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Fatty Acid Composition of Two Candidate Species of Aquaculture, *Fenneropenaeus merguiensis* and *F. penicillatus* (Crustacea: Decapoda) in Pakistan

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Abstract.- Fatty acid composition was determined in two candidate aquaculture species *Fenneropenaeus merguiensis* and *F. penicillatus* sampled from Karachi, Pakistan. Five fatty acids, that is, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), oleic acid (18:1n-9), palmitic acid (16:0) and stearic acid (18:0) were particularly abundant in the muscle tissues of both species. Palmitic acid and oleic acid were the most abundant saturated and monounsaturated fatty acids, respectively in *F. merguiensis* and *F. penicillatus*. Among PUFA, the predominant fatty acid were docosahexaenoic acid (DHA; 22:6n-3), eicosapentaenoic acid (EPA; 20:5n-3) and arachidonic acid (ARA; 20:4n-6). No significant differences were observed in the contents of total saturated, monounsaturated and polyunsaturated fatty acids among two species. The *n*-3/*n*-6 fatty acid ratios, DHA/EPA ratios and ARA/EPA ratios were similar in two species. Fatty acid profiles have been reported to show seasonal changes in penaeid shrimps, thus there is a need to study the fatty acid composition of *F. merguiensi*s and *F. penicillatus* in various seasons as the present study was conducted in spring only.

Key words: Fatty acids, *Penaeus* spp., aquaculture in Pakistan.

INTRODUCTION

Seafood products are an important source of nutrients in the human diet. Crustaceans such as shrimps, crabs and lobsters, are good sources of amino acids, protein and other nutrients. Shrimp muscle is an excellent source of protein (Yanar and Celik, 2006) and contains good amount of unsaturated fatty acids (HUFA), such as eicosapentaenoic (20:5n3, EPA) and docosahexaenoic (22:6n3, DHA) acids, which are essential in human health and nutrition (Feliz *et al*., 2002; Simopoulos, 2004) especially for the prevention of cardiovascular disease (Dyerberg, 1986; Kinsella, 1987; Bruckner, 1992; Connor, 2000) and other diseases (Innis, 2000). Shrimp muscle is also a good source of calcium (Yanar and Celik, 2006).

Twenty-seven species of penaeid shrimps have been reported from the coast of Pakistan, among these, *Fenneropenaeus merguiensis, F. penicillatus, P. semisulcatus, Metapenaeus affinis, M. monoceros* and *Parapeneaopsis stylifera* are of commercial importance (Tirmizi, 1967; Gololobov and Grobov, 1969; Tirmizi and Bashir, 1973; Ahmed, 1985). Sufficient biological and fisheries information on these commercially important species is available from Pakistan (Zupanovic, 1971; Hussain, 1974; Karim and Rehman, 1975; Ahmed, 1977; Tirmizi and Bashir, 1973; van Zalinge *et al.*, 1987; Ayub and Ahmed, 1991, 1992a,b, 2001, 2002a,b) but the data related to biochemical composition is scarce (Nisa *et al.*, 1993; Nisa and Asadullah, 2006; Nisa and Sultana, 2010). Throughout the world, several studies have dealt with the fatty acid profile of various penaeid species (Bottino *et al.*, 1980; Saglık and Imre, 1997; Bragagnolo and Rodriguez-Amaya, 2001; Rosa and Nunes, 2003; Yanar and Celik, 2005; Sriket *et al*., 2007; Oksuz *et al*., 2009; Zlatanos *et al*., 2009). These studies do not include two species, *F. merguiensis* and *F. penicillatus*, which are now the

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preferred cultured species in Asia (Qingbo *et al.*, 1988; Chen *et al*., 1998; Hoang, 2001; Hoang *et al.*, 2002, 2003; Zacharia and Kakati, 2002). The objective of this study was to provide a detailed description of the fatty acid profile in muscle tissue of *F. merguiensis* and *F. penicillatus* and compare it with already reported fatty acid composition of both species (Nisa and Asadullah, 2006) from same area, Karachi. The present information on the fatty acid composition of *F. merguiensis* and *F. penicillatus* can be utilized as guideline for preparation of appropriate diets for these two species in aquaculture programs.

MATERIALS AND METHODS

Adult shrimps of *F. merguiensis* and *F. penicillatus* were procured from the fishermen operating their trawler in the vicinity of Karachi in the month of May. They were brought to the laboratory in ice container where they were washed with freshwater and deheaded. After the removal of shell, the shrimps were deveined and the muscle tissues of shrimps was utilized for the fatty acid analyses.

Determination of fatty acid profiles

Tissue (2g) of *F. merguiensis* (n= 6) and *F. penicillatus* (n= 4) was soaked in 20 ml of chilled solvents, chloroform: methanol (2:1, v/v). The samples were kept at -20ºC until further analyses. Lipid in the shrimp muscle tissue was extracted by the method described by Folch *et al.* (1957). Lipid concentrations were determined by measuring mg of lipid g^{-1} wet tissue weight. The fatty acid compositions were determined as fatty acid methyl esters (FAME) using a Gas-Chromatograph (Fisons MD800) equipped with a phenomenex ZB-WAX column (30mt x 0.32mm x 0.25), and cold on-column injection system, using helium as carrier gas at a flow rate of 2.0 ml/ min. Initial oven temperature was kept at 50°C then raised to 225°C at a ramping temperature of 40°C/ min to 150°C then at 2°C/ min to 225°C and finally held for 5 minutes at 225°C and 1ml of solution in iso-hexane was injected. Peaks were recorded and integrated on a personal computer using Chrom Card software (Fisons) and FAMES were identified by comparison with known fish oil standard 'Marinol' (AOAC, 1999). All samples were analyzed in triplicate.

Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) using the software of the SPSS 14.0 for Windows.

RESULTS

Lipid content

Lipid in the muscle tissue of *F. penicillatus* varied from 0.92 to 1.0% and of *F. merguiensis* from 0.87 to 0.98 percent.

Fatty acid profile

A total of 33 individual fatty acids were identified in the muscle of *F. merguiensis* and *F. penicillatus* (Table I). Five fatty acids, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), oleic acid (18:1n-9), palmitic acid (16:0) and stearic acid (18:0) were particularly abundant in the muscle tissue of both species. Palmitic acid was the most abundant saturated fatty acid and oleic acid was abundant among monounsaturated fatty acid. Palmitic acid and stearic acid accounted for 54.34% and 34.83% of the total amount of saturated fatty acids in *F. merguiensis*, respectively and 56.92% and 34.12% in *F. penicillatus*, respectively. Oleic acid constituted 41.40% and 42.65% of the total amount of monounsaturated fatty acids in *F. merguiensis* and *F. penicillatus*, respectively. No significant differences were found in total contents of saturated fatty acids (F= 0.00; df= 1; P= 0.996) or monounsaturated fatty acids (F= 0.00; df= 1; P= 0.948) between two species, *F. merguiensis* and *F. penicillatus.*

Polyunsaturated fatty acids (PUFA) were the most common fatty acids (Table I). Among the n-6 PUFA the predominant fatty acid was arachidonic acid (20:4n-6), which showed no significant difference in *F. merguiensis* (5.95%) and *F. penicillatus* (6.04%). Arachidonic acid (ARA) accounted for 56.97% and 57.09% of the total amount of n-6 PUFA in *F. merguiensis* and *F. penicillatus*, respectively. Among n-3 PUFA, the predominant fatty acids were docosahexaenoic acid, DHA (22:6n-3) and eicosapentaenoic acid, EPA

Table I.- Fatty acid composition (% of total fatty acid wet weight) of *F. merguiensis* and *F. penicillatus* muscle tissue. The data denotes the mean and standard deviation of 6 samples of *F. merguiensis* and 4 samples of *F. penicillatus.* Each sample was analyzed in triplicate.

Fatty acids	*F. merguiensis* (n=6)	*F. penicillatus* (n=4)
14:0	1.19 ± 0.26	1.04 ± 0.08
15:0	0.98 ± 0.12	0.90 ± 0.13
16:0 (Palmitic acid)	16.90 ± 0.93	17.31 ± 0.54
18:0 (Stearic acid)	10.64 ± 0.47	10.38 ± 0.35
20:0	0.35 ± 0.03	0.35 ± 0.02
22:0	0.38 ± 0.07	0.39 ± 0.12
24:0	0.10 ± 0.09	0.05 ± 0.10
16:1n-9	0.02 ± 0.05	0.03 ± 0.07
16:1n-7 (Palmitoleic acid)	7.28 ± 0.96	8.15 ± 0.34
18:1n-9 (Oleic acid)	9.61 ± 0.67	10.35 ± 1.24
18:1n-7 (Vaccenic acid)	3.82± 0.68	3.30 ± 0.09
20:1n-11	1.07± 0.19	0.88± 0.04
20:1n-9	0.38± 0.24	0.47 ± 0.11
20:1n-7	0.52± 0.04	0.48 ± 0.12
22:1n-11	0.34± 0.16	0.29 ± 0.10
24:1n-9	0.17± 0.05	0.31 ± 0.05
18:2n-6	1.26 ± 0.13	1.49 ± 0.39
18:3n-6	0.24 ± 0.03	0.26 ± 0.02
20:2n-6	0.82 ± 0.12	0.79 ± 0.14
20:3n-6	0.21 ± 0.03	0.20 ± 0.03
20:4n-6 (Arachidonic acid)	5.97± 1.31	6.04 ± 0.48
22:4n-6	0.97 ± 0.15	0.83 ± 0.06
22:5n-6	1.02 ± 0.11	0.96 ± 0.03
18:3n-3	0.64 ± 0.08	0.61 ± 0.02
18:4n-3	0.36± 0.03	0.33 ± 0.03
20:4n-3	0.20 ± 0.01	0.19 ±0.03
20:5n-3 (Eicosapentaenoic acid, EPA)	14.57 ± 0.45	13.95± 0.63
22:5n-3	1.64 ± 0.05	1.56 ± 0.06
22:6n-3 (Docosahexaenoic acid, DHA)	12.90 ±1.40	12.79 ± 0.54
16:2	0.17 ± 0.06	0.17 ± 0.00
16:3	1.31 ± 0.27	1.24 ± 0.02
16:4	3.68 ± 0.28	3.66 ± 0.38
18:0 Dimethyl acetals	0.28 ± 0.08	0.24 ± 0.01
∑ Saturated fatty acids	30.54±1.12	30.41 ± 0.96
∑ Monounsaturated fatty acids	23.21 ± 1.76	24.27 ± 1.38
∑ Polyunsaturated fatty acids	40.82 ± 2.68	40.01± 0.88
EPA+DHA	27.47	26.74
n 3/n 6 polyunsaturated ratio	2.89	2.78
DHA/EPA ratio	1.13	1.09
ARA/EPA ratio	2.44	2.31

(20:5n-3). DHA and EPA were found at levels of 12.90% and 14.57%, respectively in *F. merguiensis* and 12.79% and 13.95%, respectively in *F. penicillatus* (Table I). The amounts of n-3 PUFA in both species were three fold greater than those of n-6 PUFA. The *n*-3/*n*-6 fatty acid ratios, DHA/EPA ratios and ARA/EPA ratios were similar in two species (Table I). Analysis of variance (F= 0.00; df= 1; P= 0.974) showed no significant differences in the composition of polyunsaturated fatty acid between *F. merguiensis* and *F. penicillatus*.

Comparison of abundant fatty acids composition between two studies

The abundant fatty acids composition between present study and Nisa and Asadullah (2006) showed differences (Table II). In present study the abundant fatty acids showed no difference between two species, while Nisa and Asadullah (2006) have reported differences in EPA and DHA between two species. Total PUFA and SFA were similar in our study between two species while PUFA was higher in *F. penicillatus* and SFA in *F. merguiensis* in the study of Nisa and Asadullah (2006). In our study, the n 3/n 6 PUFA ratio was similar in both species, while it was dissimilar in study of Nisa and Asadullah (2006), being 4.1 and 5.0 in *F. merguiensis* and *F. penicillatus*, respectively.

DISCUSSION

In the present study, the abundant fatty acids in the muscle tissue of *F. merguiensis* and *F. penicillatus* showed no significant differences, which contradict the study of Nisa and Asadullah (2006) which showed that some constituents were different in two species, collected from the same location, Karachi. Our study was conducted in May (spring), whereas, Nisa and Asadullah (2006) did not mention the month in which their study was conducted. It is difficult to draw any clear conclusion to this contradiction since fatty acid composition of shrimps may be affected by such environmental factors as season, depth, geographic location of catch or size. However, present study is similar to the study of Bottino *et al.* (1980) who reported that three species, *Penaeus setiferus*, *P. aztecus* and *P. duorarum* collected at the same time of the year differed very little from each other in their fatty acid patterns.

Krzynowek and Panunzio (1989) studied 11 species of shrimps and found lipid to range between 0.8-1.1%, classifying crustaceans as low-lipid foods. In the present study low lipid content was found in muscle tissue of *F. merguiensis* and *F. penicillatus.*

Table II.- Comparison of abundant fatty acids (% of total fatty acid wet weight) of *F. merguiensis* and *F. penicillatus.*

Fatty acids	*F. merguiensis* Mean ± STD (Present study)	*F. merguiensis* Mean ± STD (Nisa and Asadullah, 2006)	*F. penicillatus* Mean ± STD (Present study)	*F. penicillatus* Mean ± STD (Nisa and Asadullah, 2006)
16:0 (Palmitic acid)	16.90 ± 0.93	14.90± 0.39	17.31 ± 0.54	14.50± 0.42
18:0 (Stearic acid)	10.64 ± 0.47	10.30± 0.12	10.38 ± 0.35	9.00± 0.05
16:1n-7 (Palmitoleic acid)	7.28 ± 0.96	5.50 ± 0.33	8.15 ± 0.34	5.00 ± 0.35
18:1n-9 (Oleic acid)	9.61 ± 0.67	8.50± 0.51	10.35 ± 1.24	9.00± 0.54
20:4n-6 (Arachidonic acid)	5.97± 1.31	4.90± 0.10	6.04 ± 0.48	4.80± 0.12
20:5n-3 (Eicosapentaenoic acid, EPA)	14.57 ± 0.45	13.90± 0.44	13.95± 0.63	16.50± 0.53
22:6n-3 (Docosahexaenoic acid, DHA)	12.90 ±1.40	12.00± 0.32	12.79 ± 0.54	15.00± 0.20
∑ PUFA	40.82	36.4	40.02	42.0
∑ n 6 PUFA	10.48	7.0	10.58	6.9
∑ n 3 PUFA	30.34	28.4	29.44	34.5
∑ SFA	30.54	31.1	30.41	27.3
∑ MUFA	23.21	24.1	24.27	22.8
EPA+DHA	27.5	25.9	26.7	31.5
n 3/n 6 PUFA ratio	2.9	4.1	2.8	5.0
DHA/EPA ratio	1.1	1.1	1.1	1.2
ARA/EPA ratio	2.4	2.8	2.3	3.4

The other study from Pakistan has also reported a low lipid content of 1.35% in *F. penicillatus* and 1.2 % in *F. merguiensis* (Nisa and Asadullah, 2006). Saglık and Imre (1997) determined total lipid in *Parapenaeus longirostris* tissue to be 0.93% and in *P. semisulcatus*, 0.58%. Yanar and Çelik (2005) reported that lipid content of *P. semisulcatus* and *Metapenaeus monoceros* ranged between 0.97-1.07% and 0.98-1.15%, respectively. Lipid level was reported as 1.1% for *P. longirostris* (Oksuz *et al*., 2009). Li *et al.* (2011) have reported a low lipid contents of 1.32% in *P. vannamei* and 1.18% in *F. chinenesis*. However, higher lipid content has been reported with value of 4.06% in *P. semisulcatus* (Diler and Atas, 2003).

The EPA content was higher than DHA in *F. merguiensis* and *F. penicillatus*, which is similar to the results reported in *P. longirostris* and *P. semisulcatus* (Saglık and Imre, 1997), in *P. brasiliensis* and *P. schimitti* (Bragagnolo and Rodriguez-Amaya, 2001), in *P. semisulcatus* and *M. monoceros* (Yanar and Celik, 2005) and in *F. merguiensis* and *F. penicillatus* (Nisa and Asadullah, 2006). However, DHA content was higher than EPA in *Xiphopenaeus kroyeri* (Bragagnolo and Rodriguez-Amaya, 2001), in *Aristeus antennatus* and *P. longirostris* (Rosa and Nunes, 2003) in *P. monodon* (Sriket *et al*., 2007) and in *P. longirostris* and *Plesionika martia* (Oksuz *et al*., 2009).

Pigott and Tucker (1990) recommended that the n-3/n-6 ratio is a better index for comparing the relative nutritional value of fish oils from different species. A ratio of 1:1 for n-3/n-6 is considered optimal for nutritional purposes (Simopoulos, 1989). In present study, the n-3/n-6 ratio of *F. merguiensis* and *F. penicillatus* was 2.9 and 2.8, a value which is lower than reported 3.8 for *Xiphopenaeus kroyeri* and 3.9 for *P. brasiliensis* (Bragagnolo and Rodriguez-Amaya, 2001), 4.06 and 5.0 for *F. merguiensis* and *F. penicillatus* (Nisa and Asadullah, 2006) 4.5 for *P. longirostris* and 5.2 for *Plesionika martia* (Oksuz *et al*., 2009). While, n-3/n-6 ratio of 2.9 and 2.8 in present study is higher than the reported values of 2.36 for *P. semisulcatus* and 1.60 for *M. monoceros* (Yanar and Celik, 2005) and 1.3 for *P. monodon* and 1.0 for *P. vannamei* (Sriket *et al*., 2007).

It has been demonstrated that EPA, DHA and ARA are present in adequate amount in brackishwater and marine crustaceans, fish and mollusk, therefore, these should be incorporated in

the diets for the broodstocks and larvae culture (Lytle *et al.*, 1990). The studies reported by Ravid *et al.* (1999) and Wouters *et al*. (1999) supported the importance of ARA in broodstock and larval culture of *P. semisulcatus* and P. v*annamei.* The ARA found in *F. merguiensi*s and *F. penicillatus* in present study is higher than reported in the same species by Nisa and Asadaullah (2006), in *P. semisulcatus* by Yanar and Celik (2005), in *P. monodon* and *P. vannamei* by Sriket *et al*. (2007), in *F. chinensis* and *P. vannamei* by Li *et al*. (2011) but is lower than reported in *M. monoceros* by Yanar and Celik (2005). Seasonal variation in fatty acid profiles have been reported in penaeid shrimps (Bottino *et al*., 1980; Iverson *et al*., 2002; Yanar and Celik, 2005), thus there is a need to study the seasonal fatty acid composition of *F. merguiensi*s and *F. penicillatus* keeping in view its importance in human nutrition and to be used as a guideline for preparation of diets for these two species in aquaculture programs.

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