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Effect of soybean phospholipid supplementation in formulated microdiets and live food on foregut and liver histological changes of *Pelteobagrus fulvidraco* larvae

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Abstract

The effect of supplementation of soybean phospholipid (PL) to *Pelteobagrus fulvidraco* larvae diets on growth and histological changes in intestine and liver were investigated. *P. fulvidraco* larvae were fed from day 6 to 21 posthatch with four diets containing the same basal diet, coated with different lipid fractions (6% diet). The lipid fractions consisted of increasing levels of soybean PL (0, 2, 4 or 6% of diet) and decreasing levels of mixed oil (fish oil: soybean oil, 2:1). A group of larvae was fed rotifers as a control. The body weight and total length of larvae increased as a result of PL supplementation. Larvae fed diet supplemented with 6% PL exhibited the best growth performance and similar to those fed rotifers. Larvae fed 6% PL and larvae fed rotifers had normal appearance of enterocytes and liver. The addition of PL to the diet caused a reduction in the degree of lipid accumulation and an increased number of goblet cells in the enterocytes of the anterior intestine. The degree of lipid accumulation in the anterior intestine and in the liver on day 21 was lower than that on day 14, which indicated that the ability of PL synthesis was enhanced as the fish aged. These results confirm that PL has a growth-promoting effect and indicate that soybean PL is suitable as a lipid and PL source in microdiets for *P. fulvidraco* larvae feed.

Keywords: *Pelteobagrus fulvidraco*; Larvae; Soybean phospholipid; Histology; Enterocytes; Hepatocytes

1. Introduction

Live food, such as rotifers and *Artemia* are widely used in fish seed production. Although sustained growth and survival are obtained in fish larvae fed live food, this feeding regimen is expensive, requiring manpower and equipment. Moreover, the production and nutritional quality of live food fluctuate with environmental conditions as they are obtained from cysts collected in the wild environment. Such problems with live food have greatly limited the development of certain farmed fish production. The way to solve this problem is to formulate a high quality and suitable artificial feed that can be substituted for live food during the larval stages.

During the past few years, much progress has been made in larval microdiets to replace live food, especially for shrimp and freshwater species, which can be fed compound diets partly or even completely substituting for live prey as early as mouth opening (Fernández-Díaz and Yúfera, 1997; Cahu et al., 1998; Takeuchi et al., 1998). Nutrition studies concerning microdiets for larval fish mainly involve lipids (Kanazawa et al., 1982; Salhi et al., 1994; González-Félix et al., 2002), protein and vitamins. Dietary lipids as a source of energy are important for normal growth and development of larval fish. The use of fish oil as the main source of lipid is common in the practical production of larval feed. But the price of fishmeal and fish oil continues to increase due to them becoming a limited resource. Thus, the use of diets formulated with lower cost plant oil such as soybean phospholipid (PL) would reduce both the demand for fish oil and feed costs. Furthermore, many studies have demonstrated the beneficial effects of PL on growth of larval fish (Coutteau et al., 1997) and it has been suggested that larvae utilize dietary phospholipid more efficiently than neutral lipid (Salhi et al., 1999; Cahu et al., 2003). Dietary PL can enhance the transport of lipid in larval fish. Previous workers have determined PL requirements using highly purified PL sources such as 95% pure phosphatidylcholine (PC) in purified diets (Coutteau et al., 1996). However, it is difficult to use purified

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PL in practical microdiets because of the high cost. Moreover, the PL requirement of larval fish is related to age and other nutrients, such as the dietary protein source, so the exact nature of the PL requirement needs to be determined.

Pelteobagrus fulvidraco are one of the important economic freshwater fish in China. It has great market demand and high price due to its tender flesh, few bones, and good taste in recent years. However, the commercial culture of this species is limited due to a lack of knowledge of their nutritional needs, especially during the larval stage. Although, success has been achieved in first-feeding larvae with live food, no specialty starter-feed is yet available. In general, fish feed is made using fish oil and/or soybean oil. China has abundant plant PL resources since soybean PL is a by-product of the processing of soybeans. The aim of this study was to investigate the effect of commercial soybean PL (feed grade) supplementation in formulated microdiets on foregut and liver histological changes of *P. fulvidraco* larvae, which can provide information on the practical use of PL in microdiets.

2. Materials and methods

2.1. Experimental fish and diets

Fertilized eggs of *P. fulvidraco* were obtained following artificial fertilization of wild captured broodstock. Larval rearing was conducted at the hatchery Center for *P. fulvidraco*, Huazhong Agriculture University and lasted 21 days, which corresponds to the end of larval development. Newly hatched larvae were transferred from incubators to 20 circular cement tanks (water volume, 5000 l) with 10,000 larvae per tank, four tanks per dietary group. Freshwater was supplied at a flow-rate of 2 l/min to each tank. During the experiment, the water temperature was maintained at 26± 2 °C and pH was 7.6±0.5. The dissolved oxygen was above 5 mg/l, ammonia-nitrogen level was less than 0.02 mg/l and nitrite level was below 0.01 mg/l. Fish were held under natural photoperiod condition throughout the feeding trail.

Three days after hatching the larvae were fed live rotifers (newly hatched rotifers, unenriched) twice a day (0900 and 1700 h) for the first 2 days at a food density of 5–10 individuals/ml. From day 6 the larvae were divided into 5 groups, one group (control) continued to feed on live food and the other four groups were fed the test diets. All experimental diets contained 94% of the same basal diet and 6% lipid with different PL levels, the lipid consisted of increasing levels of soybean phospholipids (purity: 30%) and decreasing levels of mixed oil(fish oil:soybean oil, 2:1). The four diets were designated as PL0, PL2, PL4 and PL6. Ingredient and nutrient contents of the experimental diets are presented in Table 1. The dietary ingredients were initially ground (below 100 μm grain size) using a super micro mill. Then all ingredients for each of the experimental diets were weighed and mixed thoroughly. Fish oil, soybean oil and soybean phospholipid were added dropwise to the appropriate diet and mixed again. The diets were sieved to obtain two sizes of particles: 150–200 μm used during the first 7 days, then 200–300 μm until the end of the experiment. Thereafter prepared diets were packed and stored at −20 °C until use.

The test diets were previously mixed with little water to form wet particles, and then were fed by hand. 1 g was dispersed near the each tank wall every hour for 8 h a day until they began actively feeding. Then the diets with added water and kneaded into a dough were put into feeding baskets suspended in the tanks for larvae to eat ad libitum. Larvae were fed in excess four times daily (0630, 1130, 1630 and 2200 h). Food ingestion was monitored by observing the larvae digestive tract under a binocular microscope. Excess feed and faeces were removed before feeding while dead larvae were removed twice per day and counted. During the experimental period, the tanks were siphoned daily in order to keep them clean.

2.2. Sample collection

To determine growth, samples of larvae ($n=50$) from each tank were collected for measurement of total length and wet weight at days 6, 8, 10, 12, 14 and 21. The final survival was calculated from daily mortality and from the final number of surviving larvae recorded in each tank. Five larvae were sampled from each tank after 5–6 h of feeding experimental diets on days 14 and 21 for histological examination.

Table 1
Formulation and composition of the experimental diets containing different phospholipid levels (g/100 g diet)

	Experimental diets			
	PL0	PL2	PL4	PL6
Ingredients (g/100 g diet)				
Basal diet [a]	94	94	94	94
Soybean phospholipid [b]	0	2	4	6
Fish oil	4	2.66	1.33	0
Soybean oil	2	1.33	0.67	0
Analyzed proximate composition (g/100 g in dry matter)				
Total PL	2.12	2.75	3.37	4.01
Moisture	7.17	7.38	7.40	7.25
Crude protein	48.04	48.10	48.09	48.11
Crude lipid	12.42	11.34	10.12	9.05
Ash	11.82	11.84	11.85	11.85
Ca	3.19	3.16	3.21	3.10
P	1.91	1.94	1.97	1.99
Gross energy (kJ/g)	19.14	19.05	18.66	18.37

Note: live feed (unenriched rotifers) used as control diet: moisture 91.42%; crude protein 4.95%; crude lipid 1.81% (wet weight basis).

[a] Basal diet (g/100 g diet) consisted of white fish meal, 48; soybean meal, 12; brewers dry yeast, 10; wheat gluten, 3; squid meal, 8; wheat flour, 2; α-Starch, 9; mineral premix/vitamin premix, 2.

[b] Commercial soybean phospholipid with the purity of 30%.

2.3. Analysis of diets

Proximate composition of the dietary ingredients was determined in triplicate according to AOAC (1990) methods as follows: moisture was determined by oven-drying at 105 °C for 24 h; crude protein (N×6.25) was determined by the Kjeldahl procedure; crude lipid was determined by etherextraction. Ash content was determined by burning the sample at 550 °C for 8 h in a muffle furnace. Gross energy was determined with an adiabatic bomb calorimeter. Calcium was determined by the EDTA titration method and total phosphorus was determined colorimetrically with a phosphomolybdate indicator using a spectrophotometer. Total phospholipid was determined by phosphorus assay of extracted lipids (Bartlett, 1959).

2.4. Histology

Sampled larvae were fixed in Bouin's solution for at least 24 h. The fixed samples were then dehydrated by increasing concentrations of ethanol and embedded in paraffin wax. Serial sections (5 μm thick) were cut and stained with hematoxylin and eosin. The changes in foregut and liver histology of larvae were examined under a light microscopy (BX41-32H02, Olympus, Japan). All microscopic images of the sections were recorded and analyzed with image processing software.

2.5. Statistical analyses

All data are presented as mean and standard deviation (SD). Statistical analysis was performed by means of one-way ANOVA followed by Duncan's multiple range test. Differences were regarded as significant when $P<0.05$. All statistical analyses were performed using SAS version 8.0.

3. Results

3.1. Growth performance

The experiment showed that the test diets were well accepted by the larvae. Larvae liked to gather at the tank wall and took bite directly out of the dough. The diets prepared in this study showed low leaching and