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Effect of dietary docosahexaenoic acid on lipogenesis and lipolysis in black sea bream, *Acanthopagrus schlegeli*

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Abstract

Hatchery-reared juvenile black sea breams are characterized by a low level of highly unsaturated fatty acids in their bodies, as compared with wild fish. To assess the effect of docosahaxaenoic acid (DHA) on lipogenic and lipolysis enzymes, one-year fish were reared on a casein-based purified diet and a DHA fortified diet (1.5% DHA ethyl ester/kg diet) for 60 d, followed with a period of 55 d for starvation. Dietary DHA was effectively incorporated into the fish body. Fortification of DHA depressed activities of glucose-6-phosphate dehydrogenase and NADP-isocitrate dehydrogenase as lipogenic enzymes in the hepatopancreas and intraperitoneal fat body. Carnitine palmitoyltransferase as lipolysis enzyme in the hepatopancreas was active in the DHA fortified fish. Starvation after feeding experiment induced increased carnitine palmitoyltransferase activity in both control and DHA fortified fish and the activity remained higher in the DHA fortified fish, while the monoenes were selectively consumed prior to highly unsaturated fatty acids. These results indicated that dietary DHA depressed lipogenesis and activated lipolysis.

Key words: DHA, enzyme, lipogenesis, lipolysis, black sea bream

1 Introduction

It is well known that varying dietary fatty acid profile affects the tissue fatty acid composition and even the growth performance in fish (Bell et al., 2002; Figueiredo-Silva et al., 2005; Harel and Place, 2003; Schulz et al., 2005; Tocher et al., 2003). Docosahexaenoic acid (DHA), an important essential fatty acid for marine species, has the effect not only on the fatty acid profile of fish body tissue, but also on biological and physiological conditions (Ishizaki et al., 2000; Montero et al., 2001). Although DHA seems to be supplemented sufficiently in a composed diet, its levels in fish organs are significantly lower than those in wild fish (Nakagawa et al., 2000). Fortification of DHA in the composed diet resulting in an equal DHA level to wild fish consequently activates lipid metabolism and vitality in the cultured black sea bream (Om et al., 2003).

Several enzymes are involved in the lipid metab-

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olism process. Synthesis of fatty acid requires NADPH as a power supplier. Glucose-6-phosphate dehydrogenase and NADP-isocitrate dehydrogenase, as lipogenic enzymes, catalyse two different reactions to generate NADPH. On the other hand, carnitine palmitoyltransferase, as lipolysis enzyme, performs a function to exchange of coenzyme A for carnitine to facilitate the transfer of acyl groups into mitochondria for β-oxidation. Although the effect of dietary fatty acids on the activities of lipogenic and lipolysis enzyme has been reported in mammals and birds (Power and Newsholme, 1997; Sanze et al., 2000), no such study related to the effect of DHA has been reported in fish. Therefore, to make certain of the mechanism of the influence of DHA on lipid metabolism in fish, while assessing its impact on fatty acid composition in different tissues, we particularly examined the effect of DHA on lipogenic and lipolysis enzymes in the present study.

2 Materials and methods

2.1 Fish and rearing conditions

One-year black sea breams produced by Kaneto Aquaculture Co (Fukuyama, Japan) were used for the experiment. A total of 64 fish were divided equally in 4 duplicate rectangular indoor aquaria (capacity 150 L). The water temperature ranged from 13.0 to 21.2 ℃, and the water flow rate was maintained at 1.8 ~ 4.7 L/min.

The fish in the control group were fed on a casein-based purified diet. The other diet was fortified with 1.5% DHA ethyl ester (DHA group). The diets were stored at −20 ℃ until used. Table 1 shows the proximate composition of diets. Table 2 shows the fatty acid composition of diets. The fish were hand fed twice daily (10 00 and 16 00) for 6 d a week at a rate of 3% ~ 6% of the total estimated biomass for 60 d.

After the feeding experiment, six fish in each tank were then starved for an additional 55 d to determine lipolysis activity. The water temperature during starvation ranged from 9.0 to 13.0 ℃.

Table 1. Ingredients and proximate composition of experimental diets

	Dietary group	
	Control	DHA
Ingredients (%)		
Casein (vitamin free)	54	54
Gelatin	12	12
Cellulose	13	13
Vitamin mixture[1)]	3	3
Mineral mixture[2)]	5	5
Amino acid mixture[3)]	4	4
Feed oil	9	7.5
DHA ethyl ester	0	1.5
Proximate composition (%)		
Moisture	67.2	68.6
Crude protein	22.2	22.0
Lipid	2.7	2.8
Ash	1.41	1.37

1) Vitamin mixture (mg/100 g): β-carotene 1.2; Vitamin D_3 0.004 5; Vitamin E (50%) 80; Menadione 4; Thiamine HCl 6; Riboflavin 20; Pyridoxine HCl 4; Nicotic acid 80; Ca-Pantothenate 28; Inositol 400; Biotin 0.6; Folic acid 1.5; p-aminobenzoid acid 40; Choline chloride 800; Cyanocobalamin 0.009; L (+)-ascorbic acid calcium salt dihydrate 5.

2) Mineral mixture (g/100g): NaCl 4.77; $MnSO_4 \cdot 3H_2O$ 7.19; Na_2HPO_4 9.38; K_2HPO_4 25.8; $Ca(H_2PO_4)_2$ 14.5; Calcium lactate 34.8; Ferric citrate 3.19; $Al(OH)_3$ 0.005; $ZnSO_4 \cdot H_2O$ 0.22; $CuSO_4$ 0.14; $MnSO_4 \cdot H_2O$ 0.082; KI 0.02; $CoSO_4 \cdot H_2O$ 0.008.

3) Amino acid mixture (g/100g): L-tryptophane 0.2; L-phenylalanine 0.6; L-aspartic acid 1.0; L-valine 0.7; L-arginine 1.5.

Table 2. Fatty acid composition (%) of diets

Fatty acid	Dietary group	
	Control	DHA
14:0	5.5	4.7
16:0	13.2	12.2
18:0	2.4	2.6
16:1n−7	8.9	7.2
18:1n−9	13.8	12.6
18:1n−7	5.7	4.6
20:1n−9	4.7	4.6
20:1n−7	4.7	2.7

To be continued

Continued from Table 2

Fatty acid	Dietary group	
	Control	DHA
22:1n - 11	7.4	3.4
22:1n - 9	2.5	3.1
18:2n - 6	1.2	1.3
20:3n - 6	0.1	0.1
20:4n - 6	0.6	0.4
18:3n - 3	0.7	0.5
18:4n - 3	2.0	1.3
20:5n - 3	10.6	7.1
22:5n - 3	0.7	0.6
22:6n - 3	6.7	23.3
Saturated	21.1	19.5
Monoenes	47.7	38.2
n - 6	1.9	1.8
n - 3	20.7	32.8

2.2 Biological measurements

After the feeding experiment, the fish were submitted to biological measurements. The muscle, hepatopancreas and intraperitoneal fat body (IPF) were measured and the following parameters were defined as follows:

$$r_m = (m_m / m_b) \times 100,$$
$$i_{hs} = (m_h / m_b) \times 100,$$
$$r_{ipf} = (m_{ipf} / m_b) \times 100,$$
$$e_f = (g_{bm} / f) \times 100,$$

where r_m (%) is muscle ratio; m_m muscle mass; m_b body mass; i_{hs} (%) hepatosomatic index; m_h hepato pancreas mass; r_{ipf} (%) IPF ratio; m_{ipf} IPF mass; e_f (%) feed efficiency; g_{bm} body mass gain; and f diet given.

2.3 Biochemical measurements

The fish were immediately frozen at -20 ℃ after sacrifice until biochemical analysis. The muscle, hepatopancreas and IPF were taken from each fish and analyzed separately.

Crude protein was determined by the Kjeldhal method. Lipid extracted with methanol - chloroform mixture according to the method of Bligh and Dyer (1959) was subjected to quantitative and qualitative analyses.

Lipid of the hepatopancreas was separated into a neutral lipid fraction and polar lipid fraction by column chromatography on Sep - Pak silica cartridges (Waters Co, Milford, USA) according to the method of Juaneda and Rocquelin (1985). Iatroscan analysis showed that the fractions of neutral and polar lipid were mainly composed of triglycerides and phospholipids, respectively. After saponification of the lipids, the fatty acids were converted into their methyl esters with methanol-HCl. A Hitachi Gas Chromatograph 163 with an FID detector (Hitachi Co Ltd, Tokyo, Japan) was used to determine the fatty acid composition. A 30 m capillary column packed with Omegawax TM 320 (30 m × 0.32 mm; Supelco, Bellefonte, PA, USA) was used. The oven temperature was kept at 200 ℃. Fatty acid methyl esters were identified using authentic standards (Sigma Co, St Louis, USA).

2.4 Enzyme assay

The sample processing for enzyme assay was conducted according to the description of Mustafa et al. (1997). Immediately after sacrifice of the fish, the hepatopancreas and IPF were sectioned out and frozen in liquid nitrogen and stored at -80 ℃. The organs were homogenized with nine volumes of 0.25 mol/dm^3 sucrose buffer (pH 7.4) containing 3 mmol/dm^3 HEPES and 1 mmol/dm^3 Na-EDTA for 1 min in an Ultra-Turrax tissue disrupter (IKA® Works Asia, Kuala Lumpur, Malaysia). The homogenate was then centrifuged at 3 800 r/min for 10 min at 0 ~ 4 ℃. The supernatant was used for the assay for carnitine palmitoyltransferase (CPT; EC 2.3.1.23) according to the method of Bieber et al. (1972).

To determine glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49), the hepatopancreas and IPF were homogenized with 0.14 mol/dm^3 KCl buffer and centrifuged at 22 000 r/min for 20 min. The supernatant was used for the assay according to the method of Glock and Mclean (1953).

To determine NADP-isocitrate dehydrogenase

(ICDH; EC 1.1.1.42), the hepatopancreas and IPF were homogenized with nine volumes of cold water for 1 min and centrifuged at 7 500 r/min for 10 min at 0 ~ 4 ℃. Then the supernatant was used for the assay according to the method of Alp et al. (1976).

All enzyme assays were carried out at 30 ℃. Enzyme activities were expressed as micromole or nanomole of substrate or coenzyme converted per minute gram organ or 100 g body mass.

2.5 Statistical analysis

Statistical comparison of differences between groups was made by one-way analysis of variance and Fisher's test. Probabilities of 0.05 and/or less were considered as significant.

3 Results

3.1 Growth, biological parameters, proximate composition and lipid class composition

No difference was found in growth and biochemical parameters after feeding and starvation (Tables 3 and 4). Feed efficiency was significantly higher in the DHA group, as shown in Table 3. Although muscle and hepatopancreas lipid tended to be lower in DHA group, the difference was not significant.

There were no significantly differences in the lipid class composition of hepatopancreas among the groups after feeding and starvation (see Table 5). Starvation induced the consumption of free acids, resulting in the increase of triglycerides, monoglycerids, phospholipids portion.

3.2 Fatty acid composition in tissues

Tables 6 and 7 show the fatty acid composition of the hepatopancreas after feeding and starvation of the fish. In the portion of polar lipid, levels of DHA and $n-3$ fatty acids were elevated significantly in the DHA group after feeding, resulting in lower monoenes level. After starvation, DHA level increased

Table 3. Effects of dietary DHA fortification on the growth performance and biological parameters at the end of feeding and after starvation

		Dietary group	
	n	Control	DHA
Feeding			
Total diet given /kg	–	1.60	1.44
Biomass increased /g	–	291	333
Survival (%)	2	96.9 ± 4.4	96.9 ± 4.4
Feed efficiency (%)	2	18.2 ± 0.8	23.1 ± 0.7[1)]
Initial body mass /g	16	44.7 ± 6.0	43.2 ± 5.8
Final body mass /g	13 ~ 16	54.2 ± 8.0	53.5 ± 7.8
body length /mm	13 ~ 16	12.5 ± 0.7	12.4 ± 0.6
Muscle ratio (%)	7 ~ 10	37.3 ± 7.0	37.8 ± 3.9
Hepatosomatic index (%)	7 ~ 10	1.90 ± 0.36	1.90 ± 0.26
IPF ratio (%)	7 ~ 10	0.80 ± 0.38	0.80 ± 0.32
Starvation			
Survival (%)	–	100	100
Body mass /g	12	51.0 ± 7.8	49.2 ± 7.2
Body length /mm	12	12.3 ± 0.7	12.1 ± 0.7
Muscle ratio (%)	12	29.4 ± 4.41	29.8 ± 7.5
Hepatosomatic index (%)	12	1.30 ± 0.57	1.14 ± 0.20
IPF ratio (%)	12	0.53 ± 0.25	0.65 ± 0.36

Notes: Values are mean and Standard deviation. 1) Significantly different from the value of control ($P < 0.05$). *n* represents the number of the samples.

Table 4. Effects of dietary DHA fortification on the proximate composition (%) at the end of feeding and after starvation

		Dietary group	
	n	Control	DHA
Feeding			
Muscle			
Moisture	4	75.2 ± 0.2	75.8 ± 1.1
Ash	4	1.7 ± 0.2	1.7 ± 0.1
Crude protein	4	21.3 ± 1.2	21.1 ± 1.6
Lipid	4	1.66 ± 0.75	1.42 ± 0.41
Hepatopancreas			
Lipid	5 ~ 6	3.26 ± 1.47	2.52 ± 0.52
Starvation			
Muscle			
Moisture	4	78.3 ± 0.5	78.9 ± 0.3
Ash	4	1.62 ± 0.12	1.64 ± 0.08
Crude protein	4	18.7 ± 0.2	19.5 ± 1.0
Lipid	4	1.36 ± 0.26	1.22 ± 0.18

Notes: Values are mean and standard deviation. *n* represents the number of the samples.

Table 5. Effects of dietary DHA fortification on the hepatopancreas lipid class composition (%) after feeding and starvation

Lipid class[1)]	Feeding		Starvation	
	Control	DHA	Control	DHA
TG	25.6 ± 11.4	27.8 ± 10.9	44.5 ± 18.4	46.8 ± 17.6
FFA	44.4 ± 19.4	41.8 ± 5.3	0.7 ± 0.3	2.6 ± 3.4
Cho	0.6 ± 0.4	0.8 ± 0.4	3.4 ± 2.3	3.8 ± 3.6
DG	1.5 ± 1.2	0.9 ± 0.2	0.3 ± 0.1	0.2 ± 0.1
MG plus PL	27.9 ± 21.3	28.7 ± 21.4	51.0 ± 16.9	46.7 ± 14.8

Notes: Values are mean and standard deviation. $n = 3$. 1) TG is abbreviated from triglycerides; FFA free fatty acids; Cho cholesterol; DG diglycerides; MG monoglycerides; and P phospholipids.

significantly in the control group, while it remained unchanged in the DHA group. The same results were found in the $n-3$ fatty acids level. The levels of $n-6$ fatty acids increased in both groups, while monoenes decreased in the control group after starvation, as compared with those after feeding. In the portion of nonpolar lipid, DHA levels were significantly higher in the DHA group after feeding and starvation, resulting in an increased level of $n-3$ fatty acids after feeding. After starvation, DHA level increased significantly in the control group, while it remained unchanged in the DHA group. The levels of $n-6$ fatty acids increased and monoenes decreased in both groups after starvation, as compared with those after feeding.

The fatty acids composition of the muscle and IPF are shown in Tables 8 and 9. The fortification of DHA effectively accelerated the accumulation of DHA in the body. In the muscle lipid, the same as that in the nonpolar lipid of hepatopancreas, DHA levels were significantly higher in the DHA group after feeding and starvation, resulting in an increased level of $n-3$ fatty acids after feeding. After starvation, DHA level increased significantly in both groups, while it remained higher only in the DHA group. The levels of monoenes decreased in both groups after starvation, as compared with those after feeding. In the IPF lipid, DHA levels were significantly higher in the DHA group after feeding and starvation, resulting in an increased level of $n-3$ fatty acids after feeding and starvation. DHA level increased significantly in both groups after starvation, while it remained higher only in the DHA group. The levels of monoenes decreased in the DHA group after starvation, as compared with that after feeding.

Table 6. Fatty acid composition (%) of polar lipid of hepatopancreas after feeding and starvation

Fatty acid	Feeding		Starvation	
	Control	DHA	Control	DHA
14:0	4.6 ± 1.5	2.2 ± 0.6	1.5 ± 0.8	1.3 ± 0.3
16:0	20.5 ± 4.3	16.8 ± 1.6	20.0 ± 1.0	18.3 ± 1.2
18:0	6.0 ± 0.5	6.4 ± 1.2	6.0 ± 0.7	5.8 ± 0.3
16:1n-7	7.1 ± 1.9	4.5 ± 0.9	3.1 ± 0.7	3.1 ± 0.5
18:1n-9	12.8 ± 4.1	9.3 ± 2.7	7.6 ± 0.6	7.4 ± 0.3
18:1n-7	5.5 ± 1.3	4.5 ± 0.3	3.1 ± 0.1	3.3 ± 0.5
18:1n-5	0.5 ± 0.6	0.5 ± 0.4	0.6 ± 0.1	0.7 ± 0.1
20:1n-9	2.7 ± 0.7	1.3 ± 0.6	0.9 ± 0.4	1.0 ± 0.2
22:1n-11	1.4 ± 0.2	0.6 ± 0.3	0.1 ± 0.1	0.3 ± 0.2
18:2n-6	1.2 ± 0.3	0.7 ± 0.2	1.7 ± 0.2	2.0 ± 0.4
20:4n-6	1.5 ± 0.7	1.8 ± 0.3	2.2 ± 0.7	2.8 ± 0.3
18:3n-3	0.3 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
18:4n-3	0.3 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
20:5n-3	8.6 ± 2.7	8.9 ± 1.5	7.5 ± 2.0	11.0 ± 4.3
22:5n-3	2.8 ± 0.8	1.7 ± 0.3	2.7 ± 0.5	4.0 ± 0.6
22:6n-3	17.8 ± 5.1	34.4 ± 5.1[1)]	35.7 ± 2.6[2)]	31.5 ± 2.6
Saturated	31.1 ± 5.9	25.4 ± 2.3	28.3 ± 3.0	25.7 ± 1.0
Monoenes	30.0 ± 7.7	20.8 ± 4.4	15.5 ± 1.8[2)]	15.7 ± 1.5
$n-6$	2.8 ± 0.5	2.4 ± 0.2	3.9 ± 0.7[2)]	4.7 ± 0.5[1),3)]
$n-3$	29.8 ± 8.3	45.2 ± 6.0[1)]	46.1 ± 4.3[2)]	46.7 ± 2.5

Notes: Values are mean and standard deviation ($n = 4$). 1) Significantly different from the value of control ($P < 0.05$). 2) Values of control after starvation are significantly different from those after feeding ($P < 0.05$). 3) Values of DHA after starvation are significantly different from those after feeding ($P < 0.05$).

Table 7. Fatty acid composition (%) of nonpolar lipid of hepatopancreas after feeding and starvation

Fatty acid	Feeding		Starvation	
	Control	DHA	Control	DHA
14:0	7.9 ± 1.1	6.5 ± 1.5	6.0 ± 1.3	5.5 ± 0.5
16:0	15.2 ± 0.8	15.2 ± 2.2	17.1 ± 0.4	16.3 ± 1.1
18:0	2.3 ± 0.4	3.3 ± 1.3	2.2 ± 0.7	2.4 ± 0.6
16:1n − 7	11.5 ± 1.2	11.7 ± 1.7	10.8 ± 1.0	10.2 ± 0.6
18:1n − 9	23.0 ± 4.1	24.0 ± 4.6	20.4 ± 1.7	20.5 ± 1.5
18:1n − 7	5.5 ± 0.9	6.3 ± 1.6	5.2 ± 1.9	4.6 ± 0.8
18:1n − 5	1.1 ± 0.2	1.2 ± 0.2	0.5 ± 0.6	0.7 ± 0.5
20:1n − 9	3.7 ± 0.7	2.7 ± 1.4	3.1 ± 1.5	2.7 ± 1.9
20:1n − 7	3.4 ± 0.6	2.9 ± 0.6	1.9 ± 1.3	2.0 ± 2.3
22:1n − 11	3.0 ± 0.6	2.1 ± 0.7	1.6 ± 0.8	1.2 ± 0.9
22:1n − 9	1.3 ± 0.9	1.6 ± 0.6	0.6 ± 0.7	1.1 ± 0.8
18:2n − 6	1.5 ± 0.4	1.6 ± 0.4	3.4 ± 0.5	3.5 ± 0.5
20:4n − 6	0.4 ± 0.2	0.2 ± 0.1	0.7 ± 0.2	0.5 ± 0.1
18:3n − 3	0.4 ± 0.0	0.4 ± 0.2	0.6 ± 0.1	0.5 ± 0.1
18:4n − 3	0.5 ± 0.3	0.2 ± 0.1	0.5 ± 0.2	0.4 ± 0.2
20:5n − 3	2.4 ± 0.3	2.5 ± 0.8	2.3 ± 0.3	2.0 ± 0.1
22:5n − 3	1.2 ± 0.2	1.5 ± 0.5	3.5 ± 0.9	3.0 ± 0.7
22:6n − 3	2.4 ± 0.9	8.4 ± 2.2[1)]	8.1 ± 1.2[2)]	12.1 ± 2.6[1)]
Saturated	25.3 ± 2.2	24.9 ± 3.0	25.4 ± 1.0	24.2 ± 1.6
Monoenes	52.6 ± 4.0	51.4 ± 2.5	44.0 ± 1.4[2)]	43.0 ± 2.1[3)]
n − 6	2.0 ± 0.4	1.7 ± 0.4	4.1 ± 0.7[2)]	4.0 ± 0.6[3)]
n − 3	6.9 ± 1.2	13.0 ± 3.5[1)]	15.0 ± 1.8[2)]	17.9 ± 2.7

Notes: Values are mean and standard deviation ($n = 4$). 1) Significantly different from the value of control ($P < 0.05$). 2) Values of control after starvation are significantly different from those after feeding ($P < 0.05$). 3) Values of DHA after starvation are significantly different from those after feeding ($P < 0.05$).

In general, fortification of DHA effectively accelerated the accumulation of DHA in the body. Starvation significantly induced the selective consumption of monoenes in different kinds of tissue lipid, except the polar lipid of DHA group and the IPF lipid of control group.

Table 8. Effects of dietary DHA fortification on the fatty acid composition (%) of muscle after feeding and starvation

Fatty acid	Feeding		Starvation	
	Control	DHA	Control	DHA
14:0	4.0 ± 0.6	3.0 ± 0.5	2.4 ± 0.5	2.2 ± 0.8
16:0	16.0 ± 1.3	16.6 ± 1.0	17.4 ± 1.2	16.2 ± 0.6
18:0	3.6 ± 0.1	4.3 ± 0.5	4.5 ± 0.7	4.4 ± 0.4
16:1n − 7	8.1 ± 1.0	6.7 ± 1.0	5.6 ± 0.8	5.1 ± 1.2
18:1n − 9	16.8 ± 2.4	14.5 ± 2.6	12.1 ± 2.0	10.8 ± 3.0
18:1n − 7	4.4 ± 0.5	5.3 ± 0.8	3.4 ± 0.6	4.0 ± 0.2
20:1n − 9	3.0 ± 1.1	2.8 ± 1.3	3.7 ± 1.0	2.2 ± 0.7
22:1n − 11	2.3 ± 1.0	2.2 ± 0.2	1.6 ± 0.2	1.2 ± 0.1
22:1n − 9	1.5 ± 1.0	1.4 ± 1.1	0.6 ± 1.2	0.5 ± 0.9
18:2n − 6	2.9 ± 0.5	2.8 ± 0.5	2.8 ± 0.6	2.3 ± 0.2
20:4n − 6	1.0 ± 0.2	1.1 ± 0.4	2.2 ± 0.5	1.6 ± 0.8
18:3n − 3	0.6 ± 0.1	0.4 ± 0.0	0.4 ± 0.2	0.3 ± 0.2
18:4n − 3	0.4 ± 0.3	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0
20:5n − 3	6.1 ± 0.5	5.1 ± 0.2	8.5 ± 1.1	5.3 ± 0.8
22:5n − 3	2.7 ± 0.7	2.3 ± 03	3.9 ± 0.4	2.8 ± 0.5
22:6n − 3	12.3 ± 2.0	24.6 ± 2.9[1)]	23.0 ± 3.8[2)]	32.6 ± 6.5[1),3)]
Saturates	23.5 ± 2.0	23.9 ± 1.2	24.3 ± 1.4	22.8 ± 1.0
Monoenes	38.1 ± 3.5	32.8 ± 4.4	27.1 ± 3.8[2)]	23.8 ± 4.7[3)]
n − 6	3.9 ± 0.6	3.9 ± 0.8	5.0 ± 1.0	3.9 ± 0.9
n − 3	22.1 ± 2.8	32.6 ± 3.2[1)]	35.8 ± 3.4[2)]	41.1 ± 7.4[3)]

Notes: Values are mean and standard deviation ($n = 4$). 1) Significantly different from the value of control ($P < 0.05$). 2) Values of control after starvation are significantly different from those after feeding ($P < 0.05$). 3) Values of DHA after starvation are significantly different from those after feeding ($P < 0.05$).

3.3 Enzyme activities

Table 10 shows the activities of ICDH and G6PDH in the hepatopancreas and IPF. In general, the activities were higher in hepatopancreas than those in IPF. No difference was found in ICDH in the IPF and hepatic G6PDH between the groups after feeding. Activities of G6PDH in the IPF and ICDH in the hepatopancreas were depressed by the fortifica-

Table 9. Effects of dietary DHA fortification on the fatty acid composition (%) of IPF after feeding and starvation

Fatty acid	Feeding		Starvation	
	Control	DHA	Control	DHA
14 0	5.1 ± 0.3	4.6 ± 0.2	5.1 ± 0.5	4.2 ± 0.1
16 0	15.5 ± 1.6	15.2 ± 1.4	15.8 ± 0.5	14.7 ± 0.5
18 0	2.9 ± 0.5	2.6 ± 1.0	3.2 ± 0.1	3.2 ± 0.2
16 1n − 7	9.6 ± 0.8	9.0 ± 0.7	9.0 ± 0.4	7.9 ± 0.3
18 1n − 9	21.0 ± 1.0	18.3 ± 1.0	17.9 ± 1.2	14.4 ± 2.0
18 1n − 7	3.5 ± 0.3	3.9 ± 0.8	3.8 ± 0.5	3.2 ± 0.2
20 1n − 9	3.0 ± 1.2	3.1 ± 0.7	3.1 ± 1.5	2.1 ± 0.4
20 1n − 7	2.1 ± 1.2	2.2 ± 1.5	3.5 ± 2.5	2.9 ± 0.3
22 1n − 11	2.3 ± 0.9	1.4 ± 0.3	2.3 ± 1.8	1.5 ± 0.3
22 1n − 9	1.9 ± 0.7	2.0 ± 1.3	1.6 ± 0.9	1.5 ± 0.7
24 1n − 9	0.7 ± 0.5	1.1 ± 0.3	0.7 ± 0.3	1.9 ± 0.2
18 2n − 6	3.4 ± 1.4	2.4 ± 0.8	2.9 ± 0.3	2.3 ± 0.4
20 3n − 6	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
20 4n − 6	0.5 ± 0.1	0.4 ± 0.1	1.2 ± 0.2	1.3 ± 0.3
18 3n − 3	1.0 ± 0.4	0.7 ± 0.3	0.8 ± 0.1	0.6 ± 0.1
18 4n − 3	0.7 ± 0.3	0.6 ± 0.4	0.2 ± 0.0	0.2 ± 0.0
20 5n − 3	4.4 ± 0.7	4.5 ± 0.7	4.4 ± 0.8	4.2 ± 0.2
22 5n − 3	2.2 ± 0.3	1.6 ± 0.6	3.0 ± 0.3	2.8 ± 0.2
22 6n − 3	7.4 ± 1.7	15.7 ± 1.0[1)]	10.3 ± 1.8	20.0 ± 0.4[1),2)]
Saturates	23.5 ± 1.9	22.4 ± 2.3	24.1 ± 0.8	22.1 ± 0.7
Monoenes	44.2 ± 2.5	40.9 ± 2.8	42.0 ± 2.9	35.2 ± 2.0[1),2)]
n − 6	4.1 ± 1.5	2.9 ± 1.0	4.3 ± 0.5	3.8 ± 0.6
n − 3	16.5 ± 0.9	23.1 ± 1.0[1)]	18.8 ± 2.9	27.8 ± 0.7[1),2)]

Notes: Values are mean and standard deviation ($n = 4$). 1) Significantly different from the value of control ($P < 0.05$). 2) Values of DHA after starvation are significantly different from those after feeding ($P < 0.05$).

tion of DHA.

Activity of CPT in the hepatopancreas was enhanced by DHA fortification, both on hepatopancreas and body mass basis, as shown in Table 11. Starvation significantly elevated the hepatic CPT activity on hepatopancreas basis in both groups. The activity remained significantly higher in the DHA group after starvation.

Table 10. Effect of dietary DHA fortification on lipogenic enzyme activities in unit hepatopancreas (HP) and unit intraperitoneal fat body (IPF)

	Dietary group	
	Control	DHA
ICDH		
HP /μmol · min^{-1} · g^{-1} HP	269.0 ± 33.3	202.0 ± 13.8[1)]
/μmol · min^{-1} · g^{-1} BM	5.480 ± 0.990	3.620 ± 0.596[1)]
IPF /μmol · min^{-1} · g^{-1} IPF	13.8 ± 10.2	17.1 ± 11.8
/μmol · min^{-1} · g^{-1} BM	0.136 ± 0.084	0.124 ± 0.104
G6PDH		
HP /μmol · min^{-1} · g^{-1} HP	197.0 ± 12.9	200.0 ± 44.0
/μmol · min^{-1} · g^{-1} BM	4.030 ± 0.488	3.990 ± 1.912
IPF /μmol · min^{-1} · g^{-1} IPF	44.6 ± 2.5	13.6 ± 3.3[1)]
/μmol · min^{-1} · g^{-1} BM	0.392 ± 0.130	0.075 ± 0.038[1)]

Notes: ICDH represents NADP-isocitrate dehydrogenase and G6PDH represents glucose-6-phosphate dehydrogenase. Values are mean and SD ($n = 3 \sim 4$). 1) Significantly different from the value of control ($P < 0.05$). BM represents body mass.

Table 11. Effect of dietary DHA fortification on the carnitine palmitoyl transferase activity in unit hepatopancreas (HP) at the end of feeding and after starvation

	Dietary group	
	Control	DHA
Feeding		
/nmol · min^{-1} · g^{-1} HP	240.2 ± 86.1	881.7 ± 184.2[1)]
/nmol · min^{-1} · g^{-1} BM	4.801 ± 1.152	15.983 ± 2.220[1)]
Starvation		
/nmol · min^{-1} · g^{-1} HP	703.4 ± 167.0[2)]	1 604.9 ± 185.6[1),2)]
/nmol · min^{-1} · g^{-1} BM	9.672 ± 3.430	15.223 ± 0.458

Notes: Values are mean and standard deviation ($n = 3 \sim 4$). 1) Significantly different from the value of control ($P < 0.05$). 2) Significantly different from the value at the end of feeding ($P < 0.05$). BM represents body mass.

4 Discussion

The effect of DHA fortification on growth performance and biological parameters was found in the zero − year black sea bream (Om et al., 2001; Om et al., 2003), but not in the one-year fish in the

present study. This might be related to the different fish sizes and rearing conditions.

Dietary DHA successfully elevated its level in different organs of the fish body in the present work. During starvation, monoenes were selectively utilized in the nonporlar portion of hepatopancreas lipid, muscle lipid and IPF lipid. Especially in the IPF lipid, DHA significantly induced the selective consumption of monoenes. These findings are in accordance with the report that the fortification of DHA at 1.5% in a composed diet successfully improved its level (Om et al., 2003), and also support the findings that certain fatty acids were selectively used as an energy source (Dave et al., 1976; Jezierska et al., 1982; Ji et al., 2003; Nakagawa et al., 1987; Takeuchi and Watanabe, 1982).

In the present study, compared with that in the control group, not only the fatty acid profile of different organs, but also the lipogenic and lipolysis enzyme activities performed differently in the DHA group. This indicated that DHA may affect lipid metabolism besides its impact on the fatty acid composition of fish body, which is in agreement with the findings of Om et al. (2003), in which it showed that both the fatty acid profile and the lipid metabolism were influenced by the dietary DHA fortification.

High dietary lipid depressed the lipogenic activity in piracanjuba *Brycon orbignyanus*, coho salmon *Oncorhynchus kisutch* and channel catfish *Ictalurus punctatus* (Borba et al., 2003; Likimani and Wilson, 1982; Lin et al., 1977). Since the difference of the lipid content between the two groups is small in this study, the effect on enzyme activities can be further considered being from the dietary DHA, instead of dietary amount of lipid.

Lipogenic enzyme activities were higher in hepatopancrease than those in IPF, which are in accordance with the results obtained by Likimani and Wilson (1982). However, although ICDH in hepatopancrease and G6PDH in IPF were depressed by dietary DHA fortification, ICDH in IPF and G6PDH in hepatopancrease were not influenced. The reason needs to be further clarified.

Torstensen et al. (2004) reported that fed with rapeseed oil, a kind of plant oil which does not contain $n-3$ highly unsaturated fatty acids, Atlantic salmon showed decreased G6PDH and increased ICDH activities in liver. But the influence of highly unsaturated fatty acids on lipid metabolism enzyme activities has not been studied.

In the present study, dietary DHA fortification depressed the activities of ICDH and G6PDH and increased the CPT activity, which are in agreement with the findings of Sanze et al. (2000), in which it was reported that dietary unsaturated fatty acids depressed the liver lipogenic enzyme activity and increased the CPT activity in broiler chickens.

S14 and fatty acid synthase mRNA levels appear to be coordinately regulated by dietary polyunsaturated fats in both the mature and weanling rats (Clarke et al., 1990). On the other hand, the influence of unsaturated fatty acids on the CPT activity has been classified into different pathways. One might be due to the lesser risk of utilization of long chain fatty acids (Power and Newsholme, 1997). Second, the presence of long-chain fatty acids in the cultured hepatocytes of rat increased the concentration of CPT-I mRNA (Chatelain et al., 1996). Ukropec et al. (2003) also indicated that the hypotriglyceridemic effect of dietary $n-3$ fatty acid is associated with the raised carnitine palmitoyltransferase-2 activity and the mRNA level in rats. Furthermore, Niot et al. (1994) suggested that slight changes in $n-3$ or $n-6$ polyunsaturated fatty acids in the mitochondria outer membranes might alter the CPT-I activity. Bremer (1981) indicated that the CPT activity increased after starvation. Therefore, the decreased ICDH and G6PDH, the increased CPT activity in the DHA fortified groups found in the present study might be derived from these pathways. Also in the

present work, the CPT activity after starvation increased in both groups. This is in agreement with the findings of Bremer (1981). And also, higher CPT activity after starvation in the DHA group implied the effect of accumulated DHA in the fish body. But, although the activity of CPT in the DHA group appeared to be three point seven times than that in the control group on hepatopancreas basis, three point three times on the body mass basis after feeding, it decreased to two point three times on the hepatopancreas basis and one point six times on the body mass basis after starvation. Moreover, the activity on the body mass basis remained unchanged in the DHA group, probably indicating a limitation of the lipolysis.

The supplementation of DHA more than its level for nutritional requirement would satisfy sustaining a normal physiological condition such as lipid metabolism and physical vitality (Om et al., 2001; Om et al., 2003). In the present study, it is found that dietary DHA fortification may affect lipogenic and lipolysis enzyme activities, suggesting its effect on reducing lipogenesis and increase lipid catabolism. Future studies regarding the effect of DHA on gene expression are needed.

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