.13^{A}
18:0	3.23 ± 0.22^{B}	4.91 ± 1.29^{A}	5.67 ± 1.12^{A}
18:1cis-9	31.89 ± 1.65^{A}	29.13 ± 4.18^{A}	21.90 ± 2.00^{B}
18:1cis-11	3.18 ± 0.08^{B}	4.15 ± 0.48^{A}	4.45 ± 0.26^{A}
18:2 n-6	10.97 ± 0.61^{A}	10.09 ± 1.34^{A}	10.50 ± 0.86^{A}
18:3 n-6	0.10 ± 0.02^{B}	0.10 ± 0.02^{B}	0.16 ± 0.02^{A}
18:3 n-3	2.98 ± 0.30^{A}	2.00 ± 0.39^{B}	1.56 ± 0.27^{C}
20:1	2.74 ± 0.28^{A}	2.68 ± 0.20^{A}	1.71 ± 0.32^{B}
20:2	0.61 ± 0.05^{A}	0.52 ± 0.09^{AB}	0.42 ± 0.04^{B}
20:3 n-6	0.54 ± 0.04^{A}	0.36 ± 0.10^{B}	0.28 ± 0.02^{B}
20:3 n-3	2.14 ± 0.24^{A}	2.23 ± 0.23^{A}	2.21 ± 0.37^{A}
22:1+20:4	0.99 ± 0.07^{A}	1.22 ± 0.22^{A}	1.10 ± 0.22^{A}
20:5 n-3	3.73 ± 0.57^{A}	2.95 ± 0.64^{A}	4.26 ± 0.71^{A}
22:5 n-3	1.87 ± 0.31^{A}	1.79 ± 0.70^{A}	1.52 ± 0.36^{A}
22:6 n-3	10.39 ± 1.71^{A}	9.78 ± 2.15^{AB}	7.53 ± 0.41^{B}
24:1	0.33 ± 0.03^{A}	0.33 ± 0.07^{A}	0.39 ± 0.07^{A}
SFA	22.17 ± 1.11^{C}	28.63 ± 3.47^{B}	35.63 ± 3.61^{A}
MUFA	43.50 ± 2.10^{A}	40.36 ± 2.59^{A}	35.00 ± 2.89^{B}
PUFA	34.33 ± 2.66^{A}	31.04 ± 4.86^{AB}	29.38 ± 1.44^{B}
n-3	21.12 ± 2.52^{A}	18.75 ± 3.47^{AB}	17.08 ± 1.32^{B}
n-6	13.21 ± 0.65^{A}	12.29 ± 1.08^{A}	12.30 ± 0.85^{A}
n-3/n-6	1.60 ± 0.20^{A}	1.52 ± 0.23^{A}	1.39 ± 0.14^{A}

$^{A, B, C}$: values in the same row with different superscript are significantly different ($p<0.05$)

Data obtained for fatty acid composition of fish lipids (Table 2) indicate that the major SFA, present in all fish samples in significant quantities, was palmitic acid. Oleic acid was the most abundant from the MUFA and linoleic acid from PUFA family. DHA and EPA were present in significant quantities, as well.

The highest content of palmitic acid was determined in trout from Pond III (24.65%) and the lowest in fish from Pond I (15.61%). The content of oleic acid was the highest in fish from Pond I (31.89%) and the lowest in fish from Pond III (21.90%). Significantly lower proportion of linoleic acid in Diet III ($p<0.05$), in relation to Diet I and II, had no influence on the share of linoleic acid in fish from all three ponds, which remained constant, with an average of 10%. The content of EPA in Diet III was significantly higher however, the content of this fatty acid in fish from all three ponds was not significantly different ($p>0.05$), (2.95-4.26%). The highest content of DHA was established in fish fed Diet I (10.39%) and the lowest in fish fed Diet III (7.53%). Generally, the content of DHA in trout was higher than in diets, while EPA content was lower, what is in accordance with data obtained by *Kalyoncu et al. (2010),* (EPA: 3.11-5.52%; DHA: 6.98-17.57%). Data we obtained reflects the fact that fish selectively deposit DHA in the body, as established by *Henderson (1996) and Sargent et al. (2002*).

The amounts of SFAs in trout were significantly different and ranged from 22.17 (Pond I) to 35.63%, (Pond III). Quantities of MUFA were in the range from 35.00 (Pond III) to 43.50% (Pond I). The proportions of PUFA ranged from 29.38 (Pond III) to 34.33%, (Pond I). Significant differences ($p<0.05$) in MUFA and PUFA content in all three diets had not greate impact on MUFA and PUFA content in fish. Fish from pond III had lower content of MUFA and PUFA ($p<0.05$) than fish from pond I and II. It was not established statistically significant difference in MUFA and PUFA content of fish from pond I and II, except difference in SFA ($p<0.05$). Generally, less signigicant diferences in SFA, MUFA and PUFA content in fish fed different diets were established in comparison to the content of these fatty acids in the feed, what was as also reported by *Hardy et al*. (1987). Significant differences ($p<0.05$) in quantities of n-3 fatty acids in trout fed Diet I (21.12%) and Diet III (17.08%) were established as well. Concerning n-6 fatty acids, their proportion in fish ranged from 12.29 to 13.21% and no statistically significant differences were established ($p>0.05$). The n-3/n-6 ratio was in the range of 1.39 (Pond III)-1.60(Pond I) and differences were not statistically significant ($p>0.05$). The obtained data for rainbow trout are in agreement with other reported data for freshwater fish (0.5-3.8), (*Henderson and Tocher, 1987*).

Results we obtained for fatty acid composition of rainbow trout reveal that fatty acid composition of fish, generally, is corelated to fatty acid composition of fish feed. However, the corelation degree is variant in different studies (*Torstensen et al., 2000; Bell and Dick, 2004*) because of selective deposition and metabolism of individual fatty acids, which depends on the type/mixture of oil in the feed, fish size and fish growth.

Conclusions

Significant differences ($p<0.05$) were established in the proportions of SFA, MUFA and PUFA between diets, as well as in the content of n-3 fatty acids. The share of n-6 fatty acids in Diet III was significantly lower ($p<0.05$) than in Diets I and II. The highest n-3/n-6 ratio was obtained in Diet III, followed by Diet II and Diet I.

Data obtained for fatty acid composition of fish lipids indicate that the major SFA in fish was palmitic acid. Oleic acid was the most abundant from MUFA family and linoleic acid from PUFA family. The most valuable fatty acids, EPA and DHA, were present in significant quantities in fish. However, due to selective deposition of DHA, its content in trout was much higher than in diets, while EPA content was lower than in diets. The n-3/n-6 ratio in fish was in the range from 1.39 to 1.60 and differences were not statistically significant ($p>0.05$).

The obtained results indicate that fatty acid profile of fish reflects the fatty acid composition of fish diets with some variabilities which indicate that the incorporation of fatty acids in fish tissue is under some metabolic effects.

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Uticaj ishrane na profil masnih kiselina kalifornijske pastrmke (*Oncorhynchus mykiss*)

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Rezime

U cilju jačanja naučnih i tehnoloških kapaciteta u akvakulturi ispitan je uticaja tri vrste komercijalne hrane na sastav masnih kiselina kalifornijske pastrmke. Komercijalna hrana za pastrmku sadržala je statistički značajno različite količine zasićenih masnih kiselina (ZMK), ($p<0,05$), (21,02- 38,50%). Statistički značajne razlike ($p<0,05$) ustanovljene su i u udelu mononezasićenih masnih kiselina (MNZMK) u hrani (29,56-45,21%), kao i u udelu polinezasićenih masnih kiselina (PNZMK), (31,95-36,43%). Količine EPA (C20:5 n-3) i DHA (C22:6 n-3) u hrani su bile 8,69; 9,13; 11,36% i 3,90; 5,30; 8,18%, respektivno. Udeo n-3 i n-6 masnih kiselina (MK) bio je od 17,97 do 26,33% i od 10,10 do 13,98%,

respektivno. Odnos n-3/n-6 bio je u opsegu 1,29- 2,61. Od ZMK (ukupno ZMK: 22,17-35,63%), u uzorcima ribe, najzastupljenija je bila palmitinska kiselina (C16:0), (15,61-24,65%). Oleinska kiselina (C18:1 *cis*-9) je bila najzastupljenija u grupi MNZMK (21,90-31,89%), (ukupno MNZMK: 35,00-43,50%). Od polinezasićenih masnih kiselina (ukupno PNZMK: 29,38-34,33%), linolenska kiselina (C18:2 n-6), (10,09-10,97%), EPA (C20:5 n-3), (2,95-4,26%) i DHA (C22:6 n-3), (7,53-10,39%) su bile prisutne u značajnim količinama u pastrmci. Količine n-3 masnih kiselina bile su u opsegu 17,08 do 21,12%, a značajne razlike ($p<0,05$) su ustanovljene samo između pastrmki hranjenih hranom I i III. Udeo n-6 MK je bio u opsegu 12,29- 13,21% i nisu ustanovljene značajne razlike ($p>0,05$). Odnos n-3 i n-6 bio je u opsegu 1,39 do 1,60 i, takođe, nisu ustanovljene značajne razlike ($p>0,05$). Dobijeni rezultati ukazuju da se sastav masnih kiselina hrane odražava na sastav masnih kiselina ribe, sa varijacijama koje ukazuju da je ugradnja masnih kiselina u tkivu ribe uslovljena određenim metaboličkim efektima.

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