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NUTRITIONAL ASPECTS AND SEASONAL INFLUENCE ON FATTY ACID COMPOSITION OF CARP (*LABEO ROHITA*) FROM THE INDUS RIVER, PAKISTAN

Nusrat N. Memon, Farah. N. Talpur, Muhammad I. Bhanger

National Center of Excellence in Analytical Chemistry, University of Sindh, Jamshoro, Pakistan

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Seasonal deviations in proximate composition and intramuscular profile of fatty acid of one of the prolific specie of fish *Labeo rohita* in Indus River, Pakistan, were studied. The lipid content was significantly higher ($p<0.05$) in spring (1.95%) followed by winter (1.36%), autumn (0.98%) and summer (0.95%). Protein fluctuates directly with lipids, highest percentage was observed in spring 23% while lowest in summer 20%. As compared to other seasons, higher contents of moisture (75.65%) were found in summer, whereas more plentiful nutrients were available in the spring, which cause an increase in the amount of parched material therefore decrease in the moisture content (72.91%) was observed. In all sampling seasons, in fish oil the most abundant fatty acids were palmitic acid (16:0), oleic acid (18:1), *n*-3 eicosapentaenoic acid (EPA 20:5) and *n*-3 docosahexanoic acid (DHA 22:6). The sum of polyunsaturated fatty acid (PUFA) was 42% in spring, 37% in winter, 36% in autumn and 32.02% in summer. In the total fatty acids the levels of DHA in spring, winter, autumn and summer were 16.63%, 15.71%, 15.12%, 13.05% and while those of EPA were 4.35%, 5.52%, 4.59% and 5% respectively. During all seasons the total *n-3* fatty acid was found higher in contrast to *n-6* fatty acid. The highest ratio *of n*-3/*n*-6 was recorded during winter 1.84, while PUFA/SFA ratio was found higher in spring 2.33.

INTRODUCTION

There is an increasing awareness about fish lipids owing to their positive effect on human health [Anon, 1992]. Prospective health benefits associated to fish eating are due to the occurrence of desired amount of proteins which contain all essential amino acids, vitamins, minerals and unsaturated essential fatty acids especially the more health favorable long chain *omega-3* PUFAs [Sidhu, 2003]. The abundance of the unsaturated fatty acids in fat is the most valuable characteristics of fish [Hedayatifard & Moeini, 2007]. In recent times there has also been an increased attention in the medical and civic health communities regarding the role of unsaturated *omega-3* fatty acid in human health and welfare. Researchers found that high fish intake societies, such as Japanese and Inuit especially Eskimo population, have significantly minor incidences of ischemic heart diseases, atherosclerosis and acute myocardial infractions [Bang & Dyerberg, 1980; Blanchet *et al*., 2000]. Moreover, the risk of impaired cognitive function was inversely correlated to the dietary ingestion of *omega-3* PUFA [Lie, 2004], it is essential in support of human fetus for normal neural and visual development [Innis & Elias, 2003] and in disorders of for instance imperfect vision, anemia and skin diseases [Mahaffey, 2004; Celik, 2008]. Additionally, an increased intake of *omega-3* PUFA from fish may cover considerable implications in favor of public health *via* diminishing the risk of coronary events and unexpected cardiac death [Schmidt *et al.,* 2000]. In composition of fish oils two members of the *omega-3* PUFA family: 20:5 *n-3* (eicosapentaenoic acid, known as EPA) and 22:6 *n-3* (docosahexaenoic acid, known as DHA) are principal fatty acids. Human bodies cannot readily synthesize long chain *omega-3* PUFAs so they have to be obtained from the diet [Alasalvar *et al*., 2002]. Thus, PUFA, particularly the longer chain *omega-3* and *omega-6* are considered vital FA. *Omega-3* PUFA such as DHA and EPA are of biomedical value and play the main function in the eicosanoids biosynthesis [Kinsella *et al*., 1990a] and also control the blood lipids [Kinsella *et al*., 1990b]. Besides, membranes of important organs possibly influencing membrane-lipid-dependent functions, especially in retina and brain contain DHA as major FA [Hoffman *et al*., 1993]. All these conclusions have produced a new market for fish oil as a nutritional supplement and food [Hjaltason, 1990]. Several products have been developed and produced commercially with technical and cosmetic applications based on fish oil fatty acids [Windson & Barlow, 1981]. Mostly, partially hydrogenated oil of fish is used for the manufacture of shortenings, margarines and compound fats. Due to these benefits the American Heart Association (AHA) now recommends every person to consume at least two servings of fish per week, besides additional food rich in alpha linolenic acid. However, fish nutrients are affected to substantial environmental transformation throughout the year and variations in compositions and availability of feed also effects their muscles proximate composition. It has been reported that in fish muscle the category and quantity of lipids and fatty acids

Author's address for correspondence: Nusrat N. Memon, National Center of Excellence in Analytical Chemistry, University, of Sindh, 76080 Jamshoro, Pakistan; tel.: (92 22) 2771379; fax: (92 22) 2775160; e-mail: nusratmemon2007@yahoo.com

differ mostly by certain factors such as feeding, age, size, geographical location and reproductive status [Alasalvar *et al*., 2002; Ackman, 1989; Henderson & Tocher, 1987].

Labeo rohita fish is the most copious freshwater specie in River Indus Pakistan which ranks as the 21st largest river globally in terms of annual flow. People living in the vicinity of river Indus use this fish species in abundance as their diet. Freshwater fish living in seasonally fluctuating conditions (temperature, oxygen access *etc*) periodically change the way of life and, consequently the contents of different compounds. To our knowledge no information is available concerning the consequence of seasonal changes on the fatty acid composition of *Labeo rohita* fish from River Indus. Therefore, in the present work concerning nutritional quality of *Labeo rohita,* we have studied the effect of season on the proximate composition and the fatty acid profile, and categorized to locate the most excellent available source of *omega-3* fatty acids throughout the year.

MATERIALS AND METHODS

Sampling

In the year 2008, samples of *Labeo rohita* fish specie were captured from the Indus River Hyderabad Sindh (Pakistan) during January (winter), April (spring), July (summer) and October (autumn). The samples were transported directly to the laboratory within one hour after capture and stored in deep freezer. The mean size for entire length for *Labeo rohita* were 41.80 cm and total weight was 1500 g. Fish samples were immediately eviscerated, beheaded, skinned and filleted. Samples were prepared in all four seasons by homogenizing the meat from twenty fish. Chemical analyses were repeated three times on each sample.

Proximate composition

The method of Folch *et al.* [1957] was used for the total lipids extraction. The mixture of chloroform methanol solvent (2:1, v/v) was added to samples in the proportion of 20:1 (v/w). Three times homogenization of samples was done at 3000–4000 rpm for 10 min. After every homogenization, the samples were cooled at 4°C for 1 h. Into the extract for 1 g of tissue 4 mL of $MgCl_2$ (0.034%) were added. The incubation of extract was done at 4°C for overnight. The base layer was washed by chloroform–methanol 2:1 (v/v). The rotary evaporator (Heidolph, W. Germany) was used for the removal of solvent at 40°C under reduced pressure (10-50 mm Hg). Total lipid contents of the sample were resoluted gravimetrically.

Crude protein was determined according to the official method of Kjeldahl [AOAC, 1998a]. The sample was heated to 420°C for 20 min with 98% H_2SO_4 and catalyst using Heating digester; then treated with 33% NaOH and 4% boric acid by distillation unit. The amount of nitrogen was estimated after titration with 0.2 N HCl. It was multiplied by the coefficient 6.25.

Moisture content in% was determined by drying the fish sample at a temperature of 105°C to constant weight [AOAC, 1998b]. The weight difference before and after drying was multiplied by 100 and divided with the original weight of the fish sample.

A 5 g sample was homogenized by weighing in a well dried porcelain basin and subjected to a low Bunsen flame. Then, the sample was subjected to 550-570°C and cooled in desiccators. The content of ash was calculated considering the difference of weight after and before using the reported procedure of AOAC [1998c].

Carbohydrate content was calculated by the difference between 100 and the sum of the crude protein, crude fat, moisture and ash. Energy values of the samples were also calculated and expressed as Kcal/100 g. The coefficients were 5.65 for protein, 9.50 for fat and 3.90 for carbohydrates [Merrill & Watt, 1973].

Fatty acid analysis

Methyl esters of fatty acids were prepared by saponification and esterification of lipid by standard IUPAC method. A layer of hexane, containing the FAME, was positioned into a GC vial; the vial was capped and kept at -20°C until analysis on GC. The FAMEs were investigated on a 8700 model of Perkin Elmer gas chromatograph (Perkin-Elmer Ltd, Buckinghamshire, England) fitted with Rt-2560 (100 m x 0.25 mm x 0.2 μm film thickness (Supelco, PA, USA), polar capillary column with stationary-phase of nonbonded biscynopropyl siloxane, and FID. Oxygen-free nitrogen was used as a carrier gas at a flow rate of 3.5 mL/min. The initial oven temperature was 150°C at a rate of 4 min which was raised to 190°C at a rate of 2°C/min and further to 220°C held for 7 min. The detector and injector temperature were set at 270°C and 260°C correspondingly. A volume of 1.0 μL of the sample was injected.

The entire quantification was completed by a built-in data-handling program supplied by the (PerkinElmer) company of the gas chromatograph as reported earlier by Talpur *et al*. [2007]. The FAME was identified by evaluating the retention time of the samples with appropriate FAME standards, obtained from Sigma (St. Louis, MO, USA).

Statistical analysis

The results obtained from this study were analysed statistically by one-way ANOVA for seasonal variation by Minitab (Minitab, 13.0, PA, USA). Tukey's studentized procedure was used to caculate the differences between least square means, which were established as a significant change at $p<0.05$.

RESULTS AND DISCUSSION

Proximate composition

The results of proximate analysis for *Labeo rohita* during four seasons are shown in Table 1. The fish were caught in all four seasons: winter, spring, summer and autumn. These results show that the moisture content was significantly higher ($p<0.05$) in the muscle of *Labeo rohita* in summer (75.65%) than in the other seasons. Due to the excessive consumption of diet (plants and humic substances) during summer, the lowest moisture content was found in spring (72.91%) when nutrients are more abundant, an increase in the fraction of dry matter is observed, while there is a decrease in the moisture content. However no significant ($p>0.05$) changes in seasons were determined in ash content of *Labeo rohita* muscle.

TABLE 1. The proximate composition (%) and energy (kcal/100 g) of *Labeo rohita* captured in different seasons.

Component	Winter	Spring	Summer	Autumn
Moisture	$74.53^{b} \pm 0.12$	$72.91^{c} \pm 0.01$	$75.65^{a} \pm 0.02$	$75.60^{a} \pm 0.01$
Protein	$22.30^{b} \pm 0.00$	$23.00^{a} \pm 0.02$	$20.00^{d} \pm 0.01$	$21.25^{c} \pm 0.03$
Lipid	$1.36^{b} \pm 0.01$	$1.95^{a} \pm 0.15$	$0.95^{c} \pm 0.01$	$0.98^{c} \pm 0.01$
Ash	$1.01^{b} \pm 0.02$	$1.15^{a} \pm 0.01$	$1.02^{b} \pm 0.12$	$1.06^{b} \pm 0.01$
Carbohydrate	$0.80^{c} \pm 0.01$	$0.99^{c} \pm 0.04$	$1.58^{a} \pm 0.01$	$1.11^{b} \pm 0.11$
Energy	$142.04^{b} \pm 0.01$	$152.34^{a} \pm 0.01$	$128.18^{d} \pm 0.12$	$133.70^{c} \pm 0.00$

Note: Values are given as mean ± S.D. from triplicate determinations. Different superscripts in the same row indicate significant differences ($p<0.05$).

The protein contents observed in the muscle of *Labeo rohita* in spring, winter, autumn, and summer, were 23%, 22.30%, 21.25%, and 20% respectively. It was observed that the protein content in muscle of *Labeo rohita* significantly increased ($p<0.05$) along with decrease in moisture content in spring. The change in the content of protein in fish muscle depends on the abundance of fish food and there is an opposite relationship among protein and moisture content [Gülsün & Abdurrahman, 2006].

Significant variation concerning the seasons was observed among the lipid level of the same species. The highest content of lipid ($p<0.05$) was observed in spring (1.95%) and lowest in summer (0.95%). Although, it is thought that the difference found in the content of lipid is not just because of food abundance, but is also associated with the reproductive behavior of fish. In the *Labeo rohita* fish species a lower level of lipid was found in the season of reproduction (summer). During sexual maturation many fish species tend to decrease their food ingestion and lipid stores are directed to use for energy [Gokce *et al.,* 2004]. Similarly, Sargent [1995] has stated that seasonal deviations in the lipid content of fish were associated mainly to the reproductive cycle, in the ordinary environment that fish save enormous contents lipid in the muscle, which were mobilized at the time of gonad maturity. It was also noticed that there is a converse correlation between the lipid and moisture contents. The moisture content decreased while lipid and protein content increased significantly.

Fatty acid analysis

Most of the fatty acids underwent changes during the four seasons. Seasonal variations of total percentages of saturated, monounsaturated and polyunsaturated fatty acids in the *Labeo rohita* lipid are shown in Table 2. We have calculated 32 fatty acids in lipids of *Labeo rohita* muscle. Most common fatty acids identified into all four seasons were palmitic (16:0), stearic (18:0), palmitoleic (16:1 *n-9*), oleic (18:1 *n-9*), linoleic (18:2 *n-6*), arachidonic AA (20:4 *n-6*), docosahexanoic DHA (22:6 *n-3*) and eicosapentaenoic acid EPA (20:5 *n-3*). Throughout all seasons, palmitic acid was found to be present at the maximum rank amongst saturated fatty acid in the lipid of *Labeo rohita*, the 16:0 its levels in spring, winter, summer and autumn were calculated to be 10.5%, 17.2%, 19.2% and 16.99%. These results were comparable with other species of fish reported by various researchers [Özyurt *et al*., 2005; Grün *et al.*, 1999; Chen *et al.,* 1995; Chanmugam *et al*., 1986]. Fish are commonly low in SFA about (<30% SFA), except some species [Guler *et al.,* 2008]. Similar results were found in this study for all seasons (18-29.84%). In the current analysis the fraction of 16:0 in total SFA was 58.33-65.51%. Alasalvar *et al.* [2002] reported that about 70% of the sum SFA content of sea bass lipid was palmitic acid. In addition, Stansby [1982] stated that 20–50% of total fatty acid in lipids of some fish was palmitic acid.

In the present work, significantly lower ($p<0.05$) sum of saturated fatty acids (Σ SFA) was observed in spring, *i.e.* 18%. The sum of SFA decreased in spring for *Labeo rohita* which is possibly due to catabolization of SFA to balance the more metabolic energy necessary during that stage. This trend was also observed in White Sea bream during a similar period [Özyurt *et al*., 2005]. In *Labeo rohita* the primary MUFA (monounsaturated fatty acid) identified for the entire time of year was C18:1 *n-9* (oleic acid). This FA in muscle of *Labeo rohita* tissue was calculated to be at levels of 19.45%, 21.66%, 16% and 21% in summer, spring, winter and autumn, correspondingly. The maximum level of C18:1 *n-9* was during spring. Correspondingly, Guler *et al.* [2007] established that C18:1 *n-9* was the most important MUFA in muscle tissue of *Sander lucioperca*, zander, living into freshwater of Turkey. According to Akpinar *et al.* [2009], C18:1 *n-9* was the main MUFA in muscle (22.4–22.1%) and liver (15.6–17.6%) of female and male *Salmo trutta macrostigma*. In the present study, the second most important MUFA was palmitoleic acid (7.68--11.6%), the results are comparable with the findings of Guler *et al.* [2008].

According to the statement of Andrade *et al.* [1995], the higher values of oleic, palmitoleic, and arachidonic acids is a good quality of freshwater fish oils. MUFA content of *Labeo rohita* muscle lipids were significantly higher ($p<0.05$) as compared to SFA in winter, autumn, summer and spring, 35%, 38%, 36% and 41%, respectively. In winter, a low level of 18:1 lowered the MUFA content while in spring the high ratio of 18:1 and palmitolic acid increased the MUFA content. Discrepancy in FA compositions may be associated with the variation in nutritional behavior of the fish [Guler *et al*., 2008]

The levels of PUFA in the ΣFA for *Labeo rohita* fillets were observed to be 32.02-42%. In this experiment the PUFA contents were found high ($p<0.05$) in comparison to MUFA and SFA in winter and spring, while MUFA content was observed higher in autumn and summer in respect of PUFA

TABLE 2. Seasonal fatty acid profile of *Labeo rohita* fish (g/100 g fat).

Fatty acids	Winter	Spring	Summer	Autumn
11:0	$0.01^{b} \pm 0.00$	$0.03^{a} \pm 0.01$	$0.02^{b} \pm 0.00$	$0.04^{a} \pm 0.01$
12:0	$0.22^{c} \pm 0.01$	$0.46^{b} \pm 0.01$	$0.03^{c} \pm 0.00$	$0.66^{a} \pm 0.02$
13:0	$0.01^{c} \pm 0.00$	$0.04^{b} \pm 0.01$	$0.04^{b} \pm 0.01$	$0.51^{a} \pm 0.03$
14:0	$1.05^{c} \pm 0.01$	$1.79^{b} \pm 0.01$	$1.56^{b} \pm 0.01$	$2.48^{a} \pm 0.02$
15:0	$1.01^{a} \pm 0.02$	$0.87^{b} \pm 0.01$	$0.32^{c} \pm 0.01$	$0.53^{c} \pm 0.01$
16:0	$17.2^{b} \pm 0.02$	$10.5^{d} \pm 0.01$	$19.2^{a} \pm 0.02$	$16.99^{c} \pm 0.02$
17:0	$1.30^{a} \pm 0.03$	$0.95^{b} \pm 0.01$	$1.05^{a} \pm 0.02$	$0.75^{b} \pm 0.01$
18:0	$6.00^{b} \pm 0.03$	$2.25^{d} \pm 0.01$	$6.47^{a} \pm 0.03$	$3.38^{c} \pm 0.01$
20:0	$0.34^{b} \pm 0.01$	$0.43^{a} \pm 0.01$	$0.30^{c} \pm 0.00$	$0.37^{b} \pm 0.01$
22:0	$0.23^{c} \pm 0.01$	$0.63^{a} \pm 0.04$	$0.27^{b} \pm 0.01$	$0.22^{c} \pm 0.01$
24:0	$0.63^{a} \pm 0.02$	$0.05^{b} \pm 0.00$	$0.58^{a} \pm 0.02$	$0.06^{b} \pm 0.00$
ΣSFA	28.0	18.0	29.84	25.99
14:1	$0.37^{d} \pm 0.01$	$0.69^{a} \pm 0.02$	$0.48^{c} \pm 0.01$	$0.55^{b} \pm 0.02$
15:1	$0.66^{a} \pm 0.03$	$0.27^{b} \pm 0.01$	$0.32^{b} \pm 0.01$	$0.24^{b} \pm 0.01$
16:1 *n-9*	$10.2^{b} \pm 0.04$	$11.06^{a} \pm 0.05$	$7.68^{d} \pm 0.01$	$9.00^{c} \pm 0.02$
16:1 *n-7*	$1.78^{c} \pm 0.01$	$2.58^{a} \pm 0.02$	$2.37^{b} \pm 0.02$	$2.17^{b} \pm 0.02$
17:1	$1.11^{a} \pm 0.02$	$0.82^{b} \pm 0.01$	$1.24^{a} \pm 0.03$	$0.95^{b} \pm 0.01$
18:1 *n-9*	$16.0^{c} \pm 0.01$	$21.66^{a} \pm 0.04$	$19.45^{b} \pm 0.02$	$21.0^{a} \pm 0.04$
18:1 *n-7*	$1.99^{a} \pm 0.01$	$1.01^{c} \pm 0.00$	$1.29^{b} \pm 0.01$	$1.83^{a} \pm 0.01$
20:1	$1.91^{a} \pm 0.02$	$1.26^{b} \pm 0.01$	$1.68^{a} \pm 0.02$	$1.22^{b} \pm 0.01$
22:1	$0.77^{b} \pm 0.02$	$0.58^{c} \pm 0.01$	$0.81^{a} \pm 0.02$	$0.55^{c} \pm 0.01$
24:1	$0.21^{c} \pm 0.01$	$0.07^{d} \pm 0.00$	$0.68^{a} \pm 0.03$	$0.49^{b} \pm 0.02$
ΣMUFA	35.0	41.0	36.0	38.0
18:2	$5.57^{a} \pm 0.04$	$3.29^{c} \pm 0.01$	$3.51^{b} \pm 0.01$	$3.21^{c} \pm 0.01$
18:3	$0.37^{d} \pm 0.01$	$0.46^{c} \pm 0.01$	$0.89^{a} \pm 0.02$	$0.60^{b} \pm 0.02$
20:2	$0.70^{c} \pm 0.01$	$1.46^{a} \pm 0.02$	$0.15^{d} \pm 0.00$	$0.98^{b} \pm 0.01$
20:4	$5.35^{b} \pm 0.01$	$9.28^{a} \pm 0.02$	$5.09^{b} \pm 0.01$	$5.96^{b} \pm 0.01$
22:4	$0.66^{b} \pm 0.02$	$0.41^{c} \pm 0.01$	$0.62^{b} \pm 0.02$	$0.73^{a} \pm 0.03$
22:2	$0.35^{c} \pm 0.02$	$0.10^{d} \pm 0.00$	$0.74^{a} \pm 0.04$	$0.52^{b} \pm 0.02$
ΣPUFA *n-6*	13.0	15.0	12.0	14.0
18:3	$0.87^{a} \pm 0.04$	$0.42^{c} \pm 0.01$	$0.58^{b} \pm 0.02$	$0.61^{b} \pm 0.02$
20:3	$0.32^{c} \pm 0.01$	$0.43^{b} \pm 0.01$	$0.85^{a} \pm 0.03$	$0.49^{b} \pm 0.01$
20:5	$4.35^{b} \pm 0.01$	$5.52^{a} \pm 0.02$	$4.59^{b} \pm 0.01$	$5.00^{a} \pm 0.02$
22:5	$2.75^{b} \pm 0.02$	$3.00^{a} \pm 0.02$	$1.95^{c} \pm 0.01$	$2.78^{b} \pm 0.02$
22:6	$15.71^{b} \pm 0.02$	$17.63^{a} \pm 0.03$	$13.05^{c} \pm 0.01$	$15.12^{b} \pm 0.02$
ΣPUFA *n-3*	24.00	27.00	20.02	22.00

Note: Each value is an average of ten samples, with its standard deviations. Values in the same line with different letters are significantly different at $p<0.05$.

and SFA. Ackman [1995] accounted that DHA and EPA of the long chain *omega-3* FA were the principal PUFA of fish oil. The similar trend is also observed in the present work that DHA (22:6) and EPA (20:5) were the predominant FA in *Labeo rohita* muscle lipids during all seasons. DHA levels in *Labeo rohita* fillets were determined as 15.71%, 16.63%, 13.05% and 15.12% in winter, spring, summer and autumn respectively. Sargent [1995] reported that *n-3* PUFAs, primarily DHA, have a quality to sustain the functional integrity and structure of fish cells. The level of EPA in *Labeo rohita* fillets was observed to be 5.52% 4.35%, 4.59% & 5% in spring, winter, summer & autumn, correspondingly. During spring, the higher ratio of EPA (5.52%) and DHA (16.63%) increased the *n-3* PUFA content. The DHA level was found higher in spring before spawning time, but it was found to be in low level during summer and autumn after the spawning period. It may be because DHA might have been shifted to eggs from the muscle for the spawning period [Abdurrahman *et al.,* 2009]. In addition to the results achieved from this experiment a number of scientists have documented that the

amount and type of FA of fish species tissue were influenced by the size and age of the fish, the maturity period and seasonal condition. Gülsün & Abdurrahman [2006] and Gokce *et al.* [2004] found that the proportion of DHA and EPA, which have a fundamental character in human nutrition, are 16.8–20% and 23.36–4.26% in the *Solea solea* [Inhamuns & Franco, 2008]. These results are comparable to those determined in the present work, *i.e.* 4.35-5 and 13.05-16.63 for *Labeo rohita* fish.

Arachidonic fatty acid (AA) from the *n-3* series is quite imperative. According to Bowman & Rand [1980], AA is an ancestor for thromboxane and prostaglandin which will control blood clot development and its connection to the endothelial tissue during injury is curative. Moreover, 20:4 acids also play a great part in growth. In the present work, the higher level of AA in *Labeo rohita* was found in spring (9.25%) which is greater than that reported for fresh water carp of Beysehir Lake (Turkey) (6.99%) [Guler *et al.*, 2008]. In the present study the level of AA in summer, winter, spring and in autumn was 5.09, 5.35%, 9.28%, and 5.96%, respectively. Guler *et al.* [2008] also recorded that carps had a higher level of AA during summer, winter, spring and autumn in the order of 6.99%, 5.57%, 5.38%, and 4.38%, respectively.

For evaluation of comparative nutritional importance of fish oils the ratio of *n-3/n-6* is an excellent indicator [Pigott & Tucker, 1990]. The present results in Table 3 show that in *Labeo rohita* the ratio of *n-3/n-6* in winter was 1.84, in spring 1.8, in summer 1.66 and in autumn it was 1.57. Our results are comparatively higher as compared to Vimba in which the ratio of *n-3/n-6* was 1.5 in summer, 1.4 in winter, 1.4 in spring, and the smallest 1.2 in autumn [Leyla *et al.,* 2009]. It is essential to increase the dietary intake of *n-3/n-6* fatty acid ratio in the human diet to reduce cancer risk and to avoid coronary heart disorders by decreasing plasma lipids. Kinsella *et al*. [1990a, b] reports from Scandinavia, Japan and The Netherlands illustrate that people who consume about twofold of fish per week *i.e.* 240 g, had minor chance of heart attacks than had people who rarely consume fish [Wardlaw *et al.,* 1992], higher value of *n-6* FA lowered the ratio of *n-3/n-6* during autumn in freshwater fish Vimba. Our analysis has revealed that the investigated fish species *Labeo rohita* had a high nutritional status for human consumption owing to its higher ratio of *n-3/n-6* and PUFA/SFA fatty acids. It was suggested that *n-3/n-6* ratio of 1:1-1:5 would constitute a healthy human diet [Zuraini *et al*., 2006], in the present study *Labeo rohita* fish had *n-3/n-6* ratio of 1.57-1.84, which is closer to the recommended ratio.

The seasonal variation shows the positive relationship between fat content and polyunsaturated fatty acid of *Labeo rohita*, it means that the PUFA percentage were high into the season of higher fat content. While, negative correlation was observed for the SFA, which decreased in the season of a higher level of fat content. This recommends varied biological roles for the variety of FA in the fish [Zlatanos & Laskaridis, 2007]. SFA are mostly used for the purpose of energy storage. Hence their concentration rose during the stage of improved feeding activity.

TABLE 3. Seasonal variation in *n*-3/*n*-6 ratio, PUFA/SFA, and arachidonic acid (AA, g/100 g) of *Labeo rohita* fish.

Seasons	*n-3/n-6*	PUFA/SFA	AA
Winter	1.84	1.32	5.35
Spring	1.80	2.33	9.28
Summer	1.66	1.07	5.09
Autumn	1.57	1.38	5.96

CONCLUSIONS

The current work shows that *Labeo rohita* is an important nutritional source of protein and vital *n-3* fatty acids throughout the entire year. An important finding is that the season with the highest PUFA content is the season with the highest fat content (spring), and the consumption of these fish especially during this season can provide the nutrition with high amount of *n-3* PUFA. The minimum value was reached in summer which coincided with the period of spawning. As in the end of result, when person health is taken into description, the Indus River Pakistan carp *Labeo rohita* materialize to be quite nourishing in requisites of fatty acid compositions and their ratios.

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