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The Effect of Dietary Soybean Lecithin on Growth, Survival and Body Amino Acid Composition of Japanese Flounder (*Paralichthys Olivaceus*) Larvae

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Abstract: ***In the study of the nutritional requirements of phospholipids in fish larvae, three diets were tested, containing 4 %, 2 % and 0 % soybean lecithin. Larvae of Japanese flounder, Paralichthys olivaceus, were cultured using these three diets. A control group was fed only rotifers, Brachionus plicatilis. Growth and survival after 30 days of culture, of larvae fed 2 % and 0% soybean lecithin were significantly lower that for larvae fed diets wit 4 % soybean lecithin and the control group. The amino acid composition of the larvae fed the different diets was very similar for all groups, and it was not dependent on the diet fed. Results indicate that larval Japanese flounder need phospholipids in the diet, which may be covered by supplementing the diet with 4% soybean lecithin.***

Keywords: larval diets, soybean lecithin, phospholipids, amino acids, Japanese flounder

1. Introduction

The production of marine fish larvae usually relies on the use of live food such as rotifers and *Artemia sp*. The culture of these organisms is throublesome due to the variability in the nutritional quality of these organisms and the uncertainty in its production due to factors beyond our control, such as climate [1]. Several authors have achieved the culture of fish larvae with microdiets [2]-[5]. In experiments with microdiets, supplementing the diets with phospholipids enhances the growth and survival of the larvae, and decreases the incidence of malformations. The aim of this research was to demonstrate the effect of several levels of dietary soybean lecithin on the growth, survival and body amino acid composition of Japanese flounder larvae.

2. Materials and Methods

2.1 Rearing Method

Larvae of Japanese flounder (10 days after hatching) were stocked in 100 l tanks at a density of 10 larvae per litre (1000 larvae per tank). Larvae were fed microdiets every half an hour from 9 am to 4 pm. Rotifers were added to all the tanks as a supplement every day at 4:30 pm at a density of 5 rotifers/ml, to provide food during the night. Water was exchanged 3-5 times per day, and gentle aeration was supplied. All groups were replicated in 3 tanks per treatment.

2.2 Diets

Zein-microbound diets were used for al treatments. The preparation of the diets followed the procedures described in [6]. The ingredient composition of the diets used are shown on table 1.

Table 1: Composition of microdiets used (g/100 g dry wt.)

Ingredient	Diet 1	Diet 2	Diet 3
casein	30.00	30.00	30.00
fish meal	20.00	20.00	20.00
Krill meal	10.00	10.00	10.00
Arginine-HCl	1.30	1.30	1.30
Lysine-HCl	1.70	1.70	1.70
vitamins	6.00	6.00	6.00
minerals	5.00	5.00	5.00
Soybean lecithin	4.00	2.00	0.00
Soybean oil	0.00	2.00	4.00
Squid liver oil	3.00	3.00	3.00
n-3 HUFAs	3.00	3.00	3.00
dextrin	6.70	6.70	6.70
attractants	1.30	1.30	1.30
zein	8.00	8.00	8.00
TOTAL	100.00	100.00	100.00

2.3 Analytical Methods

At the end of the experimental trials, samples from all treatments were taken, and tissue and dietary amino acids were determined using a Shimadzu HPLC after hydrolysis of the protein at 110 ºC for 22 hours with methane sulphonic acid. N-leucine was used as internal standard. The crude protein of the diets were determined using the Kjeldhal method. Lipid class composition was determined using an Iatroscan system. Fatty acids were analysed by gas chromatography, after esterification to methyl esters. All data were analysed using Statview.

3. Results

3.1 Growth and survival

Growth and survival of the larvae fed the four experimental diets are shown on figures 1 and 2. Growth was best for larvae fed rotifers. Survival of larvae fed on rotifers was similar to the survival of larvae fed diet 1, with 4% soybean lecithin. However, larvae fed diets with 2% soybean lecithin (diet 2) and 0% soybean lecithin (diet 3) had significantly

lower survival. This indicates the need of phospholipids in the diets for Japanese flounder larvae.

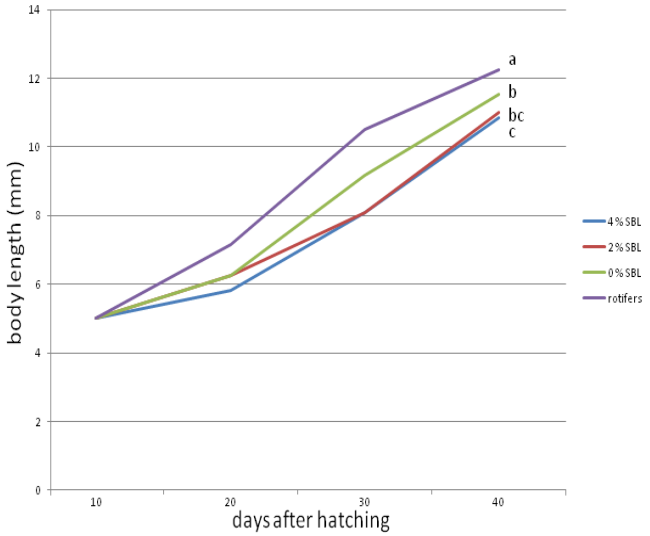

Figure 1: Mean body length (mm) of the larvae fed the different diets after 10, 20, 30 and 40 days.

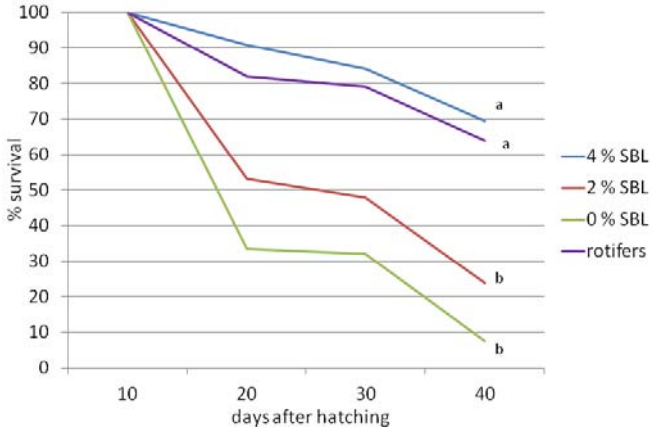

Figure 2: Survival of red sea bream larvae fed the different diets after 10, 20, 30 and 40 days.

3.2 Biochemical analysis

The gross biochemical composition of the diets used are shown on table 2. Crude protein and total lipids were similar in all diets used.

Table 2: Proximate composition of microdiets used with Japanese flounder larvae (% dry weight)

	Diet 1	Diet 2	Diet 3
Crude protein	61.69	62.75	61.50
Total lipids	12.31	12.12	12.89

The lipid fractions of the diets are shown on table 3.

Table 3: Lipid fractions of the diets used with flounder larvae, as % of total lipids (spectrophotometric method)

Lipid fraction	Diet 1	Diet 2	Diet 3
Neutral lipids	76.89	84.47	86.22
Polar lipids	23.11	15.53	13.78
Total lipids	100.00	100.00	100.00

It is outstanding the high proportion of polar lipids in diet 1, with 4% soybean lecithin.

Lipid classes in each lipid fraction of the diets are shown on table 4. The polar fraction of diet 1 has a higher amount of phosphatidil choline and phosphatidil inositol than for diets 2 and 3.

Table 4: Lipid class composition of the lipid fractions of diets used

Lipid class	Diet 1	Diet 2	Diet 3
Neutral lipids:			
Steryl esters	1.41	0.73	0.49
Triglycerides	92.07	91.62	96.93
Free fatty acids	3.15	2.96	1.13
Cholesterol	2.08	0.71	n.d.
Diglycerides	0.70	n.d.	n.d.
Monoglycerides	0.58	3.98	1.45
Total	100.00	100.00	100.00
Polar lipids:			
Phosphatidyletanolamine	3.29	3.52	3.57
Phosphatidylinosotol	10.54	6.97	9.93
Phosphatidylcholine	82.75	79.36	17.52
Sphingomyeline	1.77	3.71	41.24
Lisophosphatidylcholine	1.65	6.44	27.73
Total	100.00	100.00	100.00

The fatty acids of the lipid fractions of the diets are shown on table 5. The essential fatty acids EPA and DHA are present at high levels in all diets.

Table 5: Fatty acids of the lipid fractions of diets, as % of total fatty acids

Fatty acid	diet	1	diet	2	diet	3
	NL	PL	NL	PL	NL	PL
14:0	4.57	1.08	3.99	1.40	3.88	2.01
14:1	0.27	n.d.	0.30	n.d.	n.d.	n.d.
15:0	0.28	0.15	0.31	n.d.	0.19	0.23
16:0	13.56	20.08	13.43	18.53	11.78	21.76
16:1	4.70	2.60	4.17	4.37	4.73	4.04
17:0	0.64	0.27	0.58	0.78	0.77	0.53
17:1	1.29	0.19	1.16	0.64	1.21	0.54
18:0	2.52	2.73	2.79	2.44	2.53	2.83
18:1	15.85	13.21	17.59	13.00	17.41	16.66
18:2n-6	12-16	31.83	18.91	23.21	24.09	7.01
18:3n-3	2.03	2.89	2.88	3.93	4.48	1.03
18:4n-3	2.01	0.23	1.67	1.11	2.17	0.64
20:1	3.51	1.32	2.98	1.93	3.10	2.25
20:2n-6	0.23	0.09	0.19	n.d.	0.12	n.d.
20:3n-3	1.08	0.73	0.94	0.75	0.62	1.38
20:4n-3	0.85	0.29	0.64	0.26	0.46	0.52
20:5n-3	13.38	12.52	10.82	13.87	11.60	3.16
22:1	2.57	n.d.	2.08	n.d.	n.d.	n.d.
22:5n-3	1.33	0.48	1.09	2.80	0.59	0.80
22:6n-3	16.01	9.06	12.65	10.97	9.96	15.52

The total amino acid composition of diets and larvae are shown on table 6 and 7. The composition is very similar in all groups of diets and larvae. It can be noticed that there seems to be no interaction between dietary lipid quality and the assimilation of dietary amino acids, since the amino acid profile of all larvae was very similar.

Table 6: Total amino acids in the diets (g/100 g dw)

Amino acid	Diet 1	Diet 2	Diet 3
Tau	0.27	0.32	0.28
Hypro	n.d.	n.d.	n.d.
Asp	10.11	12.02	9.95
Thr	1.73	2.03	1.69
Ser	2.59	3.58	3.06
Glu	9.51	11.20	9.47
Pro	4.01	4.73	3.98
Gly	1.53	1.77	1.52
Ala	2.38	2.82	1.16
Cys	0.04	0.06	0.04
Val	2.15	2.69	2.17
Met	0.82	0.79	0.84
Ile	2.15	2.35	1.84
Leu	4.92	5.53	4.58
Tyr	1.99	2.64	2.16
Phe	2.11	2.63	2.17
His	1.14	1.36	1.13
Lys	3.92	4.72	3.93
Trp	1.13	0.93	0.99
Arg	2.95	3.51	2.94

Table 7: Total amino acids in the larvae after 30 days feeding on the different diets (g/100 g dw)

Amino acid	Diet 1	Diet 2	Diet 3	Diet 4
Tau	n.d.	0.04	n.d.	0.09
Hypro	n.d.	0.31	n.d.	0.36
Asp	15.65	4.67	13.88	15.29
Thr	2.50	2.15	2.30	2.39
Ser	3.52	2.67	3.22	3.43
Glu	8.28	7.44	7.57	8.03
Pro	2.63	2.54	2.46	2.71
Gly	3.10	2.96	3.14	3.24
Ala	3.15	2.72	2.70	3.11
Cys	0.17	n.d.	n.d.	n.d.
Val	2.24	2.01	3.14	2.31
Met	1.96	1.62	1.54	1.65
Ile	1.76	1.61	1.72	1.80
Leu	3.98	3.49	3.69	3.92
Tyr	2.04	1.78	1.56	1.97
Phe	2.29	2.00	2.08	2.26
His	1.51	1.39	1.35	1.39
Lys	4.02	3.37	3.58	3.82
Trp	0.55	n.d.	n.d.	0.13
Arg	3.68	3.31	3.09	3.66

4. Discussion

Best growth and survival was achieved when diets were supplemented with 4 % soybean lecithin, compared to 2 % and 0 % dietary soybean lecithin. Final length of larvae fed 0 % soybean lecithin was nominally (but not significantly) higher than for larvae fed a 4 % soybean lecithin diet, but this group (0 % SBL) had a survival of only 7 %, so only the most fit larvae survived and grew. These results are similar to the results found by other researchers [7] who found that 4 % soybean lecithin had a positive effect on growth performance of rainbow trout fry. Even though the nutritional requirements of Japanese flounder and rainbow trout are very different, this result is in line with the results of the present research. Results with Japanese flounder juveniles (initial weight 6.3 g) showed that 2 % soybean lecithin had no substantial effect on growth and feed [8] but in that research all diets had high amounts of squid liver oil, probably masking the effects of soybean lecithin, and only one level of soybean lecithin was tested (2 %). Researchers working with smaller Japanese flounder juveniles (1 g initial weight) found that 5.2 % Soybean lecithin improved growth [9], confirming the results of the current research.

In other studies [10], researchers also found increased survivals of yellow croaker larvae when dietary soybean phospholipids increased from 2.6 to 5.7 %. Similar results were obtained for loach *Misgurnus anguillicaudatus* larvae, obtaining increased growth and survival with dietary soybean phospholipid levels above 2 % [11]. In studies with pikeperch, *Sander lucioperca* larvae, dietary levels of 5 and 9 % significantly increased growth and reduced skeletal deformities [12]. The effect of dietary soybean lecithin in sea bream, (*Sparus aurata*) larvae was also studied by other authors [13]. They found that increasing dietary soybean lecithin up to 8 % significantly improved digestive enzymes activities, enterocyte maturation, utilization and deposition of dietary essential fatty acids and larval growth. Soybean lecithin has also proven to be effective in crustaceans. In trials with *Litopenaeus vannamei* fed feeds with 1 and 4 % soybean lecithin and three levels of fish oil, shrimp fed diets with 4 % soybean lecithin had better growth and lipid utilization [14].

Phospholipids are essential nutrients in diets for fish larvae, since they form part of cell membranes [15]. There seems to be no interaction between dietary lipid quality and the assimilation of dietary amino acids, since the amino acid profile of all larvae was very similar. The results found in this research indicate that lecithin supplementation improves the nutritional quality of the diet for Japanese flounder larvae, suggesting the need of dietary phospholipids by Japanese flounder larvae. This need may be provided by supplementing the feeds with 4 % soybean lecithin.

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