

COMPOSITIONAL PROPERTIES OF THREE FRESHWATER CARP SPECIES GROWN IN BRACKISH WATER

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ABSTRACT

Three freshwater fish species viz. *Labeo rohita*, *Cirrhinus mrigala* and *Gibelion catla*, grown in brackish water ponds were analyzed for compositional properties to assess the potential of this habitat to produce nutritionally adequate fish for human consumption. Overall, the unsaturated fatty acids were lower in *L. rohita* (46.6%) than saturated fatty acids; while in *C. mrigala* and *G. catla*, the unsaturated fatty acids were 50.4% and 58.2%, respectively. The most abundant saturated fatty acid in examined species was palmitic acid (C_{16:0}), 23.7 to 34.1%; mono-unsaturated fatty acid was oleic acid (C_{18:1}) 19.6 to 31.7% and poly-unsaturated fatty acid linoleic acid (C_{18:2}) 9.46 to 13.3%. A reasonable amount of essential fatty acids ω-3 (5.80 to 9.26%) and ω-6 (9.46 to 13.3%) was also found in these species while growing in brackish water on salt tolerant forages like *Leptochloa fusca*, *Brachiaria mutica* and *Kochia indica* as supplemental feed. The ω-3/ω-6 ratio was calculated as 0.46, 0.80 and 0.69 in *L. rohita*, *C. mrigala* and *G. catla*, respectively. The maximum EPA (eicosapentaenoic acid, C_{20:5}) was observed in *C. mrigala* (2.23%), followed by *G. catla* (1.62%) and *L. rohita* (0.98%). While the DHA (docosahexaenoic acids, C_{22:6}) was found maximum in *G. catla* (1.97%) and minimum in *C. mrigala* (0.95%). The results of body composition indicated that *L. rohita* found maximum protein contents (19.2%) with minimum total fats (1.28%) while *C. mrigala* found maximum total fats (2.11%) but minimum protein contents (18.3%). Overall results indicated that the Indian carps grown in brackish water have comparable chemical composition and nutritive value with the same species grown in freshwater medium.

Keywords: Indian carps, brackish water, chemical composition, fatty acids profile

INTRODUCTION

In Asian countries, the Indian carps are commonly cultured in freshwater ponds under semi-conservative system. These carp species contribute a major proportion of fish production with over 1.8 million tonnes (FAO, 2003). Fish is considered as the fastest growing food source in Asia as it provides 26.2% of total meat (Delgado *et al.*, 2002). The most preferred Indian carp is *Labeo rohita* which is extensively cultured in the Indian sub-continent (Misra and Samantaray, 2004). Fish is widely consumed in Pakistan but still very low per capita consumption (1.7 kg). Only 26% of total fish production is consumed domestically, 19% being exported and 55% used for fish meal (Wasim, 2007).

Fish is a good source of dietary protein and high in unsaturated fatty acids, essential amino acids, vitamins and minerals. Fish lipids have protective role against cardiovascular diseases, stroke, hypertension, diabetes, depression and rheumatoid arthritis (Karanth *et al.*, 2009; Hussain *et al.*, 2011). Coronary heart disease has been identified as a main cause of death in various parts of the world including Pakistan and those peoples who consume more fish have less chances of this disease (Hassan *et al.*, 2010; Afkhami *et al.*, 2011). The quantity of total lipids varies among different fish species. The type of fatty acids present as free acid or as neutral lipid also differs from species to species and or its aqueous medium (Saify *et al.*, 2000). Fish quality is important for rural communities because it contributes to their healthy diets and livelihoods (Jabeen and Chaudhry, 2011).

The nutritional parameters are attributed to the diet which they consume and their ecological conditions. Marine fish and its products are high in health promoting fatty acids but required in very small amount in human diet (Sargent, 1997). The fatty acid composition in fish oil is affected by several factors including geographical location, temperature and water salinity. The concentration of eicosapentaenoic acid in fish oil increased due to increase in temperature and decrease in water salinity (Razak *et al.*, 2001). Fish is also a good source of antimicrobial peptides used for defense against pathogens (Ravichandran *et al.*, 2010). The most important feature in the fatty acid profile of fish is the high content of essential poly-unsaturated fatty acids such as eicosapentaenoic acid and docosahexaenoic acid (Wu and Peter, 2008; Ravichandran *et al.*, 2011). The supplemental feed plays a significant role in growth and chemical composition of fish (Jankowska *et al.*, 2007). The body composition is a good

indicator to assess the nutritional status and condition of fish which are essential parameters to check the quality characteristics of farmed fish for human consumption (Ali *et al.*, 2005). An attempt has been made to study the nutritional value and fatty acids composition of brackish water fish fed on salt-tolerant forages under semi-intensive polyculture system. The present study will provide baseline data on some of the important chemical constituents as there is limited data on chemical properties of brackish water fish.

MATERIALS AND METHODS

Study site

The study was conducted at Bio-Saline Research Station, Pakka Anna (lat. 31°24' N and long. 73°05'E) of the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan, which is located at a distance of 50 km in South-West of the Faisalabad city, with an elevation of 190 m above sea level. The climate is semi-arid with an annual average rainfall of 325 mm and evaporation exceeds 1600 mm. The annual average temperature in the area is 32°C.

Fish sampling

Three fish species viz. *Labeo rohita*, *Cirrhinus mrigala* and *Gibelion catla*, grown in brackish water ponds (dimensions 25 m × 13 m × 1.5 m) under semi-intensive polyculture system, were selected to study their chemical composition. Fish samples with average weight 217±9.89 g and length 27.3±0.30 cm, were collected (twelve specimens of each species) in 2013. These fishes were fed on salt-tolerant forages including *Leptochloa fusca* (Kallar grass), *Brachiaria mutica* (Para grass) and *Kochia indica* (Kochia) as supplemental feed on daily basis. The ponds were also fertilized with goat droppings and nitrophos @ 6000 and 7.5 kg ha⁻¹, respectively.

Morphometric measurements

The fish samples were immediately transported to the laboratory where morphometric measurements including wet weight, head length, snout length, eye

diameter, length of pectoral, dorsal, ventral, anal and caudal fins, standard length, forked length, total length, number of fin rays and scales area of each sample were carried out. After morphometric measurements, each fish sample was dissected, its internal organs removed and then washed to remove residual blood. After removing skin, the fish fillets were obtained by cutting the fish lengthwise along the backbone to obtain maximum flesh without including the backbone. The fillets were cut in to small pieces and transferred into labeled sterilized polythene bags and stored in freezer at -20°C until further analysis.

Proximate composition

Whole body composition analysis was carried out by using standard methods (AOAC, 2003). Water content was determined by placing whole fish in pre-weighed aluminum foil tray for drying in an electric oven at 105°C till constant weight. Total ash contents were calculated gravimetrically following ignition of samples in muffle furnace at 550°C until constant weight. Crude protein (N×6.25) was determined using the Kjeldahl method. Lipids were extracted by adopting the procedure as described by Bligh and Dyer (1959) with some modification by Kinsella et al. (1977). The total carbohydrates were calculated by the difference method. Sodium and potassium were measured with flame photometer (Model PFP-7, Jenway) while phosphorus was spectrophotometer (Model U-2800, Hitachi).

Preparation of methyl esters

Fish oil samples were converted to their constituent fatty acid methyl esters by using an improved, rapid base-catalyzed transesterification method described by Ichibara et al. (1996). Up to 10 mg of fish oil was dissolved in 2 ml hexane followed by the addition of 0.2 M methanolic potassium hydroxide. The tube was vortexed for 2 minutes at room temperature. After a light centrifugation, an aliquot of the hexane layer was separated and transferred in capped test tubes for gas chromatographic analysis. Same procedure was adopted for the preparation of all samples.

Gas chromatography

A GC system (Agilent, 7890A, USA), equipped with FID and DB-23 capillary column (length 30 m, I.D 0.25 mm, film thickness 0.25 µm) was used for the analysis of methyl esters of fish oils. The injector and detector temperatures were set at 250 and 300°C, respectively. The column oven temperature was programmed from 180 to 210°C at the rate 20°C per minute; initial and final temperatures were held for 5 and 14 minutes, respectively. Nitrogen was used as carrier gas with a flow of 2 ml per minute. Hydrogen and synthetic air were used as supportive gases. A sample of 1.0 µL was injected by using split mode (split ratio 1:75). Fatty acid methyl esters were analyzed by using external standards. Retention times and peak area percentages were automatically computed by Agilent ChemStation program. The composition was reported as a relative percentage of the total peak area.

Statistical analysis

All the data is expressed as means ± standard deviation. The values of proximate composition and fatty acid profile of each species were compared by one-way analysis of variance followed by Duncan’s multiple range tests. All analyses were conducted by using MSTAT-C program (Freed and Scott, 1989).

RESULTS AND DISCUSSION

Table 1 shows the morphometric measurements of fish samples collected from brackish water ponds at Bio-saline research Station, Pakka Anna (Faisalabad). The brackish groundwater was used throughout the growing period. Results of water analysis indicated that the water temperature varied from 16.2 to 33.1°C. The pH and water salinity (electrical conductivity-EC) ranged from 8.23 to 8.80 and 5.17 to 6.37 desi Siemens per meter (dS m⁻¹), respectively. The pH of water remained within limit of 7.5 to 9.0 (Pailwan et al., 2008). Yadav (2006) investigated the lethal alkaline limit for fish which is pH 11. The average dissolved O₂ was observed 5.81 mg L⁻¹. Banarjee and Lal (1990) reported 5 to 10 mg L⁻¹ dissolved O₂ as an ideal range for fish production. The free CO₂ was fluctuated between 8.57 to 11.6 mg L⁻¹. Similarly, the pond’s soil up to 150 cm depth was also analyzed. The maximum value of EC (19.6 dS m⁻¹) was observed in soil profile at 120-150 cm depth. The overall soil texture of all fish ponds indicated the textural class as sandy loam.

Table 1 Morphometric measurements of three carp species, grown in brackish water

Variables	<i>Labeo rohita</i>	<i>Cirrhinus mrigala</i>	<i>Gibelion catla</i>
Wet weight (g)	235.4±9.89	210.3±11.5	206.7±8.62
Head length (cm)	5.80±0.14	5.23±0.20	6.23±0.25
Snout length (cm)	1.85±0.07	1.47±0.11	2.23±0.24
Eye diameter (mm)	9.50±0.15	10.1±0.10	8.80±0.12
Standard length (cm)	21.9±0.14	22.7±0.25	19.5±0.30
Forked length (cm)	23.1±0.15	25.5±0.17	21.7±0.23
Total length (cm)	26.6±0.10	29.8±0.30	25.5±0.50
Length of pectoral fin (cm)	4.15±0.12	4.63±0.19	3.77±0.21
Length of dorsal fin (cm)	4.55±0.07	5.03±0.08	4.63±0.11
Length of ventral fin (cm)	4.10±0.14	3.86±0.11	4.07±0.05
Length of anal fin (cm)	4.45±0.11	4.20±0.21	4.12±0.10
Length of caudal fin (cm)	5.65±0.05	6.30±0.08	6.10±0.10
No. of pectoral fin rays	11.3±0.57	14.0±0.34	12.3±0.58
No. of dorsal fin rays	12.4±0.57	13.3±0.57	10.7±0.58
No. of ventral fin rays	14.3±0.57	8.33±0.57	6.67±0.58
No. of anal fin rays	7.33±0.57	7.67±0.57	7.33±1.53
No. of caudal fin rays	25.6±1.53	21.3±1.53	20.3±0.58
Area of scales (cm ²)	0.36±0.02	0.52±0.01	0.51±0.02

Values are mean of six samples ± Standard deviation

Results of the chemical composition of three carp species are presented in Table 2. *C. mrigala* and *G. catla* showed comparable moisture contents but differed significantly ($p \leq 0.05$) from *L. rohita*. The moisture contents in the examined fish species were in the range observed by other authors (Abii et al., 2007). The *L. rohita* was highest in moisture and crude protein content but lowest in total fats whereas *C. mrigala* was lowest in moisture and crude protein content but highest in total fats. Fish proteins contain all essential amino acids for the human beings (Vladau et al., 2008). In mammals and fish, the protein usually ranges from 14 to 20% (Cirkovic et al., 2010). The lipid contents vary depending on fish type, time of year and feed used for fish (Guler et al., 2008). Rahman et al. (1995) classified fish on the basis of fat contents as lean fish (fat <5%), medium fat fish (fat 5-10%) and fatty fish (fat >10%). Based on this classification, the examined fish species are classified as lean fish. Results were contrary to Jabeen and Chaudhry (2011) who studied these fish species grown in freshwater medium.

This difference may be due to sampling season, supplemental feed type and growth medium. The difference is clear that in the present study the supplemental feed source was salt-tolerant forages and the growing medium was brackish pond water. May be due to this reason the examined species were lean with <5% fat while the same species grown in freshwater were medium fat with 5-10% fat. Similarly, the protein contents in *L. rohita* and *G. catla* were slightly higher than the level reported by FAO (2008).

The mean ash contents in *L. rohita* and *G. catla* were similar but differed significantly ($p \leq 0.05$) with *C. mrigala*. The phosphorus concentration was observed maximum (60.8 mg g⁻¹) in *G. catla* and minimum (43.3 mg g⁻¹) in *C. mrigala*. Similarly, the sodium was found maximum (11.6 mg g⁻¹) in *G. catla* while minimum (10.8 mg g⁻¹) in *L. rohita*. There was no significant difference in potassium concentration among the examined fish species (Table 2).

Table 2 Chemical composition of three carp species, grown in brackish water

Body constituents	<i>Labeo rohita</i>	<i>Cirrhinus mrigala</i>	<i>Gibelion catla</i>
Moisture (%)	75.1±2.45 ^a	72.7±3.14 ^b	73.8±2.71 ^{ab}
Dry matter (%)	24.6±1.52 ^{ab}	28.3±1.96 ^a	28.2±1.03 ^a
Crude protein (%)	19.2±3.21 ^a	18.3±2.01 ^b	19.1±2.50 ^a
Total fats (%)	1.28±0.21 ^b	2.11±0.56 ^a	1.84±0.10 ^{ab}
Total ash (%)	3.32±0.19 ^b	5.56±0.35 ^a	3.45±0.11 ^b
Carbohydrates (%)	1.10±0.08 ^b	1.33±0.11 ^b	1.81±0.15 ^a
Phosphorus (mg g ⁻¹)	58.7±3.34 ^b	43.3±3.71 ^b	60.8±4.21 ^a
Sodium (mg g ⁻¹)	10.8±2.15 ^{ab}	11.1±1.09 ^a	11.6±1.14 ^a
Potassium (mg g ⁻¹)	7.30±1.02 ^a	7.70±0.89 ^a	7.30±0.62 ^a

Values are mean of six samples ± Standard deviation; Means within a row with different superscript letter are significantly different ($p \leq 0.05$)

Table 3 Fatty acid profile (%) of three carp species, grown in brackish water

Fatty acids	Retention time (min.)	<i>Labeo rohita</i>	<i>Cirrhinus mrigala</i>	<i>Gibelion catla</i>
Saturated fatty acids				
Caproic acid, C _{6:0}	2.315	0.03±0.01 ^b	0.25±0.01 ^{ab}	0.54±0.02 ^a
Caprylic acid, C _{8:0}	2.517	0.32±0.02 ^a	0.09±0.01 ^b	0.25±0.01 ^{ab}
Capric acid, C _{10:0}	2.773	0.00	0.02±0.00 ^a	0.04±0.01 ^a
Lauric acid, C _{12:0}	3.256	0.01±0.00 ^b	0.23±0.02 ^a	0.62±0.01 ^a
Tridecanoic acid, C _{13:0}	3.604	0.56±0.02 ^a	0.08±0.01 ^c	0.29±0.01 ^b
Myristic acid, C _{14:0}	4.023	3.25±0.14 ^a	2.32±0.01 ^b	2.92±0.05 ^{ab}
Pentadecanoic acid, C _{15:0}	4.493	1.68±0.02 ^a	0.85±0.02 ^b	0.73±0.01 ^b
Palmitic acid, C _{16:0}	5.036	31.97±1.10 ^a	34.12±1.12 ^a	23.66±2.12 ^b
Heptadecanoic acid, C _{17:0}	5.652	3.02±0.03 ^a	1.58±0.02 ^b	2.60±0.01 ^{ab}
Stearic acid, C _{18:0}	6.385	11.25±0.15 ^a	8.94±0.21 ^{bc}	9.80±0.11 ^b
Arachidic acid, C _{20:0}	8.279	0.43±0.01 ^a	0.71±0.01 ^a	0.24±0.02 ^b
Behenic acid, C _{22:0}	8.641	0.84±0.01 ^a	0.45±0.01 ^{ab}	0.12±0.01 ^b
Total SFAs	-	53.37	49.64	41.83
Mono-unsaturated fatty acids				
Myristoleic acid, C _{14:1} n-5	4.242	0.67±0.01 ^{ab}	1.22±0.01 ^a	1.08±0.02 ^a
Palmitoleic acid, C _{16:1} n-7	5.250	7.02±0.04 ^a	5.12±0.03 ^b	2.23±0.01 ^c
Oleic acid, C _{18:1} n-9	6.643	19.61±1.01 ^c	26.23±1.25 ^b	31.71±1.17 ^a
Erucic acid, C _{22:1} n-9	11.716	0.96±0.04 ^a	0.74±0.02 ^a	0.57±0.01 ^a
Total MUFAs	-	28.27	33.31	35.59
Poly-unsaturated fatty acids				
Linoleic acid, C _{18:2} n-6	7.097	12.57±0.18 ^a	9.46±0.25 ^b	13.32±0.14 ^a
Linolenic acid, C _{18:3} n-3	7.721	3.15±0.2 ^{ab}	4.41±0.02 ^{ab}	5.67±0.05 ^a
Eicosapentaenoic acid, C _{20:5} n-3	8.641	0.98±0.02 ^b	2.23±0.01 ^a	1.62±0.01 ^b
Docosahexaenoic acid, C _{22:6} n-3	11.945	1.67±0.01 ^a	0.95±0.01 ^a	1.97±0.01 ^a
Total PUFAs	-	18.36	17.05	22.58
ω-3/ω-6 ratio	-	0.46	0.80	0.69
ω-6/ω-3 ratio	-	2.16	1.25	1.44
SFT:USFA ratio	-	1.14:1	1:1.01	1:1.39

Values are mean of six samples ± Standard deviation; Means within a row with different superscript letter are significantly different ($p \leq 0.05$)

The results of fatty acid profiles of all examined carp species are presented in Table 3. Total saturated fatty acids (SFAs) in lipids were 53.4, 49.6 and 41.8% in *L. rohita*, *C. mrigala* and *G. catla*, respectively. Among SFAs, myristic, palmitic, heptadecanoic and stearic acids were dominating fatty acids. Palmitic acid was the primary SFA, contributing about 56 to 68% to the total SFA contents of lipids in all species. Total mono-unsaturated fatty acids (MUFAs) in *L. rohita*, *C. mrigala* and *G. catla* were calculated as 28.3, 33.3 and 35.6%, respectively, where the palmitoleic and oleic acids were the dominating MUFAs. Oleic acid contributed about 69-85% to the total MUFAs similar to the results of Osman et al. (2007). Among SFAs and MUFAs, the highest palmitic acid followed by oleic acid and stearic acid was comparable with the findings of Luczynska et al. (2008) and Jabeen and Chaudhry (2011).

Total poly-unsaturated fatty acids (PUFAs) were 18.4, 17.1 and 22.6% in *L. rohita*, *C. mrigala* and *G. catla*, respectively with highest concentrations of linoleic and linolenic acids. This may be due to phytoplankton feeding habit as they are good source of these essential fatty acids. The maximum amount of EPA (2.23%) was observed in *C. mrigala* and minimum (0.98%) in *L. rohita*. Similarly, the maximum percentage of DHA was observed in *G. catla* (1.97), followed by *L. rohita* (1.67) and *C. mrigala* (0.95).

The values of EPA and DHA were lower in *L. rohita* as compared to other experimental fish species which is may be due to low fat contents. Simopoulos (2002) recommended the ω-3: ω-6 appropriate balance from 1.1 to 1.4 depending on the disease. The ratio of ω-3 and ω-6 fatty acids has been suggested a useful indicator to compare the nutritional values of fish oils which depends on species, period of year, age, size, reproduction period and the fatty acids in diet (Sirot et al., 2008). In the present study, the amount of ω-3 fatty acids and ω-3: ω-6 ratio was lower than the findings of Ugoala et al. (2009). The levels of ω-6 fatty acids were higher than ω-3 fatty acids in examined fish species similar to the findings of Aras et al. (2003). The recommended daily intake of ω-3 fatty acids is ranged from 0.5 to 1.6 g for healthy adults, infants, pregnant and lactating women (Cejas, 2000).

CONCLUSION

The Indian carps cultured in brackish water using salt-tolerant forages as supplemental feed are characterized by high protein content, reasonable amount of essential fatty acids (ω-3 and ω-6) and satisfactory proportion of n-3 and n-6 poly-unsaturated fatty acids especially linoleic acid. So, it is concluded that these carp species have comparable proximate compositions and fatty acid profiles

with the same species grown in freshwater medium and is nutritionally adequate for human consumption.

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