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Effects of dietary linoleic and linolenic acids on growth of prawn

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ABSTRACT

Ten groups of juvenile prawn, *Penaeus japonicus*, were each given a different test diet containing the following lipids: 1) 8% soybean oil; 2) 7% soybean oil + 1% linoleic acid; 3) 7% soybean oil + 1% linolenic acid; 4) 8% pollack (*Theragra chalcogramma*) residual liver oil (PRO); 5) 7% PRO + 1% linoleic acid; 6) 7% PRO + 1% linolenic acid; 7) 7% PRO + 1% glycerol monolinoleate; 8) 8% short-necked clam (*Tapes philippinarium*) lipids (*Tapes* lipids); 9) 7% *Tapes* lipids + 1% linoleic acid; 10) 7% *Tapes* lipids + 1% linolenic acid.

The highest weight gain was attained with the diet containing 7% *Tapes* lipids + 1% linolenic acid, and the lowest, with the diet containing 8% soybean oil alone. The addition of 1% linolenic acid (18 : 3ω3) to the diets containing 7% levels of soybean oil, PRO, or *Tapes* lipids markedly improved the weight gain of prawns.

The addition of 1% linoleic acid to the diet containing 7% PRO also resulted in weight gain. The fatty acid composition of prawn lipids was affected by that of the dietary lipids: the proportions of ω3-series of polyunsaturated fatty acids were elevated in those prawns fed diets containing an abundance of linolenic acid.

These data indicate that both linoleic and linolenic acids are effective for satisfactory survival and growth of the prawn *P. japonicus*, and that the effect of linoleic acid is inferior to that of linolenic acid.

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RÉSUMÉ

Effet de l'acide linoléique et de l'acide linolénique sur la croissance de la crevette

Dix lots de juvéniles de *Penaeus japonicus* ont été nourris à l'aide d'aliments expérimentaux contenant les lipides suivants : 1) 8 % d'huile de soja; 2) 7 % d'huile de soja + 1 % d'acide linoléique; 3) 7 % d'huile de soja + 1 % d'acide linolénique; 4) 8 % d'huile de résidu de foie de morue du Pacifique occidental *Theragra chalcogramma* (pollack residual liver oil : PRO); 5) 7 % PRO + 1 % d'acide linoléique; 6) 7 % PRO + 1 % d'acide linolénique; 7) 7 % PRO + 1 % de monolinoléate de glycérol; 8) 8 % de lipides extraits de *Tapes philippinarium*; 9) 7 % de lipides extraits de *T. philippinarium* + 1 % d'acide linoléique; 10) 7 % de lipides extraits de *T. philippinarium* + 1 % d'acide linolénique.

Les plus fortes augmentations de poids ont été obtenues à l'aide de l'aliment contenant les lipides extraits de *T. philippinarium* + 1 % d'acide linolénique, et les moins fortes correspondent à l'emploi de l'aliment contenant comme source de lipides seulement 8 % d'huile de soja. L'addition de 1 % d'acide linolénique (18 : 3ω3) à l'aliment contenant un taux de 7 % d'huile de soja, PRO ou des lipides extraits de *T. philippinarium* améliore aussi nettement le gain de poids des crevettes. D'autre part, l'ajout de 1 % d'acide linoléique à l'aliment contenant 7 % de PRO conduit également à un gain de poids. La composition en acides gras des lipides des

crevettes est modifiée par la composition des lipides contenus dans les aliments : les proportions des acides gras polyinsaturés de la série en ω3 sont augmentés chez les crevettes nourries à l'aide d'aliments enrichis en acide linolénique.
Ces résultats indiquent que l'acide linoléique et l'acide linolénique sont tous deux efficaces pour la survie et la croissance de la crevette *P. japonicus*, et que l'effet de l'acide linoléique est inférieur à celui de l'acide linolénique.

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INTRODUCTION

It has been known for several years that the lipids of marine animals differ in composition from those of terrestrial animals. The former contain a large proportion of long-chain polyunsaturated fatty acids, and this fact is of considerable interest to specialists working in different fields: ecologists concerned with food chains; biochemists engaged in the study of the metabolism of marine animals or aquatic organisms; fish farmers or nutritionists looking for efficient and inexpensive foods for artificially-raised aquatic animals.

Recent progress in nutritional studies concerning fish indicates that fish growth is affected by the types and level of lipids supplied in their diets. Investigations in several laboratories have shown by feeding trials that certain fatty acids such as linoleic (18 : 2ω6) *, linolenic (18 : 3ω3); eicosapentaenoic (20 : 5ω3) and docosahexaenoic (22 : 6ω3) acids are essential for the growth of chinook salmon (Nicolaides, Woodall, 1962) rainbow trout (Castell *et al.*, 1972 *a*, 1972 *b*, 1972 *c*; Lee *et al.*, 1967; Watanabe *et al.*, 1974 *a*, 1974 *b*, 1974 *c*; Yu, Sinnhuber, 1972, 1975), carp (Watanabe *et al.*, 1975 *a*, 1975 *b*) red sea bream (Yone, Fujii, 1975 *a*, 1975 *b*; Fujii, Yone, 1976), and turbot (Cowey *et al.*, 1976).

Information concerning the lipid nutritional requirements of Crustacea is, however, scanty.

In an earlier study, the authors demonstrated that the addition of 1% linoleic or linolenic acids to a diet containing 4% oleic acid (18 : 1ω9) as a sole lipid source improved the growth of the prawn, *Penaeus japonicus* and suggested that both play an important role as essential fatty acids (Kanazawa *et al.*, 1977 *a*). This prawn was subsequently found to attain a high weight gain when fed diets containing 8% lipids such as pollack (*Theragra chalcogramma*) residual liver oil (abbreviated as PRO in this text) or short-necked clam (*Tapes philippinarium*) lipids (Kanazawa *et al.*, 1977 *b*). In the present study, therefore, the effects of linoleic and linolenic acids on the growth of prawn were examined with the use of diets containing 8% lipid. The effects of dietary fatty acids on the fatty acid composition of prawn lipids were also evaluated. The present paper describes these effects and discusses their significance.

* 18 : 2 ω 6 : 18 carbon atoms-2 double bonds-ω 6 : greek letter ω indicate the position of the ultimate double bond between the 6th and the 7th carbon atom counted from the terminal methyl group.

MATERIALS AND METHODS

Prawn and feeding methods

The prawn, *P. japonicus*, hatched in Mitsui Nohrin Kaiyo Sangyo Co, were transported to the Kamoike Marine Production Laboratory, Faculty of Fisheries, University of Kagoshima, where they where maintained until use on a commercial pellet diet for prawn. Prawns, averaging 0.5 g in body weight, were used for the feeding trials (Experiments I, II, and III) with the test diets. Fifteen or twenty prawns were placed in each trough (30 l) and reared as described previously (Kanazawa *et al.*, 1977 *b*). Animals receive food *ad libitum* and the excess is removed each morning. Water temperature, 24° ± 1°C is controlled by thermostat, and circulates in a closed system containing a sandy bottom, 6 cm thick as filter and air lifts. Two thirds of the water volume is changed every week. There are only traces of reduction in the sediment by the end of the experiments. Dietary treatments were run in duplicate.

The body weight gain was measured every 10 days and at the conclusion of the feeding trials.

Test diets

The basal diet was identical with that reported in the previous paper (Kanazawa *et al.*, 1977 *b*), and the

Table 1
Composition of the basal diet for the prawn.

Ingredient	Composition (g)
Casein (lipid and vitamin free)	50.0
Glucose	5.5
Starch	4.0
Glucosamine hydrochloride	0.8
Sucrose	10.0
Sodium citrate	0.3
Sodium succinate	0.3
Minerals (*a*)	8.6
Vitamins (*b*)	2.7
Cholesterol	0.5
Cellulose powder	9.3
Lipids (*c*)	8.0
Agar	3.0
Distilled water (ml)	130-135

(*a*) Minerals (*g*) : K_2HPO_4, 2.60; KCl, 0.80; $MgSO_4$, 1.30; $FeSO_4$, $7H_2O$, 0.13; $Ca_3(PO_4)_2$, 2.35; $MnSO_4$, $7H_2O$, 0.02; $CaCO_3$, 1.40.

(*b*) Vitamins (mg) para-amino-benzoic acid, 5; biotin, 0.2; folic acid, 0.4; nicotinic acid, 20; Ca pantothenate, 30; thiamine-HCl (B_1), 2; riboflavine (B_2), 4; pyridoxine-HCl (B 6), 6; cyanocobalamine (B_{12}), 0.04; choline HCl, 300; inositol, 200; ascorbic acid (C), 2 000; menadione (K), 2; β-carotene (provitamin A), 4.9; calciferol (D), 0.6; α-tocopherol (E), 10.

(*c*) In the test diets, several types of lipids were used as shown in Table 2.

composition of ingredients is as shown in Table 1. PRO and glycerol monolinoleate were supplied by Riken Vitamin Oil Co, Ltd (Japan). Linoleic and linolenic acids were purchased from Sigma Chemical Co (USA) and their purity was determined by gas-liquid chromatography (GLC) to be of the order of 99%. Soybean oil was obtained from Nakarai Chemicals Co, Ltd (Japan). Lipids of short-necked clam, *Tapes philippinarum*, (abbreviated as *Tapes* lipids in this text) were extracted from the whole bodies with chloroform-methanol-water, 2 : 2 : 1 (Bligh, Dyer, 1959).

In the test diets, the lipids of the basal diet were replaced by various lipids as shown in Table 2. The test diets so prepared contained 8% levels of lipids. The fatty acid composition of soybean oil, PRO and *Tapes* lipids was determined by GLC and listed in Table 3.

Table 2
Composition of dietary lipids in the test diets.

Diet No.	Dietary lipid
Experiment I	
1	8% soybean oil
2	7% soybean oil + 1% linoleic acid
3	7% soybean oil + 1% linolenic acid
Experiment II	
4	8% PRO (pollack residual oil)
5	7% PRO + 1% linoleic acid
6	7% PRO + 1% linolenic acid
7	7% PRO + 1% glycerol monolinoleate
Experiment III	
8	8% *Tapes* lipid (*a*)
9	7% *Tapes* lipid + 1% linoleic acid
10	7% *Tapes* lipid + 1% linolenic acid

(*a*) Lipids extracted with chloroform-methanol from the whole body of the short-necked clam, *Tapes philippinarum*.

Analysis of fatty acid composition

To examine the effect of dietary fatty acids on the fatty acid composition of prawn lipids, lipids were extracted from the prawns at the conclusion of the feeding trials, and separated into neutral and polar lipids by thin-layer chromatography with ether-benzene-ethanol-acetic acid 40 : 50 : 2 : 0,2, as described previously (Kanazawa *et al.*, 1977 *b*). The fatty acid compositions was determined as the methyl esters-derivatives by direct transesterification as in an earlier paper (Teshima *et al.*, 1976): to 10 mg of lipids, 4 ml of HCl (5%)-methanol and 0,5 ml of dry benzene were added, and this mixture was refluxed over calcium chloride at 60°C for 2 hours. After cooling, the reaction mixture was diluted with 2 volumes of distilled water and the fatty acid methylesters were extracted with petroleum ether 3 times, dehydrated over anhydrous sodium sulphate and concentrated to dryness under nitrogen gas. Gas liquid chromatography (GLC) was carried out on a Shimadzu Gas Chromatograph GC-4BP; column length is 3 m, internal diameter 4 mm, packing 10% diethylene glycol succinate (DEGS) polyester on 60-80 mesh Shimalite W. The column temperature was 185 or 195°C and carrier gas (nitrogen) flow was 40 ml/mn. A flame ionization detector was used and sample size was 1 µl. Identification of peak in GLC was accomplished by comparing relative retention times (RRT) to stearic acid methylester of samples with standards; a semi-log plot of RRT of fatty acid methylesters vs. the number of carbon atoms (Herb *et al.*, 1962) and the end carbon chain (Ackman, 1963 *a*), equivalent chain length (Hofstetter *et al.*, 1965) values and separation factors (Ackman, 1963 *b*). An aliquot of samples was hydrogenated with platinum oxide in *n*-hexane and the hydrogenates were analysed by GLC to confirm the identity of peaks. In addition, the peaks of chromatograms were compared with those of the methylesters prepared from cod liver oil as secondary reference standard (Ackman, Burger, 1965).

Table 3
Composition of basal dietary lipids.

Main fatty acid	Soybean oil	Composition (%) Dietary lipids PRO (*a*)	*Tapes* lipids (*b*)
14 : 0	0.1	7.8	3.4
16 : 0	13.3	15.0	26.3
16 : 1	0.1	14.2	7.6
18 : 0	4.4	2.6	5.9
18 : 1ω9 (*c*)	20.9	20.5	6.8
18 : 2ω6	49.4	1.8	0.5
18 : 3ω3	8.0	0.2	0.9
20 : 1ω9 (*c*)	1.2	12.2	7.3
20 : 4ω6	–	–	3.3
22 : 1ω9 (*c*)	–	11.2	2.3
20 : 5ω3	–	10.9	13.7
22 : 6ω3	–	2.2	13.3

(*a*) Pollack residual oil.

(*b*) Lipids extracted with chloroform-methanol from the whole body of the short-necked clam, *Tapes philippinarum*.

(*c*) Other isomers probably present.

Results

The survival rate and percentage weight gain of prawns receiving the test diets are shown in Table 4 and Figure 1.

Comparisons between Table 2 content and the three groups of curves in Figure 1 show clearly that soybean oil containing diets 1), 2) and 3) give low results, even supplemented with linoleic and linolenic acids. PRO and *Tapes* lipid may contain more effective components on growth than soybean oil. 1% glycerol monolinoleate gives better results than soybean oil diets and even 8% PRO supplemented diets. The weights gain was the highest in the group given diet (10) containing 7% *Tapes* lipids + 1% linolenic acid, and the lowest in the group given the diet 1) containing 8% soybean oil alone. The addition of 1% linolenic acid to diets containing 7% soybean oil, PRO, or *Tapes* lipids, distinctly improved the weight gain.

The addition of linoleic acid to 7% levels of either soybean oil or *Tapes* lipids did not increase weight gain, but its addition to the diet prepared with PRO increased the weight gain to a small degree. The authors assume that the ineffectiveness of 1% linoleic acid addition to the diets containing 7% soybean oil or *Tapes* lipids may be attributed to the pre-existence of either linoleic or arachidonic (20 : 4ω6) acids in the lipids used (*cf.* Table 3). The PRO diet contained extremely small amounts of linoleic acid and no arachidonic acid and the addition of 1% linoleic acid to 7% PRO resulted in a slight increase of weight gain. These data suggest that linoleic acid has a growth promoting effect on the prawn similar to that observed in the case of linolenic acid. The survival rate was inferior in the groups receiving diets 1) and 2), containing soybean oil with or without 1% linoleic acid in comparison with that of the other groups. Variation in the survival rate was less marked among the groups given diets 3), 4), 5), 6), 7), 8), 9) and 10).

These results lead to the conclusion that both linoleic and linolenic acids are effective for satisfactory survival and growth of the prawn, but that the activity of linoleic acid seems to be inferior to that of linolenic acid.

Table 4

Effects of dietary linoleic and linolenic acids on the survival and growth of prawn.

Diet No.	Feeding period (days)	Number of prawn	Survival (%)	Average body weight Initial (g)	Average body weight Final (g)	Weight gain (%)
			Experiment I			
1	30	15	60	0.51	0.81	59
2	30	15	60	0.50	0.81	63
3	30	15	87	0.53	0.92	73
			Experiment II			
4	30	20	90	0.53	1.05	98
5	30	20	90	0.52	1.09	110
6	30	20	85	0.51	1.16	128
7	30	20	85	0.50	1.09	117
			Experiment III			
8	30	20	95	0.50	1.06	111
9	30	20	95	0.51	1.09	113
10	30	20	90	0.50	1.15	129

Tables 5 and 6 show the fatty acid composition of the polar and neutral lipid fractions from the whole body of prawn fed with the test diets for 30 days. In the prawns receiving diets supplemented with 1% linoleic acid, the percentage of linoleic acid increased in both polar and neutral lipid fractions, but those of arachidonic acid (20 : 4ω6) did not do so. This suggests that the bioconversion of linoleic to arachidonic acid does not take place in the prawn. When linolenic acid (18 : 3ω3) is fed, its level in prawn increases. In polar lipids, 18 : 3ω3 increases from 0.4% (diet 4 : 8% PRO) to 6,0% (diet 6 : 7% PRO + 1% linolenic acid). In neutral lipids, 18 : 3ω3 increases from 1,8% (diet 4) to 5.4% (diet 6). Similar results are observed comparing the effects of diets 8 and 10 on prawn linolenic acids. Same variations less marked, occurs for 20 : 5ω3 increasing by a factor of 3 to 9, and for 22 : 6ω3, affected by a ratio comprised between 0.95 and 1.3.

This suggests also that linolenic acid may be converted to the ω3-series of long chain polyunsaturated fatty acid in the prawn, *P. japonicus*. Since the weight gain of prawn was large in the groups given diets 4) and 8) containing 20 : 5ω3 and/or 22 : 6ω3, and small in the group given diet 1) lacking in both fatty acids, it is likely

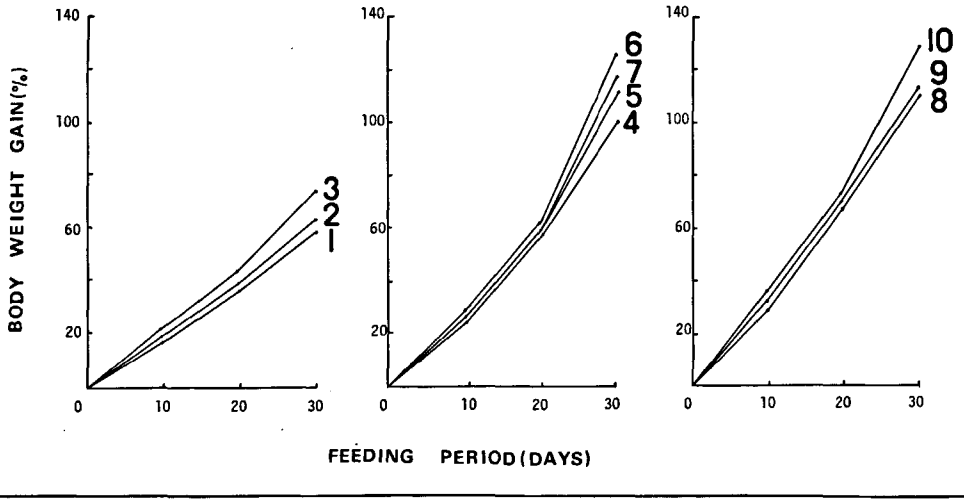

Figure 1

Weight gain of prawns fed diets containing 8% levels of various lipids with or without 1% linoleic or linolenic acids.

Table 5
Major fatty acids of polar lipids from the whole body of prawns after feeding of the test diets.

Fatty acid	Fatty acid composition (%) of polar lipids from prawns Diet supplied					
	4	5	6	8	9	10
14 : 0	0.9	0.6	0.8	0.4	0.6	0.3
16 : 0	16.3	17.6	16.3	21.7	16.9	17.1
16 : 1	3.2	3.3	3.9	3.0	2.0	1.8
18 : 0	9.5	9.8	9.2	11.1	14.0	12.4
18 : 1ω9	25.3	21.8	22.5	17.8	14.3	16.2
18 : 2ω6	3.3	10.4	4.9	6.3	15.2	3.6
18 : 3ω3	0.4	0.4	6.0	0.6	0.5	5.9
20 : 1ω9	6.1	4.8	1.0	2.9	2.3	1.0
20 : 4ω6	1.6	1.5	1.6	4.5	3.9	3.2
22 : 1ω9	0.1	0.2	0.3	–	0.4	0.2
20 : 5ω3	16.1	13.2	18.4	13.4	15.7	19.6
22 : 6ω3	10.5	10.3	14.9	10.7	10.8	14.2
Σω9/Σω6	6.42	2.25	3.66	1.92	1.83	2.56
Σω9/Σω3	1.17	1.12	0.61	0.84	1.30	0.44
$\frac{18:0+18:1\omega9}{20:5\omega3}$	2.16	2.39	1.72	2.16	1.80	1.46
$\frac{18:0+18:1\omega9}{22:6\omega3}$	3.31	3.06	2.13	2.70	2.62	2.01
$\frac{18:0+18:1\omega9}{20:5\omega3+22:6\omega3}$	1.31	1.34	0.94	1.23	1.07	0.85

Separation of 20 : 4ω6 from 20 : 3ω3 was not completely achieved.

Table 6
Major fatty acids of neutral lipids from the whole body of prawns after feeding of the test diets.

Main fatty acid	Fatty acid composition (%) of neutral lipids from prawns Diet No. supplied					
	4	5	6	8	9	10
14 : 0	1.5	0.9	1.8	1.2	0.4	0.7
16 : 0	12.1	12.8	12.1	14.1	13.4	17.1
16 : 1	3.7	3.3	4.6	3.4	2.3	2.1
18 : 0	6.2	9.1	5.4	12.0	13.0	10.1
18 : 1ω9	20.3	21.1	23.4	14.1	12.6	14.3
18 : 2ω6	2.4	10.6	1.4	6.3	19.8	1.2
18 : 3ω3	1.8	0.2	5.4	0.5	0.5	4.8
20 : 1ω9	7.7	7.5	11.8	3.3	2.3	3.1
20 : 4ω6	2.5	2.7	1.7	6.2	3.2	3.1
22 : 1ω9	0.8	0.2	0.8	0.5	0.4	0.1
20 : 5ω3	16.8	11.1	16.7	11.6	11.9	18.1
22 : 6ω3	8.5	7.6	8.1	6.1	5.8	8.1

Separation of 20 : 4ω6 from 20 : 3ω3 was not completely achieved.

that the ω3-series of long chain polyunsaturated fatty acids such as 20 : 5ω3 and 22 : 6ω3 are more effective than linolenic acid as required fatty acids in the prawn, *P. japonicus*.

In examination of variations in the fatty acid compositions of dietary and tissue lipids, the ratio of 20 : 3ω9/20 : 4ω9 has been used as an index of satisfied essential fatty acid requirement (EFA index) in mammals. In the case of aquatic animals such as fish, however, the use of ratios of 20 : 3ω9/22 : 6ω3 for carp has been recommended (Castell *et al.*, 1972*b*; Takeuchi, Watanabe, 1977). On the other hand, Owen and co-wokers (Owen *et al.*, 1972, 1975) have pointed out that EFA indices were not available for the plaice, *Pleuronectes platessa*, and turbot, *Scophthalmus maximum*. Hence, in the present study, the possibility of using the EFA index was investigated. However the tissue lipids of the prawn generally contained extremely small amounts of 20 : 3ω9, and the satisfied essential fatty acid requirements should therefore be assessed by the ratio of other fatty acids. To this end, several ratios of fatty acids were calculated and the results are shown in Table 7. It has been indicated predecently by Guary *et al.* (1976), that 16 : 0, 16 : 1, 18 : 1 and presumably 18 : 0 fatty acids were synthesized *de novo* by juvenile *Penaeus japonicus*. With regard to the fatty acids of polar lipids extracted from the prawns after the feeding trials, the ratios of

$$\frac{18:0+18:1\omega9}{20:5\omega3},$$

or

$$\frac{18:0+18:1\omega9}{22:6\omega3},$$

Table 7
Evaluation of the effects of dietary lipids on the fatty acid compositions of polar and neutral lipids in the whole body of prawn.

Fatty acids of prawn tissues	Polar lipids Diet No. supplied						Neutral lipids Diet No. supplied					
	4	5	6	8	9	10	4	5	6	8	9	10
Fatty acids (%)												
A. 18 : 0 + 18 : 1ω9	34.8	31.6	31.7	28.9	28.3	28.6	26.5	30.2	28.8	26.1	25.6	24.4
B. 18 : 1ω9 + 20 : 1ω9 + 22 : 1ω9	31.5	26.8	23.8	20.7	17.0	17.4	28.8	28.8	36.0	17.9	15.3	17.5
C. 16 : 0 + 18 : 0 + 18 : 1ω9 + 20 : 1ω9 + 22 : 1ω9	57.3	54.2	49.3	53.5	47.9	46.9	47.1	50.7	53.5	44.0	41.7	44.7
D. 18 : 2ω6 + 20 : 4ω6	4.9	11.9	6.5	10.8	19.1	6.8	4.9	13.3	3.1	12.5	23.0	4.3
E. 20 : 5ω3 + 22 : 6ω3	26.6	23.5	33.3	24.1	26.5	33.8	25.3	18.7	24.8	17.7	17.7	26.2
F. 18 : 3ω3 + 20 : 5ω3 + 22 : 6ω3	27.0	23.9	39.3	24.7	27.0	39.7	27.1	18.9	30.2	18.2	18.2	31.0
Ratio of fatty acids												
A/20 : 5ω3	2.2	2.4	1.7	2.2	1.8	1.5	1.6	2.7	1.7	2.3	2.2	1.3
A/22 : 6ω3	3.3	3.1	2.1	2.7	2.6	2.0	3.1	4.0	3.6	4.3	4.4	3.0
A/F	1.3	1.2	0.8	1.2	1.0	0.7	1.0	1.6	1.0	1.4	1.4	0.8
A/E	1.3	1.3	0,9	1.2	1.1	0.8	1.0	1.6	1.2	1.5	1.4	0.9
A/D	7.1	2.7	4.9	2.7	1.5	4.2	5.4	2.3	9.3	2.1	1.1	5.7
B/D	6.4	2.3	3.7	1.9	0.9	2.6	5.9	2.2	11.6	1.4	0.7	4.1
B/F	1.2	1.1	0.6	0.8	0.6	0.4	1.1	1.5	1.2	1.0	0.8	0.6
C/D	11.7	4.6	7.6	5.0	2.5	6.9	9.6	3.8	17.3	3.5	1.8	10.4
C/F	2.1	2.3	1.3	2.2	1.8	1.2	1.7	2.7	1.8	2.4	2.3	1.4

or

$$\frac{18:0+18:1\omega9}{20:5\omega3+22:6\omega3},$$

were low in the groups given diets containing an abundance of linolenic acid and ω3-long chain polyunsaturated fatty acids such as 20 : 5ω3 and 22 : 6ω3 in comparison with other groups.

A similar tendency was also observed in the ratio of

$$\frac{16:0+18:0+20:1\omega9+22:1\omega9}{18:3\omega3+20:5\omega3+22:6\omega3}.$$

As far as the fatty acids of neutral lipids are concerned, however, the ratios of the above mentioned fatty acids were not always matched to the growth rate of prawns in the feeding trials, especially in the groups receiving PRO as a basal lipid. These data indicate that the ratio of some ω9/ω3 acid(s) in the polar lipids of prawn tissues might reflect the satisfied essential fatty acid requirements. However, further detailed studies to establish the EFA index for the prawn, *P. japonicus*, are warranted.

DISCUSSION

The present study indicates that the addition of linolenic acid to the diets containing 7% levels of soybean oil, PRO, or *Tapes* lipids resulted in an improved weight gain, and this confirms that linolenic acid is one of the required fatty acid for satisfactory survival and growth of the prawn, *P. japonicus*. However, the growth-promoting effect of linoleic acid was not so clearly demonstrated in this study as in the previous study (Kanazawa *et al.*, 1977 *b*) in which 4% oleic acid was used as a basal lipid. The authors suggest that the prawn, *P. japonicus*, requires dietary sources of both linoleic and linolenic acids for normal growth, although the activity of linoleic acid as a required fatty acid appears to be inferior that of linolenic acid. In crustaceans, the growth-promoting effect of linolenic acid has also been reported in other species of prawn, *Penaeus duorarum* (Sick, Andrews, 1973) and *Penaeus aztecus* (Shewbart, Mies, 1973). Sandifer and Joseph (1976) obtained better growth in juvenile *Macrobrachium rosenbergii* fed with 3% oil extracted from *Penaeus setiferus*. This procedure leads to a reduction in the level of ω6 fatty acids and an increase in the percentages of ω3 fatty acids and saturated acids in the diets. In this experiment, these authors observed that ω3 fatty acids remain constant indicating their conservation and/or their biosynthesis. The animals contained, in contrast, much less 18 : 2ω6 than in their diets by the end of experiment, suggesting that this fatty acid may have been utilised as an energy source. Guary *et al.* (1976) using various oils and 4% clam oil found that diets rich in ω3 polyunsaturated fatty acids give the best growth and a high level of ω3 polyunsaturated fatty acids in juvenile (0.3 to 0.6 g) *Penaeus japonicus*. Favourable effects of ω3 fatty acids have been demonstrated also in *Homarus gammarus* by Castell and Covey (1976). However, the growth rate of *Palaemon serratus* seems to be maximal when the ratio of 18 : 3ω3/18 : 2ω6 is 0.5 in the diet (Martin, 1978). On the basis of the information available so far, the requirements of aquatic animals in fatty acids would appear to vary from species to species. Thus the rainbow trout (Castell *et al.*, 1972 *a*, 1972 *b*, 1972 *c*; Watanabe *et al.*, 1974 *a*, 1974 *b*, 1974 *c*) requires linolenic rather than linoleic acid, whereas the carp (Watanabe *et al.* 1975 *a*, 1975 *b*; Takeuchi, Watanabe, 1977) requires both fatty acids. In the case of the turbot (Cowey *et al.* 1976) both linoleic and linolenic acids exert a growth-promoting effect, although the effect of linoleic acid is considerably inferior to that of linolenic acid. The turbot would appear to ressemble the prawn, *P. japonicus*, with regard to fatty acid requirements. On the other hand, the red sea bream has been reported to require neither linoleic nor linolenic acids, but long chain polyunsaturated fatty acids such as 20 : 5ω3 and 22 : 6ω3 (Yone, Fujii, 1975 *a*, 1975 *b*; Fujii, Yone, 1976). Variation in requirement for dietary linoleic and linolenic acids for growth among the above mentioned aquatic animals may be an interesting subject of future study from the point of view both of comparative biochemistry and of nutrition.

As far as the study of food chains is concerned, detailed knowledge of the qualitative requirements of each of the main species is as important as knowledge of the quantitative requirements at each stage. The growth of aquatic animals depends firstly on the quantity of foodstuffs found in their biotope, secondly on the chemical composition of these foodstuffs, and finally on seasonal variation in this composition. The modelling of food chains should thus incorporate data of this type, which are at present lacking in the scientific literature.

CONCLUSION

The addition of 1% linolenic acid to diets containing 7% levels of soybean oil, PRO, or *Tapes* lipids markedly improved the weight gain of prawn, *P. japonicus*. Addition of 1% linoleic acid to the diet containing 7% PRO also resulted in enhanced weight gain.

GLC analysis of fatty acids from the whole body of prawns after the feeding trials showed that the addition of linolenic acid (18 : 3ω3) to the diets increased the proportion of the ω3-series of polyunsaturated fatty acids, such as 20 : 5ω3 and 22 : 6ω3.

These data indicate that both linoleic and linolenic acids improved the growth of the prawn, *P. japonicus*, but that the effect of linoleic acid as a necessary fatty acid for satisfactory survival and growth is inferior to that of linolenic acid.

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