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Increased Pellet Water Stability by the Supplementation of Phospholipid and Its Effects on Shrimp Growth

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Abstract

The effects of phospholipid supplementation on feed pellet water stability and growth performance of juvenile *Penaeus penicillatus* were studied. Pellet leaching rates were used to indicate pellet water stability. The results show that the presence of phospholipid in the diets significantly improved pellet water stability and shrimp growth. Increased supplements resulted in corresponding increase in pellet stability but not additional weight gain. Improved diet water stability is one important reason why phospholipid supplementation enhances shrimp growth.

Introduction

The most important difference between aquatic animal feeds and land animal feeds is the durability of the aquatic feeds. Effective feed for aquatic animals must hold together before being eaten. The ability of the feed to maintain its physical stability in water is often referred to as *water stability*. The criteria of water stability for each diet type are very much related to the feeding behavior and digestion ability of a particular animal. Trout and channel catfish are instant feeders. Feed particles are quickly consumed after delivery. The required water stability (or resident time) for these instant feeders is thus, several minutes. Crustacean feed, on the other hand, requires good water stability, as crustaceans such as shrimp are slow feeders. Feed particles usually stay in the water for a considerable time before being approached by shrimp. The manipulation of feed particles by the animals during feeding further impedes the stability of the feed.

Published data concentrate on the characteristics and development of suitable binders (Meyers et al. 1972; Meyers and Zein-Eldin 1972). Little is known about the effect of feed components on water stability. Hysmith et al. (1972) suggested that water stability and composition density are the most important physical characteristics of shrimp feed. A more compact feed produced by the steam pelleting process attained better shrimp growth than less compact diets (Parker and Hilcomb 1973).

The use of phospholipids in aquatic feeds has received increasing attention. In addition to their essentiality in crustacean lipid nutrition (D'Abramo et al. 1981; Kanazawa et al. 1985; Briggs et al. 1988), phospholipids are believed to increase water stability. Chien (1986) proposed that the water stability of a dried pellet can be measured by immersing feed pellets in water for a fixed period of time. The weight of the remaining portion of the pellets can effectively indicate the water stability of the pellets. This study utilized the immersion method to compare the effect of supplementary phospholipid on the water stability of test pellets. The results of the water stability analysis were related to the growth-promoting effects of these test diets.

Materials and Methods

Two experiments were conducted.The first experiment examined the difference between a phospholipid-supplemented diet and an unsupplemented diet. Both diets had the same ingredients, except 5% of phosphatidylcholine (PC) in one diet (the basal diet, Table 1) was replaced by an equal amount of soybean oil in the other diet. Phosphatidylcholine was chromatographically purified and contained 90% of PC and 10% lyso-PC.

The second experiment had four test diets with graded PC levels (0, 1.25, 2.5 and 5%). The same basal diet was used. Soybean oil was added to make the adjustment so that the total amount of PC and soybean oil was 5% in each diet.

All the test diets were prepared and processed into pellets according to a same procedure (Meyers and Zein-Eldin 1972). Measured ingredients were thoroughly blended for at least 15 minutes in a mixer. Sodium alginate (HV type, Kelco, San Diego, CA) and sodium hexametaphosphate were dissolved and stirred in 65-75°C

Table 1. Percentage composition of the basal diet.[1]

Ingredient	Per cent
Casein, vitamin-free	45.7
Dextrin	20.0
Mineral mixture	8.6
Fish oil	5.0
Phosphatidylcholine	5.0
Cellulose	4.6
Amino acid mixture[2]	3.0
Vitamin mixture	2.7
Sodium alginate	2.5
Sodium hexametaphosphate	1.0
Glucosamine	0.8
Cholesterol	0.5
Sodium succinate	0.3
Sodium citrate	0.3

[1]Chen and Jenn (1991)
[2]Alanine : glycine : glutamic acid : betaine = 1:1:1:2 (as attractant)

distilled water. The resulting colloidal liquid was added to the dry components to form a stiff dough, which was hand-extruded as 2-mm diameter spaghetti-like strands. The strands were chilled in a refrigerator for 2 hours and freeze-dried. Dried strands were broken into 5-mm pellets and stored in plastic bags at -20°C.

Pellets with a consistent physical appearance were selected from the stocked feeds by sieving and by eye. Five grams of the selected pellets were dropped into a 1-l beaker which contained 800 ml filtered seawater. The immersion times examined were 4 and 8 hours. After immersion, the undissolved solids and water were filtered through a Toyo No. 1 qualitative filter paper (Toyo Roshi Kaisha, Japan) and were dried in 105°C to obtain dry weight. Each observation was duplicated. The leaching rate was calculated as:

$$\text{Leaching rate} = \frac{A \times (1-r) - R}{A \times (1-r)} \times 100\%$$

where A = weight of pellets before immersion;
r = moisture content of pellets; and
R = dry weight of the remaining solid.

Thus, a pellet with better water stability has a lower leaching rate. A less stable pellet has a higher leaching rate.

Juvenile *Penaeus penicillatus* were obtained from outdoor ponds of a commercial farm in Kaohsiung, Taiwan. Prior to stocking into the 60W x 60L x 46.5 H cm experimental aquaria, the acclimatized (three weeks in indoor condition) animals were weighed and ranked so that the biomass in each tank was generally similar. The initial body weights of the shrimp ranged between 0.9 and 1.2 g. The first experiment used 26 juveniles divided into two groups of 13 for each dietary treatment. The second experiment used 30 animals divided into two groups of 15 for each treatment. Each separate group was housed in an aquarium. The aquaria were fitted with under-gravel filters. The filter bed consisted of crushed oyster shell and sand. Salinity was maintained at 30 ppt. Temperature was not controlled but monitored and mean temperature ranged between 23.6 and 26.2°C. The juvenile shrimp were fed to excess three times daily at approximately 0900, 1500 and 2100 hours. Uneaten food, fecal waste and molt exuviae were removed before each feeding. The feeding experiments were conducted for four weeks. The shrimp were weighed individually to the nearest 0.01 g in the beginning and at the end of the trials.

Student's t-test was used to examine the results of the first experiment and an ANOVA program from the Statistical Analysis System for the results of the second experiment.

Results

In both experiments, phospholipid supplementation significantly enhanced the water stability of the test diets and the growth of the juvenile shrimp. In the first experiment, the diet containing phosphatidylcholine (PC diet) had lower leaching rates than the unsupplemented (no PC) one (Table 2). The leaching rates of the PC diet observed after either 4 or 8 hours immersion were significantly ($P<0.05$) lower than those of the no-PC diet. The final body weights and weight gains of the shrimp fed the PC diet were significantly higher than the unsupplemented group. Food conversion results showed the similar contrast.

Results of the second experiment confirmed the conclusion obtained in the first experiment in that PC supplementation increased pellet water stability and shrimp growth (Table 3). Increased PC supplementation resulted in improved pellet stability but not additional weight gain. Table 3 shows a corresponding improvement in pellet stability by increased PC at 4 hours, but the difference was not significant ($P>0.05$) at 8 hours. When the feeding behavior of the shrimp was followed, the feed offered in each feeding was mostly consumed within the first 4 hours. PC supplementation

Table 2. Pellet water stability (measured by leaching rate at 4 and 8 hours) and final body weights, weight gains, food conversion ratios (FCR) and survival of *P. penicillatus* after 4 weeks of feeding the test diets with or without 5% phosphatidylcholine (PC).

Diet	Leaching rate (%)[ss]		Final body weight (g)[s]	Weight gain (%)[s]	FCR[s]	Survival (%)[ns]
	4 hours	8 hours				
no-PC	27.3	28.2	2.51	134	1.62	96
PC	24.5	25.6	2.72	161	1.45	98

[s]Significantly different ($P<0.05$).
[ss]Significantly different ($P<0.01$).
[ns]Not significantly different ($P>0.05$).

Table 3. Pellet water stability (measured by leaching rate at 4 or 8 hours) and final body weights, weight gains, food conversion ratios (FCR) and survival of *P. penicillatus* (initial mean body weight: 0.93 g) after fed test diets containing graded levels of phosphatidylcholine (PC) for 4 weeks[1,2].

PC (%)	Leaching rate (%)		Final body weight (g)	Weight gain (%)	FCR[ns]	Survival (%)[ns]
	4 hours	8 hours[ns]				
0	15.8[a]	17.9	2.05[a]	118.0[a]	3.15	72.2
1.25	15.0[b]	16.8	2.24[b]	142.5[b]	2.67	66.7
2.5	15.3[b]	17.4	2.23[b]	133.5[b]	2.80	72.2
5	13.9[c]	16.2	2.16[b]	139.0[b]	2.81	77.8

[1]Means in the same column with the same superscript are not significantly different ($P>0.05$).
[2]Part of the growth and survival data has been published elsewhere (Chen and Jenn 1991).
[ns]Not significantly different ($P>0.05$).

significantly improved fish growth, but increased supplementation from 1.25 to 5% did not yield additional weight gain. There was no significant difference in food conversion ratios and survival of the test shrimp over the dietary range (Table 3).

Discussion

When approaching the question of a nutrient requirement, it is important to recognize the interactions among ingredients.

Supplementation of phospholipids increased shrimp growth and this growth-promoting effect is partly related to the increased pellet water stability. Phospholipids have been shown to be essential for growth and survival of many crustacean species (D'Abramo 1981; Teshima et al. 1986; Briggs et al. 1988; Chen and Jenn 1991). The data presented in the present study clearly suggest that the improvement in growth by phospholipid supplementation is partly contributed by the reduced leaching.

The leaching rates used in this study seem to be an informative pellet water stability indicator. But it alone cannot directly provide the qualitative or quantitative value of how the diet is leached or what is made unavailable to the ingesting animals. Growth of slow feeders such as shrimp could be greatly affected by reduced food ingestion because of unstable food particles. The difference in 4-hour leaching rates between supplemented and unsupplemented was generally less than 2% in weight. This rather insignificant difference could be greatly amplified because of manipulation during shrimp feeding. Slight agitation during leaching experiments to imitate shrimp manipulation might have yielded a better estimation of leaching. No agitation was applied in the present study.

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