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Effects of Phospholipids on Growth, Survival Rate, and Incidence of Malformation in the Larval Ayu*[1]

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Abstract

The effects of dietary phospholipids on the growth, survival, and incidence of malformation were investigated on the larvae of Ayu, *Plecoglossus altivelis*, with the purified diets. The inclusion of lecithin, soybean-lecithin (SB-lecithin) or chicken-egg lecithin (CE-lecithin) in the diets markedly improved the survival rate of the 10-day larvae, indicating that SE-lecithin was more effective than CE-lecithin. Also, the incidence of malformation, especially scoliosis and twist of jaw, was reduced by the supplement of lecithin. Furthermore, the weight gain of the 100-day larvae was also improved by the supplement of 3% SB-lecithin or bonito-egg lecithin to the diet containing 6% refined pollack liver oil-oleic acid (1:1, w/w). These results suggest that the addition of lecithin is indispensable for growth and survival of the larval Ayu.

The seedling of Ayu, *Plecoglossus altivelis*, has been produced by using the rotifer, *Brachionus plicatilis*[1]). The juveniles of Ayu have been successfully reared with the formula diets. As for the early stages of Ayu larvae, however, none have developed the formula diets with a high dietary value comparable to the rotifer, a live food. Recently, we have succeeded in rearing the prawn, *Penaeus japonicus*, from zoea$_1$ to postlarvae$_1$ (P–1) on the microparticulate diets with zein as a binder (Zein-MCD)[2]). The success encouraged us to attempt rearing larvae of the Ayu with a similar type of diet (Zein-MBD) consisting of all chemically known substances and zein as a binder. The present study was planned as a part of investigating the nutritional requirements of the larval Ayu by using the Zein-MBD. Now, we report that the addition of phospholipids, especially soybean-lecithin (SB-lecithin) and bonito-egg lecithin (FE-lecithin), to the diet markedly improved growth and survival rate and also reduced the incidence of malformation in the larval Ayu.

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Materials and Methods

Eggs of the Ayu were hatched at the Oyano Branch, Fisheries Experimental Station of Kumamoto Prefecture. Feeding trials (experiments 1, 2, and 3) were carried out under the conditions listed in Table 1. Ten test diets (Zein-MBD) contained all chemically defined ingredients and 9% level of various lipids (Table 2). The basal diet was the essentially same as reported previously[3] and contained the following ingredients (g/100 g): casein 52, dextrin 8, amino acids 10, minerals 8, vitamins 6, cellulose 7, and lipids 9. To the basal diet, zien (5%) was added, mixed well, and heated at 100°C. The diets were dried in an oven and then powdered to give the Zein-MBD (125–250 μm diameters).

Fifteen groups of the larval Ayu were fed the diets as shown in Table 3 and the dietary values were evaluated in terms of total length, body weight gain, survaival rate, and index of dietary value. Regarding groups 14 and 15, the incidence of malformation was checked 116 days after hatching.

Fatty acid composition (%) of lipids was determined by gas-liquid chromatography (GLC) on 10% DEGS[4].

Table 1. Rearing and feeding methods of the Ayu larvae.

Experimental condition	Experiments 1 and 3	Experiment 2
Larvae used		
Age (days after hatching)	10-day larvae	100-day larvae
Total length	12.4 mm	30.2 mm
Body weight	2.4 mg	87.2 mg
Number of specimens/tank	2000 (or 1500*1)	50
Rearing system		
Aquarium	1 ton	30 l
Supply of sea water	Running water (3 rounds/day)	Circulating water
Water temperature	16.7–23.2°C	20°C
Feeding method		
Diet supplied*2	Test diet or test diet plus rotifers	Test diet
Frequency of feeding	10 times/day (10-day—40-day larvae) 4–8 times/day (41-day—116-day larvae)	4 times/day

*1 Experimental groups 14 and 15

*2 See Tables 2 and 3

Table 2. Composition of dietary lipids in the diets.

Diet*1	Dietary lipid
1	3% Refined pollack liver oil (PLO)*2+6% Oleic acid (18: 1ω9)*3
2	3% Soybean oil*4+6% 18: 1ω9
3	3% PLO+3% 18: 1ω9+3% Chicken-egg lecithin (CE-lecithin)*5
4	3% PLO+3% 18: 1ω9+3% Soybean-lecithin (SB-lecithin)*4
5	3% PLO+6% 18: 1ω9
6	3% PLO+3% 18: 1ω9+3% CE-lecithin
7	3% PLO+3% 18: 1ω9+3% SB-lecithin
8	3% PLO+3% 18: 1ω9+3% Bonito-egg lecithin (FE-lecithin)*6
9	3% PLO+5% 18: 1ω9+1% CE-lecithin
10	3% PLO+1% 18: 1ω9+5% CE-lecithin
11	Unknown (commercial diet)*7

*1 Except for diet 11, all diets are the Zein-MBD.
*2 Riken Vitamin Co., Japan
*3 Sigma Chemicals Co., U.S.A.
*4 Wako Pure Chemicals Co., Japan
*5 Merck, West Germany
*6 Isolated from the eggs of bonito by the similar method to that described previously[8)]
*7 Oriental Yeast Kogyo Co. Japan

Table 3. Feeding experiments and the diets used.

Experiment	Group	Feeding period (days)	Diet supplied
Experiment-1*1	1	20	Diet 1
	2	20	Diet 2
	3	20	Diet 3
	4	20	Diet 4
Experiment-2*2	5	33	Diet 5
	6	33	Diet 6
	7	33	Diet 7
	8	33	Diet 8
	9	33	Diet 9
	10	33	Diet 10
Experiment-3*1	11	50	Commercial diet+Rotifers
	12	50	Diet 1+Rotifers
	13	50	Diet 3+Rotifers
	14	106	Commercial diet+Rotifers
	15	106	Diet 3+Rotifers

*1 After hatching, the Ayu larvae were maintained on the rotifers cultured with a marine *Chlorella* for 10 days. Subsequently, the larvae were fed the particulate diet (1–3 g/tank/day) and a combination of particulate diet and rotifer (500–1500 individuals/larva/day) in experiments 1 and 3, respectively.
*2 After hatching, the Ayu larvae were maintained on the rotifers for 100 days and then the particulate diet (feeding level; 10% of body weight per day).

Results

In experiment 1, the feeding trials were carried out by using the 10-day larvae. The results are shown in Table 4 and Fig. 1. When the 10-day larvae were maintained on the artificial diets (groups 1, 2, 3, and 4), the high values on the total length, body weight, survival rate, and index of dietary value were obtained on the diets containing 3% levels of SB-lecithin (group 4) and chicken-egg lecithin (CE-lecithin) (group 3) besides 3% refined pollack liver oil (PLO) and 3% oleic acid (18: 1ω9). Especially, the survival rate of larvae were markedly improved by the supplement of SB-lecithin or CE-lecithin. SB-lecithin was shown to be more effective in the improvement of survival rate than CE-lecithin. Presumably, the superior growth and survival rate on groups 3 and 4 were attained by some effects of lecithin itself. This assumption was also supported by the results on groups 11, 12, and 13 in experiment 3. When the 10-day larvae were maintained on both the artificial diets and rotifers, the larvae receiving 3% CE-lecithin (group 13) gave the higher values on the total length, body weight gain, survival rate, and index of dietary value than that receiving the diet supplemented no phospholipid (group 12) (Table 4 and Fig. 3).

In experiment 2, the feeding trials were carried out by using the 100-day larvae. The results are given in Table 4 and Fig. 2. The weight gain of larvae were notably

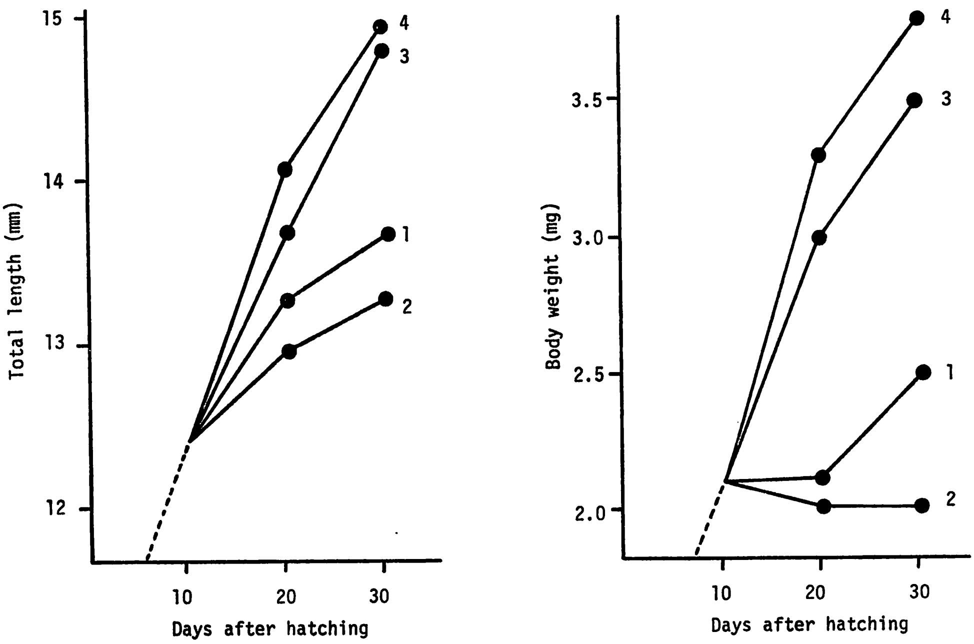

Fig. 1. Growth of the Ayu larvae in experiment 1.
Numerical letters show experimental groups.

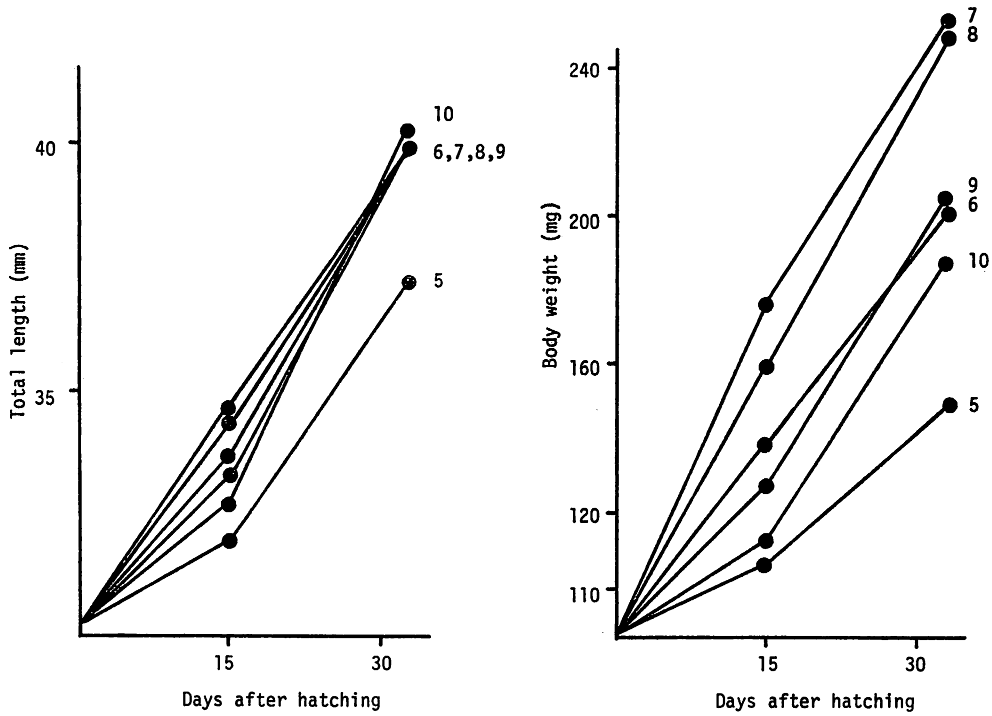

Fig. 2. Growth of the Ayu larvae in experiment 2. Numerical letters show experimental groups.

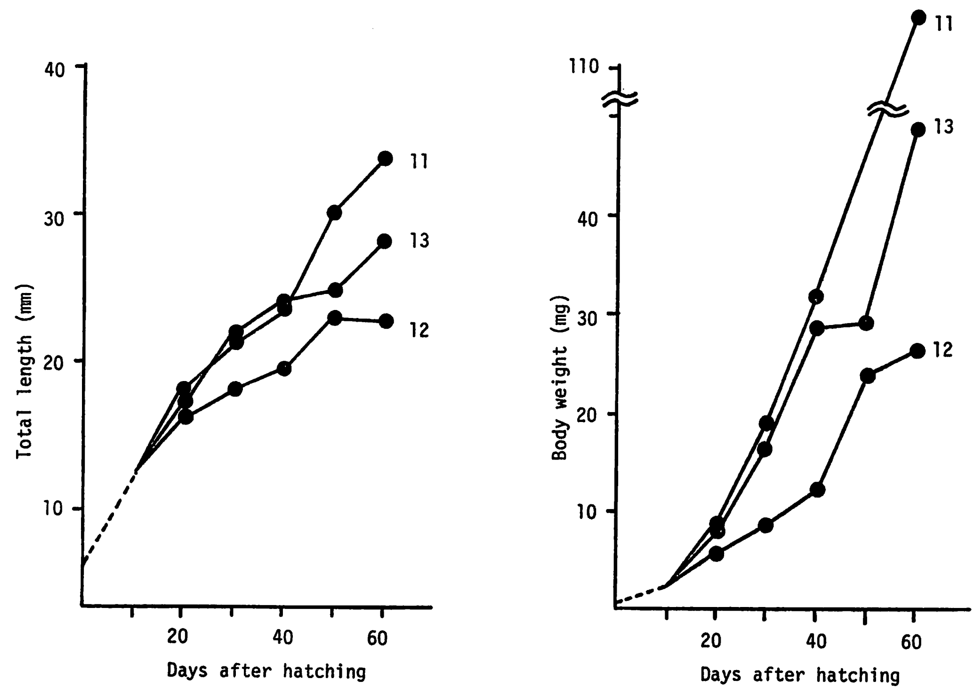

Fig. 3. Growth of the Ayu larvae in experiment 3. Numerical letters show experimental groups.

increased by the supplement of 3% levels of SB-lecithin (group 7) and FE-lecithin (group 8) to the diet containing 6% PLO-18: 1ω9 (1: 1, w/w). The supplement of CE-lecithin also improved the weight gain, but the growth-promoting effect of it was inferior to those of SB-lecithin and FE-lecithin.

Table 5 shows the incidence of malformation, together with the growth and survival rate, 116 days after hatching in groups 14 and 15. The larval Ayu receiving both the rotifers and commercial diet (group 14) showed a superior growth and survival rate to those receiving both the rotifers and diet 3 containing 3% CE-lecithin and 6% PLO-18: 1ω9 (group 15). But, the incidence (%) of malformation was clearly lower in group 15 than in group 14. Especially, the incidence of scoliosis and twist of caudal region was low in the Ayu larvae fed the diet containing 3% CE-lecithin and 6% PLO-18: 1ω9 besides the rotifers. These results suggest that the lack of phospholipids, especially lecithin, in the diet is related to the incidence of scoliosis and twist of jaw in the larval Ayu.

Table 4. Effects of dietary lipids on growth and survival rate of the Ayu larvae.

Experiment	Group	Dietary lipid	Total length (mm)		Body weight (mg)			Survival rate (%)	Index of dietary value*1
			Initial	Final	Initial	Final	Gain (%)		
1	1	3% PLO+6% 18: 1ω9	12.4	13.7	2.4	2.5		3.6	0.5
	2	3% Soybean oil+6% 18: 1ω9	12.4	13.3	2.4	2.0		3.0	—
	3	3% PLO+3% 18: 1ω9 +3% CE-lecithin	12.4	14.8	2.4	3.5		37.5	56
	4	3% PLO+3% 18: 1ω9 +3% SB-lecithin	12.4	14.9	2.4	3.8		52.8	100
2	5	3% PLO+6% 18: 1ω9	30.2	37.3	87.2	149	71	86	39
	6	3% PLO+3% 18: 1ω9 +3% CE-lecithin	30.2	39.8	87.2	201	131	76	64
	7	3% PLO+3% 18: 1ω9 +3% SB-lecithin	30.2	39.8	87.2	253	190	80	98
	8	3% PLO+3% 18: 1ω9 +3% FE-lecithin	30.2	39.8	87.2	248	184	84	100
	9	3% PLO+5% 18: 1ω9 +1% CE-lecithin	30.2	39.8	87.2	203	133	72	62
	10	3% PLO+1% 18: 1ω9 +5% CE-lecithin	30.2	40.2	87.2	189	117	62	47
3	11	Lipids from commercial diet and rotifers	12.4	33.9	2.4	115	4790	59.2	100
	12	3% PLO+6% 18: 1ω9 +lipids from rotifers	12.4	22.9	2.4	26.3	1100	18.5	7
	13	3% PLO+3% 18: 1ω9 +3% CE-lecithin +lipids from rotifers	12.4	28.3	2.4	48.7	2030	34.4	24

*1 Survival and growth indices are the relative values when the maximum values of survival rate and weight gain are expressed as 100, respectively. Index of dietary value is the relative value when the product of survival index by growth index were expressed as the relative value to the maximum value in the respective experiments.

Table 5. Growth and the incidence of malformation in the Ayu larvae (groups 14 and 15).

Growth and malformation	Group 14*1	Group 15*2
Days after hatching	116	116
Number of larvae		
1-day larvae	1500	1500
116-day larvae	407	113
Number of specimens examined	75	64
Total length (mm)		
1-day larvae	6.5	6.5
116-day larvae	45.6	36.6
Body weight (mg)		
1-day larvae	0.3	0.3
116-day larvae	355.0	113.0
Incidence of malformation		
Pughead	1	0
Protrusion of thrax	1	0
Twist of jaw	8	3
Scoliosis (trunk region)	10	0
Twist of caudal peduncle	35	0
Incidence (%)	63	5

*1 Dietary lipids: lipids from the rotifers and commercial diet
*2 Dietary lipids: 3% PLO+3% 18: 1ω9+3% CE-lecithin and lipids from the rotifers

Table 6. Composition (%) of main fatty acids of lipids used.

Fatty acid	Soybean oil	PLO*1	CE-lecithin*2	SB-lecithin*3	FE-lecithin*4	Lipids from commercial diet
14: 0	0.2	2.3	0.2	0.2	3.5	3.1
16: 0	14.8	6.7	36.4	20.0	9.7	21.4
16: 1	–*5	1.3	1.3	0.3	2.8	5.0
18: 0	3.2	2.4	13.1	3.9	10.5	4.8
18: 1ω9	22.9	12.8	26.0	11.0	9.5	25.7
18: 2ω6	51.4	0.9	13.9	55.7	1.2	11.9
18: 3ω3	6.6	0.4	—	5.4	0.5	0.9
20: 1ω9	—	18.3	—	0.4	0.4	8.4
20: 4ω6	0.4	1.1	3.9	—	3.4	1.0
20: 5ω3	—	38.0	0.7	0.7	6.7	10.0
22: 4ω6	—	2.2	0.3	—	1.7	0.4
22: 5ω3	—	3.3	0.2	0.2	1.8	1.0
22: 6ω3	—	7.4	1.8	—	39.8	5.3
Σω3–HUFA	—	48.7	2.7	0.9	48.3	16.3

*1 Refined pollack liver oil *2 Chicken-egg lecithin *3 Soybean-lecithin
*4 Bonito-egg lecithin *5 Less than 0.1%

Table 7. Fatty acid composition (%) of polar lipids from the whole bodies of Ayu larvae after the feeding trials in experiment 2.

Fatty acid	Experimental group			
	5	6	7	8
14: 0	1.4	1.0	1.4	1.3
16: 0	24.2	18.5	16.2	15.1
16: 1	4.2	2.1	5.1	5.3
18: 0	6.2	7.5	7.7	7.6
18: 1ω9	26.6	27.0	24.8	22.5
18: 2ω6	3.5	5.8	8.1	4.9
18: 3ω3	0.1	0.2	0.6	0.1
20: 1ω9	1.2	1.2	1.1	1.4
20: 4ω6	2.5	5.0	2.6	3.2
20: 5ω3	5.2	5.8	5.2	6.4
22: 4ω6	0.3	0.4	0.3	0.4
22: 5ω3	3.8	3.9	3.4	3.8
22: 6ω3	20.3	20.0	22.5	25.4

Table 6 and 7 show the fatty acid compositions of dietary lipids and body lipids isolated from the Ayu larvae after feeding trials.

Discussion

Several workers[5-7] have shown that the larval Ayu grew well when they were fed *Artemia salina* containing high levels of ω3-highly unsaturated fatty acids (HUFA), suggesting the necessity of ω3-HUFA for growth. Quite recently, we have demonstrated by using the purified diets that the Ayu larvae (90 days after hatching) required ω3-fatty acids such as linolenic acid (18: 3ω3) and eicosapentaenoic acid (20: 5ω3) but not linoleic acid (18: 2ω6) as essential fatty acids (EFA), indicating that the EFA-activity of 18: 3ω3 almost equal to that of 20: 5ω3[3].

The results of the present study show that the addition of SB-lecithin improved the growth and survival rate of the 10-day larvae, and also that both SB-lecithin and FE-lecithin were effective in the improvement of weight gain of the 100-day larvae. Especially, the inclusion of lecithin in the artificial diets is likely to be essential for the survival of early stages of larval Ayu. Furthermore, the present study suggests that the lack of phospholipids, lecithin, is one of possible causes for the incidence of malformation such as scoliosis and twist of jaw.

We assume that the effectiveness of SB-lecithin and probably FE-lecithin is related to some effect of lecithin itself rather than to the increase in amounts of EFA, because SB-lecithin contained only low levels of ω3-fatty acids such as 18: 3ω3, 20: 5ω3, and docosahexaenoic acid (22: 6ω3) as shown in Table 6. As shown in Table 7, polar lipids isolated from the whole bodies of larvae after the feeding trials were found to contain high levels of ω3-fatty acids such as 20: 5ω3 and 22: 6ω3. This may imply that every diet contained satisfying amounts of EFA for the larvae.

Regarding the crustaceans, we have shown the growth-promoting effect of lecithin and cephalin from the short-necked clam, *Tapes phillipinarum*, for juveniles of the prawn, *P. japonicus*[8]. Also, CONKLIN *et al*[9]. and D'ABRAMO *et al.*[10] have demonstrated the necessity of phophatidyl choline for the survival of juvenile lobster, *Homarus americanus*, indicating that phosphatidyl choline molecules containing polyunsaturated fatty acids were the most effective. In the crustaceans, the physiological role of lecithin has been assumed to be associated with hemolymph lipoproteins and the transport of lipids[10,11]. In the case of the larval Ayu, however, the role of lecithin is obscure.

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