Necessity of Dietary Phospholipids for Growth of the Larval Ayu

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Necessity of Dietary Phospholipids for Growth of the Larval Ayu

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Abstract

The effects of dietary phospholipids on growth of the larval Ayu *Plecoglossus altivelis* were investigated by using the microparticulate diets with a carrageenan as a binder. The 10-day larvae showed better growth on the diets with 3 % levels of chicken-egg lecithin (CEL), soybean lecithin (SBL), or bonit -egg lecithin (BEL) than with a phospholipid-deficient diet. The nutritive value of SBL for the 10-day larvae was superior to that of bonito-egg cephalin.

The growth and survival of 10-day larvae were improved with the increasing levels of phospholipids from 0 % to 5 % when SBL was used as a phospholipid source. As for the 30-day larvae, the addition of 3 % phospholipids to the diets was also found to improve growth and survival, indicating the inferior nutritive value of soybean cephalin to those of CEL, SBL, and BEL. As for the 10-day and 30-day larvae, the nutritive values of CEL and BEL were slightly higher than that of SBL when 3 % levels of respective phospolipids were added to the diets.

Previously, we have reared successfully the 10-day and 100-day larvae of the Ayu *Plecoglossus altivelis* with the microparticulate diets with zein as a binder, showing the necessity of inclusion of dietary phospholipids such as soybean lecithin (SBL), chicken egg lecithin (CEL), and bonito egg lecithin (BEL) for good growth[1,2]. Interestingly, the addition of phospholipids to the diets has also been shown to reduce the incidence of malformation such as scoliosis and twist of jaw in the larval Ayu[2].

The present study is planned to reconfirm the growth-enhancing effect of several phospholipids on the larval Ayu and also to examine the efficacy of varying dietary levels of SBL. To this approach, the feeding trials were conducted using the 10-day and 30-day larvae of the Ayu with the microparticulate diets containing carrageenan as a binder (carrageenan MBD).

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Materials and Methods

Eggs of the Ayu were hatched at the Oyano Branch of the Fisheries Experimental Station of Kumamoto Prefecture, and the newly hatched Ayu larvae were reared with the rotifer *Brachionus plicatilis* during the period of 1-19th day and with the combination of *Artemia* nauplii, formula diet, and egg yolk during the 20-60 day (see Table 2 for details). Feeding trials were conducted using the 10-day and 30-day larvae with the test diets (Table 2) under the conditions listed in Table 1. The artificial diets used were all the carrageenan MBD[3,4], which were sieved through the mesh of 125 μm or 250 μm agreeably to the size of Ayu larvae. In experiment I, the feeding trials were conducted using the 10-day Ayu larvae to compare the dietary values of BEL, bonito egg cephalin (BEC), CEL, and SBL at the 3% dietary level, and also to examine the growth-enhancing effects of SBL at the dietary levels of 1%, 3%, and 5%. In experiment II, the dietary values of the above 4 phospholipids at the 3% level were also tested using the slightly grown (30-day) Ayu larvae, and the incidence of malformation was investigated at the end of the feeding trial.

Results and Discussion

In experiment I, the feeding trials were conducted by using the 10-day larvae. The results are shown in Figs. 1 and 2. The 10-day larvae grew well on the diets supplemented with 3% levels of BEL (diet 2), SBL (diet 5), bonito-egg cephalin (BEC, diet 3), and CEL (diet 4) in comparison with the phospholipid-deficient diet (diet 1). The

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

Condition	Experiment I	Experiment II
Larvae used		
Age (days after hatching)	10-day larvae	30 day larvae
Total length (mm)	9.6	22.1
Number of fish/tank	1000	500
Rearing and feeding methods		
Aquarium (capacity)	500 liters	500 liters
Supply of water	0.5 - 4 rounds/day	4 rounds/day
Water temperature (℃)	13.0 - 25.4	9.0 - 13.0
Feeding period (days)	50	60
Feeding rate (mg diet/larva):		
10 - 20 day larvae	3	—
21 - 30 day larvae	4.5	4.5
31 - 40 day larvae	6.0	6.0
41 - 50 day larvae	8.0	8.0
51 - 60 day larvae	10.0	10.0

Table 2. Composition of the diets used in the feeding trials.

Experiment *1	Exptl. group	Diet used *2	Dietary lipids *3
I	1	1	3% PLO (pollack liver oil) + 6% 18:1ω9
	2	2	3% PLO + 3% 18:1ω9 + 3% BEL
	3	3	3% PLO + 3% 18:1ω9 + 3% BEC
	4	4	3% PLO + 3% 18:1ω9 + 3% CEL
	5	5	3% PLO + 3% 18:1ω9 + 3% SBL
	6	6	3% PLO + 3% 18:1ω9 + 1% SBL
	7	7	3% PLO + 3% 18:1ω9 + 5% SBL
	8	8	Lipids from the control diet *4
II	9	2	3% PLO + 3% 18:1ω9 + 3% BEL
	10	4	3% PLO + 3% 18:1ω9 + 3% CEL
	11	5	3% PLO + 3% 18:1ω9 + 3% SBL
	12	9	3% PLO + 3% 18:1ω9 + 3% SBC
	13	10	Lipids from the control diet *5

*1 The 10-day and 30-day larvae of the Ayu were reared with the diets in experiments I and II, respectively.

*2 Except for the control diets, all the diets are the carrageenan MBD containing the following ingredients (g/100 g dry diet): casein 52, dextrin 8, amino acids 10, minerals 8, vitamins 6, lipids 9, and cellulose (equal to 100), and carrageenan (binder) 5. The basal composition of these diets are similar to those reported previously[2].

*3 Abbreviated as follows: 18:1ω9 (olecic acid), BEL (lecithin from the bonito eggs), BEC (cephalin from the bonito eggs), CEL (chicken egg lecithin; Merck, West Germany), SBL (soybean lecithin; Wako Pure Chemicals Co.), SBC (soybean cephalin; Wako Pure Chemicals Co.)

*4 Experimental group 8 (control): the Ayu larvae were given 500-1000 rotifers/larva during the period of 1-19 days after hatching and then the combination of 1500 rotifers, 50 *Artemia* nauplii, 2.7-6.0 mg of a formula diet/larva during the period of 20-60 days after hatching.

*5 Experimental group 13 (control): The Ayu larvae were given the combination of 1500 rotifers, 55-165 *Artemia* nauplii, 3.6-10.8 mg of a formula diet, and 0.6-1.8 mg of egg yolk during the period of 30-90 days after hatching.

survival rates of the lavae were also higher on the diets supplemented with 3% levels of CEL, BEL, and SBL than with the phospholipid-deficient diet. However, the addition of 3% BEC did not result in the improvement of survival rates in contrast with the above 3 types of lecithins. The results of experiment I confirmed the previous findings that the 10-day larvae of larval Ayu necessitate dietary phospholipids such as CEL, BEL, and SBL for their normal growth, although a slight discrepancy in the effects of CEL and SBL on the survival rates was found between the results of the present and previous studies[2]. When the 10-day larvae were fed the diets containing several levels of SBL as a phospholipid, better growth and survival were attained on the diets with 3

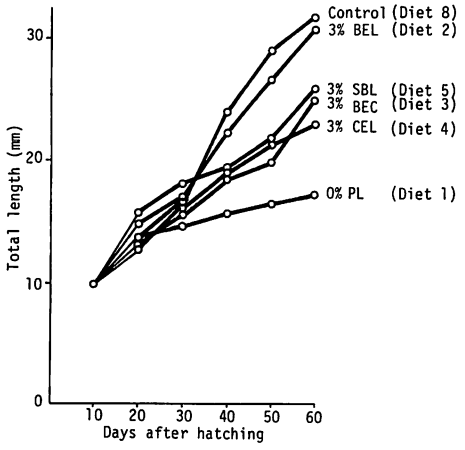

Fig. 1. Effect of several phospholipids on the total length of the 10-day larvae of Ayu. 0 % PL, no supplemental phospholipids.

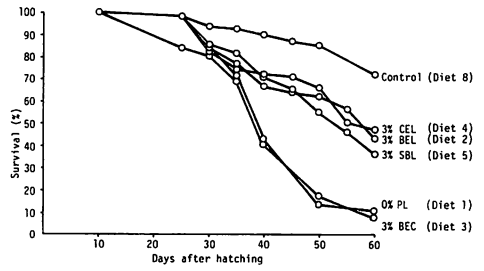

Fig. 2. Effect of several phospholipids on the survival rate of the 10-day larvae of Ayu. 0 % PL, no supplemental phospholipids.

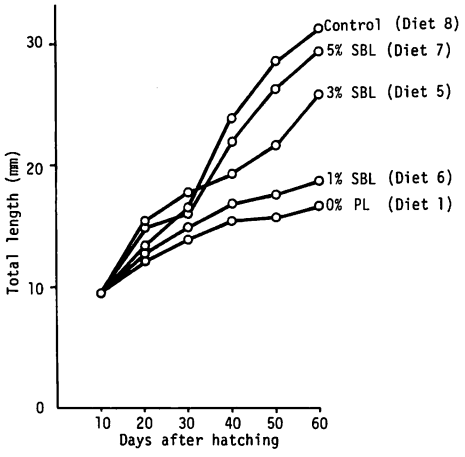

Fig. 3. Effect of SBL levels on the total length of the 10-day larvae of Ayu. 0 % PL, no supplemental phospholipids.

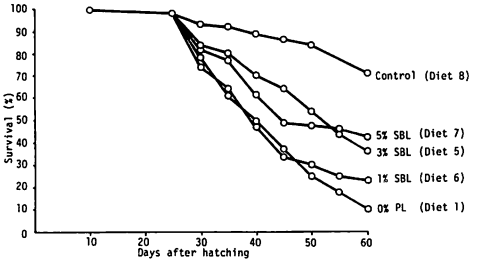

Fig. 4. Effect of SBL levels on the survival rate of the 10-day larvae of Ayu. 0 % PL, no supplemental phospholipids.

% and 5 % SBL than on the diets with 1 % SBL (Figs. 3 and 4).

In experiment II, the feeding trials were conducted by using the 30-day larvae. The results are shown in Figs. 5 and 6. The 30-day larvae grew well with the diets supplemented with 3 % levels of CEL (diet 4), BEL (diet 2), and SBL (diet 5), but growth

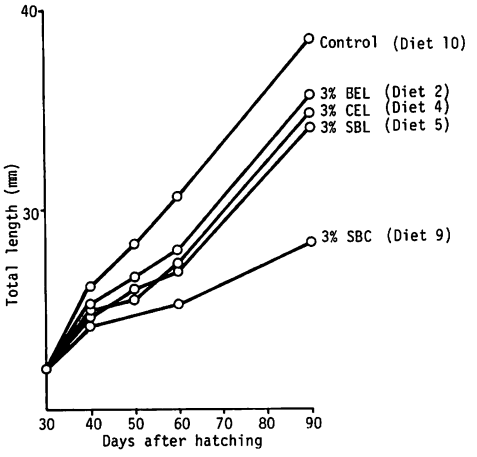

Fig. 5. Effect of several phospholipids on the total length of the 30-day larvae of Ayu.

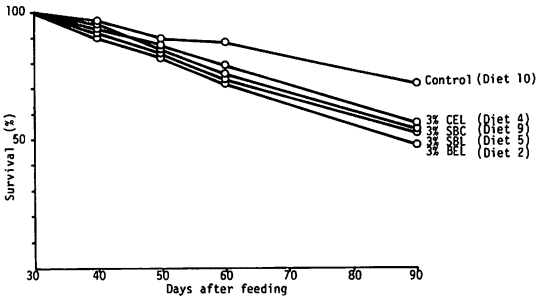

Fig. 6. Effect of several phospholipids on the survival rate of the 30-day larvae of Ayu.

Table 3. Effects of dietary phospholipids on the incidence (%)*1 of malformation in the larval Ayu.

Malformation	Dietary phospholipid *2				
	Control	CEL	SBL	SBC	BEL
Protrusion of lower jaw	36	47	39	41	41
Twist of lower jaw	0	2	1	0	1
Shortening of lower jaw	3	0	0	0	0
Twist of caudal peduncle	6	0	4	0	0
Scoliosis	10	0	0	11	2

*1 At the end of the 60-day feeding trial in experiment 2, the incidence of malformation was checked with 100 random samples.
*2 See Table 1 for abbreviations.

was poor on the diet containing 3 % soybean cephalin (SBC) as also observed in the 10 -day larvae in experiment I. Concerning the survival rates, however, a marked difference was not found among the experimental groups receiving CEL, BEL, SBL, and SBC.

Table 3 shows the incidence (%) of malformation in the 90-day larvae in experiment II. The incidence of some malformation in the Ayu larvae was reduced when reared with the diets containing lecithins. Particularly, the incidence of scoliosis was reduced when the larvae were reared with the diets containing 3 % levels of CEL, SBL, and possibly BEL, suggesting that the scoliosis of the Ayu larvae may be attributable partly

or entirely to the deficiency of lecithins in diets.

Apart from the studies on essential fatty acid requirements[5-8], little information is available with the lipid nutrition of the larval Ayu. In the preceding study, we have demonstrated by the feeding trials using the 10-day or 100-day larvae that the inclusion of phospholipids such as CEL, SBL, and BEL in the diets are indispensable for their growth and survival[2]. The present study also shows the necessity of dietary sources of lecithins such as CEL, SBL, and BEL for growth of the 10-day and 30-day larvae. However, the cephalins such as SBC and BEC did not enhance growth of the 10-day or 30-day larvae. This suspects that the choline group of lecithin may be related to the effectiveness for the Ayu larvae. In future, it is desirable to examine the growth-enancing effects of dietary phospholipids on the Ayu larvae by using the lecithin with known fatty acid moieties or purified other phospholipid classes. Larval or juvenile crustaceans such as the prawn *Penaeus japonicus*[9,10] and the American lobster *Homarus americanus*[11,12] have also been shown to require dietary sources of phospholipids, especially lecithins, for their growth and survival. We postulate that the physiological role of dietary phospholipids in the larval Ayu and other fish may be different with that suspected in crustaceans[13].

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