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Comparisons of DHA and EPA contents in skin and muscle between normal and albino *Paralichthys olivaceus*

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Abstract: In this study, the variations of contents of DHA (22:6n - 3) and EPA (20:5n - 3) in the skin and muscle of normal and albino Japanese flounder growing under the same environment and fed with the same diets were determined by using gas-chromatographer. The results showed that the contents of EPA are similar in the skin and muscle of both normal and albino fish, while the content of DHA in the skin of albino fish is significantly lower than that in the skin of normal fish. The contents of DHA in muscle of normal and albino fish show no difference. Moreover, the ratios of DHA/EPA in the skin and muscle of albino fish are a little lower than those in the skin and muscle of the normal ones. So we suggest that the skin of albino fish has low capability of cumulating and utilizing DHA. This result is helpful for further understanding the utilization of fatty acids in fish bodies, and evaluating the effects of diets enriched with n - 3 unsaturated fatty acid in reducing the albinism of flatfish.

Key words: DHA; EPA; skin; *Paralichthys olivaceus*; albinism

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1 Introduction

Malpigmentation of hatchery-reared flatfish juveniles remains a major problem for mariculturists. Its irreversible nature and undesirable appearance will reduce the market value of suffering fishes[1]. Larval nutrition has been considered as one of possible factors determining normal pigmentation patterns, since flatfish larvae fed with natural diets of zooplankton generally exhibit a higher rate of normal pigmentation in comparison with those fed with *Artemia nauplii*[2-3].

The absolute and relative contents of docosahexaenoic acid (DHA, 22 : 6n - 3) and eicosapentaenoic acid (EPA, 20:5n - 3) in the diets of marine larvae have received considerable attention recently[4-7]. In addition to the maintenance of structural and functional integrity of fish cell membranes, DHA is particularly important for normal development and function of neural systems and retina[8], and altered EPA contents affect the production and modulation of eicosanoids[9-11] which are involved in central neural system of mammals controlling the release of hormones at the hypothalamus - pituitary axis and playing an important role in neural transmission.

In flatfish, the metamorphosis and development of pigmentation are controlled both neurally and hormonally[12], thus the abnormality of DHA and EPA contents in fish body could cause serious

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developmental consequences including malpigmentation. A few researchers have reported the positive correlation between the decreasing of albinism of flatfish and the increasing of levels of DHA or ratio of DHA/EPA in diets[5]. In these studies, larvae were divided into several groups and fed with different diets, such as zooplankton, rotifer and *Artemia* enriched with marine oil micellar emulsions in different levels, and were measured the contents of fatty acids in different tissues of fish. The rates of malpigmentation of these groups were compared, and the effects of fatty acids such as DHA and EPA were evaluated. It is not clear why some fish are albino and the others are normal in the same group fed with the same diets and grew under the same environmental conditions.

The aim of this study is to evaluate the contents of EPA and DHA in muscle and skin of Japanese flounder juveniles fed with same diets and under the same rearing environment. The possibilities of inherent factors of fish responsible for albinism are discussed as well.

2 Materials and Methods

2.1 Samples

Two groups of Japanese flounder juveniles (group A: 5.0cm total length in average) and young fishes (group B: 15.0cm total length in average) were collected randomly from Huaxin Seafood Product Corporation in Weihai, Shandong Province, China. These juveniles were fed with commercial micro-particle diets and the young fish were with frozen fresh food. Each group included 10 individuals of normal fishes and 10 individuals of albino fishes which grew in the same rearing tank. These live fishes were transported to Lab. of Fish Behavior, Ocean University of China. All the skin of ocular side in each fish was peeled off. The muscle in the same section of normal and albino fish bodies were collected too. All specimens of skin and muscle were frozen at $-20℃$.

2.2 Determination of fatty acid contents

The sampled skin and muscle were lyophilized at $-35℃$ for 24 hours, and were ground into powder respectively. 80.0mg powder was put into tube and dissolved in chloroform/methanol (2∶1 v/v) with stirring, and 1mL NaOH – CH_3OH solution was added. The solution was incubated at 75℃ for 15 minutes. After cooling, 2mL HCl – CH_3OH solution was added in the tube to adjust pH value less than 2, then the solution was incubated at 75℃ for another 15 minutes making the fatty acids be methylated. Finally 1mL of hexane was added with stirring. The extract was used for analysis.

The extract was put into a HP6890 Ⅱ (HP, USA) gas chromatographer equipped with a BPX 70 column 50m × 0.2mm, using hydrogen as carrier gas at a pressure of 100skg Pa. The injection and detection temperatures were 25℃. Peak identification and quantification were done in a calibrated plotter integrator by comparing with known standards and well-characterized fish oil.

2.3 Statistical analysis

The contents of lipid acids were calculated. Variance of the data is given as the standard deviation of the mean of three replicates with differences being tested for statistical significance ($P < 0.05$) by one-way ANOVA. Frequency data were standardized using a square root transformation before analysis. Regression curves and correlation coefficients were calculated in order to test the incorporation of dietary fatty acids into body tissues.

3 Results

Average percent contents of DHA and EPA in skin and muscle of normal and albino fish are presented in Tab.1.

Tab.1 Percent contents of DHA and EPA and the ratios of DHA / EPA in skin and muscle of normal and albino *P. olivceus* (group A, B) **% dry matter**

samples	skin			muscle		
	DHA	EPA	DHA/EPA	DHA	EPA	DHA/EPA
group A normal	14.510±0.179	7.322±1.357	1.982	16.263±1.001	4.923±0.148	3.303
albino	6.870±1.900	3.598±1.268	1.909	13.165±1.312	5.372±0.486	2.627
ANOVA	$P<0.01$	$P>0.05$		$P>0.05$	$P>0.05$	
group B normal	13.150±0.910	4.574±0.194	2.869	20.879±0.536	4.501±0.142	4.720
albino	9.001±0.850	4.420±0.180	1.663	19.008±2.089	4.563±0.721	4.166
ANOVA	$P<0.01$	$P>0.05$		$P>0.05$	$P>0.05$	

Tab.1 showed that the absolute contents of EPA ranged from 3.598 to 7.322 per cent of total fatty acid in skin and muscle of sampled albino and normal fish. No significant differences ($P>0.05$) were observed in EPA contents among skin and muscle in these two groups of fish (group A, B). The absolute contents of DHA were 2 – 4.5 times higher than those of EPA in the same tissues of albino or normal fish. However, in skin and muscle of normal and albino fish, the absolute contents of DHA changed dramatically. Both in normal and albino fish, the absolute contents of DHA in muscle were over 13% of total fatty acids, but the absolute contents of DHA in muscle of albino fish were slightly lower than that of normal fish. There is still no significant difference between them at the level of $P<0.05$. The highest content was obtained (20.879%) in muscle of normal fish (15.0cm in average TL). The absolute contents of DHA in skin were generally lower than those in muscle of the same fish. But the largest difference of DHA contents was obtained between the skin of albino and normal fish. It was noticed that the absolute contents of DHA in skin of albino fish were significantly lower, and about half of that value in skin of normal fish in the same group. The statistical analysis showed that there was significant difference between them at the level of $P<0.01$.

In comparison with the ratio of DHA to EPA in these tissues (Tab.1), the results showed that in these two groups of fish, the ratios of DHA/EPA in skin and muscle of albino fish were a little lower than those in normal skin and muscle. But the ratios of DHA/EPA in albino and normal muscle of fish were both higher than those in sampled skin.

4 Discussion

A number of studies have shown that pigmentation of Japanese flounder is greatly affected by the quality of diets given during larval stage[2, 7, 13], especially by (n – 3) highly unsaturated fatty acids ((n – 3) HUFA), including DHA and EPA[14]. Fatty acid compositions of tissues are partly determined by the levels of fatty acids available from the diets. The living feeds, such as *Artemia* and Rotifer, enriched with DHA and EPA rich fish oil, could improve larval pigmentation significantly[12, 15-17]. However, such conclusion was challenged by the observation that larval pigmentation is not determined by DHA absolute content, but rather the ratio of DHA/EPA in larval body[18]. Kanazawa[14] suggested that lacks of pigmentation accompanied with abnormal responses to light stem from the retinal abnormality caused by deficient experimental diets. Low levels of dietary vitamin A and DHA may interfere with the rhodopsin synthesis in the retina. This defect could in turn disrupt transmission of signals from the retina to the central nervous system, which is required for triggering pituitary production of melanophore stimulating hormone (MSH). Without this endocrine signal, melanin synthesis can not continue, leading to pigmentation deficiency finally. The presumptions mentioned above were not evidenced by direct experimental data till today. It is well known that the contents of DHA in tissues of flatfish show direct relationship with the formation of pigment in fish body.

In this study, the sampled *P. olivaceus* young

fish were reared in the same living environment and with the same diets. Our results have shown that the content of DHA in the skin of albino fish is significantly lower than that in the skin of the normal, and the content of DHA in muscle of albino fish is higher than that in skin, which is similar to that in the muscle of the normal. It can be presumed that the cumulation and utilization of DHA in the skin of albino fishes is lower than that in the skin of the normal. Further studies are needed to clarify whether the deficiency of DHA in the diets causes the albinism, or the albino fish can't absorb and utilize efficiently the DHA during the process of pigmentation. It was observed that the absolute contents of EPA in the skin and muscle showed no significant difference respectively between normal and albino fish, but it is interesting to note that the ratios of DHA/EPA in skin and muscle of albino fish were a little lower than those in normal skin and muscle of fish. Therefore it is desirable to get more detailed information on different fatty acid compositions in different tissues of both normal and albino *P. olivaceus* fed under the same conditions, in order to determine the approach of fatty acid transformation in different tissues, and evaluate the effects of diets enriched with n－3 unsaturated fatty acid for reducing the albinism of flatfish. Efficient procedures may be developed based on such studies to prevent the occurrence of malpigmentation in hatchery-reared flatfish culture.

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正常和白化褐牙鲆皮肤和肌肉中 DHA 和 EPA 含量的比较

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摘要:利用气相色谱法对相同养殖条件下生长的正常和白化褐牙鲆(*Paralichthys olivaceus*)的皮肤和肌肉组织中的 DHA 和 EPA 含量进行了比较分析。结果显示,正常和白化褐牙鲆的皮肤和肌肉中 EPA 含量相近,但白化个体皮肤中 DHA 含量显著低于正常个体,约为后者的 1/2。而两者肌肉中的 DHA 含量则没有显著差异。同时,白化个体皮肤和肌肉组织中的 DHA/EPA 比值略低于正常个体。这一结果证明白化褐牙鲆皮肤蓄积或利用 DHA 的能力要低于正常褐牙鲆。关于鲆鲽类白化现象同鱼体对饵料中脂肪酸类物质的利用率之间的关系有待深入研究。

关键词: DHA; EPA; 皮肤; 褐牙鲆; 白化

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