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Proximate composition and fatty acid profile in different tissues of juvenile *Siganus guttatus* fed with *Enteromorpha prolifera*

C. Song, F. Zhao, J.Y. Liu, Y. Wang, X.R. Huang, P. Zhuang

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ABSTRACT

The proximate composition and fatty acid profile of five tissues (head, skin, bones, muscle and liver) of juvenile *Siganus guttatus* fed with *Enteromorpha prolifera* were investigated. The results indicated that: (1) The content of protein and fat among these five tissues showed a significant differences ($P<0.05$). The muscle has the highest content of protein and the lowest content of fat. (2) The highest content of fatty acid in SFA is 16:0 in all five tissues, the highest content of fatty acid in MUFA is18:1n-9c (cis) in skin, bones, muscle and liver, while the highest content of PUFA is DHA in muscle. Muscle contained the highest value of DHA and EPA, the total content was more than 100 times in bones. Muscle tissue is rich in many important polyunsaturated fatty acids such as EPA, DPA, DHA, ARA and ALA, which has better nutritional quality and immune and health function.

Key words: *Enteromorpha prolifera*, Fatty acid profile, Proximate composition, PUFA, *Siganus guttatus*.

Abbreviations: SFA: saturated fatty acids, MUFA: mono-unsaturated fatty acids, PUFA: poly unsaturated fatty acids, HUFA: highly unsaturated fatty acid, DHA: docosahexaenoic acid, EPA: eicosapentaenoic acid, DPA: docosapentaenoic acid; ARA: arachidonic acid; ALA: a-linolenic acid.

INTRODUCTION

Siganus guttatus is a member of the family Siganidae within the order Perciformes, is mainly found in the tropical and subtropical Indian, Pacific and South China Sea (Woodland, 1990). It is an omnivorous fish, tolerant to a wide range of salt concentration, with high adaptability to the environment (Zhao *et al*., 2013; Song *et al*., 2015 b). *S. guttatus* can be cultured preferably due to its rapid growth and excellent disease resistance and thus brings considerable economic benefits to the farmers (Wang *et al*., 2011; Song *et al*., 2012). It is an environmental friendly fish that is able to restore ecosystem (Wang *et al*., 2015). *S. guttatus* takes a key role in cage cleaning due to its habitat of ingesting algae attached on cage, especially *Enteromorpha prolifera* (Zhao *et al*., 2009; Song *et al*., 2012).

E. prolifera, a common green alga near the top of the shore, on rocks or other algae, on open coasts or in estuaries and harbours, belongs to the family Ulvaceae of the order Ulvales within the phylum Chlorophyta (Wang *et al*., 2006). Due to global warming and water eutrophication, *E. prolifera* have grown rapidly in recent years, leading to the frequent occurrence of green tide, and great losses to aquaculture in the surrounding areas (Wu *et al*., 2009).

At present, *S. guttatus* that feed on *E. prolifera* are used to control the overgrown of *E. prolifera* in aquaculture waters (Wang *et al*., 2015). To date, there are studies focusing on growth, digestive enzymes, serum biochemical, antioxidant enzymes and the muscle nutrition of *E. prolifera* (Hu *et al*., 2015; Song *et al*., 2015 a; Song *et al*., 2016; Song *et al*., 2017 b), while the proximate composition and fatty acid profile in different tissues of *S. guttatus* have not been reported. The general aim of the present study was to analyze the effects of *E. prolifera* on proximate composition and fatty acids profile in different tissues of *S. guttatus*, to reveal the utilization value of *E. prolifera* and to evaluate the edible value of different tissues of *E. prolifera*-*feeding S. guttatus*.

East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai, 200 090, China.

Corresponding Author: P. Zhuang, F. Zhao, East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai 200090, China. Email: pzhuang@ecsf.ac.cn; zhaof@ecsf.ac.cn

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MATERIALS AND METHODS

The *S. guttatus* juveniles were artificially bred by Qionghai Research Center of East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences and air-transported to Weihai in Shandong Province. The juveniles those were healthy and similar in size were selected for test. Their average body length was (3.99±0.23) cm, and average body weight was (2.1± 0.2) g. The *E. prolifera* was collected from the coast of the Yellow Sea in China, with the proximate composition of crude protein, fat and ash was 22.58%, 0.26% and 15.88%, respectively.

Experimental design

The experiment was carried out in the sea cucumber farming bay in Xiaoshidao National Special Marine

Protected Area in Weihai City, Shandong Province. The *S. guttatus* juveniles were reared in net pens of 2.5 m × 1.5 m × 1.5 m and mesh size of 0.5 mm. The *S. guttatus* juveniles were equally assigned into three replicates with 30 fish in each. The experiment lasted for 90 d, from June 24 to September 19. The *S. guttatus* juveniles were fed to satiation with enough fresh *E. prolifera* at 05:30, 09:00, 13:00 and 17:00 every day, and the feed unconsumed after every feeding was removed in time.

Sample preparation

At the end of the experiment, the fish were fasted for 24 h. Thirty individuals were randomly selected (ten from each net pen), anesthetized with 200 mg/L tricaine mesylate (MS-222). The body length and body weight of each fish were measured. The results showed that the fish body length was (6.60 ± 0. 58) cm, and the body weight was (9.61 ± 0.61) g. The 30 fish were randomly assigned into six subgroups, with five individuals in each subgroup, and immediately dissected on an ice plate. The fresh muscle samples of each subgroup were collected and mashed together before the measurement of the proximate composition and fatty acids components.

Sample determination

Moisture, protein, fat and ash contents were determined according to the AOAC method (AOAC, 1990); fatty acids were extracted according to the ISO5509 method (ISO, 2000)(Song *et al*., 2017 a).

Statistical analysis

One-way ANOVA was used to determine the differences among these five different tissues. Data are presented as means ± SD and $P<0.05$ was regarded as the statistically significant level. All statistics were performed using SPSS package (version 15.0).

RESULTS AND DISCUSSION

Proximate composition

Table 1 shows the content of the proximate composition in different tissues of juvenile *S. guttatus* fed with *E. prolifera*. The highest moisture content was in liver and the lowest in the bones. The highest protein content was observed in muscle and the lowest in liver. The highest fat content was present in liver and the lowest was detected in muscle. The highest ash content was noted in head, followed by bones and lowest in the muscle. The content of protein and fat among these 5 tissues showed a significant differences ($P<0.05$).

Fatty acid profile

Table 2 shows the fatty acid profile in different tissues of juvenile *S. guttatus* fed with *E. prolifera*. The highest content fatty acid in the SFA was 16: 0, with the same results reported for *Scorpaena porcus* muscle (Kaya and Kocatepe, 2014); followed by 14: 0 in head, skin and bones and followed by 18: 0 in muscle and liver, respectively. Comparison of the content of 14: 0, 16: 0 and 18: 0 in these 5 tissues showed that the content of 14:0 in bones was significantly higher than that in other tissues ($P<0.05$), while the content of 16: 0 and 18: 0 in liver was significantly higher than that in the rest tissues ($P<0.05$). Among MUFAs, oleic (18:1) and palmitoleic (16:1) acids were the predominant fatty acids, with the same results reported for *Oncorhynchus mykiss* muscle (Sarma *et al.*, 2015). The highest content fatty acid in MUFA was 16:1 in head, while that was 18:1n9c in other tissues. The content of 18:1n9c in skin were highest among all these 5 tissues ($P<0.05$). The highest content fatty acid in PUFA was 22:6n3 (DHA) in muscle, while that was 18:2n6c in other tissues. Muscle contained the highest value of DHA and EPA, the total content was almost 4 times more than that in liver, 6 times in skin, 25 times in head, and 100 times in bones. In addition, the content of 22:5n3 (DPA) in muscle was also higher with the content of (5.61±0.30)%. The DPA content in the muscle was significantly higher than that in the rest tissues ($P<0.05$).

Comparison of the total amount of different kinds of fatty acids, the SFA in the liver and bones were significantly higher than that in the rest tissues ($P<0.05$), and the MUFA in bones was significantly higher than that in the rest tissues ($P<0.05$). The muscle contained the highest content of PUFA, while the bones contained the lowest. The amounts of n3 PUFA decreased gradually according to the order of muscle, liver, skin, head and bones. In the head, skin and bones, the amount of n3 PUFA were significantly lower than that of n6 PUFA, while in muscle and liver, the amount of n3 PUFA were significantly higher than that of n6 PUFA.

This study showed that the contents of proximate contents in different tissues were significantly different. The liver has the highest content of moisture and fat, the muscle has the highest content of protein, and the head has the highest content of ash. The nutritive components in muscles of juvenile *S. guttatus* fed with *E. prolifera* and

Table 1: Proximate composition in different tissues of juvenile *S. guttatus* fed with *E. prolifera.*

Proximate (%)	Head	Skin	Bones	Muscle	Liver
Moisture	76.34±0.29^{a}	75.74±0.01^{a}	70.43±0.24^{b}	79.78±1.29^{c}	81.65±1.18^{d}
Protein	46.03±0.58^{a}	79.50±2.41^{b}	56.29±1.48^{c}	87.88±1.14^{d}	35.93±0.11^{e}
Fat	31.85±1.46^{a}	14.35±2.95^{b}	22.06±0.82^{c}	9.42±0.40^{d}	51.55±0.40^{e}
Ash	22.12±1.59^{a}	6.15±1.54^{b}	21.65±0.96^{a}	2.70±0.20^{c}	12.52±0.29^{d}

All values are represented as Means ± SD (n = 6). Values in the same row that do not share common letters indicate significant difference (P< 0.05).

artificial feed have been conducted (Song *et al*., 2017 b). In this study, the content of fat in the muscle of juvenile *S. guttatus* not only was significantly lower than that of juvenile *S. guttatus* fed with artificial feed (Song *et al*., 2017 b), but also lower than that of the other tissues. As the *E. prolifera* has a lower content of fat (0.26%), which was significantly lower than that in the artificial feed (7.94%) (Song *et al*., 2017 b). So the *S. guttatus* had to spend more energy to cut algae during feeding, which avoid the excessive accumulation of fat in their body (Song *et al*., 2017 b). The above results showed that the muscle of S. *guttatus* fed with *E. prolifera* has the highest content of protein and the lowest content of fat, which is consistent with the composition characteristics of protein and fat in *E. prolifera*. The contents of protein and fat are two indices commonly used to assess the quality of fish meat. Fish meat with a

Table 2: Fatty acids profile in different tissues of juvenile *S. guttatus* fed with *E. prolifera.*

Fatty acids (%)	Head	Skin	Bones	Muscle	Liver
12:0	0.46±0.02^{a}	-	0.59±0.01^{b}	-	-
14:0	9.49±0.49^{a}	6.49±0.10^{b}	10.35±0.02^{c}	3.77±0.04^{d}	3.40±0.22^{e}
15:0	0.95±0.06^{a}	0.65±0.06^{b}	0.92±0.01^{a}	0.38±0.03^{c}	0.56±0.04^{d}
16:0	38.67±0.91^{a}	37.07±1.21^{b}	40.78±0.07^{c}	27.67±0.61^{d}	43.43±0.29^{e}
17:0	0.70±0.02^{a}	-	0.70±0.00^{a}	0.44±0.03^{b}	0.95±0.07^{c}
18:0	5.92±0.03^{a}	6.25±0.18^{b}	5.61±0.03^{c}	6.38±0.07^{b}	12.06±0.41^{d}
20:0	0.30±0.00^{a}	0.37±0.02^{b}	0.35±0.00^{c}	0.21±0.01^{d}	0.33±0.02^{e}
21:0	0.08±0.00	-	-	-	-
22:0	0.36±0.00^{a}	0.36±0.00^{a}	0.44±0.01^{b}	0.20±0.01^{c}	-
23:0	0.08±0.00	-	-	-	-
24:0	0.32±0.00^{a}	-	0.37±0.01^{b}	-	-
16:1	17.27±0.13^{a}	14.93±0.60^{b}	16.65±0.02^{a}	9.15±0.41^{c}	10.61±1.01^{d}
17:1	0.54±0.35^{a}	-	0.35±0.17^{a}	0.06±0.03^{b}	-
18:1n9t	-	-	-	-	0.17±0.00
18:1n9c	16.20±0.44^{a}	19.12±0.93^{b}	17.67±0.03^{c}	14.23±0.17^{d}	15.53±1.08^{a}
20:1n9	0.59±0.03^{a}	0.70±0.04^{b}	0.52±0.00^{c}	0.69±0.02^{b}	0.78±0.10^{d}
22:1n9	0.22±0.01^{a}	-	0.04±0.04^{b}	-	-
24:1n9	0.21±0.00^{a}	-	0.37±0.11^{b}	-	-
18:2n6t	0.13±0.01	-	-	-	-
18:2n6c	2.95±0.19^{a}	4.55±0.54^{b}	2.23±0.05^{c}	5.12±0.05^{d}	2.15±0.17^{c}
20:2	0.29±0.00^{a}	1.02±0.08^{b}	0.24±0.06^{a}	1.91±0.14^{c}	0.60±0.02^{d}
22:2	0.38±0.00^{a}	0.78±0.08^{b}	-	2.25±0.04^{c}	0.63±0.04^{d}
18:3n6	0.36±0.01^{a}	0.59±0.03^{b}	-	1.01±0.04^{c}	0.31±0.01^{d}
18:3n3(ALA)	1.91±0.01^{a}	2.38±0.09^{b}	1.40±0.00^{c}	3.17±0.15^{d}	1.61±0.11^{e}
20:3n6	0.24±0.01^{a}	0.51±0.06^{b}	-	1.10±0.01^{c}	0.46±0.02^{d}
20:3n3	0.35±0.00^{a}	0.41±0.04^{b}	0.30±0.03^{c}	0.55±0.01^{d}	0.25±0.03^{e}
20:4n6(ARA)	0.26±0.00^{a}	0.98±0.01^{b}	-	3.60±0.18^{c}	1.40±0.09^{d}
20:5n3(EPA)	0.23±0.00^{a}	0.87±0.01^{b}	0.05±0.00^{c}	4.33±0.12^{d}	0.94±0.13^{b}
22:5n3(DPA)	0.31±0.01^{a}	0.98±0.11^{b}	-	5.61±0.30^{c}	1.80±0.04^{d}
22:6n3(DHA)	0.25±0.00^{a}	0.99±0.13^{b}	0.06±0.00^{a}	8.21±0.71^{c}	2.04±0.16^{d}
SFA	57.32±0.49^{a}	51.19±1.53^{b}	60.11±0.08^{c}	39.05±0.57^{d}	60.73±0.25^{c}
MUFA	35.02±0.71^{a}	34.75±0.37^{a}	35.61±0.00^{b}	24.09±0.21^{c}	27.09±0.16^{d}
PUFA	7.66±0.22^{a}	14.06±1.16^{b}	4.28±0.08^{c}	36.86±0.77^{d}	12.19±0.41^{e}
EPA+DHA	0.49±0.00^{a}	1.86±0.12^{b}	0.12±0.00^{c}	12.54±0.62^{d}	2.98±0.28^{e}
HUFA	3.92±0.02^{a}	7.70±0.46^{b}	1.81±0.03^{c}	27.58±0.93^{d}	8.81±0.26^{e}
n3 PUFA	3.05±0.00^{a}	5.62±0.36^{b}	1.81±0.03^{c}	21.87±0.78^{d}	6.64±0.18^{e}
n6 PUFA	3.95±0.22^{a}	6.63±0.64^{b}	2.23±0.05^{c}	10.83±0.12^{d}	4.32±0.25^{a}
EPA/DHA	0.92	0.87	0.86	0.53	0.46
n3/n6 PUFA	0.77	0.85	0.81	2.02	1.54

n3 PUFA including ALA, 20:3n3, EPA, DPA, DHA; n6 PUFA including 18:2n-6t, 18:2n-6c, 18:3n-6, 20:3n-6, ARA; t (trans) = 'on different side'; c (cis) = 'on same side'; –, 'not detected'

All values are represented as Means ± SD (n = 6). Values in the same row that do not share common letters indicate significant difference ($P < 0.05$).

high fat content may lead to the high intake of fat by human. On the other hand, a high fat content will lead to loose muscle texture and reduce the taste quality of meat (Thakur *et al*., 2002; Thakur *et al*., 2003).

This study showed that fat and fatty acid composition was different in these 5 tissues. Fat, especially PUFA-rich fat, is an indispensable substance that makes meat aromatic and juicy (Bing *et al*., 2005). PUFA is effective in lowering blood lipid level and blood pressure, inhibiting platelet aggregation, improving biomembrane fluidity and anti-tumor and immune regulation (Bing *et al*., 2005). The *S. guttatus* in this study all ingested *E. prolifera*, which is rich in protein, fatty acids, amino acids, minerals and other nutrients, has been attracted wide attention as a new feed resource (Sun *et al*., 2011; Lin *et al*., 2015). *E. prolifera* has significantly higher content of EPA and DHA of (8.76%), but much lower content of 18:2n6c and 18:3n-3 (ALA) (8.06%) (Song *et al*., 2017 b). According to previous studies, herbivorous marine fish is able to convert 18:2n6c and ALA to ARA, EPA, DHA and other highly unsaturated fatty acids (Zheng *et al*., 2004; Li *et al*., 2008). In this study, there were not enough precursors (like 18:2n6c and ALA) in *E. prolifera* for the biosynthesis of ARA, EPA and DHA. However, the results revealed that the content of EPA and DHA in the muscle (12.54%) of *S. guttatus* fed with *E. prolifera* was much higher than that in liver (2.98%). As the liver is the fatty acid synthesis tissue, in which the content of the synthetic precursor substance of 18:2n6c and ALA (3.76%) was significantly lower than that in the muscle (8.29%), which further explained that the high level of n3 PUFAs, like EPA and DHA, in the muscle of *S. guttatus* was enriched from the diet of *E. prolifera.* EPA and DHA are essential fatty acids for human and animal growth and development, and effective for the prevention and treatment of heart disease and diabetes (Zhang *et al*., 2010). Therefore, the quality of fish fat depends largely on the content of HUFA, as EPA and DHA (Xu *et al*., 2010). The ratio of n3/n6 PUFAs in head, skin, bones, muscle and liver tissues of *S. guttatus* accounted for 0.77, 0.85, 0.81, 2.02 and 1.54, respectively. The ratio is a good index for comparing relative nutritional value of fish oils in different tissues, and a higher ratio of n3/n6 PUFAs has often been cited as an index of high nutritional value (Das *et al*., 2009). In addition, human body is unable to synthesize ALA, the precursor of n3PUFA, but can obtain it though diet. ALA can improve human intelligence, inhibit allergic reactions and brain hemorrhage, lower blood lipid level and blood pressure, etc. (Li *et al*., 2001). In this study, the content of ALA was highest in the muscle, which showed that the muscle has the better ALA nutritional quality than other tissues.

CONCLUSION

On the whole, the content of PUFA, n3 PUFA, EPA, DHA, DPA, ARA, ALA and n3/n6 PUFAs were the highest in the muscle tissue of juvenile *S. guttatus*, therefore, the muscle tissue is the main feeding part, which has better nutritional quality and immune and health function. In short, the muscle of *E. prolifera*-fed *S. guttatus* is characterized by high crude protein and low fat content, and is also rich in EPA, DPA, DHA, ARA and ALA and thus has a positive effect on human immunity and health.

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