クルマエビの栄養要求に関する研究7

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Nutritional Requirements of Prawn—VII Effect of Dietary Lipids on Growth

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The effects of dietary lipids on the growth and fatty acid composition of the prawn, *Penaeus japonicus*, were examined. The survival and growth of prawn kept on soybean oil diet were extremely low. However, all diets containing pollack residual oil were found to improve both survival and growth. The highest growth was obtained in the prawn group receiving 8% short necked clam lipid. Dietary lipids affected the fatty acid composition of prawn lipids. The diets containing either pollack residual oil or short necked clam lipid elevated 20: 5ω3 and 22: 6ω3 levels as compared with soybean oil diet.

Many reports have been shown about the effect of dietary lipids on the growth of marine animals. Especially, the essential fatty acids in the diets of rainbow trout[1-8], carp[9,10], and red sea bream[11-13] have been recently investigated.

In crustaceans, it has been reported that the prawn, *Penaeus japonicus*[14-16], and the lobster, *Homarus americanus*[17], require the dietary sterol for normal growth owing to lacking the ability to synthesize sterol from acetate. However, regarding the effect of other lipids on the growth of crustaceans, relatively little information has been obtained. KANAZAWA *et al.*[18] have used 8% soybean oil as a lipid in the artificial diet for prawn. SHUDO *et al.*[15] have reported that the highest growth rate of prawn was obtained on the diet containing 4.4% squid liver oil. Also, 8% oil mixture which consists of 33.3% corn oil, 33.3% hydrolyzed vegetable and animal fats, and 33.3% menhaden oil has been used in the diet of Penaeid shrimp by SICK *et al.*[19]. DESHIMARU and KUROKI[20] have shown that the diet containing 6% soybean oil-cod liver oil (1: 1) was effective for growth of prawn.

The present study describes the effects of dietary lipids on the growth and fatty acid composition of the prawn, *P. japonicus*, and also deals with the modification of the artificial diet to establish a high growth rate.

Materials and Methods

Prawn The prawn, *P. japonicus*, 0.5 g in body weight, hatched in Mitsui Nohrin Kaiyo Sangyo Co., Ltd. was used in this experiment.

Lipids The liquid and powdered pollack residual oils were supplied from Riken Vitamin Oil Co., Ltd. (Japan). Liquid pollack residual oil was obtained by distilling

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away vitamin A from pollack liver oil. Powdered pollack residual oil was made by drying liquid pollack liver oil with an adsorbent, and contained about 67% of oil content. Consequently, 12% powdered pollack residual oil corresponded to 8% liquid pollack residual oil. Soybean oil was purchased from Nakarai Chemicals Co., Ltd. (Japan). Short necked clam lipid was isolated from *Tapes philippinarum*, by the method of BLIGH and DYER[21].

Artificial diet In order to obtain a high growth rate of prawn, the artificial diet (Diet A) devised by KANAZAWA *et al.* previously[14,18,22] was further modified as shown in Diet B of Table 1. Chitin was removed from the artificial diet due to be ineffective for the growth of prawn. Glucosamine was reduced to a half amount. Milk casein was substituted for soybean casein. As the supplemental effect of amino acid was not clear, methionine, tryptophan, glutamic acid, and glycine were tentatively removed. Mineral mixture (8.6%) and vitamin mixture (2.7%) with the composition as shown in Tables 2 and 3 gave a superior growth. Morin, feeding factor of the silkworm, was excluded. Agar has been used by KANAZAWA *et al.*[18] as a binder of the artificial diet for prawn. Recently, FORSTER[23] has also reported that agar was one of binders desirable for prawn diet. For the purpose of making an insoluble diet in the sea water, the dry ingredients were powdered to a size of 10–50 μ diameter and thoroughly mixed with water of 130–135 m*l* per

Table 1. Composition of the artificial diet for prawn

Ingredient	Diet A*1	Diet B*2
Glucose	5.5	5.5
Sucrose	10.0	10.0
Starch	4.0	4.0
Chitin	4.0	—
Glucosamine	1.5	0.8
Casein (Lipid and vitamin free)	50.0	50.0
Methionine	1.0	—
Tryptophan	0.2	—
Na-Glutamate	0.2	—
Glycine	0.1	—
Na-Citrate	0.3	0.3
Na-Succinate	0.3	0.3
Soybean oil	8.0	—
Pollack residual oil (liquid)*3	—	8.0
Cholesterol	0.5	0.5
Mineral mixture	7.7	8.6
Vitamin mixture	2.6	2.7
Morin	0.1	—
Cellulose powder	4.0	9.3
Agar	5.0	3.0
Water	100 m*l*	130–135 m*l*

*1 Artificial diet presented in 1970[18]

*2 Modified artificial diet

*3 Residual oil obtained by distilling away vitamin A from pollack liver oil

Table 2. Composition of mineral mixture

Mineral	(g/100 g of dry diet)
K_2HPO_4	2.000
$Ca_3(PO_4)_2$	2.720
$MgSO_4 \cdot 7H_2O$	3.041
$NaH_2PO_4 \cdot 2H_2O$	0.790
Total	8.551

Table 3. Composition of vitamin mixture

Vitamin	(mg/100 g of dry diet)
p-Aminobenzoic acid	10.00
Biotin	0.40
Inositol	400.00
Nicotinic acid	40.00
Ca-Pantothenate	60.00
Pyridoxine-HCl	12.00
Riboflavin	8.00
Thiamine-HCl	4.00
Menadione	4.00
β-Carotene	9.60
α-Tocopherol	20.00
Cyanocobalamine	0.08
Calciferol	1.20
Na-Ascorbate	2000.00
Folic acid	0.80
Choline chloride	120.00
Total	2690.08

100 g of dry diet. After adjusting to pH 6.8, the diet was heated at 100°C for 20 min, and sealed in plastic casing (Kurehalon), and again heated for 10 min.

The modified artificial diet (Diet B) thus prepared indicated a high growth rate as shown in Table 4. In the present study, the lipid free diet lacking pollack residual oil from the Diet B was used as a basal diet. The addition of different levels of lipids was arranged by replacing lipids with cellulose powder.

Table 4. Survival and growth of prawn fed on the modified artificial diet (Diet B)

Supply of sea water	Capacity of tank (liters)	Feeding period (days)	No. of prawn at start	Survival (%)	Percent gain
Circulating sea water (renewed at ratio of 150 liters per day)	30	30	20	95	147
Running sea water (5 liters per min)	1000	30	100	94	198

Feeding method The prawns were maintained in the troughs (30 liters) equipped with circulating systems by filtration through a sand bed. The troughs were further supplied with the running sea water at the rate of 150 liters per day. After the 30-day feeding of each test diet, the average weight and survival of animals were determined.

Determination of lipids Lipids were extracted with chloroform-methanol-water[21], and then fractionated into the polar and neutral lipids by thin layer chromatography on Kiesel gel G-Kiesel gel GF_{254} (4: 1) with ethylether-benzene-ethanol-acetic acid (40: 50: 2: 0.2) as a solvent system. In this chromatography, the polar and neutral lipids were eluted with chloroform-methanol (1: 1) and chloroform-methanol (9: 1), respectively. The fatty acid composition was analyzed by gas-liquid chromatography (GLC) after methylation of each fraction with 5% of anhydrous hydrogen chloride in methanol[24]. GLC was performed with a Shimadzu GC-4BPF equipped with a flame ionization de-

tector and stainless steel column (4 mm i.d.×3 m long), packed with 10% DEGS on Shimalite W (60–80 mesh).

Results

The effects of soybean oil and pollack residual oil on the growth of prawn are as shown in Table 5. Feeding diet with soybean oil resulted in poor growth, but powdered pollack residual oil diet remarkably improved weight gain. Also, the growth rate of prawn approached a maximum when 12% powdered pollack residual oil was supplemented in the diet.

Table 5. Effect of pollack residual oil levels on growth of prawn

Diet no.	Dietary lipid	Feeding period (days)	No. of prawn at start	Percent gain
1	8% Soybean oil	30	15	33.1
2	8% Powdered pollack residual oil*	30	15	97.7
3	12% Powdered pollack residual oil*	30	15	128.8
4	16% Powdered pollack residual oil*	30	15	82.1

* Powdered pollack residual oil contained about 67% of oil content

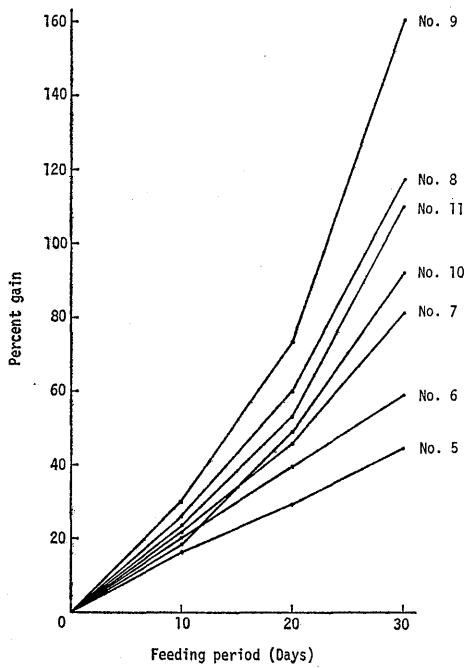

Fig. 1. Effect of various dietary lipids on growth of prawn.
No. 5, Lipid free diet; No. 6, 8% Soybean oil diet; No. 7, 8% Liquid pollack residual oil diet; No. 8, 12% Powdered pollack residual oil diet; No. 9, 8% Short necked clam lipid diet; No. 10, 4% Soybean oil+4% Liquid pollack residual oil diet; No. 11, 4% Soybean oil+6% Powdered pollack residual oil diet.

The effects of various dietary lipids on the survival and percent gain are summarized in Table 6 and Fig. 1. The survival and growth of prawn received lipid free diet or 8% soybean oil diet were extremely low. All diets containing pollack residual oil were found to increase the survival and growth rates. The highest growth was obtained by the group of prawn receiving 8% short necked clam lipid. The effect of powdered pollack residual oil on the growth of prawn was slightly superior to that of liquid. The effect of a mixture of soybean oil and pollack residual oil on the

Table 6. Effect of dietary lipids on survival and growth of prawn

Diet no.	Dietary lipid	Survival (%)	Percent gain
5	Lipid free	33.0	44.6
6	8% Soybean oil	60.0	59.0
7	8% Liquid pollack residual oil	87.0	81.2
8	12% Powdered pollack residual oil*	93.0	117.0
9	8% Short necked clam lipid	93.0	160.3
10	4% Soybean oil+4% Liquid pollack residual oil	93.0	92.1
11	4% Soybean oil+6% Powdered pollack residual oil*	93.0	110.0

* Powdered pollack residual oil contained about 67% of oil content

Table 7. Effect of various dietary lipids on lipid contents in the whole body of prawn

Diet no.	Total lipid (%)*	Polar lipid (%)	Neutral lipid (%)
5	0.8	54.8	45.2
6	0.9	61.9	38.1
7	1.2	68.2	31.8
8	1.0	76.7	23.3
9	1.4	63.7	36.3
10	1.2	70.6	29.4
11	0.8	70.5	29.5

* Percentage to fresh weight

Table 8. Fatty acid composition (%) of polar lipid fraction from the whole body of prawn

Fatty acid	Diet no.						
	5	6	7	8	9	10	11
12:0	0.7	0.6	0.1	0.8	0.2	0.3	0.5
12:1	0.6	0.1	trace	0.5	0.2	0.1	0.5
13:0	—	0.1	0.1	0.2	0.1	—	0.1
14:0	0.8	1.1	0.6	0.9	0.4	1.0	0.8
14:1	trace	0.1	0.1	—	trace	0.5	trace
15:0	0.2	0.8	0.3	0.6	0.3	0.9	0.5
15:1	0.8	0.2	0.5	0.4	0.8	1.5	1.0
16:0	18.7	15.0	17.0	16.3	21.7	16.2	15.1
16:1ω7	5.6	7.6	5.1	3.2	3.0	3.9	3.6
17:0	0.8	0.1	0.8	0.2	0.3	2.4	0.6
16:2ω4	0.7	0.7		0.8	0.5		
16:3ω4	1.6	0.8	0.7	0.9	1.5	1.7	1.2
16:3ω3	0.1	0.1	0.1	0.3	0.6	0.4	0.7
18:0	10.3	10.1	8.3	9.5	10.1	9.1	9.3
18:1ω9	24.7	26.1	24.3	25.2	17.8	21.4	24.6
18:2ω6	5.3	9.2	2.2	3.3	6.3	6.5	10.0
18:2ω4	0.1	0.1	0.2	0.1	—	0.7	—
18:3ω3	0.9	0.9	0.3	0.4	0.6	0.1	1.3
18:4ω3	—	—	0.2	0.2	—	0.3	0.2
20:1ω9	3.7	6.6	5.7	6.1	2.9	5.1	4.5
20:2ω6 20:3ω9	1.5	1.2	0.8	0.7	1.1	1.3	1.0
20:3ω3 20:4ω6	3.7	1.1	2.5	1.6	4.5	1.9	1.3
22:1ω9	0.2	0.4	0.9	0.1	—	0.5	0.2
20:5ω3	11.0	11.4	14.9	16.1	13.4	13.9	13.6
22:3ω6	0.4	0.1	—	0.2	0.5	0.1	0.1
22:4ω6	—	—	—	—	0.6	0.5	0.1
22:5ω6	0.2	trace	—	0.1	0.3	0.1	trace
22:5ω3	0.1	trace	1.9	0.8	0.6	0.4	0.8
22:6ω3	7.3	5.5	12.4	10.5	11.7	9.2	8.4

Table 9. Fatty acid composition (%) of neutral lipid fraction from the whole body of prawn

Fatty acid	Diet no.						
	5	6	7	8	9	10	11
12:0	1.2	0.4	0.3	2.5	trace	0.8	1.0
12:1	1.0	0.3	0.3	2.3	0.9	0.6	0.7
13:0	0.4	0.1	0.2	0.6	0.5	0.2	trace
14:0	0.9	0.2	0.4	1.5	1.2	0.6	1.3
14:1	—	0.8	2.1	0.4	0.2	2.7	trace
15:0	0.7	0.6	1.1	0.2	0.3	0.9	0.2
15:1	0.6	0.4	0.5	0.8	0.6	0.6	0.3
16:0	15.1	10.6	14.5	12.1	15.1	14.2	11.0
16:1ω7	6.5	4.2	4.5	3.7	3.4	4.9	2.9
17:0	0.7	0.7	0.6	0.5	0.8	0.9	0.4
16:2ω4	0.9			0.5	1.1		0.3
16:3ω4	0.6	0.7	1.1	0.5	0.3	0.4	0.4
16:3ω3	—	0.5	trace	0.1	0.2	0.2	0.1
18:0	11.4	7.1	9.8	6.2	12.0	8.4	7.5
18:1ω9	25.8	24.7	24.0	20.3	16.1	22.9	24.2
18:2ω6	5.3	20.9	12.9	2.5	6.3	10.7	16.6
18:2ω4	trace	0.4	—	0.2	0.6	0.4	—
18:3ω3	0.9	0.2	0.6	1.8	0.5	0.5	1.4
18:4ω3	—	0.2	0.8	0.4	0.3	0.2	0.2
20:1ω9	6.0	8.6	10.9	7.7	4.3	9.0	7.8
20:2ω6 20:3ω9	0.9	0.8	0.5	0.7	1.5	0.4	0.8
20:3ω3 20:4ω6	5.2	0.7	0.2	2.5	6.2	—	1.3
20:4ω3	—	0.7	0.7	—	—	—	—
22:1ω9	—	0.4	1.0	0.8	0.5	0.2	0.2
20:5ω3	10.1	10.0	14.3	16.8	14.2	11.6	15.0
22:3ω6	—	0.1	—	2.6	—	—	0.3
22:5ω6	0.2	0.4	0.5	1.5	0.5	—	—
22:5ω3	0.5	1.0	—	1.8	1.6	1.1	0.2
22:6ω3	5.1	4.3	9.8	8.5	10.8	7.6	5.9

survival and growth of prawn was almost similar to pollack residual oil alone.

The lipid contents in the whole body of prawn at the end of feeding experiment are given in Table 7. The definite relation between the kind of dietary lipid and lipid content of the whole body was not seen, although the prawn receiving 8% short necked clam lipid diet showed a high lipid content. The amounts of polar lipids from prawn kept on lipid free diet or 8% soybean oil diet were 54.8% and 61.9% of total lipid, respectively, however the diets containing pollack residual oil increased the percentage of polar lipids (68.2–76.7%). These results suggest that the ratio of polar to neutral lipid in the whole body of prawn greatly varies with the kind of dietary lipids.

The fatty acid cmoposition of polar and neutral lipids from the whole body of prawn is shown in Tables 8 and 9. The fatty acids of polar and neutral lipids were mainly composed of 16:0, 16:1ω7, 18:0, 18:1ω9, 18:2ω6, 20:1ω9, 20:5ω3 and 22:6ω3,

and the percentage of 22: 6ω3 in polar lipid was generally higher than that in neutral lipid. The addition of pollack residual oil or short necked clam lipid increased the percentage of 20: 5ω3 and 22: 6ω3 in lipids of the whole body, compared with soybean oil. From these data, it is considered that the fatty acid composition in lipids of the prawn is affected with dietary lipids, and that the influence is more remarkable in polar lipid than in neutral lipid.

Discussion

TSUKAHARA *et al.*[25] and YONE *et al.*[26] have reported that the growth rate in groups of marine fish fed the diets containing corn oil or soybean oil was inferior to those in fish fed pollack residual oil or cod liver oil. The present study also elucidated a high nutritive value of pollack residual oil for the prawn, *P. japonicus*. Compared with liquid pollack residual oil, the effective result of powdered oil may be attributed to the elevation of digestibility, homogeneous dispersion of oil in the diet, etc. The fatty acid composition of dietary lipids used in this experiment is as shown in Table 10. A low nutritive value of soybean oil for prawn seemed to be due to a very small amount of polyunsaturated fatty acids, especially 20: 5ω3 and 22: 6ω3. On the other hand, pollack residual oil and short necked clam lipid effective for prawn contained a large amount of ω3 polyunsaturated fatty acids. Judging from these results, it is assumed that in the prawn polyunsaturated fatty acids such as 20: 5ω3 and 22: 6ω3 may be more essential than that of 18: 2ω6 and

Table 10. Fatty acid composition (%) of dietary lipids

Fatty acid	Soybean oil	Pollack residual oil	Short neck clam lipid
14: 0	0.1	7.8	3.4
15: 0	trace	0.5	0.1
15: 1	trace	—	0.3
16: 0	13.3	15.0	26.0
16: 1ω7	0.1	14.2	7.6
17: 0	0.1	—	1.0
16: 2ω7 } 16: 2ω6 }	0.1	0.9	1.2
18: 0	4.4	2.6	5.9
18: 1ω9	20.9	20.5	6.8
18: 2ω6	49.4	1.8	0.5
18: 3ω3	8.0	0.2	0.9
20: 1ω9	1.2	12.2	7.3
20: 2ω6	—	—	1.7
20: 4ω6	—	—	3.3
22: 1ω9	2.4	11.2	2.3
20: 5ω3	—	10.9	13.7
22: 3ω6	—	—	1.9
22: 4ω6	—	—	1.1
22: 5ω3	—	—	1.7
22: 6ω3	—	2.2	13.3

18: 3ω3, similarly to red sea bream[11,13] and rainbow trout[27,28]. Also, it is suggested that dietary lipids affect the fatty acid composition in prawn, as well as in fish[3,4,6-8,10,12,29-32].

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References

1) J. D. CASTELL, R. O. SINNHUBER, J. H. WALES, and D. J. LEE: *J. Nutrition*, **102**, 77–86 (1972).
2) J. D. CASTELL, R. O. SINNHUBER, D. J. LEE, and J. H. WALES: *ibid.*, **102**, 87–92 (1972).
3) J. D. CASTELL, D. J. LEE, and R. O. SINNHUBER: *ibid.*, **102**, 93–100 (1972).
4) T. C. YU and R. O. SINNHUBER: *Lipids*, **7**, 450–454 (1972).
5) T. WATANABE, F. TAKASHIMA, and C. OGINO: This Bull., **40**, 181–188 (1974).
6) T. WATANABE, I. KOBAYASHI, O. UTSUE, and C. OGINO: *ibid.*, **40**, 387–392 (1974).
7) T. WATANABE, C. OGINO, Y. KOSHIISHI, and T. MATSUNAGA: *ibid.*, **40**, 493–499 (1974).
8) T. C. YU and R. O. SINNHUBER: *Lipids*, **10**, 63–66 (1975).
9) T. WATANABE, O. UTSUE, I. KOBAYASHI, and C. OGINO: This Bull., **41**, 257–262 (1975).
10) T. WATANABE, T. TAKEUCHI, and C. OGINO: *ibid.*, **41**, 263–269 (1975).
11) Y. YONE and M. FUJII: *ibid.*, **41**, 73–77 (1975).
12) Y. YONE and M. FUJII: *ibid.*, **41**, 79–86 (1975).
13) M. FUJII and Y. YONE: *ibid.*, **42**, 583–588 (1976).
14) A. KANAZAWA, N. TANAKA, S. TESHIMA, and K. KASHIWADA: *ibid.*, **37**, 211–215 (1971).
15) K. SHUDO, K. NAKAMURA, S. ISHIKAWA, and K. KITABAYASHI: *Bull. Tokai Reg. Fish. Res. Lab.*, No. 65, 129–137 (1971).
16) O. DESHIMARU and K. KUROKI: This Bull., **40**, 421–424 (1974).
17) J. D. CASTELL, E. G. MASON, and J. F. COVEY: *J. Fish. Res. Bd. Can.*, **32**, 1431–1435 (1975).
18) A. KANAZAWA, M. SHIMAYA, M. KAWASAKI, and K. KASHIWADA: This Bull., **36**, 949–954 (1970).
19) L. V. SICK, J. W. ANDREWS, and D. B. WHITE: *Fish. Bull.*, **70**, 101–109 (1972).
20) O. DESHIMARU and K. KUROKI: This Bull., **40**, 413–419 (1974).
21) E. G. BLIGH and W. J. DYER: *Can. J. Biochem. Physiol.*, **37**, 911–917 (1959).
22) A. KANAZAWA, S. TESHIMA, and N. TANAKA: *Mem. Fac. Fish., Kagoshima Univ.*, **25**, 47–51 (1976).
23) J. M. R. FORSTER: *J. Cons. int. Explor. Mer.*, **34**, 200–216 (1972).
24) S. TESHIMA, A. KANAZAWA, and H. OKAMOTO: *Mem. Fac. Fish., Kagoshima Univ.*, **25**, 41–46 (1976).
25) H. TSUKAHARA, A. FURUKAWA, and K. FUNAE: *Bull. Naikai Reg. Fish. Res. Lab.*, No. 24, 29–50 (1967).
26) Y. YONE, M. FURUICHI, and S. SAKAMOTO: *Report Fish. Res. Lab., Kyushu Univ.*, No. 1, 49–60 (1971).
27) T. WATANABE and T. TAKEUCHI: This Bull., **42**, 893–906 (1976).
28) T. TAKEUCHI and T. WATANABE: *ibid.*, **42**, 907–919 (1976).
29) R. REISER, B. STEVENSON, M. KAYAMA, R. B. R. CHOUDHURY, and D. W. HOOD: *J. Amer. Oil Chem. Soc.*, **40**, 507–513 (1963).
30) R. R. BRENNER, D. V. VAZZA, and M. E. DE TOMÁS: *J. Lipid Res.*, **4**, 341–345 (1963).
31) D. J. LEE, J. N. ROEHM, T. C. YU, and R. O. SINNHUBER: *J. Nutrition*, **92**, 93–98 (1967).
32) R. R. STICKNEY and J. W. ANDREWS: *ibid.*, **101**, 1703–1710 (1971).