Effect of Seasonal Changes in Fatty Acids Profile of Orange Fin Pony Fish (Leiognathus bindus) and Sulphur Goatfishes (Upeneus sulphureus)

Khadijeh Nabi Ghahfarrokhi 1, Mansoreh Ghaeni 2, Ladan Zaheri 3
1. Young Researchers and Elite Club, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran
2. Department of Fisheries, College of Agriculture, Islamic Azad University, Ahvaz Branch, Ahvaz, Iran
3. Fisheries Scientific Association, Islamic Azad University, Ahvaz, Iran

Corresponding author email: mansoreh.ghaeni@gmail.com

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Abstract
In this study, the fatty acids composition and the related health lipid indices (IA, atherogenic and IT thrombogenic) of Orange fin pony fish (Leiognathus bindus) and sulphur goatfishes (Upeneus sulphureus) were studied in different season in Mahshahr port in south of Iran. Fatty acids were analyzed by Gas Chromatograph (FID). The fatty acid composition of L. bindus and U. sulphureus showed a relevant proportion (26.95% and 25.34% respectively) of poly-unsaturated fatty acids (PUFAs) with a prevalence of the n – 3 series. The IA and IT indices resulted comparable in Spring and Fall. The ratio of W3/W6 was different in Fall and Spring for both species.

Keywords
Leiognathus bindus; Upeneus sulphureus; Biochemistry; Atherogenic and Thrombogenic

Introduction
Fish is consumed as food all over the world. It is generally recognized that polyunsaturated fatty acids (PUFA) composition might vary among species of fish (Muhammad and Mohamad, 2012). It is well known that PUFA’s in fish have a beneficial effect on health by, for example, decreasing the risk of stroke, reducing serum triacylglycerol levels, reducing blood pressure, and insulin resistance and modulating the glucose metabolism. Among the PUFA’s are known components with anti-atherogenic action like LA (18:2n – 6) belonging to the n – 6 PUFA’s class, and in the more important n – 3 PUFA’s class, components such as LNA (18:3n – 3), EPA (20:5n – 3) and DHA (22:6n – 3) which are appreciated for their anti-thrombogenetic effect. On the contrary, amongs the saturated fatty acids (SFAs), lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0), are recognized as health risk factors (Garaffo et al., 2011; Sanfilippo et al., 2011).

Most fatty acids can be synthesized in the body, however human body lacks the enzymes required to produce the two essential fatty acids EPA and DHA. These fatty acids must be taken from the diet (Öksüz et al., 2011). The lipid content of fish is highly variable between and within species. Many factors appear to contribute to this variability, including food availability; catch location, fish size, maturity stage, biological variations, sampled tissue, ration size and starvation (Rueda et al., 2001).

U. sulphureus (sulphur goatfishes) is from Mullidae family in the order Perciformes (Randall and Kulbicki, 2006) distributed from Red Sea, Persian Gulf, Madagascar, Seychelles, Réunion, Pakistan, India, Sri Lanka,Andaman Sea, Indonesia, New Guinea, Fiji, NewCaledonia, Philippines and southern Japan (Uiblein et al., 2010).

L. bindus (Orange fin pony fish) known as slip mouths or slimy are a small family, Leiognathidae, of fishes in the order Perciformes (Froese and Pauly, 2006). They reported in the Red Sea (Port Sudan), Persian Gulf, coasts of India and Sri Lanka, and elsewhere in the eastern Indian Ocean; eastward to the western Central Pacific (Woodland et al., 2001).
2009). Even though these species have less demand in fresh condition, there is considerable market for dry fish and also as fishmeal especially in poultry industry.

In this study we selected two marine fish; goat fish and pony fish because they are trash fish in our trawlers, also the world total catch of them is 2643 and 111448 ton respectively (FAO, 2010).

Fishery, distribution, biology and population dynamics of them have been studied in detail (Rajkumar, 2006; Shadi et al., 2011; Sparks, 2006; Kwak and Klumpp, 2004; Ra et al., 2005; Golani et al., 2011; Murty, 1986; Abraham et al., 2011; Chakrabarty, 2008; Gholami and Zoriasatein, 2005) Since no detailed information is available on fatty acid profile from these species so the objective of this study was to determine fatty acid profiles of goatfish and pony fish and to compare their nutritional content in Fall and Spring season in Mahshahr Port.

1 Method and Materials

1.1 Samples

Goat fish and pony fish were obtained from fishermen at Mahshahr port randomly in spring and Fall season, because in other season they didn’t catch in their net. In every season, 30 fish have been collected from every species. The fish were caught the night before the procedure, kept in ice and transferred to the laboratory for analyses. The fishes were weighed, deheaded, eviscerated and cleaned prior to freezing. In an attempt to obtain a homogeneous sample from each species, their flesh were removed from their backbones, minced, blended and immediately extracted. The mean weights and total length of the fishes were: 78.25±11.6 g and 17±0.8 cm for goat fish and 13.25±5.7 gram 6.5±1.7 cm for pony fish.

1.2 Lipid Extraction

Lipid extractions were performed on minced fish samples (25g each) using the extraction methods of Folch et al. (1957): chloroform-methanol. Methylene chloride (100µL) and 1 mL 0.5M NaOH in methanol were added to oil extracts in a test-tube and heated in a water bath at 90°C for 10 min. The test tubes were removed from the water bath and allowed to cool before addition of 1 mL 14% BF3 in methanol. The test tubes are heated again in a water bath for 90°C for 10 min, and cooled to room temperature. One mL distilled water and 200-500µL hexane was added to the test tubes and then FAME (Fatty Acid Methyl Ester) was extracted by vigorous shaking for about 1 min. Following centrifugation (2000rpm), the top layer was transferred into a sample bottle for GC analysis (Chukwuemwka et al., 2008).

1.3 Fatty Acids Analysis

Fatty acid analyses were carried out using the IUPAC II.D.19 method (IUPAC, 1979). Fatty acids were analyzed using a HP Agilent 5890 system Gas Chromatograph equipped with SP-2330 and a flame ionization detector (FID). Separation of fatty acid methyl esters was achieved by using fused silica capillary column (30m × 0.25 mm × 0.20µm film thickness). The oven temperature was set at 120°C for 2 min then reached to 220°C with a ramp rate of 5°C /min, and then held for 15 min. The injector and detector temperatures were maintained at 155°C and 260°C, respectively. The carrier gas was helium 10psi with a split ratio of 1/50. The air and hydrogen of pressure were 338 ml/min and 45 ml/min respectively. Fatty acids were identified by comparing the retention times of fatty acid methyl esters(FAME) with a standard 37 component FAME mixture (Supelco-Catalog No: 18919-1Amp.).Results were expressed as the percentage of each fatty acid with respect to the total fatty acids. (Turan et al., 2007; Kaya and Turan, 2008).

1.4 Indexes of Lipid Quality

The saturated/unsaturated fatty acids (SFA/UFA) ratios were calculated including trans fatty acids in the UFA group. The atherogenicity (AI) and thrombogenicity (TI) indices were also calculated according to the following equations (Larqué et al., 2003).

From the data on the fatty-acid composition, the following were calculated:

1) Index of atherogenicity (IA): indicating the relationship between the sum of the main saturated fatty acids and that of the main classes of unsaturated, the former being considered pro-atherogenic (favoring the adhesion of lipids to cells of the immunological and circulatory system), and the latter anti atherogenic (inhibiting the aggregation of plaque and diminishing the levels of esterified fatty acid, cholesterol, and phospholipids, there- by preventing the appearance of
micro- and macro- coronary diseases).

\[
IA = \frac{[(4 \times C14:0)+C16:0+C18:0]}{\sum \text{MUFA}+\sum \text{PUFA-n6}+\sum \text{PUFA-n3}}
\]

2) Index of thrombogenicity (IT): showing the tendency to form clots in the blood vessels. This is defined as the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenic fatty acids (MU-FAs, PUFAs – n6 and PUFAs – n3) (Garaffo et al., 2011; Kaya and Turan, 2008).

The following equation was applied:

\[
IT = \frac{(C14:0+C16:0+C18:0)}{(0.5\text{MUFA}+0.5\text{PUFA-n6}+3\text{PUFA-n3}+\text{PUFA-n3}/\text{PUFA-n6})^{1.5}}
\]

1.5 Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan’s test. Statistical analysis was performed with SPSS. Significance was established at P<0.05.

2 Results

2.1 Fatty acid profile

The fatty acid composition of goatfish and pony fish are shown in Table 1 and Figure 1.

Table 1 Fatty acid profile (%) of *L. bindus* and *U. sulphureus* in Fall and Spring

| Fatty acid | *Leiognathus bindus* | *Upeneus sulphureus* |
|------------|-----------------------|-----------------------|
|            | Fall                  | Spring                | Fall                  | Spring                |
| (12:0)     | 0.1±0.02              | ND                    | 0.25±0.01             | ND                    |
| (14:0)     | 5.49±0.01             | 5.95±0.00             | 2.9±0.03              | 2.45±0.01             |
| (15:0)     | 0.47±0.00             | ND                    | 0.34±0.00             | ND                    |
| (16:0)     | 29.57±0.03            | 33.97±0.01            | 27.49±0.02            | 39.7±0.05             |
| (17:0)     | 1.04±0.02             | 1.36±0.00             | 0.92±0.05             | 1.76±0.02             |
| (18:0)     | 10.75±0.02            | 10.25±0.02            | 16.41±0.03            | 11.97±0.03            |
| (20:0)     | 0.15±0.03             | 1.78±0.02             | 1.97±0.01             | ND                    |
| (21:0)     | 0.43±0.00             | ND                    | 0.53±0.00             | ND                    |
| (22:0)     | 0.6±0.01              | 1.06±0.04             | ND                    | 1.8±0.00              |
| (23:0)     | 0.08±0.01             | ND                    | ND                    | ND                    |
| (24:0)     | 1.32±0.02             | ND                    | 1.59±0.02             | ND                    |
| (14:1)     | 1.57±0.10             | 1.08±0.02             | 1.36±0.04             | 0.89±0.02             |
| (16:1)     | 9.59±0.05             | 13±0.01               | 5.27±0.03             | 6.97±0.03             |
| (18:1)     | 12.09±0.01            | 18.4±0.00             | 15.40±0.09            | 24.45±0.06            |
| (20:1)     | 0.71±0.01             | 1.11±0.03             | 0.83±0.03             | ND                    |
| (22:1)     | 0.08±0.03             | ND                    | 0.35±0.02             | ND                    |
| (24:1) \(\omega 9\) | 0.87±0.02             | ND                    | 0.29±0.00             | ND                    |
| (18:2) \(\omega 6\) | 0.66±0.03             | 0.8±0.03              | 1.12±0.03             | 0.85±0.00             |
| (20:2) \(\omega 6\) | 0.29±0.05             | ND                    | 0.25±0.01             | ND                    |
| (18:3) \(\omega 3\) | 0.36±0.05             | 0.61±0.06             | 0.29±0.00             | 0.33±0.01             |
| (20:3) \(\omega 3\) | 1.07±0.00             | 0.47±0.01             | 1.54±0.01             | 0.5±0.00              |
| (20:4) \(\omega 6\) | 0.27±0.02             | ND                    | 0.23±0.01             | ND                    |
| (22:5) \(\omega 3\) | 2.99±0.01             | 4.58±0.02             | ND                    |
| (22:6) \(\omega 3\) | 1.66±0.00             | 1.74±0.01             | 0.46±0.00             |
| EPA \(\omega 3\) | 5.7±0.01              | 4.63±0.02             | 4.65±0.03             | 2.43±0.01             |
| DHA \(\omega 3\) | 3.35±0.02             | 3.78±0.04             | 4.5±0.00              | 4.33±0.02             |
| SFA        | 50.004±0.02           | 54.37±0.02            | 52.4±0.12             | 57.68±0.05            |
| MUFA       | 24.919±0.03           | 33.59±0.00            | 23.5±0.03             | 33.31±0.01            |
| PUFA       | 17.27±0.01            | 11.95±0.03            | 18.9±0.04             | 8.9±0.02              |
| HUFA       | 14.62±0.02            | 10.07±0.01            | 15.47±0.02            | 7.22±0.05             |

Note: \(n=3\) ± standard error; SFA: saturated fatty acid, MUFA: mono unsaturated fatty acid, PUA: poly unsaturated fatty acid, HUFA: high unsaturated fatty acid; ND: Non-detected
The amount and number of fatty acid were different in Fall and Spring for each species. A total of 24 fatty acids in goatfish and 26 fatty acids for pony fish were identified in this study in Fall. The composition of saturated (SFA), mono-unsaturated (MUFA), poly-unsaturated fatty acid (PUFA) and high unsaturated fatty acid (HUFA) of goatfish were found to be 52.4%, 23.5%, 18.9% and 15.47% in Fall, while those of pony fish were found to be 50.004%, 24.919%, 17.27% and 14.62% respectively. Accordingly, the SFA level in goatfish and the MUFA level in pony fish were the highest among the other fatty acid groups. The PUFA/SFA ratios for goatfish and pony fish were 0.36 and 0.34 respectively in Fall.

### 2.2 Lipid quality indices

Table 2 and Figure 2 show total lipids IA and IT values for *L. bindus* and *U. sulphureus* fat. IT values were significantly higher (1.154±0.0) in *U. sulphureus* than *L. bindus* in spring. The ratio of W3/W6 was significantly different in fall and spring for both species (p=0.05). IA value for *U. sulphureus* was the lowest (1.376).

### 3 Discussion

Fish consumption is increasingly recommended by health authorities, not only for its high-quality protein content, but also for being a source of fatty acids considered highly beneficial for human health (n3 and n6). Therefore, it is not surprising that there is higher demand for fish with a growing concern for the health aspects of the diet (Senso et al., 2007). Marine fish have a higher fraction of monounsaturated fatty acid (MUFA) and PUFA than freshwater fish (Chedoloh et al., 2011).

Previous studies have revealed that the lipid and moisture composition of fish can differ depending on seasonal changes, age, maturity, sex, availability of food, and spawning period (Yeannes and Almandos, 2003; Ackman, 1989).

It was observed that the concentrations of C12:0, C15:0, C21:0, C23:0 and C24:0 saturated fatty acids were found to disappear. Also palmitic acid (C16:0) observed as a major constituent of lipid, showed an increasing in spring for both species. Our findings are in close agreement with the results reported by Öksüz et al., (2011) in both gold band goatfish and striped red mullet and also by Hassan et al., (2010) for the saturated fatty in wild *Catla catla*.

In mono-unsaturated fatty acids (MUFA), C18:1 fatty acid was observed in the highest concentration and ranged from 12.09±0.01 to 18.4±0.00 for *L. bindus*
and $15.40\pm0.09$ to $24.45\pm0.06$ for *U. sulphureus* in fall and spring respectively. It is similar to those previously reported in gold band goatfish, striped red mullet (Öksüz et al., 2011) and wild *Catla catla* (Hassan et al., 2010). This result may be attributed to the effect of fish digestion.

The level of PUFA ($18.08\pm0.01$) and HUFA ($15.04\pm0.03$) in fall were found to be higher than that of them in spring ($10.45\pm0.02$ and $8.64\pm0.00$ respectively). Even in low concentrations, these polyunsaturated fatty acids are an important group of lipid metabolism and are bases for the formation of arachidonic acid C20:4 (n-6) and eicosapentaenoic acid 20:5 (n-3), precursors for eicosanoic acid and the relative levels of these two fatty acids have profound effect on the formation of very active substances metabolically (Sargent et al., 1995). Polyunsaturated fatty acids (PUFA-n-6) which dominate structural lipids and, like monoenes, are highly affected by factors suchas lipid contents, growth levels and body weight.Particularly as precursor fatty acids to eicosanoids and arachidonic acid, PUFA (n- 6) are metabolized from adipose tissues (Voss et al., 1991; Sargent et al. 1995).

In our study two distinct indexes were investigated: 1) Atherogenic index (IA); and 2) Thrombogenic index (IT). These indexes take into account the different effects that single fatty acid might have on human health and in particular on the probability of increasing the incidence of pathogenic phenomena, such as atheroma and/or throm-bus formation. In this research *Leiognathus bindus* was the best IT indices ($0.886\pm0.001$ and $0.810\pm0.01$ in spring and fall respectively) .The IT and IA indices in both *L. bindus* and *U. sulphureus* determined in this study is quite different from the level of them previously measured in products of *Thunnus thynnus* by Garaffo et al. (2011).Those indices was lower and better that these indices.

In conclusion *L. bindus* and *U. sulphureus* showed a considerable amount of saturated and unsaturated fatty acids in both fall and spring. Polyunsaturated fatty acids (PUFA) are found to be one of the highest compounds found in these fishes and n-3 PUFA is studied extensively in both types of fishes. The n-3 polyunsaturated fatty acid which is primarily docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) was found in appreciable amounts (Muhamad and Mohamad, 2012).

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