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Short communication

Effects of dietary fatty acid composition on muscle composition and hepatic fatty acid profile in juvenile *Synechogobius hasta*

By Z. Luo[1,2], X. Li[2], H. Bai[2] and S. Gong[1]

[1]*Fishery College, Huazhong Agricultural University, Wuhan;* [2]*Postdoctoral Research Base, Panjin Guanghe Fishery Co. Ltd, Panjin, China*

Introduction

Synechogobius hasta has been identified as a species destined for diversification of Chinese marine aquaculture. In recent years, commercial farming of this fish has become of increasing interest in northern China because of its euryhalinity, rapid growth, good taste and high market value. At present, farming of *S. hasta* relies heavily on raw fish, resulting in high feed costs for commercial operation, as well as high waste output – an increasingly important concern in some regions due to the high farm densities encountered. The development of high quality formulated feed, based on the nutrient requirements of the species, will help improve economical and environmental sustainability of aquaculture in the country. However, to our knowledge, information on nutritional requirements of *S. hasta* is absent. Investigation of the quantitative and qualitative nutritional composition is therefore urgently needed to develop cost-effective and environmentally friendly diets for this fish species.

Dietary lipids play not only important roles as potential sources for energy, essential fatty acids (EFA), and fat-soluble vitamins, but also have important physiological functions and can significantly affect flesh quality such as the FA profile of fish body lipids. It is therefore suggested that body composition is strongly related to the FA composition of the diet (Cowey, 1993). Like other vertebrates, fish are unable to synthesize long-chained polyunsaturated FA (PUFA) *de novo* and must obtain these substances via the food. The requirements of EFA for growth and health of some cultured fish species have been thoroughly investigated, and most have a species-dependent demand for n-3 and n-6 FAs (Sargent et al., 1999). Most marine fish have a limited ability to convert linolenic acid (18:3n-3) into longer-chained eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Thus the dietary sources must supply these EFAs, usually with the addition of fish oil, which contains high amounts of EPA and DHA (Sargent et al., 1999).

As part of a series of investigations on nutrient requirements of juvenile *S. hasta*, the purpose of this study was to determine the effects of varying dietary FA compositions on the muscle composition and hepatic FA profile of juvenile *S. hasta* under controlled experimental environmental conditions.

Materials and methods

The trial was conducted in the experimental facilities of Panjin Guanghe Fishery Co., Ltd, with 400 juvenile *S. hasta* obtained from a local fish farm. Prior to the experiment, they were acclimated to the experimental conditions for 2 weeks by feeding a commercial pelleted diet (as described in Table 1). At the beginning of the experiment, 20 fish (initial body weights: 17.26 ± 0.75 g, mean ± SD, total sample size n = 180) of equal size and in good condition were randomly distributed in fiberglass tanks supplied with sand-filtered seawater at a flow rate of 2 L min^{-1} and with continuous aeration to maintain the dissolved oxygen level near saturation. Each experimental food (a commercial pelleted diet, iced fish and iced shrimp) was randomly allotted to three different tanks (three replicates × three diets = nine tanks in total). The pelleted diet (closed formula) was supplied by the Hefeng Feed Company (Shenyang, China). The composition (on a dry matter basis) in decreasing order was as follows: fishmeal (65% protein), soybean meal (44% protein), wheat flour, vitamin C, mineral and vitamin premix. The iced fish and iced shrimp were stored at −20°C until used. The proximate compositions of the pelleted diet, iced shrimp and iced fish are shown in Table 1. The pelleted diet had crude protein of 36% and crude fat at 10%. The iced shrimp and iced fish had similar levels of crude protein, fat and moisture at 15–18%, 2.7–3% and 78–80%, respectively. Iced shrimp contained the highest contents of 20:5n-3; iced fish contained the highest contents of 22:6n-3 and total n-3 FAs. In contrast, the pelleted diet had the highest amounts of linoleic acid (18:2n-6), oleic acid (18:1n-9) and total n-6 FAs, and lowest amounts of EPA and DHA.

During the experiment, *S. hasta* were fed to near satiation and food consumption was recorded daily. Mortalities were recorded daily and dead fish were subjected to necropsy. Tanks were cleaned of excess feed and feces daily. A natural 14 h light / 10 h dark cycle was used. Water quality parameters were monitored in the morning (09.00 hours) following standard methods (CEPB, 1989). Seawater temperature from 20 to 25°C (n = 30) and salinity 26–28‰ (n = 30) were monitored daily, while pH 8.2–8.5 (n = 8), ammonia nitrogen 0.4 mg L^{-1} (n = 8) and nitrite nitrogen 0.06 mg L^{-1} (n = 8) were monitored twice weekly. The trial continued for 30 days.

At the conclusion of the experiment, all fish were counted and weighed to determine survival and body weight. After obtaining the final total weight of fish in each aquarium, four fish per tank were randomly selected, weighed and measured, and body weight and body length individually recorded for calculations of condition factor (CF). They were then dissected to obtain liver and white muscle samples, and viscerosomatic

Table 1
Proximate analysis of food fed to juvenile *Synechogobius hasta*

	Iced shrimp	Iced fish	Pelleted diet
Crude protein	15.4 ± 0.52	17.3 ± 0.23	36.6 ± 0.65
Crude lipid	2.96 ± 0.13	2.72 ± 0.05	10.41 ± 0.53
Moisture	79.4 ± 0.56	78.8 ± 0.42	10.1 ± 0.27
Ash	2.96 ± 0.10	2.61 ± 0.14	9.70 ± 0.29
Fatty acid compositions (% of total fatty acids)			
C14:0	1.3	1	1.1
C16:0	18.9	22.4	19.1
C17:0	3.0	2.5	0.4
C18:0	8.1	11.9	2.2
C20:0	nd	nd	0.3
∑SFA	31.3	37.8	23.1
C16:1	5.8	4.1	1.8
C17:1	1.3	0.6	–
C18:1	21.0	16.6	20.4
C20:1	1.5	1.7	1.1
∑MUFA	29.6	23.0	23.3
C18:2n-6	3.3	1.2	34.4
C20:2n-6	0.5	0.4	0.1
C20:4n-6	4.4	2.1	0.3
∑n-6	8.2	3.7	34.8
C18:3n-3	2.3	0.5	3.2
C20:5n-3	10.0	6.6	2.8
C22:6n-3	8.7	18.3	3.6
∑n-3	21.1	25.4	9.6
∑n-3 ⁄ ∑n-6	2.56	6.86	0.28

nd, not detected; SFA, saturated fatty acid; MUFA, mono-unsaturated fatty acids.
Crude protein, lipid and ash contents from iced shrimp and iced fish expressed as percentage live weight; parameters from pelleted diet expressed on basis of dry matter.

index (VSI) and hepatosomatic index (HSI) values were calculated.

Liver and muscle samples were kept at −70°C prior to analysis. Proximate analysis consisted of determining moisture, ash, crude protein, and lipid contents of muscle and hepatic FA profiles according to the AOAC (1995) methods described in our previous studies (Luo et al., 2005, 2007). For analysis of the hepatic FA profile, total lipid was extracted in triplicate samples per treatment of about 5 g of wet weight after homogenization in chloroform ⁄ methanol (2 : 1, v ⁄ v) containing 0.01% butylated hydroxytoluene as an antioxidant, according to Bligh and Dyer (1959). They were then methylated and transesterified with boron trifluoride in methanol (AOAC, 1995). FA methyl esters were resolved and analyzed by gas–liquid chromatograph (Shimadzu GC-14A, Japan) equipped with a flame ionization detector and a Shimadzu C-R6A Chromato – Integrator. The conditions were as follows: Omegawax fused silica capillary column (30 m × 0.25 mm ID, 0.25 μm film thickness) (Supelco, Bellafonte, PA), temperature programmed from 100 to 250°C at 3°C min^{-1}, held for 10 min. Carrier gas was helium at 1.0 ml min^{-1}, inlet pressure 12 psi. FAs were identified by comparing retention time with those of known standards (Supelco 37 Component FAME Mix; Supelco), and areas below the identified chromatographic peaks were calculated by integration. The individual FA methyl esters were identified by comparing the retention times of authentic standard mixtures and quantified relative to the internal standard.

All data were subjected to one-way ANOVA and Duncan's multiple range test using the software STATISTICA (release 5.0, Tulsa, OK, USA). Differences were considered significant at $P < 0.05$.

Results

The best growth was observed in *S. hasta* fed the iced fish and the pelleted diet, respectively (Table 2). Survival attained up to 60–65% and was not influenced by the different foods. HIS of fish fed the pelleted diet was significantly lower compared to the iced fish group ($P < 0.05$) (Table 3). However, CF and VSI showed no significant differences among the treatments ($P > 0.05$). Muscle composition was affected by varying diets ($P < 0.05$). Highest lipid content and the lowest protein content were found in *S. hasta* fed the pelleted diet ($P < 0.05$). The hepatic FA profile of *S. hasta* is shown in Table 4. *Synechogobius hasta* fed the pelleted diet showed generally increased proportions of 18:2n-6 and total n-6 PUFA, and decreased proportions of EPA and DHA compared to those fed the iced fish and iced shrimp ($P < 0.05$). The liver tissue of *S. hasta* fed the iced fish contained the highest levels of DHA,

Table 3
Effect of practical diets on morphometrical parameters and muscle composition (% live weight) of juvenile *Synechogobius hasta* reared for 30 days

	Iced shrimp	Iced fish	Pelleted diet
Morphometrical parameters			
CF	0.91 ± 0.04	0.89 ± 0.07	0.90 ± 0.03
VSI	8.1 ± 0.24	8.2 ± 0.70	7.5 ± 0.41
HSI	1.83 ± 0.06b	2.02 ± 0.05b	1.08 ± 0.08a
Muscle			
Crude protein	17.8 ± 0.34a	17.6 ± 0.54a	15.6 ± 0.25b
Crude lipid	2.6 ± 0.10ab	2.3 ± 0.17b	2.7 ± 0.18a
Moisture	80.3 ± 0.54b	80.5 ± 0.57b	82.9 ± 0.34a
Ash	1.25 ± 0.07	1.24 ± 0.09	1.34 ± 0.07

Values are mean ± SD of three replicate tanks (four fish × three tanks, n = 12).
Values within same row with different letters are significantly different ($P < 0.05$).
CF (condition factor) = 100 × (live weight, g) ⁄ (body length, cm)^3; HSI (hepatosomatic index) = 100 × (liver weight) ⁄ (body weight); VSI (viscerosomatic index) = 100 × (viscera weight) ⁄ (body weight).

Table 2
Effect of practical diets on growth and feed utilization of juvenile *Synechogobius hasta* reared for 30 days

Diets	Growth performance IBW	WG (n)	FBW (n)	Survival
Iced shrimp	17.1 ± 0.40	108.5 ± 5.0b (39)	35.5 ± 0.18b (39)	65.0 ± 8.66
Iced fish	17.4 ± 1.17	163.3 ± 9.9c (37)	46.1 ± 4.60c (37)	61.7 ± 2.89
Pelleted diet	17.3 ± 0.66	56.1 ± 3.5a (36)	26.9 ± 1.48a (36)	60.0 ± 10.0

Values are mean ± SD of three replicate tanks.
Values within same column with different letters are significantly different ($P < 0.05$).
IBW (g per fish), initial mean body weight; FBW (g per fish), final mean body weight; WG (weight gain, %) = (initial mean weight − initial mean weight) ⁄ initial mean weight; survival = 100 × (final fish number) ⁄ (initial fish number).

Table 4
Effect of practical diets on fatty acid compositions (% total fatty acids) of liver in juvenile *Synechogobius hasta* reared for 30 days

	Iced shrimp	Iced fish	Pelleted diet
C14:0	2.33 ± 0.12a	1.97 ± 0.15b	0.37 ± 0.06c
C16:0	22.00 ± 0.40a	19.10 ± 1.05b	23.13 ± 0.91a
C17:0	1.50 ± 0.10a	0.93 ± 0.06b	0.63 ± 0.06c
C18:0	4.40 ± 0.26b	4.07 ± 0.15b	10.67 ± 0.50a
C20:0	nd	nd	0.33 ± 0.06
∑SFA	30.23 ± 0.29b	26.07 ± 1.31c	35.13 ± 0.96a
C16:1	12.77 ± 0.65a	13.47 ± 0.57a	3.73 ± 0.61b
C17:1	2.13 ± 0.23a	0.93 ± 0.12b	0.17 ± 0.06c
C18:1	16.17 ± 0.45a	13.90 ± 0.78b	15.50 ± 0.75a
C20:1	1.27 ± 0.12a	0.50 ± 0.10b	0.40 ± 0.10b
∑MUFA	32.33 ± 0.64a	28.77 ± 1.31b	19.80 ± 0.35c
C18:2n-6	7.53 ± 0.32b	7.13 ± 0.21b	21.37 ± 0.65a
C20:2n-6	0.23 ± 0.06b	0.23 ± 0.06b	0.73 ± 0.06a
C20:4n-6	2.57 ± 0.15a	1.50 ± 0.10c	2.10 ± 0.17b
∑n-6	10.33 ± 0.42b	8.87 ± 0.15c	24.20 ± 0.46a
C18:3n-3	1.47 ± 0.12a	0.93 ± 0.06b	0.53 ± 0.06c
C20:5n-3	5.57 ± 0.21a	5.10 ± 0.30a	3.87 ± 0.25b
C22:6n-3	12.73 ± 0.42b	20.10 ± 0.70a	8.13 ± 0.25c
∑n-3	19.77 ± 0.47b	26.13 ± 0.85a	12.53 ± 0.15c
∑n-3 ⁄ ∑n-6	1.91 ± 0.03b	2.95 ± 0.06a	0.52 ± 0.01c

nd, not detected; SFA, saturated fatty acid; MUFA, mono-unsaturated fatty acids.
Values are mean ± SD of three replicate tanks (three subsamples ⁄ tank × three tanks, n = 9).
Values within same row with different letters are significantly different ($P < 0.05$).

total n-3 FA and n-3 ⁄ n-6 ratio ($P < 0.05$). The percentages of 20:4n-6 and sum of MUFAs were the highest for *S. hasta* fed the iced shrimp ($P < 0.05$). Significant differences were also observed in the liver ⁄ diet ratios of 18:2n-6 among dietary treatments ($P < 0.05$). *Synechogobius hasta* fed the iced fish showed the highest 18:3n-3 ratio, whereas the liver ⁄ diet ratio was quite similar for oleic acid in the other dietary groups.

Discussion

Mortality was not influenced by the different feeds and varied between 35 and 40%. This seemed to be a reasonable rate of loss in the early stages because *S. hasta* is a rather sensitive species and is easily stressed. The culture methods for this species are not yet well established. The fish became extremely excited while being handled and during cleaning operations. Fish sometimes also attacked each other, causing injury and possible mortality. Bite marks could often be observed on dead fish. Similarly, Molnar et al. (2004) reported higher mortalities of 50.4–55.8% in pike perch reared at different stocking densities. Although apparent physical EFA-deficient signs such as fin erosion and bacterial disease (Sargent et al., 1995, 1999) were not observed in any groups during the feeding trial, the best growth was observed in *S. hasta* fed the iced fish and the pelleted diet, respectively (Table 2), indicating that iced fish rich in n-3 highly unsaturated FA (HUFA), especially EPA and DHA, had better nutritional values than that of 18:2n-6 and 18:3n-3 FAs for juvenile *S. hasta,* similar to other reports (Kalogeropoulos et al., 1992; Wilson and Moreau, 1996). In the present study, varying diets significantly influenced HSI and muscle composition ($P < 0.05$). Fish fed the pelleted diet showed the lowest HSI, the highest lipid content and the lowest protein content ($P < 0.05$).

In the present experiment, the hepatic FA profile of *S. hasta* generally reflected the dietary FA composition, as pointed out in other studies (Arzel et al., 1994; Tocher et al., 2002; Schulz et al., 2005). *Synechogobius hasta* fed the pelleted diet showed generally increased proportions of 18:2n-6 and total n-6 PUFA due to increased dietary content, and decreased proportions of EPA and DHA compared to those fed the iced fish and iced shrimp (Table 4). The effect of dietary EFA deficiencies is a reduction in n-3 HUFA in tissues, which has been described for several fish species (Nematipour and Gatlin, 1993; Ibeas et al., 1994; Agradi et al., 1995). Liver tissue of *S. hasta* fed the iced fish contained the highest levels of DHA, total n-3 FA and n-3 ⁄ n-6 ratio ($P < 0.05$), again reflecting dietary FA composition. A higher proportion of 20:4n-6 in iced shrimp was reflected in a higher percentage of 20:4n-6 in the liver, and the sum of MUFAs was the highest ($P < 0.05$) proportionally to their dietary levels (Table 5). In the present study, the ranges for percentages of saturated and unsaturated FAs in the liver lipids were narrower than those for dietary lipids, suggesting selective metabolism of some of the saturated FAs and of oleic acid and linoleic acid to meet energy demands of the fish, as reported by Dosanjh et al. (1988, 1998). For the pelleted diet group, liver ⁄ dietary ratios of DHA and arachidonic acid (ARA, 20:4n-6) were the highest due to their low dietary contents, but liver ⁄ diet ratios for oleic acid were lower than one (0.76–0.84). The increased values of 20:4n-6 and 22:6n-3 in the liver of *S. hasta* fed the pelleted diet indicated a preferential utilization of n-3 and n-6 FAs even if 18:1n-9 was present in the diets in higher amounts than 18:3n-3, in agreement with that of Caballero et al. (2002). Thus, although PUFA synthesis, namely elongation and desaturation of 18 carbon precursors, was found for all groups tested, it seems to be more evident in those fish fed lower n-3 HUFA diets balancing in this way the DHA concentration in liver and denoting the importance of DHA as a structural component of the cell membrane (Izquierdo et al., 1989; Watanabe et al., 1989; Montero et al., 2001). This preferential conservation of n-3 HUFA has also been described in other species (Izquierdo, 1996; Montero et al., 2001) as helping fish to maintain normal membrane functions even in the absence of dietary EFAs (Kanazawa, 1985). However, the results still have to be taken with caution because the test diets were not truly iso-caloric as the experiment was intended to gain initial insight from scenarios of the actual feeding regimes currently used by the industry. The results are of practical value but need further verification and improvements under standardized nutrition procedures commonly used in finfish nutrition research.

In conclusion, dietary FA composition can significantly influence the muscle composition and hepatic FA profile of

Table 5
Effect of practical diets on liver fatty acid (FA) ⁄ dietary FA ratios for main fatty acids of fish reared for 30 days

	Diets		
Liver FA ⁄ dietary FA	Iced shrimp	Iced fish	Pelleted diet
18:1n-9	0.77 ± 0.02ab	0.84 ± 0.05a	0.76 ± 0.04b
18:2n-6	2.28 ± 0.10b	5.94 ± 0.17a	0.62 ± 0.02c
18:3n-3	0.64 ± 0.05b	1.87 ± 0.12a	0.17 ± 0.02c
ARA (20:4n-6)	0.58 ± 0.03b	0.71 ± 0.05b	7.00 ± 0.58a
EPA (20:5n-3)	0.56 ± 0.02c	0.77 ± 0.05b	1.38 ± 0.09a
DHA (22:6n-3)	1.46 ± 0.05b	1.10 ± 0.04c	2.26 ± 0.07a

ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.
Values are mean ± SD of three replicates.
Values within same row with different letters are significantly different ($P < 0.05$).

S. hasta. Iced fish rich in n-3 HUFA, especially, EPA and DHA, had a better nutritional value than that of 18:2n-6 and 18:3n-3 FAs in the pelleted diet (rich in 18:3n-3 and 18:2n-6) for juveniles. *Synechogobius hasta* could preferentially conserve n-3 HUFA, helping it to maintain normal membrane functions in the absence of dietary EFA. Thus, the present study is the first report involving nutrient physiology of *S. hasta* under controlled experimental conditions.

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Author's address: Zhi Luo, Fishery College, Huazhong Agricultural University, Wuhan 430070, China.
E-mail: luozhi99@yahoo.com.cn