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Fatty Acid Composition of Three Species of *Siphonaria* (Gastropoda: Pulmonata) in Pakistan

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Abstract.- Fatty acid composition was determined in three pulmonate gastropod species, *Siphonaria ashgar, S. belcheri* and *S. kurracheensis* inhabiting the high, mid and low tidal zones on the coast of Mubarak Village, Karachi, Pakistan. Eleven fatty acids were abundant in all three species, that is, palmitic acid, stearic acid, palmitoleic acid, oleic acid, vaccenic acid, gadoleic acid, linoleic acid, arachidonic acid, docosatetraenoic acid, α-linolenic acid and eicosapentaenoic acid. The analysis revealed no significant qualitative differences in the fatty acid profile of three *Siphonaria* species, however the difference in quantities of different fatty acids in tissue of *S. ashgar*, *S. belcheri* and *S. kurracheensis* may be related to their distribution in different tidal zones and thus availability of different microalgal diets. The presence of excellent quantity of polyunstaurated fatty acid, that is, linoleic acid, α-linolenic acid, Arachidonic acid and eicosapentaenoic acid in three species of *Siphonaria* can be utilized as potential fishery resource for nutrition, particularly in developing countries.

Key words: Fatty acids, *Siphonaria* spp.

INTRODUCTION

The Siphonariidae (Gastropoda: Pulmonata) is a diverse family with over 60 species occurring globally (Hubendick, 1946). The majority of these species are found in the Indo-Pacific region especially in the southern hemisphere (Hubendick, 1946). Earlier reported species of *Siphonaria* from Pakistan include *Siphonaria kurracheensis, S. javanica siphon* and *S. lecanium* (Melvill and Standen, 1901). Recently Bano *et al.* (2011) reported three species of *Siphonaria*, *viz.*, *S. ashgar*, *S. belcheri* and *S. kurracheensis* from the rocky shores of Karachi.

Siphonariids are capable of feeding on a wide range of algae including microalgae, lichens, cyanobacteria and diatoms (Hodgson, 1999), while cyanobacteria was considered as principal food source in tropical regions (Chan, 2003). The shell structure and the tidal distribution of three species of false limpets, *Siphonaria ashgar, S. belcheri* and *S. kurracheensi* are different. The shell of *S. ashgar* is thick and conical and found attached on rocks at 3 meters above the mean sea level. The shell of *S. belcheri* is thick and low conical and found at 1-2 meters above the mean sea level and the shell of *S. kurracheensis* is thick and flattened and found at 0.5 meter above the mean sea level (Bano *et al.,* 2011). As the three species are found in three different tidal zones, therefore, there would probably be qualitative differences in their diets. Bano and Siddiqui (2003) have reported a difference in the distribution and diversity of cyanobacteria with respect to tidal heights on rocky shore of Buleji, the lower tidal zone exhibited higher number of cyanobacterial species than the high tidal zone. Some species were present either at high or low tidal zone only (Bano and Siddiqui, 2003). Limpets are also herbivorous grazers and considerable variation in their diets has been reported (Branch, 1981). In literature the fatty acid composition of several species of marine algae is available (Chuecas and Riley, 1969; Ben-Amotz *et al.,* 1987; Sargent *et al.,* 1989; Sukenik *et al.,* 1993; Brown *et al.,* 1997). The presence of a particular fatty acid in the tissue of marine animal may allow the researchers to make assumption regarding the microalgal diet of that animal.

In recent years, polyunsaturated fatty acids (PUFAs) have been recognized as useful factors in human health and nutrition, especially for cardiovascular diseases (Dyerberg, 1986; Kinsella, 1987a,b; Bruckner, 1992). Gastropods appear to be an excellent source of palmitic acid (16:0), oleic acid (18:1n-9), arachidonic acid (20:4n-6)

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eicosapentaenoic acid (EPA, 20:5n-3) and docoshexaenoic acid (DHA, 22:6n-3) (Joseph, 1982). Studies on the lipid and fatty acid composition of gastropod species have been reported (Voogt, 1983; Dunstan *et al.,* 1996; Brazão *et al.,* 2003; Morais *et al.,* 2003; Su *et al.,* 2004; Babu *et al.,* 2010, 2011). From Pakistan coast the fatty acid composition of *Xancus pyrum* (Usmanghani, 1989) and *Thais carinifera* (Afsar *et al.,* 2012) has been reported. However, some of these gastropods are carnivores while others are herbivore grazers. Among the *Siphonaria* species, *S. diemenensis* was reported to possess palmitic, oleic and eicosenoic acid (Johns *et al.,* 1980) and *S. denticulata* showed the novel fatty acids, 17-methyl-6-octadecenoic acid and 17-methyl-7-octadecenoic acid (Carballeira *et al.,* 2001). Though siphonariid limpets are not yet exploited for the purpose of food or bait as the patellid limpets are exploited in different parts of the world but with the increasing world population siphonariid limpets can be considered as a potential fishery resource and can be utilized as a food, particularly in developing countries.

The present study aims at (1) providing qualitatively the fatty acid composition in tissue of three species of *Siphonaria, S. ashgar, S. belcheri* and *S. kurracheensis*; (2) comparing the fatty acid composition in tissue of *S. ashgar, S. belcheri* and *S. kurracheensis* residing in three different tidal zones; and (3) assuming the microalgal diet of *S. ashgar, S. belcheri* and *S. kurracheensis* on the basis of fatty acid composition in their tissues.

MATERIALS AND METHODS

Specimens of S*iphonaria ashgar*, *S. belcheri* and *S. kurracheensis* were collected from the rocky shore of Mubarak Village, Karachi in the month of May 2008. The specimens of size ranging between 16-20 mm were removed from the substratum with the help of spatula, hand-picked and were brought to the laboratory in ice container.

Sample preparation

The samples were thoroughly washed with distilled water in the laboratory. The length of each specimen was taken and the whole of the animal containing the tissue, gonad and digestive gland was removed from the animal. The size of three species is small and the weight varied between 0.11 to 0.43 g. In order to get the desired quantity of tissue, the samples were pooled. Tissue (2 g) of S*iphonaria ashgar*, *S. belcheri* and *S. kurracheensis* was soaked in 20 ml of chilled solvents, chloroform: methanol (2:1, v/v). The samples were stored at -20ºC until further analyses.

Determination of fatty acid profiles

Lipid in the tissue was extracted by the method of Folch *et al.* (1957). Estimation of total lipid was done by gravimetric method and expressed as mg of lipid per gm of wet tissue weight. The fatty acid compositions were determined as fatty acid methyl esters (FAME). For this purpose a Gas-Chromatograph (Fisons MD800) equipped with a phenomenex ZB-WAX column dimension of 30 meter x 0.32 mm x 0.25 µm was used and fitted with cold on-column injection system, using helium as carrier gas at a flow rate of 2.0 ml/ min as fatty acid. Initial oven temperature was kept at 50°C then raised to 225°C at an inclining temperature of 40°C/ min to 150°C then at 2°C/min to 225°C and finally held for 5 min 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. To eliminate quantitative differences between the analysed samples and to get better picture of the proportion of different fatty acids, the fatty acid composition was presented as relative amount (%) of total fatty acids.

Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) at P α 0.05 using the software of the SPSS 14.0 for Windows.

RESULTS

Lipid content in Siphonaria *spp.*

Lipid in the tissue of *S. ashgar* varied from 12.4 to 17.3 mg g^{-1}, of *S. belcheri* from 16.7 to 17.2 mg g^{-1} and *S. kurracheensis* 11.8 to 15.7 mg g^{-1}.

Table I.- Fatty acids composition (% total fatty acids) from whole tissue of *Siphonaria* species. The data denotes the mean and standard deviation of samples of *S. ashgar, S. belcheri* and *S. kurracheensis*. Each sample was analyzed in triplicate.

Fatty acids	*S. ashgar*	*S. belcheri*	*S. kurracheensis*
14:0 (Myristic acid)	1.82±0.24	1.75±0.17	1.17±0.02
15:0	0.88±0.08	0.83±0.06	0.67±.013
16:0 (Palmitic acid)	19.11±0.99	19.01±1.41	17.79±0.88
18:0 (Stearic acid)	6.20±0.35	5.98±0.33	7.09±0.33
20:0	0.29±0.00	0.29±0.02	0.39±0.02
22:0	0.45±0.01	0.16±0.00	0.17±0.02
24:0	0.24±0.00	0.06±0.09	0.11±0.16
16:1n-9	1.22±0.74	3.16±0.25	2.64±.1.15
16:1n-7 (Palmitoleic acid)	5.27±0.38	4.68±0.81	3.44±0.27
18:1n-9 (Oleic acid)	4.53±1.25	4.17±0.61	3.89±.0.09
18:1n-7 (Vaccenic acid)	5.55±0.60	4.70±0.40	4.58±0.72
20:1n-11 (Gadoleic acid)	5.20±0.01	4.42±0.26	5.13±.0.24
20:1n-9	0.0±0.00	1.15±0.05	1.83±0.21
20:1n-7	2.15±0.17	2.27±0.14	2.41±0.16
22:1n-11	0.95±0.25	0.63±0.11	0.80±0.00
24:1n-9	0.17±0.00	0.14±0.02	0.04±0.06
18:2n-6 (Linoleic acid)	4.12±0.54	4.09±0.04	3.12±0.10
18:3n-6	0.20±0.06	0.27±0.06	0.14±0.04
20:2n-6 (Eicosadienoic acid)	3.15±0.00	3.08±0.53	3.60±0.22
20:3n-6	1.24±0.06	1.18±0.03	1.37±0.03
20:4n-6 (Arachidonic acid, ARA)	7.83±1.07	7.57±0.10	7.30±0.84
22:4n-6 (Docosatetraenoic acid)	3.42±0.77	5.01±0.41	5.45±0.59
22:5n-6	0.08±0.11	0.07±0.10	0.06±0.09
18:3n-3 (α-linolenic acid, ALA)	6.95±1.16	4.89±0.02	4.38±0.24
18:4n-3	0.75±0.36	0.77±0.16	0.55±0.07
20:3n-3	1.10±0.05	0.98±0.29	1.49±0.30
20:4n-3	1.01±0.13	1.32±0.24	1.91±0.10
20:5n-3 (Eicosapentaenoic acid, EPA)	3.82±0.49	4.67±0.31	5.03±0.08
22:5n-3	0.91±0.13	1.39±0.08	1.65±0.06
22:6n-3 (Docoshexaenoic acid, DHA)	0.07±0.10	0.41±0.02	0.53±0.01
16:2	1.11±0.02	0.43±0.15	0.24±0.01
16:3	0.18±0.03	0.36±0.13	0.18±0.00
16:4	2.84±0.74	3.18±0.47	4.12±0.33
20:2NMID	0.47±0.14	0.36±0.00	0.42±0.09
22:2NMID	2.69±0.78	3.05±0.18	2.57±0.77
22:2NMID	3.35±1.49	2.90±0.15	3.19±1.30
22:3NMIT	0.67±0.00	0.62±0.05	0.56±0.12

Fatty acid profile of Siphonaria *spp.*

A total of 37 individual fatty acids were identified in the muscle of *S. ashgar*, *S. belcheri* and *S. kurracheensis* (Table I). Eleven fatty acids were abundant in all three species, that is, palmitic acid (16:0), stearic acid (18:0), palmitoleic acid (16:1 n-7), oleic acid (18:1n-9), vaccenic acid (18:1n-7), gadoleic acid (20:1 n-11), linoleic acid (18:2 n-6), arachidonic acid (20:4 n-6), docosatetraenoic acid (22:4 n-6), α-linolenic acid, ALA (18:3 n-3) and eicosapentaenoic acid (20:5 n-3). On the whole, among these species the polyunsaturated fatty acids (PUFA) were found dominant (varied from 45.97 to 47.84%) followed by the SFA, saturated fatty acids (varied from 27.40 to 28.99%) (Table II).

Table II.- Comparison of abundant fatty acids (% of total fatty acid) of *Siphonaria ashgar, S. belcheri* and *S. kurracheensis*.

Fatty acids	*S. ashgar*	*S. belcheri*	*S. kurracheensis*
Total saturated	28.99	28.08	27.40
Total monounsaturated	25.04	25.31	24.76
Total n-6 PUFA	20.05	21.27	21.05
Total n-3 PUFA	14.63	14.42	15.51
Other PUFA	11.29	10.90	11.28
Total PUFA	45.97	46.59	47.84
n-3PUFA/n-6PUFA	0.73	0.68	0.74
DHA/EPA ratio	0.02	0.09	0.11
ARA/EPA ratio	2.03	1.62	1.46

In the tissues of three *Siphonaria* species, palmitic acid was the most abundant SFA, followed by stearic acid. The quantity of palmitic acid was similar in *S. ashgar* and *S. belcheri* and lower in *S. kurracheensis* but stearic acid was higher in *S. kurracheensis* than *S. ashgar* and *S. belcheri*. Among MUFA, palmitoleic acid, vaccenic acid, gadoleic acid and oleic acid were abundant but there was variation in their quantity in three species of *Siphonaria*, like palmitoleic acid was highest in *S. ashgar* and lowest in *S. kurracheensis* (Table I).

Analysis of variance (F= 0.001; df= 2; P= 0.998) showed no significant difference in total content of SFA of three species of *Siphonaria*. Similarly, no significant difference was found in the total content of MUFA (F= 0.001; df= 2; P= 0.998) of three *Siphonaria* species.

Among the n-6 PUFA the predominant fatty acid were linoleic acid, eicosadienoic acid, arachidonic acid (ARA) and docosatetraenoic acid. ARA showed no difference in *S. ashgar* (7.83%) *S. belcheri* (7.57%) and *S. kurracheensis* (7.30%). Among n-3 PUFA, the predominant fatty acids was α-linolenic acid (ALA) and eicosapentaenoic acid (EPA). ALA was higher (6.95%) in *S. ashgar* and similar in *S. belcheri* (4.89%) and *S. kurracheensis* (4.38%). EPA was highest in *S. kurracheensis* and significantly ($P<0.05$) different from *S. ashgar*. Analysis of variance ($F= 0.015$; $df= 2$; $P= 0.984$) showed no significant difference in total content of PUFA among three species of *Siphonaria*.

The amounts of n-6 PUFA in three species of *Siphonaria* were greater than those of n-3 PUFA. The *n*-3/*n*-6 PUFA ratios and DHA/EPA ratios were similar in three species while, ARA/EPA ratio was highest in *S. ashgar* and significantly ($P<0.05$) different from *S. kurracheensis* (Table II).

DISCUSSION

The present study throws light on the fatty acid composition in the tissue of three *Siphonaria* species in the month of May, that is, summer season. There is need to study the fatty acid profile in different seasons as seasonal variation in lipid and fatty acid composition has been reported in mollusk (Abad *et al*., 1995; Pazos *et al*., 1996).

The presence of particular fatty acids so called ‘‘marker’’ fatty acids in the tissue of different marine animal has allowed researchers to postulate their microalgal diet. For example, diatoms are rich in 16:0, 14:0, 16:1n-7 and EPA while dinoflagellates are richer in 16:0, 14:0, 18:4n-3 and DHA, which is rare in other microalgae. Green algae are low in EPA and DHA but are rich in 16:0, 18:2n-6 and 18:4n-3, whereas red algae are rich in 16:0 and (n-3) PUFA and ARA. In brown microalgae the main PUFA are ARA and EPA, but they also contain significant levels of (n-3) PUFA (Chuecas and Riley, 1969; Sargent *et al.,* 1989; Zhukova and Aizdaicher, 1995; Dunstan *et al.,* 1996; Lang *et al.,* 2011). Among the cyanobacteria species, the 16:0, 18:0, 16:1n-7, 16:1n-9 and 18:3n-3 were abundant (Vargas *et al.,* 1998; Lang *et al.,* 2011).

In the present study quantitatively the most abundant SFA in three species of *Siphonaria* was 16:0 followed by 18:0. The SFA, 14.0 was comparatively in very low quantity in *Siphonaria* spp., which differed from the quantity of SFA reported in limpet species, *Patella vulgata, P. rustica*, *P. depressa* and *P. ulyssiponensis* from central region of Portuguese coast where 16:0 was most abundant in all four species while 18:0 and 14:0 SFA were equally similar and lower in quantity (Brazão *et al.,* 2003). The fatty acid analysis of the *P. depressa* tissues from Portugal revealed that the quantitatively most important SFA was 16:0 while the quantity of 18:0 and 14:0 was equally similar (Morais *et al.,* 2003). Taking into account the fatty acid composition typical of each class of microalgae, we may say that *Siphonaria* species are taking cyanobacteria along with other microalgae in their diet as the tissue of *Siphonaria* showed the abundance of 18:0, which is abundant in cyanobacteria species while negligible in other microalgae. *Siphonaria karacheensis* living in low tidal zone showed higher SFA 18:0 than *S. belcheri* and *S. ashgar*, which may be due to the fact that in low tidal zone more cyanobacterial species are found than mid and high tidal zone (Bano and Siddiqui, 2003).

The major MUFA found in the tissues of pulmonate gastropods, *S. ashgar, S. belcheri* and *S. kurrachensis* were 16:1n-7, 18:1n-7, 18:1n-9 and 20:1n-9 and the quantity of these four MUFA was almost similar in each species. The earlier work on fatty acid composition in *S. diemenensis* (Johns *et al.,* 1980) revealed the presence of high amounts of 18:1n-9 and 20:1n-9 fatty acids, as well as the non-methylene interrupted fatty acids 20:2 and 22:2. The abundant MUFA reported in herbivore grazer limpets from Portuguese waters were 18:1n-9 and 18:1n-7 (Brazão *et al.,* 2003).

The PUFA, EPA and DHA have been regarded as essential fatty acids and important tissue components in a variety of molluscs, particularly bivalves (Galap *et al.,* 1999). In the present study in the tissue of *Siphonaria* spp., DHA was present in very low quantity, while EPA and ARA were found in larger quantities. The EPA has been reported to be abundant in diatoms and red microalgae while in low quantity in dinoflagellate and green microalgae

while ARA is highest in red microalgae. Brazão *et al*. (2003) reported DHA in very low amounts and EPA and ARA in larger quantities in soft bodies of *Patella* spp. from Portuguese coast. Similary, Morais *et al.* (2003) also reported that the main PUFA were EPA and ARA in *P. depressa* from Portugal. The major PUFA in marine gastropod species was ARA and EPA (Joseph, 1982) while in freshwater species was ARA and DHA (Freid *et al.,* 1993). The major PUFA in marine gastropod species was ARA while high percentages of DHA were found in freshwater species from Red Sea, Mediterranean Sea and Sea of Galilee (Go et al., 2002). However, in freshwater snail, *Melanopsis praemorsa* Ekin *et al.* (2011) reported the abundance of ARA and EPA while DHA were in low quantity. The presence of different PUFA in different species suggested that each species probably have different dietary requirements for essential fatty acids, which has been described in *Haliotis* species (Dunstan *et al.,* 1996).

The analyses of the fatty acid composition of three *Siphonaria* spp. revealed no significant qualitative differences in the fatty acid profile of three species and like other gastropods were found to be an excellent source of PUFA, that is, linoleic acid, ALA, ARA and EPA and thus can be utilized as potential fishery resource for nutrition, particularly in developing countries. The observed difference in quantities of different fatty acids in the tissue of *S. ashgar*, *S. belcheri* and *S. kurracheensis* may be related to their distribution in different tidal zones and thus availability of different microalgal diets to these species.

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