Requirements of Prawn, Penaeus japonicus, for Essential Fatty Acids

著者	KANAZAWA Akio, TESHIMA Shin-ichi, ENDO Minoru
journal or publication title	鹿児島大学水産学部紀要=Memoirs of Faculty of Fisheries Kagoshima University
volume	28
page range	27-33
別言語のタイトル	クルマエビにおける必須脂肪酸要求量
URL	http://hdl.handle.net/10232/13142

Mem. Fac. Fish., Kagoshima Univ.
Vol. 28 pp. 27~33 (1979)

Requirements of Prawn, *Penaeus japonicus*, for Essential Fatty Acids

Akio KANAZAWA, Shin-ichi TESHIMA
and Minoru ENDO*

Abstract

Feeding trials using purified diets were carried out to estimate the requirements of prawn, *Penaeus japonicus*, for essential fatty acids (EFA) such as linoleic acid (18: 2ω6), linolenic acid (18: 3ω3), eicosapentaenoic acid (20: 5ω3), and docosahexaenoic acid (22: 6ω3). For 18: 2ω6 and 18: 3ω3, the optimum weight gain was attained on diets containing 1.0% of either 18: 2ω6 or 18: 3ω3. However, the weight gain on diets containing 18: 2ω6 or 18: 3ω3 was inferior even at the optimum levels to that on the diets containing lower levels of 20: 5ω3 or 22: 6ω3. This indicated that 18: 2ω6 and 18: 3ω3 are not as effective EFA for the prawn as 20: 5ω3 and 22: 6ω3. For 20: 5ω3 and 22: 6ω3, the optimum weight gain was attained on the diets containing 1% of either 20: 5ω3 or 22: 6ω3.

Recent advances in nutritional studies on lipids for aquatic animals have demonstrated the unique aspects of essential fatty acids (EFA) requirements in the rainbow trout, *Salmo gairdnerii*, the carp, *Cyprinus carpio*, the eel, *Anguilla japonica*, the red sea bream, *Chrysophrys major*, the turbot, *Scophthalmus maximus*, the yellow tail, *Seriola quinqueradiata*, the black sea bream, *Acanthopagurus schlegelii*, the opaleye, *Girella punctata*, etc. The nutritive values of dietary lipids from the viewpoint of EFA in fresh-water and marine fish have been reviewed by TAKEUCHI[1] and by YONE[2], respectively.

As for crustaceans, we have also shown that the prawn, *Penaeus japonicus*, requires EFA such as linoleic acid (18: 2ω6)[3,4], linolenic acid (18: 3ω3)[3,4], eicosapentaenoic acid (20: 5ω3)[5], and docosahexaenoic acid (22: 6ω3)[6] for normal growth, suggesting that 20: 5ω3 and 22: 6ω3 probably exert a higher EFA activity than 18: 2ω6 and 18: 3ω3. In the present study, we intend to estimate the requirements of the prawn, *P. japonicus*, for 18: 2ω6, 18: 3ω3, 20: 5ω3, and 22: 6ω3.

Materials and Methods

The specimens of the prawn, *P. japonicus*, were obtained from the Mitsui-Nohrin Kaiyosangyo Co. and maintained on a commercial diet until use. Oleic acid (18: 1ω9), 18: 2ω6, and 18: 3ω3 were purchased from Sigma Chemicals,

* Faculty of Fisheries, University of Kagoshima, 4-50-20 Shimoarata, Kagoshima

and 20: 5ω3 and 22: 6ω3 were prepared from the squid liver oil by the same method as described previously[7]. The purities of 18: 1ω9, 18: 2ω6, 18: 3ω3, 20: 5ω3, and 22: 6ω3 were 99, 98, 99, 90, and 94%, respectively, determined by gas-liquid chromatography (GLC) on 10% DEGS[8].

In Experiment I, 7 groups of the prawns (each group, 15 prawns), 0.65 g in average body weight, were maintained on the diets 1–7 (Table 1). In Experiment II, 8 groups of the prawns (each group, 30 prawns), 0.15 g in average body weight, were maintained on the diets 1 and 7–14 (Table 1). The composition of the basal diet (lipid-free) was the same as reported previously[9]. Each group of prawns was reared in an aquarium (30 liters) for 50 days by the similar methods to those reported previously[9].

Table 1. Composition of dietary lipids in Experiment I and Experiment II

Diet	Dietary lipids*
Experiment I	
1	5.0% Oleic acid
2	4.5% Oleic acid + 0.5% Linoleic acid
3	4.0% Oleic acid + 1.0% Linoleic acid
4	3.0% Oleic acid + 2.0% Linoleic acid
5	4.5% Oleic acid + 0.5% Linolenic acid
6	4.0% Oleic acid + 1.0% Linolenic acid
7	3.0% Oleic acid + 2.0% Linolenic acid
Experiment II	
1	5.0% Oleic acid
8	4.5% Oleic acid + 0.5% Eicosapentaenoic acid
9	4.0% Oleic acid + 1.0% Eicosapentaenoic acid
10	3.0% Oleic acid + 2.0% Eicosapentaenoic acid
11	4.5% Oleic acid + 0.5% Docosahexaenoic acid
12	4.0% Oleic acid + 1.0% Docosahexaenoic acid
13	3.0% Oleic acid + 2.0% Docosahexaenoic acid
14	5.0% Pollack liver oil

* Fatty acids except oleic acid were added to the diets as a form of methylester.

The body weight and survival rate were measured every 10 days and at the end of feeding trials. After the feeding trials, lipids were extracted with chloroform-methanol-water[10] from the whole body of the prawns, and the content of lipids, neutral lipids (NL), and polar lipids (PL) were determined as described previously[11]. Also, the fatty acid composition of NL and PL was analyzed by GLC on 10% DEGS[8].

Results

Table 2 shows the results of feeding trials in Experiment I and Experiment

II. Experiment I was planned to estimate the requirements of 18: 2ω6 and 18: 3ω3. In Experiment I, the weight gain of prawns reached a maximum in the groups receiving either 1.0% 18: 2ω6 or 1.0% 18: 3ω3. Elevation of 18: 2ω6 or 18: 3ω3 levels from 1.0 to 2.0% did not result in the improvement of weight gain. The groups of prawns receiving 1.0 and 2.0% levels of either 18: 2ω6 or 18: 3ω3 gave a high survival rate as compared with those receiving the diets containing no EFA and 0.5% levels of 18: 2ω6 or 18: 3ω3. These results indicate that optimum levels of dietary 18: 2ω6 or 18: 3ω3 for the prawn, *P. japonicus*, are about 1%, respectively, under the experimental conditions adopted. However, the weight gain of prawns on the diets containing 1.0% 18: 2ω6 or 1.0% 18: 3ω3 was clearly inferior to that on the diets containing 0.5 and 1.0% levels of 20: 5ω3 or 22: 6ω3 (see Experiment II). These results imply that 18: 2ω6 and 18: 3ω3 are not as effective EFA as 20: 5ω3 and 22: 6ω3 for the prawn, *P. japonicus*. Although the prawn has been shown to convert 18: 3ω3 to 20: 5ω3 and 22: 6ω3[12)], the bioconversion does not seem to proceed rapidly enough to satisfy the requirements of 20: 5ω3 and 22: 6ω3.

Experiment II was conducted to estimate the requirements of 20: 5ω3 and 22: 6ω3. The weight gain of prawns reached a maximum in the groups receiving either 1.0% 20: 5ω3 or 1.0% 22: 6ω3. Elevation of 20: 5ω3 or 22: 6ω3 levels

Table 2. Results of the feeding trials and the lipid content of prawn after feeding trials

Diet	Average body weight (g)		Weight gain (%)	Survival rate (%)	Lipid content (%)*1	% of lipids	
	Initial	Final				NL*2	PL*2
Experiment I							
1	0.707	0.976	38.0	33	1.17	35.3	64.7
2	0.699	1.189	70.1	60	1.33	35.4	64.7
3	0.637	1.114	75.0	67	1.29	37.4	62.6
4	0.644	1.125	74.8	73	1.39	38.5	61.5
5	0.685	1.193	74.2	53	0.92	44.8	55.2
6	0.688	1.238	80.0	67	0.88	37.6	62.4
7	0.690	1.256	82.1	87	0.95	38.8	61.2
Experiment II							
1	0.168	0.234	39.3	20	1.26	27.8	72.2
8	0.167	0.313	79.2	50	1.23	26.2	73.8
9	0.158	0.385	144	80	1.27	24.2	75.8
10	0.162	0.405	150	85	1.33	24.4	75.6
11	0.154	0.278	80.3	70	1.13	29.4	70.6
12	0.172	0.407	138	83	1.33	27.0	73.0
13	0.133	0.319	140	89	1.36	26.3	73.7
14	0.167	0.468	180	90	1.31	28.5	71.5

*1 % of fresh prawn
*2 NL, neutral lipids; PL, polar lipids

from 1.0 to 2.0% gave no further improvement of weight gain. The survival rates of prawns receiving 1.0 and 2.0% levels of either 20:5ω3 or 22:6ω3 were high and almost comparable to that of prawns receiving 5% pollack liver oil as lipid sources. These results indicate that optimum levels of dietary 20:5ω3 or 22:6ω3 for the prawn, *P. japonicus*, are about 1%, respectively.

The lipid content of prawn bodies did not vary markedly with the dietary levels of EFA such as 18:2ω6, 18:3ω3, 20:5ω3, or 22:6ω3, although it seemed to be slightly low in the prawns fed the diets containing 18:3ω3. Also, the ratio of NL to PL was slightly lower in the prawns receiving either 20:5ω3 or 22:6ω3 than in the prawns receiving 18:2ω6 or 18:3ω3. Tables 3 and 4 show the effect of dietary levels of EFA on the composition of main fatty acids in NL and PL fractions from the prawn. The addition of 18:2ω6 increased the proportion of 18:2ω6 in both PL and NL and 20:4ω6 in PL, but not that of ω3 HUFA (highly unsaturated fatty acids) such as 20:5ω3 and 22:6ω3 in PL and NL. The addition of 18:3ω3 also elevated the proportion of 18:3ω3 in both PL and NL and 22:6ω3 in PL. The increment of 18:2ω6 and 18:3ω3 proportions in the prawn bodies induced by supplementation with the respective fatty acids was proportional to the increment in dietary levels of 18:2ω6 and 18:3ω3. Also, the addition of 20:5ω3 or 22:6ω3 resulted in the elevation of ω3

Table 3. Effect of dietary levels of EFA on the percentage composition of main fatty acids in polar lipids from the whole body of prawn

Fatty acid and dietary level		% Composition							
		16:0	18:0	18:1ω9	18:2ω6	18:3ω3	20:4ω6	20:5ω3	22:6ω3
Experiment I									
EFA-free*1		12.9	5.7	48.3	3.2	0.1	0.2	10.5	2.5
	0.5%	14.1	7.1	47.9	3.8	0.1	2.6	9.5	2.6
18:2ω6	1.0%	14.9	6.2	44.3	7.1	0.1	3.1	8.3	2.3
	2.0%	12.4	6.2	40.7	13.8	0.4	4.0	8.0	1.6
	0.5%	13.0	4.5	33.7	2.7	1.0	4.1	13.0	11.9
18:3ω3	1.0%	13.3	4.7	32.7	2.5	2.7	2.6	12.1	11.1
	2.0%	12.1	3.4	35.5	2.5	6.1	3.6	12.0	11.6
Fxperiment II									
EFA-free*1		15.3	7.1	48.8	1.7	t*2	2.6	5.0	2.6
	0.5%	14.3	7.1	42.8	t	0.1	3.0	11.4	5.3
20:5ω3	1.0%	13.7	4.6	39.4	t	0.1	3.6	15.3	5.3
	2.0%	10.6	5.3	39.3	0.3	0.3	3.7	16.6	7.9
	0.5%	12.6	5.4	37.0	t	0.1	4.0	11.0	8.6
22:6ω3	1.0%	13.7	3.9	40.8	t	0.2	2.4	10.7	12.8
	2.0%	9.6	3.5	40.4	0.2	0.1	2.6	10.7	19.7

*1 Diet contained 5% 18:1ω9 as a lipid source
*2 Less than 0.1%

Table 4. Effect of dietary levels of EFA on the percentage composition of main fatty acids in neutral lipids from the whole body of prawn

Fatty acid and dietary level		% Composition							
		16: 0	18: 0	18: 1ω9	18: 2ω6	18: 3ω3	20: 4ω6	20: 5ω3	22: 6ω3
Experiment I									
EFA-free*1		14.1	6.0	30.9	0.5	0.6	5.0	7.7	2.5
	0.5%	14.8	8.6	25.0	1.5	0.1	6.3	7.6	2.2
18: 2ω6	1.0%	10.2	8.9	17.5	4.5	0.2	5.8	8.1	3.2
	2.0%	8.2	8.3	19.3	5.3	0.2	5.2	8.0	2.5
	0.5%	11.4	8.1	18.8	3.1	2.1	9.0	9.4	2.7
18: 3ω3	1.0%	11.6	6.0	22.3	2.6	6.1	6.6	9.7	3.2
	2.0%	12.0	6.5	24.9	1.2	9.7	9.5	9.9	2.5
Experiment II									
EFA-free*1		16.3	7.3	45.6	4.0	0.5	t*2	3.3	1.6
	0.5%	13.4	7.2	45.1	2.2	0.7	t	11.0	3.4
20: 5ω3	1.0%	10.8	6.9	45.7	2.4	t	t	15.1	2.9
	2.0%	10.3	8.3	42.9	3.3	0.4	0.1	14.8	2.7
	0.5%	10.7	5.3	45.3	3.1	0.7	0.1	8.2	6.5
22: 6ω3	1.0%	14.4	5.1	40.3	3.3	0.7	t	6.0	6.6
	2.0%	8.6	6.0	39.4	1.6	t	t	8.9	15.3

*1 Diet contained 5% 18: 1ω9 as a lipid source
*2 Less than 0.1%

HUFA such as 20: 5ω3 and 22: 6ω3 in both PL and NL.

Discussion

There are several reports on the requirements of EFA for fish. YU and SINNHUBER[13] have shown that 1% 18: 2ω6 attained the best weight gain for the rainbow trout. Also, the optimum levels of 18: 3ω3 for the rainbow trout have been reported to be 1% and 0.86–1.66% in the diets by CASTELL *et al.*[14] and by WATANABE *et al.*[15], respectively. In the case of rainbow trout[13], however, the addition of 18: 3ω3 to a diet rich in 18: 2ω6 has been found to reduce the weight gain due to the competitive inhibition between 18: 2ω6 and 18: 3ω3 as also observed in the chicken[16] and rat[17-20], whereas the simultaneous supplementation of both 18: 2ω6 and 18: 3ω3 has been reported to exert an additive effect for the growth of carp[21] and eel*. The best weight gain was attained when the diets contained both 1% 18: 2ω6 and 1% 18: 3ω3 for the carp[21] and both 0.5% 18: 2ω6 and 0.5% 18: 3ω3 for the eel[21]. The optimum levels of 18: 2ω6 and 18: 3ω3 for the prawn, *P. japonicus*, determined in the present study

* S. ARAI, T. WATANABE, T. TAKEUCHI, and Y. SHINMA: the Autumn Meeting of Japanese Society of Scientific Fisheries, p. 92 (1975).

are similar to those of 18: 2ω6 and 18: 3ω3 for the rainbow trout[14,15]. However, a marked difference was perceived between the prawn and the rainbow trout with respect to the growth enhancing effects of 18: 2ω6 and 18: 3ω3. The weight gain of the prawn on the diets containing 18: 2ω6 or 18: 3ω3 was inferior even at the optimum levels to that on the diets containing lower levels of 20: 5ω3 or 22: 6ω3, differing from the rainbow trout[14-15] and also the carp[21].

On the other hand, neither 18: 2ω6 nor 18: 3ω3 has been shown to exert an EFA efficiency for the red sea bream[22-24], the black sea bream*[1], and the opaleye*[1]. In the red sea bream[25]*[2], the best weight gain and feed conversion have been attained on diets containing 0.5% ω3 HUFA or 0.5% 20: 5ω3. Generally, ω3 HUFA such as 20: 5ω3 and 22: 6ω3 are more effective as EFA than 18: 2ω6 and 18: 3ω3 not only for the red sea bream but also for other fish. Yu and Sinnhuber[26] have reported that the EFA efficiency of 1% 22: 6ω3 was almost equal to that of 1% 18: 3ω3 for the rainbow trout. Later, Takeuchi and Watanabe[27] demonstrated that not only 0.5% 20: 5ω3 but also 0.5% 22: 6ω3 gave a higher weight gain in the rainbow trout than 1.0% 18: 3ω3, indicating the additive effect between 20: 5ω3 and 22: 6ω3 in the promotion of growth. They have further shown that the supplementary effect of 0.5% ω3 HUFA for the carp slightly exceeded that of 1.0% 18: 3ω3[21]. In the present study, the requirements of the prawn, *P. japonicus*, for 20: 5ω3 and 22: 6ω3 were estimated to be about 1% in the diet. This value was high as compared with the requirements of 20: 5ω3, 22: 6ω3, or ω3 HUFA which had been reported for the rainbow trout, the carp, and the red sea bream. Since the requirements of EFA seem to vary with the levels and types of dietary lipids[28,29], the estimated requirements of 20: 5ω3 and 22: 6ω3 for the prawn, *P. japonicus*, should be evaluated in this light.

References

1) Takeuchi T.: in "Fish Culture and Dietary Lipids" (ed. by Japan. Soc. Sci. Fish.), Suisangaku Series No. 22, Koseisha Koseikaku, Tokyo, 1978, pp. 23–42 (in Japanese).

2) Yone Y.: in "Fish Culture and Dietary Lipids" (ed. by Japan. Soc. Sci. Fish.), Suisangaku Series No. 22, Koseisha Koseikaku, Tokyo, 1978, pp. 43–59 (in Japanese).

3) Kanazawa A., S. Tokiwa, M. Kayama, and M. Hirata: *Bull. Japan. Soc. Sci. Fish.*, **43**, 1111–1114 (1977).

4) Kanazawa A., S. Teshima, S. Tokiwa, and H. J. Ceccaldi: *Oceanol. Acta*, **2**, 41–47 (1979).

5) Kanazawa A., S. Teshima, M. Endo, and M. Kayama: *Mem. Fac. Fish., Kagoshima Univ.*, **27**, 35–40 (1978).

6) Kanazawa A., S. Teshima, S. Tokiwa, M. Kayama, and M. Hirata: *Bull. Japan. Soc.*

*1 K. Yamada and Y. Yone: the Spring Meeting of Japanese Society of Scientific Fisheries, p. 190 (1977).

*2 H. Nakayama and Y. Yone: the Spring Meeting of Japanese Society of Scientific Fisheries, p. 189 (1977).

Sci. Fish., **45**, 1151-1153 (1979).
7) TESHIMA S., A. KANAZAWA, and S. TOKIWA: *Bull. Japan. Soc. Sci. Fish.*, **44**, 927 (1978).
8) TESHIMA S., A. KANAZAWA, and H. OKAMOTO: *Mem. Fac. Fish. Kagoshima Univ.*, **25**, 41-46 (1976).
9) KANAZAWA A., S. TESHIMA, and S. TOKIWA: *Bull. Japan. Soc. Sci. Fish.*, **43**, 849-856 (1977).
10) BLIGH E. G. and W. J. DYER: *Can. J. Biochem. Physiol.*, **37**, 911-917 (1959).
11) TESHIMA S. and A. KANAZAWA: *Bull. Japan. Soc. Sci. Fish.*, **42**, 1129-1135 (1976).
12) KANAZAWA A., S. TESHIMA, and K. ONO: *Comp. Biochem. Physiol.*, **63B**, 295-298 (1979).
13) YU T. C. and R. O. SINNHUBER: *Lipids*, **10**, 63-66 (1975).
14) CASTELL J. D., R. O. SINNHUBER, J. H. WALER, and D. J. LEE: *J. Nutr.*, **102**, 77-86 (1972).
15) WATANABE T., C. OGINO, Y. KOSHIISHI, and T. MATSUNAGA: *Bull. Japan. Soc. Sci. Fish.*, **40**, 493-499 (1974).
16) CENTURY B. and M. K. HORWITT: *Arch. Biochem. Biophy.*, **104**, 416-422 (1964).
17) MOHRHAUER H. and R. T. HOLMAN: *J. Lipid Res.*, **4**, 34-35 (1963).
18) MOHRHAUER H. and R. T. HOLMAN: *J. Nutr.*, **81**, 67-74 (1963).
19) RAHM J. J. and R. T. HOLMAN: *J. Nutr.*, **84**, 15-19 (1964).
20) GARCIA R. T. and R. T. HOLMAN: *J. Am. Oil Chem. Soc.*, **42**, 1137-1141 (1965).
21) TAKEUCHI T. and T. WATANABE: *Bull. Japan. Soc. Sci. Fish.*, **43**, 541-551 (1977).
22) YONE Y. and M. FUJII: *Bull. Japan. Soc. Sci. Fish.*, **41**, 73-77 (1975).
23) YONE Y. and M. FUJII: *Bull. Japan. Soc. Sci. Fish.*, **41**, 79-86 (1975).
24) FUJII M. and Y. YONE: *Bull. Japan. Soc. Sci. Fish.*, **42**, 583-588 (1976).
25) FUJII M., H. NAKAYAMA, and Y. YONE: *Rep. Fish. Res. Lab. Kyushu Univ.*, **3**, 65-86 (1976).
26) YU T. C. and R. O. SINNHUBER: *Lipids*, **7**, 450-454 (1972).
27) TAKEUCHI T. and T. WATANABE: *Bull. Japan. Sco. Sci. Fish.*, **43**, 947-953 (1977).
28) YU T. C. and R. O. SINNHUBER: *Aquaculture*, **8**, 309-317 (1976).
29) TAKEUCHI T. and T. WATANABE: *Bull. Japan. Soc. Sci. Fish.*, **43**, 893-898 (1977).