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Chemical composition and fatty acids profile of farmed Big head carp (*Hypophthalmichthys nobilis*) and Grass carp (*Ctenopharyngodon idella*) filet

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Abstract. This study investigated the chemical composition and fatty acid profiles of big head (*Hypophthalmichthys nobilis*) and grass carp (*Ctenopharyngodon idella*) in dashte azadegan farm. Results showed: amount of saturated fatty acids (SFA) in big head and grass carp were $26.8\pm1\%$ and $28.06\pm1\%$ respectively, SFA in big head was higher compared to grass carp (P<0.05). Levels of polyunsaturated fatty acids (PUFA) in the big head and grass carp were $16.5\pm0.83\%$ and $17.8\pm0.9\%$ respectively and there was significant difference between the two species (P<0.05). Monounsaturated fatty acids (MUFA) in the big head and grass carp were $47.2\pm0.95\%$ and $52.3\pm1.02\%$ respectively. There was higher significant difference in MUFA between two species (P<0.05). This study showed in grass carp PUFA was higher than SFA, while in big head SFA was higher than PUFA (P<0.05). Also oleic acid (C18: 1n-9) and ecosapantanoic acids (C20:5n-3) had the maximum percentage of mono and poly unsaturated fatty acids. In big head and grass carp were also found to differ in the n-3/n-6 ratio, n-3 and n-6 fatty acids series, PUFA/SFA, but have a similar values in IA and IT indexes. There were significant differences in ash, lipid and moisture content in both studied species (P<0.05) but there was no significant difference in the level of fiber and protein in the two fish species (P>0.05).

Key Words: Chemical composition, fatty acid, big head carp, *Hypophthalmichthys nobilis*, grass carp, *Ctenopharyngodon idella.*

Introduction. Fish have essential concentration unsaturated fatty acids, protein with high biological value, observational studies concerning the role of fatty acids in minerals and vitamins that make them distinguished from human health have revealed that saturated and trans-fatty other creatures (Stolyhwo et al 2006). Fish is a major source of food for mankind, providing a significant amount of the animal protein diet in many countries. As compared to red meat, fish flesh is easily digestible because it contains long have muscle fibers. The high nutritional value of fish meat is reflected in favorable content of proteins, carbohydrates, minerals and vitamins (Cirković et al 2002). Some polyunsaturated fatty acids, characterized as essential, are of extreme biological importance for human health. The essential and long-chain polyunsaturated fatty acids have an indispensable role in the synthesis of prostaglandins, tromboxanes and leukotrienes, and eicosanoids. Some recent studies have demonstrated the great importance of the n-3fatty acids, so that the quality of lipids is presently evaluated on the basis of the n-6/n-3 ratio of essential fatty acids, as well as the ratio of a-linolenic acid (18:3n-3) eicosapentaenoic acid (EPA) (20:5n-3) and docosahexaenoic acid (DHA) (22:6n-3) according to the recommendations of the World Health Organization (Newton 1996). As for the intake of a-linolenic acid, the recommended value is from 0.8 to 1.1 g/d, whereas the recommended total intake of EPA and DHA is in the range of 0.3-0.4 g/d. It is also thought that daily intake of 2 g/d ofn-3 polyunsaturated fatty acids may completely satisfy the daily needs of the human organism (Peredi 1995). Increasing demand for fish and fish protein will be met by aquaculture (Queméner et al 2002). Food

quality is important for nutritionists whereas the farmer growth and final weight is concerned. In these connections (Sahu et al 2000) reported that among the commercial characteristics of fish, flesh quality is becoming more important to the aquaculture industry. Results referring to meat quality of *Hypophthalmichthys nobilis* and *Ctenopharyngodon idella* are different in communication by various authors as Memon et al (2011), Afkhami et al (2011), Ojagh et al (2009), Mieth et al (1989), Rahman et al (1995). So, because of no reports has yet been published about the fatty acid composition and comparison of this important species in Khuzestan province. In view of these facts, it seemed necessary to carry out a study on lipid profile of highly consumed fish, bighead and grass carp, in this location. The objective of this work is therefore to characterize carp in terms of their lipid and fatty acid in different species.

Material and Method

Fish samples. Samples including 20 number of *C. idella* and *H. nobilis* from dashte azadegan warm water fish pond. They were kept in iced boxes and transported to the laboratory where they were washed with cold water, weighed and measured.

Analyses of protein. Total protein was determined by using Kjeldahl method (Ritzmann & Daniels 1975). A conversion factor of 6.25 was used to convert total nitrogen to crude protein for all varieties of fish.

Moisture and ash analyses. Moisture content of 5 g of homogenized sample was determined by drying the sample in oven at 105 °C until constant mass was obtained (Sidhu 2003). Ash was determined by using the basic AOAC method (Bligh & Dyer 1959) by heating the samples in the furnace at 550 °C for 8-12 h. Each sample analyzed three times.

Lipid analyses. Total lipids were extracted by the method of Folch et al (1957) and measured gravimetrically. The formation of FAME was carried out according to the procedure described by Desvilettes et al (1994). The sample was saponified with methanolic sodium hydroxide and the fatty acids were esterified with methanolic sulfuric acid. FAME were analysed with a 6890 N GC–FID (Agilent Technologies, Wilmington, DE, USA) fitted with a J&W DB-Wax capillary column (30m, 0.25 mm i.d., 0.25 mm film thickness), a split–splitless injector with Agilent tapered liner (4mm id) and flame ionization detector. The initial column temperature was maintained at 100 °C for 1 min and then raised at 25 °C/min to 190 °C and held for 10 min and then raised to 220 °C and held for 5 min. Nitrogen was used as carrier and makeup gas, at flow rates of 1.0 and 45 mL/min, respectively. The injector and detector temperature were held at 250 and 260 °C, respectively. ChemStation software was used for online data collection and processing. Individual FAME was identified by comparison with known standards (Sigma, Chemical Co. St. Louis).

Nutritional quality. The propensity of crap's tissue to promote the incidence of coronary heart disease, atherogenic (IA) and thrombogenic (IT) indices were calculated by using the Ulbricht & Southgate (1991) equations.

 $IA = [(12: 0) + (4 \times 14: 0) + (16: 0)] \times [(PUFA n-6 and n-3) + MUFA)] - 1$

 $IT = [(14: 0) + (16: 0) + (18: 0)] \times [(0.5 \times MUFA) + (0.5 \times n - 6) + (3 \times n - 3) + (n - 3 \times n - 6 - 1)] - 1$

Statistical Analysis. Statistical analysis of data was carried out with the SPSS 16. Values is expressed as mean±SD. A Student's t-test (independent variables) was used to check for differences between two means, at 95% significance level was used to evaluate the effects of species on the chemical compositions and filet fatty acids of two carp species.

Results. Fish biometry showed the average length and weight were $(0.7\pm0.05$ kg) (45±3cm) in *H. nobilis* and (1.8±0.08kg) (58.5±1.5cm) in *C. idella* respectively. Results of chemical compounds show in muscle tissue of them in figures 1, 2, 3, 4, 5, and 6.



Figure 1. Lipid content in cultured *Hypophthalmichthys nobilis* and *Ctenopharyngodon idella* muscles (% mean±SD).



Figure 2. Protein content in cultured *Hypophthalmichthys nobilis* and *Ctenopharyngodon idella* muscles (% mean±SD).



Figure 3. Ash content in cultured *Hypophthalmichthys nobilis* and *Ctenopharyngodon idella* muscles (% mean±SD).



Figure 4. Moisture content in cultured *Hypophthalmichthys nobilis* and *Ctenopharyngodon idella* muscles (% mean±SD).



Figure 5. Fiber content in cultured *Hypophthalmichthys nobilis* and *Ctenopharyngodon idella* muscles (% mean±SD).



Figure 6. Carbohydrate content in cultured *Hypophthalmichthys nobilis* and *Ctenopharyngodon idella* muscles (% mean±SD).

Table 1

Fatty acids as % of total fatty acids profile		Big head	Grass carp	F(S)
Myristic acid	C14:0	2.80 ± 1.53	1.80±0.90	0.57**
Tetrasenoic acid	C14:1n-5	0.02 ± 0.15	0.08 ± 0.61	0.01*
Palmitic acid	C16:0	20.80 ± 1.00	18.60 ± 0.95	4.92**
Palmitoleic acid	C16:1n-7	10.50 ± 1.70	14.20 ± 1.44	3.08*
Stearic acid	C18:0	3.40 ± 0.84	5.70 ± 1.40	1.1*
Oleic acid	C18:1n-9	36.80 ± 0.90	38.00 ± 1.00	9.35**
Linoleic acid	C18:2n-6	6.50 ± 1.10	8.00 ± 1.30	1.8*
a-Linolenic acid	C18: 3n-3	4.10 ± 1.00	3.40 ± 0.87	0.93 ^{ns}
Arachidic acid	C20:0	0.70 ± 1.00	0.24 ± 036	0.11**
Linolenic acid	C18: 3n-6	0.77 ± 0.66	0.91 ± 0.78	0.21 ^{ns}
Stearidonic acid	C18:4n-3	0.31 ± 0.59	0.28 ± 0.53	0.07 ^{ns}
Behenic acid	C22:0	7.10 ± 3.60	0.27 ± 0.13	0.92**
Dihomo- gamma - linolenic acid	C20: 3n-6	0.41 ± 0.64	0.77 ± 1.20	0.14*
Eicosatrienoic acid	C20: 3n-3	1.06 ± 1.60	0.28 ± 0.43	0.16 ^{**}
Arachidonic acid	C20: 4n-6	0.74 ± 1.00	0.54±0.72	0.16 [*]
Eicosapentaenoic acid	C20:5n-3	1.50 ± 1.40	0.24 ± 0.23	0.21**
Docosapentaenoic acid	C22:5n-6	0.22 ± 0.84	0.27 ± 1.03	0.06 ^{ns}
Docosapentanoat acid	C22:5n-3	0.44 ± 2.20	0.24 ± 1.20	0.08*
Docosahexaenoic acid(DHA)	C22:6n-3	1.50 ± 1.20	0.68 ± 0.86	0.19*
saturated fatty acids	SFA	28.06 ± 1.00	26.80 ± 1.00	6.85**
monounsaturated fatty acids	MUFA	47.20 ± 0.95	52.30 ± 1.02	12.4**
polyunsaturated fatty acids	PUFA	17.80 ± 0.90	16.50 ± 0.83	4.28**
ω 6	n-6	9.10 ± 0.86	10.60 ± 1.00	2.46 [*]
ω 3	n-3	8.70 ± 1.00	7.50 ± 0.86	2.02*
ω 3 /ω 6	(n-3/n-6)	0.95 ± 0.18	0.70 ± 0.25	0.2 ^{ns}
DHA/EPA	DHA/EPA	1.08 ± 1.30	0.76 ± 0.93	0.23*
PUFA/SFA	PUFA/SFA	0.63 ± 0.87	0.61 ± 0.84	0.15 ^{ns}
IA	IA	0.50 ± 0.22	0.44 ± 0.25	0.11 ^{ns}
IT	IT	0.50 ± 1.40	0.47 ± 0.97	0.12 ^{ns}

Fillets fatty acids profile compression of *Hypophthalmichthys nobilis* and *Ctenopharyngodon idella*

Significance level: * P<0.05, ** P<0.01, ns= non-significant.

Amount of protein in *C. idella* (17.23±0.0%) was more than in *H. nobilis* (17.13±0.10%) and had non significant difference (p > 0.05). Lipid in big head was 3.4 ± 0.85 and in grass carp 5.57±1.39 percent. Amount of ash and carbohydrate in grass carp was 0.6±0.29, 1.8±0.70% and in big head 0.47±0.42, 1.2±1.30 %, there was high significant difference in muscle tissue of both fish. Moisture content percent in C. idella (74.8 ± 3.00) was less than in *H. nobilis* (77.8 ± 0.00) (p < 0.05) but there was non significant difference in amount of fiber (0.3 ± 0.10) in two filet species (p > 0.05). Amount of saturated fatty acids (SFA) and mono unsaturated fatty acids (MUFA) in H. nobilis muscle was orderly 28.06±1.00 and 47.2±0.95%, also the amount of PUFA in big head was 17.8±0.9%. In C. idella the levels of SFA and MUFA was orderly 26.8±1.00 and 52.3±1.02 percent, and about PUFA in it was 16.5±0.83% (Table 1). The results of statistical analysis showed the total amount of MUFA in C. idella have the most significant difference with its amount in *H. nobilis*. Also the amount of PUFA and SFA in grass carp it is less than in big head (p < 0.05). Amount of n-3/n-6 in C. idella was 0.7 ± 0.25 and in *H. nobilis* 0.95 ± 0.18 , there was significant difference between two species (p < 0.05). Also in stearidonic (C18:4n-3), linolenic (C18:3n-3), alpha linolenic (C18:3n-3), dicosapatanoic acids (C22:5n-6), PUFA / SFA ratio, IT and IA indexes had non significant difference (P \geq 0.05). In tetrasoenic (C14:1n-5), palmetolitic (C16:1n-7), stearic (C18:0), di-homogamalinolenic (C20:3n-6), arachidonic (C20:4n-6), dicusapantanoat acids (C22:5n-3), DHA and omega 3, omega 6 series of fatty acids and DHA / EPA ratio had significant difference (p < 0.05) between their filets fatty acids contain (Table 1).

Discussion. Chemicals properties of fresh water fishes investigation is very important, because useful information for experts related to food resources having low fat, high protein and being easily accessible. In addition, composition of *C. idella* compared the present study with Ojagh et al (2009) showed high difference in fat ($2.71\pm1.56\%$) because of our survey approached (5.57%) and compared the content of ash ($0.94\pm0.02\%$) and moisture ($82.66\pm0.65\%$) of this study by the last one demonstrated high difference brightly ($p \le 0.05$) but in protein levels ($15.18\pm0.61\%$) this two study had significant difference (p < 0.05).

Amount of chemical composition of grass carp in Crikovic et al (2011) studies contain 76.29 \pm 1.03% moisture, 14.8 \pm 0.12% protein, percentage of fat was 3.43 \pm 0.08 and ash content 0.98 \pm 0.03% as compared by this study put on upper level of moisture but in other contents were in lower level and have significant difference in the same specie and *H. nobilis*. In the other hands for ash and moisture content of big head had non difference (p > 0.05). The fatty acid composition big head and grass carp is presented in Table 1.

Among SFA, the 16:0 and 18:0 acids were the two most dominant (ranging from $18.60\pm0.95\%$ to $20.80\pm1\%$ and $3.40\pm0.84\%$ to $5.70\pm1.40\%$) in meat tissue analyzed.

In the present study MUFA content was higher than SFA and PUFAs in all tissues which is similar to findings of Afkhami et al (2011) ($27.18\pm0.5\%$ SFA and $31.55\pm3.1\%$ PUFA), Circovick et al (2011) (29.03% and 18.26%) for grass carp and Mieth et al (1989) (29.4% and 21.7%) for big head. Different authors demonstrated that MUFA is the most important group of FA in carps.

Afkhami et al (2011), Circovick et al (2011) and Mieth et al (1989) reported (48.3%), (51.29%) and (35.12 \pm 0.9%) MUFA in grass carp and big head. In the other hand Ojagh et al (2009) viewed that the PUFA level (37.5 \pm 3.8%) is higher than MUFA (25.02 \pm 4.6%) and SFA (34.06 \pm 3.6%) in *C. idella*. So comparison of this by them shows significant difference in their levels (P < 0.05).

The amount of SFA in big head (27.3%) from Rahman et al (1995) and Memon et al (2011) studies is lower than our report, but shows no difference (p > 0.05); while (29.4%) SFA in Meilth et al (2006) was upper than ours and show difference (P < 0.05) (Table 1). Also there is a significant difference in MUFA (26.7%) for Rahman et al (1995) and Memon et al (2011) studies and 48.3% of MUFAs in Mieth et al (1989).

Generally these differences refer to distinction environmental factors such as temperature (Cordier et al 2002; Tocher et al 2004), pH and salinity are known to influence the composition of lipids in fish (De Torrengo & Brenner 1976). Also Different climate, age, weight of samples, time and place of examined.

Compared this study with Ojagh et al (2009) in some factor like DHA ($12.61\pm2.9\%$), EPA ($2.52\pm0.7\%$), linoleic acid ($9.67\pm1.47\%$), a- linolenic ($5.38\pm1.7\%$) and archidonic acid ($2.5\pm1\%$) shows high differences with new report and this differ affected from climate change, plankton species variation or grass carp fed barely here. This comparison with Memon et al (2011) about the big head is shown this difference except in archidonic acid (C20:4n-6) (4.8%) level, that may be refer to capability of omega3 FA long chain changing to archidonic acid (Steffens & Wirth 2007).

Compared amount of n-3 and n-6 in two groups of carp showed the maximum levels of them for grass carp in this case (Table 1), which had lower level than in Ojagh et al (2009) research ($12.23\pm1.2\%$ n-6) and ($25.31\pm2.91\%$ n-3) PUFA. While the total percentage of n-6 PUFA ($10.6\pm1\%$) is higher than the n-3 (7.5-086%) on the contrary, referred in earlier research reports for big head. According to these results can said that the ω 6 FA is probably incorporated into the tissue lipids of herbivorous fish *via* the food chain, but the elevated SFA may be the result of *de novo* synthesis within the fish, as well as the availability of large quantities of food in the tropics. On the other hand, marketable carp reared on the basis of natural food in ponds exhibit high contents of n-6 as well as n-3 fatty acids, on the other hand carp fed supplementary wheat, which is characterized by a low content of n-3 PUFA (Steffens et al 1998), resulted in somewhat lower concentrations of these acids and higher oleic acid content. Carp groups probably ingested in higher rate of natural pond foods containing more EPA and DHA. The content of n-3 PUFA (especially DHA) is high when cryptophytes and DHA-rich copepods become

an important group of plankton. Carp grown on natural food had a high content of both n-6 and n-3 fatty acids, while carp fed grains, which are characterized by low levels of n-3 PUFA (Buchtová et al 2010; Ćirković et al 2010), contained lower concentrations of these fatty acids, because of a higher concentration of oleic acid (Steffens et al 1998). The above statements are in agreement with our results, where higher content of oleic acid (38±1%) was observed in the grass carp fed corn and marginal and drift herb of lakes, as dominant energy source than the lowest percentage of oleic acid has been reported in carp fed a complete mixture (41.96%) (Buchtová et al 2010; Ćirković et al 2011). Other wise the European Food Safety Authority proposed labeling reference intake values: 2 g of n-3 PUFA ALA per day and 250 mg of EPA and DHA per day (EFSA 2009). Mozaffarian & Rimm (2006) recommend 250 mg of EPA and DHA per day for the general population in order to reduce mortality from cardiac and heart disease (CHD).

Also n-3/n-6 ratio in this study compared with Ojagh et al (2009) ($0.48\pm0.04\%$) and Afkhami et al (2011) ($0.52\pm0.3\%$) shows difference for grass carp, but comparison this ratio in big head shows high differences and repeated the results to compared (5.4%) by Memon et al (2011) researches.

Increase in the human dietary of n-3/n-6 fatty acid ratio is essential in the diet and nutritionists believe that this ratio should be 0.1-0.2 and considers higher ratios (>0.2) more beneficial to human health (FAO/WHO 1994). However, Okuyama et al (1996) recommend an n-3/n-6 PUFA ratio of 1:1–2 for prevention of certain chronic diseases instead of the amount of individual FA. Simopoulos (2008) suggested that the n-3/n-6 ratio should be kept between 1:1 and 1:4. It has been proposed that the western diet is deficient in omega three fatty acids, with an n-3/n-6 ratio of 1:15–20 (Eaton & Konner 1985; Simopoulos 1991).

Furthermore nutritionists believed that PUFA/SFA, IA and IT indexes which are indicators of filet lipids quality and the indices of atherogenicity and thrombogenicity were calculated using the fatty acid composition of the muscle to determine the potential health impact on human consumers. Amount of $0.44\pm0.25\%$ to $05\pm0.22\%$ IA and IT $0.47\pm0.97\%$ to $0.5\pm1.4\%$ indexes in this two species illustrated no difference (p > 0.05) allowing an integrated assessment of dietary lipid on human coronary health. Higher values of IT and IA (>1.0) are detrimental to human health (Ouraji et al 2009) this values are suitable for human nutrition and tells the difference amount of FA muscle (Table 1).

Conclusions. Proximate constituents in the whole body as well as the fillet are readily manipulated by feed composition and feeding strategies, whereas the sensory parameters are less affected by these variables. Different rearing systems generate products having variable quality level. However, there were significant differences in the production system influence on lipid quality, showing that non-supplemented and rapeseed supplemented carp contained higher amounts of beneficial n-3 and total PUFA.

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