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Effects of Eicosapentaenoic Acid on Growth and Fatty Acid Composition of the Prawn, *Penaeus japonicus*

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Abstract

In the present study, the effects of eicosapentaenoic acid (20: 5ω3) on growth and fatty acid composition of the prawn, *Penaeus japonicus*, were investigated and compared with those of linoleic (18: 2ω6), linolenic (18: 3ω3), and docosahexaenoic (22: 6ω3) acids. Six groups of the prawns were maintained on the diets containing lipids as mentioned bellow: 1, 5% oleic acid (18: 1ω9) alone; 2, 4% 18: 1ω9+1% 18: 2ω6; 3, 4% 18: 1ω9+1% 18: 3ω3; 4, 4% 18: 1ω9+1% 20: 5ω3; 5, 4% 18: 1ω9+1% 22: 6ω3; 6, lipid free.

The highest weight gain and survival rate were attained in the group of prawns fed the diet containing 4% 18: 1ω9+1% 20: 5ω3. The prawns receiving the diets with 18: 2ω6 or 18: 3ω3 revealed lower weight gains and survival rates than those receiving 20: 5ω3 or 22: 6ω3. The supplementation of either 1% 20: 5ω 3 or 22: 6ω3 to the diet containing 4% 18: 1ω9 resulted in the increase of the proportion of ω3 fatty acids such as 20: 5ω3 and 22: 6ω3 in the body of prawns. These data suggest that 20: 5ω3 and/or 22: 6ω3 possess higher activity as essential fatty acids in the prawn, *P. japonicus*, than 18: 2ω6 and 18: 3ω3.

Linoleic acid (18: 2ω6) is an important essential fatty acid (EFA) for mammals, and it is converted *in vivo* to arachidonic acid (20: 4ω6) exerting more high EFA activity. However, recent investigations on the nutritional studies of lipids have shown that fatty acids of the linolenic family (ω3) are more important for fish[1~3] and crustaceans[4~8] than those of the linoleic family (ω6). Also WATANABE *et al.*[9] have reported that the carp, *Cyprinus carpio*, requires both 18: 2ω6 and linolenic acid (18: 3ω3). Recent studies on EFA in aquatic animals were reviewed by TAKEUCHI[10], YONE[11], and TESHIMA[12], and it has been suggested that EFA requirements of aquatic animals probably vary with species.

Previously, we have shown that growth of prawn, *Penaeus japonicus*, was improved by the supplementation of 18: 3ω3 to the diets containing oleic acid (18 : 1ω9) as a sole lipid source[7] and other lipids[13] such as pollack residual oil

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(PRO) and short-necked clam lipids. In the case of the prawn, *P. japonicus*, a high weight gain is generally attained when PRO and short-necked clam lipids, containing ω3 highly unsaturated fatty acids (HUFA), were used as a lipid source. Therefore, we suspect that the ω3 series of HUFA such as eicosapentaenoic acid (20: 5ω3) and docosahexaenoic acid (22: 6ω3) probably possess higher activity as EFA in the prawn rather than 18: 3ω3 and 18: 2ω6, because the prawn, *P. japonicus*, converts 18: 3ω3 to 20: 5ω3 and 22: 6ω3.

The purpose of the present study is to examine the effects of dietary 20: 5ω3 on growth and fatty acid composition of the prawn, *P. japonicus*.

Materials and Methods

Prawn and feeding methods The prawn, *P. japonicus*, was obtained from the Fisheries Research Laboratory, Faculty of Fisheries, Kagoshima University, and maintained on a commercial diet for prawn (Ebian, Kyowahakko Co., Japan) until use. Twenty prawns, weighing 0.5 g in average body weight, were placed in each tank (30 liters) and fed the diets (see Table 1) for 65 days by the similar feeding method to that described previously[14].

Diets The basal diet (diet No. 6, lipid free) was the same as reported previously[8] and composed of the following ingredients: casein (milk casein, lipid and vitamin free) 50.0 g, glucose 5.5 g, starch 4.0 g, glucosamine hydrochloride 0.8 g, sucrose 10.0 g, sodium citrate 0.3 g, sodium succinate 0.3 g, minerals 8.6 g, vitamins 2.7 g, cholesterol 0.5 g, cellulose powder 9.3 g, agar 3.0 g, and distilled water 130–135 ml. The test diets (diets No. 1 to No. 5) were prepared by adding 5% levels of lipids to the basal diet as shown in Table 1.

Fatty acids used The 18: 2ω6 and 18: 3ω3 were obtained from Sigma Chemical Co. and their purities were about 98–99% by gas-liquid chromatography (GLC) on 10% DEGS. The 20: 5ω3 and 22: 6ω3 were prepared from the squid

Table 1. Effects of dietary lipids on growth, survival, and lipid content of the prawn, *P. japonicus*

Diet No.	Dietary lipid	Feeding period (days)	Number of prawns used	Survival rate (%)	Weight gain (%)	Lipid content (%)*1	Neutral lipid (%)*2	Polar lipid (%)*2
1	5%18: 1ω9	65	20	65	43.6	1.13	28.3	71.7
2	4%18: 1ω9+ 1%18: 2ω6	65	20	70	82.1	1.37	27.5	72.5
3	4%18: 1ω9+ 1%18: 3ω3	65	20	80	97.4	1.44	28.7	71.3
4	4%18: 1ω9+ 1%20: 5ω3	65	20	90	141	1.32	25.3	74.7
5	4%18: 1ω9+ 1%22: 6ω3	65	20	85	130	1.42	25.5	74.5
6	Lipid free	65	20	70	39.8	1.16	29.1	70.9

*1 After feeding trials, lipids were extracted from the whole body of prawns. Lipid content was expressed as % of fresh weight.

*2 % of total lipids.

liver oil according to the method of TESHIMA *et al.*[15]. After saponification of squid liver oil, the fatty acids so obtained were methylated with 3% (w/v) HCl-methanol and then chromatographed on 5% (w/w) $AgNO_3$-silicic acid with hexane, hexane-ether, and hexane-ether-acetic acid. Since the 20: 5ω3 and 22: 6ω3 fractions so obtained contained small amounts of unknown polar substance(s) as impurities, they were respectively purified by using column chromatography on Kieselgel 60 with petroleum ether-ether (95: 5,v/v). The resultant 20 : 5ω3 and 22: 6ω3 methylesters had the 95-96% purities by GLC. As to 20: 5 ω3 methylester, the purity was further checked by nuclear magnetic resonance spectrometry (chemical shifts are given in δ); 0.95-1.10 (3H, triplet, methyl CH_3-CH_2-CH=CH-), 1.65-1.85 (2H, quartet, methylene CH_3-CH_2-CH=), 2.0-2.5 (6H complex signals, methylene =CH-CH_2-CH_2-CH_2-$COOCH_3$), 2.86 (8H, methylene =CH -CH_2-CH=), 3.68 (3H, singlet, methyl CH_3-O-CO-), and 5.50 (10H, methin -CH= CH-). The hydrolysis of 20: 5ω3 and 22: 6ω3 methylesters with 10% KOH in methanol gave 20: 5ω3 and 22: 6ω3, respectively.

Fatty acid composition After the feeding trials, lipids were extracted with chloroform-methanol-water (2: 2: 1, v/v)[16] from the whole body of prawns and separated into neutral and polar lipid fractions by thin-layer chromatography (TLC) on Kieselgel G with ether-benzene-ethanol-acetic acid (40: 50: 2: 0.2, v/v)[8]. The fatty acid composition of each lipid fraction was determined by GLC on 10% DEGS (3m×4mm i. d., column temperature 190°C)[17].

Results and Discussion

In the present study, the effects of 20: 5ω3 on growth and fatty acid composition of the prawn, *P. japonicus*, were investigated and the results were compared with those of 18: 2ω6, 18: 3ω3, and 22: 6ω3. The weight gain was low in the groups of prawns maintained on the diets free from ω6 and ω3 fatty acids (diets No. 1 and No. 6) (Fig. 1). The addition of 1% 18: 2ω6 (diet No. 2) or 1% 18: 3ω3 (diet No. 3) to 4% 18: 1ω9 improved the weight gain and survival rate of prawns as observed in the previous studies[7]. However, the highest weight gain and survival rate were attained when the diet contained 4% 18: 1ω9+1% 20: 5ω3 (diet No. 4) as a lipid source, and the diet No. 5 containing 4% 18: 1ω9+1% 22: 6ω3 followed to this. The results of feeding trials suggest that the ω3 HUFA such as 20: 5ω3 and 22: 6ω3 are more important as EFA for the prawn, *P. japonicus*, rather than 18: 2ω6 and 18: 3ω3.

The difference in the dietary lipids resulted in the variation of lipid content and fatty acid composition of prawn, *P. japonicus*, as shown in Tables 1 and 2. The lipid content (% of fresh weight) was higher in the groups of prawns fed the diets containing ω6 and ω3 fatty acids (diets No. 2, 3, 4, and 5), than in those fed the diets containing 18: 1ω9 and free from lipids. Also, the proportion of polar lipids to total lipids seemed to be high in the prawns received 20:

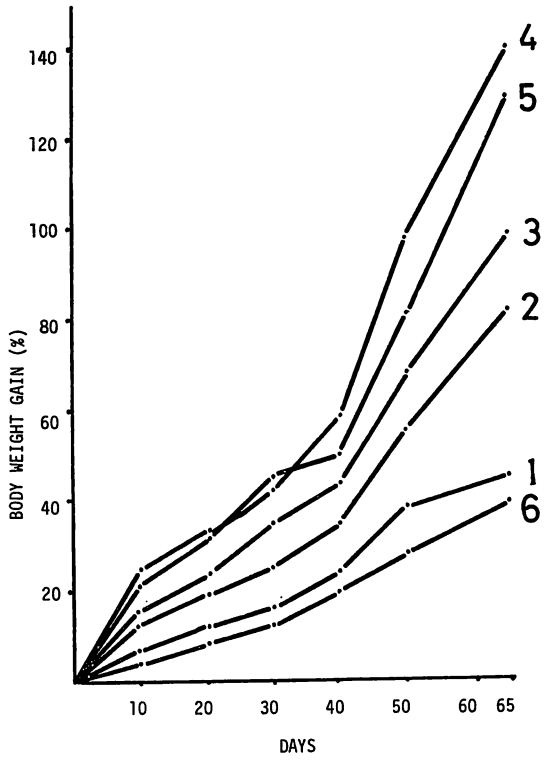

Fig. 1. Growth of the prawns fed the diets containing several fatty acids

The diets (1 to 6) contained the following fatty acids as lipid sources: 1, 5%18: 1ω9; 2, 4%18: 1ω9+1%18: 2ω6; 3, 4%18: 1ω9+1%18: 3ω3; 4, 4%18: 1ω9+1%20: 5ω3; 5. 4%18: 1ω9+1%22: 6ω3; 6, lipid free.

5ω3 or 22: 6ω3. Table 2 shows the percentage composition of fatty acids in the polar and neutral lipids from the whole body of prawns after the feeding trials. The supplementation of ω3 fatty acids such as 18: 3ω3 (diet No. 3), 20: 5ω3 (diet No. 4), and 22: 6ω3 (diet No. 5) resulted in the marked increase in the proportions of 20: 5ω3 and 22: 6ω3 in the polar lipids. As to the neutral lipids, the supplementation of 20: 5ω3 and 22: 6ω3 also brought about the elevation of proportions of 20: 5ω3 and 22: 6ω3, whereas that of 18: 3ω3 increase only the proportion of 22: 6ω3. Except for the increase in the proportion of 18: 2ω6, the supplementation of 18: 2ω6 (diet No. 2) did not cause noticeable variation in the fatty acid composition of both neutral and polar lipids.

In our previous studies, the prawn, *P. japonicus*, has been shown to be incapable of synthesizing 18: 2ω6, 18: 3ω3, and HUFA such as 20: 5ω3 and 22: 6ω3 from acetic acid-$^{14}C^{18)}$ and palmitic acid (16: 0)-$^{14}C^{19)}$, but capable of converting exogenous 18: 3ω3-$^{14}C^{12)}$ to 20: 5ω3 and 22: 6ω3. These tracer experiments

Table 2. Effects of dietary lipids on the fatty acid composition (%) of body lipids in the prawn, *P. japonicus*

Main fatty acid of body lipids from prawns		Diet supplied					
		No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Neutral lipid	12: 0	2.9	1.3	2.1	0.8	0.6	1.2
	14: 0	4.7	3.3	4.4	2.1	2.0	5.9
	15: 0	1.8	0.8	1.1	0.4	1.0	0.9
	16: 0	16.5	16.7	18.2	14.8	11.8	19.6
	16: 1ω7	8.3	7.5	7.5	5.7	5.6	17.7
	16: 2ω4	0.8	0.7	1.2	1.2	1.3	0.6
	17: 0	0.5	0.3	0.5	0.4	0.5	0.6
	18: 0	6.7	0.6	8.8	7.0	6.3	6.4
	18: 1ω9	31.8	31.9	28.0	35.8	34.6	20.4
	18: 2ω6	3.5	9.2	1.5	1.1	3.2	1.8
	18: 3ω3	0.1	0.1	1.5	0.1	0.2	0.5
	20: 1ω9	0.5	0.7	1.5	0.6	0.8	1.2
	20: 4ω6	3.9	4.4	2.8	4.3	5.2	2.6
	20: 5ω3	6.2	7.1	7.7	12.2	10.1	8.5
	22: 1ω9	2.2	1.1	1.9	1.2	1.2	1.2
	22: 6ω3	2.5	1.8	5.1	8.6	9.7	4.7
Polar lipid	12: 0	0.1	0.1	0.1	0.4	0.1	0.1
	14: 0	0.8	0.8	1.0	0.9	0.4	1.4
	15: 0	0.8	0.7	0.8	0.5	0.5	0.5
	16: 0	15.8	16.5	14.5	15.4	14.8	20.0
	16: 1ω7	4.5	4.2	3.6	3.6	2.5	11.7
	16: 2ω4	0.9	1.1	0.8	0.9	1.1	0.6
	17: 0	0.9	1.0	0.8	1.4	0.6	1.3
	18: 0	5.0	4.1	4.5	5.4	8.4	10.1
	18: 1ω9	44.9	41.7	38.6	39.9	37.9	24.8
	18: 2ω6	2.3	6.2	2.8	2.2	2.7	3.6
	18: 3ω3	0.2	0.1	1.7	0.3	0.1	0.3
	20: 1ω9	0.2	1.4	0.3	0.5	0.3	1.1
	20: 4ω6	3.1	3.7	3.2	2.0	3.3	2.8
	20: 5ω3	8.6	7.9	13.1	10.9	11.2	8.3
	22: 1ω9	1.5	0.7	0.7	0.9	0.6	0.3
	22: 6ω3	5.4	5.1	10.0	11.0	12.2	7.1

have suggested that crustaceans such as the prawn, *P. japonicus*, probably requires EFA for their normal growth. In fact, the feeding trials have shown that the addition of 18: 2ω6 and/or 18: 3ω3 to the diets improved the weight gain of prawn, *P. japonicus*[7,13]. SHEBART and MIES[5] also have reported the growth-promoting effect of 18: 3ω3 for *Penaeus aztecus*. The results of the present study showed that the weight gain and survival rate of the prawns receiving either 18: 2ω6 or 18: 3ω3 were inferior to those of the prawns receiving 20: 5

ω3 or 22: 6ω3. Therefore, it is likely that 20: 5ω3 and 22: 6ω3 play an important role as EFA in the prawn, *P. japonicus*, more than 18: 2ω6 and 18: 3ω3.

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