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Effect of Dietary n-3 Highly Unsaturated Fatty Acids on Growth, Feed Efficiency and Fatty Acid Composition of Juvenile Silver Bream *Rhabdosargus sarba* (Sparidae)

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Abstract

An experiment was conducted to investigate the response of juvenile silver bream (*Rhabdosargus sarba*) to dietary n-3 highly unsaturated fatty acids (n-3 HUFA). White fishmeal and casein were the protein sources with 7.0% soybean oil as the major lipid component in the basal diet. Different levels of n-3 HUFA (n-3 HUFA, 85%) ranging from 0.5 to 2.0% (at 0.5% increment) were supplemented to the basal diet to replace equivalent levels of soybean oil. Test diets were fed to silver bream in duplicate 500-l fiberglass tanks for 8 weeks. Fish fed the basal diet exhibited the poorest weight gain (639.45%) and feed efficiency (91.93%). With increasing levels of dietary n-3 HUFA (0.61-1.64%), weight gain and feed efficiency improved (639.45-789.42% and 91.93-104.60%, respectively); but, at 2.19% n-3 HUFA, weight gain and feed efficiency were slightly suppressed (739.75% and 92.97%, respectively). However, statistically, there were no significant differences ($P>0.05$) in weight gain among 1.31, 1.64 and 2.19% dietary n-3 HUFA, and feed efficiency among 0.91, 1.31, 1.64 and 2.19% dietary n-3 HUFA. There were no significant differences ($P>0.05$) in survival and condition factor among dietary treatments. Body lipid composition of fish was af-fected by dietary lipid composition. Feeding a diet low in the n-3 HUFA level resulted in an in-crease of 18:1n-9 and 18:2n-6. Elevation of dietary n-3 HUFA levels effectively reduced the 18:1n-9 and 18:2n-6 levels and increased n-3 HUFA levels. This indicates that the dietary n-3 HUFA for juvenile silver bream should be above 1.31% in order to achieve better growth and feed efficiency.

Introduction

The silver bream *Rhabdosargus sarba* is common in subtropical and tropical inshore waters and estuaries throughout the Indo-Pacific (Smith 1965). Due

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to its high commercial value, the fish is considered a desirable species for culture in Taiwan (Lin et al. 1989; Leu 1994). In conventional silver bream culture, trash fish have been the major food source. However, poor quality fish are produced due to the overaccumulation of tissue lipid. Pollution of fishponds can also occur due to unconsumed food and nutrient leaching from trash fish. Therefore, it is necessary to develop effective and stable artificial feeds to replace trash fish for silver bream culture.

Research on nutrient requirements of cultured fish is important to develop an artificial feed. Although there have been many studies on fatty acids requirement of finfish, no research related to that of silver bream has been done. Essential fatty acid (EFA) requirements have been reported to vary among fish species (Watanabe 1982). Several studies have shown that marine finfish require n-3 highly unsaturated fatty acids (n-3 HUFA), such as eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA), as EFAs for their normal growth (Owen et al. 1972; Cowey et al. 1976a, b; Fujii and Yone 1976; Leger et al. 1979; Watanabe 1982; Kanzawa 1985; Sargent et al. 1989). EFA deficiency causes growth retardation and high mortality. The present study examined the effect of dietary n-3 HUFA levels on growth, feed efficiency and fatty acid composition of juvenile silver bream.

Materials and Methods

Experimental Design

Juvenile silver bream were produced from captive broodstock maintained in the Taihsi Branch of the Taiwan Fisheries Research Institute. Before the experimental period, the fish were fed the basal diet for 2 weeks to acclimate them to the experimental conditions. At the start of the experimental period, the fish (average weight of 1.1±0.2 g) were randomly stocked into ten 500-l circular fiberglass tanks (25 fish per tank) and provided with aeration. Waste was siphoned out and replaced new every morning. Water quality was checked periodically: pH was 7.8-8.2, salinity 30-34 ppt, and water temperature 23.4-27.6°C. The experiments were conducted under natural photoperiod. Fish were fed 5% of their body weight per day, and the daily ration was divided into four equal feedings between 800 and 1600 h. All the fish in each tank were individually weighed every 2 weeks (no feed was offered on sampling day), and the amount of diet was adjusted accordingly. The feeding trial was conducted for 8 weeks.

Experimental Diets

Five experimental diets (Table 1) were formulated containing different levels of n-3 HUFA. The n-3 HUFA used was Ester 85 (Nippon Chemical Feed Co. Ltd., Japan), a kind of methyl ester mixture containing about 85% n-3 HUFA. All diets were mechanically mixed, pelleted, air dried and stored frozen at -20°C prior to their use. Diet A with 7% soybean oil was the basal diet. Different levels

of n-3 HUFA (0.5-2.0% at 0.5% increment) were supplemented to the basal diet to replace equivalent levels of soybean oil. The fatty acid compositions of the diets are given in Table 2. Each diet was tested in duplicate in randomly assigned groups of fish.

Table 1. Composition of the experimental diets ($g \cdot 100g^{-1}$ dry weight).

Ingredients	Diet				
	A	B	C	D	E
Casein	30.0	30.0	30.0	30.0	30.0
White fishmeal[1]	30.0	30.0	30.0	30.0	30.0
∝-starch	12.0	12.0	12.0	12.0	12.0
Wheatflour	10.0	10.0	10.0	10.0	10.0
Vitamin mixture[2]	2.0	2.0	2.0	2.0	2.0
Mineral mixture[3]	4.0	4.0	4.0	4.0	4.0
Soybean oil	7.0	6.5	6.0	5.5	5.0
n-3 HUFA[4]	0	0.5	1.0	1.5	2.0
Attractant mixture[5]	3.0	3.0	3.0	3.0	3.0
CMC[6]	2.0	2.0	2.0	2.0	2.0

[1] Not defatted
[2] Halver (1957)
[3] Sakamoto (1981)
[4] Methyl esters containing 85% n-3 highly unsaturated fatty acids (20:5n-3 25.0%, 22:6n-3 47.6%, 20:4n-3 5.1% and 22:5n-3 7.3%). The n-3 HUFA was a product of Nippon Chemical Feed Co. Ltd., Tokyo, Japan.
[5] Attractant mixture ($g \cdot 100g^{-1}$ diet): 0.6 methionine, 1.0 lysine, 0.4 glycine, 0.4 alanine, 0.1 nucleotide, 0.1 betain, 0.4 glutamate-Na
[6] CMC=carboxymethyl cellulose

Table 2. Fatty acid (% of total lipid) composition of the diets.

Fatty acid	Diet				
	A	B	C	D	E
14:0	1.71	1.67	1.98	2.66	2.07
16:0	14.23	14.21	14.40	15.21	14.77
16:1	0.43	tr	tr	0.72	0.70
18:0	3.35	2.56	2.07	2.89	2.85
18:1n9	20.60	20.56	19.88	17.20	16.31
18:2n6	43.72	42.32	40.91	36.44	32.92
18:3n6	tr	tr	tr	tr	tr
18:3n3	5.96	6.98	5.33	4.88	4.02
20:0	0.26	0.09	0.05	0.50	0.48
20:1	2.76	3.18	2.10	2.50	2.80
20:2	tr	tr	tr	0.02	tr
20:3	tr	tr	tr	0.12	tr
20:4n6	0.11	tr	0.20	0.36	0.43
22:1	tr	tr	tr	0.19	0.38
20:5n3	3.96	5.34	7.16	7.51	9.78
22:5n3	tr	tr	tr	0.34	0.90
22:6n3	1.92	3.44	5.49	7.67	10.47
Crude lipid (%)	10.45	10.38	10.39	10.56	10.39
Σn-3 HUFA in lipid[1] (%)	5.88	8.78	12.65	15.52	21.15
Σn-3 HUFA in diet[1] (%)	0.61	0.91	1.31	1.64	2.19

[1]HUFA: carbon chain length ≥20 with three or more double bonds.

Sample Collection and Analyses

After the experiment, all the fish were starved for 1 d, then collected and frozen at -20°C for subsequent polar lipid fatty acid composition analysis of whole bodies. All the fish from the same tank were pooled and homogenized using a polytron homogenizer (Kinematica GmbH Uttau, Switzerland).

The whole body lipids were extracted using chloroform and methanol (Folch et al. 1957). The lipid extracts were subsequently separated into polar and neutral fractions by silicic acid column chromatography (Christie 1982). After separation, the fractions were subjected overnight to methylation in 1% sulfuric acid in methanol at 50°C (Koven et al. 1989). The methyl esters were analyzed in a Hitachi 163 gas-liquid chromatograph equipped with a flame ionization detector and glass column (3 x 2 mm) packed with 5% shinchrom E70 coated on shimalite (AW, 80-100 mesh, Shimadzu, Tokyo, Japan). Fatty acids were identified by comparison with the retention times of a reference standard (GLC-68A, Nu-Check Prep., Elysian, MN, USA) consisting of a mixture of saturated and unsaturated fatty acids. The magnitude of each peak of each chromatograph was quantified by a Hitachi D-2000 recording integrator. Cod liver oil served as the secondary reference standard.

Statistical Analysis

All data were analyzed following the completely randomized design (one-way analysis of variance), and Duncan's new multiple-range test was used to resolve the differences among treatment means (Duncan 1955). Prior to this analysis, the percent weight gain, feed efficiency and survival were normalized through $(\arcsin\%)^{-2}$ transformation (Snedecor and Cochran 1967).

Results and Discussion

The growth data in Table 3 indicated that, with increasing levels of dietary n-3 HUFA (0.61-1.64%), the weight gain and feed efficiency improved (639.45-789.42% and 91.93-104.60%, respectively). It has been reported that an excess amount of EFA may induce ill effects like growth rate and poor feed efficiency in rainbow trout *Salmo gairdneri* (Takeuchi and Watanabe 1979), red sea bream *Pagrus major* (Takeuchi et al. 1992a) and striped jack *Pseudocaranx dentex* (Takeuchi et al. 1992b). However, in this study, there were no significant differences ($P>0.05$) in weight gain and feed efficiency among juvenile silver bream fed diets with 1.31, 1.64 and 2.19% n-3 HUFA. However, at 2.19% n-3 HUFA, weight gain and feed efficiency were slightly suppressed (739.75% and 92.7%, respectively). There was a significant increase ($P<0.05$) in feed efficiency of silver bream fed 1.31 and 1.64% dietary n-3 HUFA compared to those fed 0.61% dietary n-3 HUFA. Therefore, using data from this growth study, the dietary n-3 HUFA requirement for juvenile silver bream is approximately 1.31%. This value is higher than 0.8% (Gatesoupe et al. 1977) and 1.0% (Cowey et al. 1976b) for turbot *Scophthalmus maximus*, 0.9% for gilthead bream *Sparus*

Table 3. Growth, feed efficiency, condition factor and survival rate of juvenile silver bream fed the experimental diets after feeding for 8 weeks[1].

Performance factor	Diet				
	A	B	C	D	E
Initial average body weight (g)	1.08 ± 0.22	1.08 ± 0.21	1.07 ± 0.22	1.09 ± 0.22	1.10 ± 0.22
Final average body weight (g)	7.99 ± 1.18	8.32 ± 1.34	8.50 ± 1.52	9.70 ± 1.77	9.25 ± 1.84
Percent weight gain[2] (%)	639.45 ± 17.02^{a}	670.29 ± 58.53^{a}	693.99 ± 18.72^{ab}	789.42 ± 15.89^{b}	739.75 ± 74.51^{ab}
Feed efficiency[3] (%)	91.93 ± 4.04^{a}	97.78 ± 1.81^{ab}	101.20 ± 0.22^{b}	104.60 ± 3.20^{b}	92.27 ± 3.88^{ab}
Condition factor[4]	14.95 ± 0.28^{a}	15.46 ± 0.19^{a}	15.36 ± 0.49^{a}	15.78 ± 0.06^{a}	15.66 ± 0.28^{a}
Survival rate (%)	94.00 ± 6.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	98.00 ± 2.00^{a}	92.00 ± 4.00^{a}

[1]Data are means ± SD of two replicates; means within a row followed by the same letter are not significantly different ($P>0.05$).
[2][Final body weight (g) - initial body weight (g)] ÷ initial body weight (g) x 100.
[3]Body weight gain (g) ÷ feed supplied (g).
[4]Body weight (g) x 1,000 ÷ (TLcm)^{3}.

aurata (Kalogeropoulos et al. 1992), and 1.0% for flounder *Paralichthys olivaceus* (Kanazawa 1988); it is lower than 2.0% for yellowtail *Seriola quinqueradiata* (Deshimaru 1984), 1.7% for striped jack (Watanabe et al. 1989) and 1.72% for seabass *Lates calcarifer* (Buranapanidgit et al. 1988), but it is close to 1.5% for red sea bream (Takeuchi et al. 1990). However, no statistical differences ($P>0.05$) were observed in survival and condition factors of silver bream fed diets supplemented with different n-3 HUFA levels.

Soybean oil contains about 50% linoleic acid (18:2n-6), linolenic acid (18:3n-3) and little n-3 HUFA (NRC 1984). Thus the n-3 HUFA extracted from the basal diet was from white fishmeal. In the present study, the data indicated that silver bream did not grow well when fed the basal diet supplemented only with soybean oil (Table 3). This result is similar to that of other marine fish fed diets supplemented with 18:3n-3 or 18:2n-6 fatty acids, such as plaice *Pleuronectes platessa* (Owen et al. 1972), turbot (Cowey et al. 1976b) and red sea bream (Fujii et al. 1976).

The fatty acid composition of polar lipids of the whole body of silver bream was affected by the types of dietary lipids (Table 4). Feeding the basal diet resulted in the highest 18:2n-6 and the lowest EPA and DHA contents; while the 2.19% n-3 HUFA group resulted in lower 18:2n-6 and the highest EPA and DHA contents. This was also true for the levels of n-3 HUFA in polar lipid fractions of the dietary treatments supplemented with n-3 HUFA. The EFA index (the ratio of 18:1 n-9 ÷ n-3 HUFA) has been proposed as a criterion for EFA status in red sea bream (Fujii et al. 1976), ratios less than 1.0 were considered indicative of sufficient levels of n-3 HUFA. However, in the present study with sil-

Table 4. Fatty acid composition (%) of the polar lipid fractions of juvenile silver bream fed the experimental diets for 8 weeks.

Fatty acid	Initial	Diet				
		A	B	C	D	E
14:0	1.33	0.74	0.72	0.81	0.92	1.23
16:0	23.50	19.89	21.93	22.73	23.71	23.45
16:1	0.92	1.07	0.88	1.62	2.03	2.35
18:0	13.65	11.19	12.66	11.41	10.03	8.28
18:1n9	12.55	15.33	12.17	11.38	11.05	10.06
18:2n6	3.77	15.75	11.47	9.00	4.71	1.93
18:3n6	0.38	0.88	0.73	0.40	0.27	tr
18:3n3	0.31	0.92	0.82	0.76	0.51	0.38
20:0	0.17	0.42	0.39	tr	0.21	0.20
20:1	2.75	2.43	2.42	2.44	2.25	3.57
20:2	0.33	1.33	0.85	0.55	0.43	1.10
20:3	0.52	1.91	1.15	0.58	0.46	tr
20:4n6	5.94	1.31	2.54	2.89	2.54	2.52
22:1	tr	0.60	0.96	1.01	1.00	0.98
20:5n3	6.17	5.37	6.44	6.73	7.45	7.81
22:5n3	1.03	1.36	1.23	1.29	1.23	1.10
22:6n3	22.16	17.23	18.17	22.74	28.15	29.78
Σn-3 HUFA[1]	29.63	23.96	25.84	30.76	36.83	38.69
18:1n-9/ Σn-3 HUFA[1]	0.42	0.64	0.49	0.37	0.30	0.26

[1]HUFA: carbon chain length $\geq$ 20 with three or more double bonds.

ver bream, lower ratios of 0.37-0.26 seemed to indicate a dietary sufficiency of n-3 HUFA. Similar results have been reported for striped jack (Watanabe et al. 1989) and gilthead bream (Kalogeropoulos et al. 1992).

Based on the results of this study, the inclusion of n-3 HUFA of at least 1.31% of diet provided good growth and feed efficiency in juvenile silver bream.

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