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# Partial and total replacement of fish meal with soybean meal and distillers' by-products in diets for pond culture of the freshwater prawn (*Macrobrachium rosenbergii*)

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## ABSTRACT

This study was conducted to evaluate the effect of partially or completely replacing fish meal with soybean meal and distillers' dried grains with solubles (DDGS) in diets for pond-raised freshwater prawns. Juvenile prawns averaging  $0.51 \pm 0.27$  g were stocked into nine 0.02-ha earthen ponds at 39,520/ha. Three isonitrogenous diets (32% crude protein) containing 15, 7.5 or 0% fish meal were fed to prawns in triplicate ponds. A variable percentage of soybean meal and a fixed percentage (40%) of DDGS replaced fish meal. Average yield, survival, individual weight, and feed conversion did not differ significantly ( $P < 0.05$ ) among shrimp fed the three diets. When averaged over the three diets, results were: yield, 1268 kg/ha; survival 78.1%; individual weight 42 g; and feed conversion 2.9. Replacement of fish meal caused increases in dietary levels of glutamine, proline, alanine, leucine, and phenylalanine, and decreases in aspartic acid, glycine, arginine, and lysine levels in the diets. Changes in fatty acid profiles of the diets were: increased concentrations of 16:0, 18:2n-6, and 20:1n-9, and decreased concentrations of 14:0, 16:1n-7, 18:1n-9, 18:3n-3, 20:4n-6, 20:5n-3, 22:5n-3 and 22:6n-3. Results indicate that fish meal can be partially or totally replaced with soybean meal and distillers' by-products in diets for pond production of freshwater prawns in temperate areas.

## INTRODUCTION

The freshwater prawn, *Macrobrachium rosenbergii*, has received considerable attention for its attractive characteristics as an aquaculture species. Although feed costs significantly impact production costs, relatively few studies have been conducted toward development of high-quality, economical diets. Fish meal is used as a major protein source in most diets for finfish and crustaceans (Lovell, 1989). Fish meal is one of the highest-quality protein sources,

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and one of the most expensive (Akiyama, 1988). To reduce production costs, the replacement of expensive animal proteins with less expensive plant proteins is desirable, as long as satisfactory growth and feed efficiency can be maintained. However, replacement of fish meal with individual plant proteins, such as soybean meal (SBM), in aquatic animal diets has generally resulted in reduced growth and lower feed efficiency (Cowey et al., 1971; Lovell et al., 1974; Jackson et al., 1982). Use of complementary combinations of plant proteins may be effective in eliminating these undesirable effects. Webster et al. (1992) demonstrated that weight gain and feed efficiency were not compromised when channel catfish were fed diets with a combination of SBM and distillers' dried grains with solubles (DDGS) completely replacing fish meal. Tidwell et al. (1993) found that diets containing as much as 40% DDGS supported excellent growth in pond-raised freshwater prawn. If fish meal could be reduced or totally replaced in practical diets for freshwater prawn, substantial savings in feed costs could be realized, as well as greater flexibility in feed formulation. The purpose of this study was to evaluate the effects of partial and total replacement of fish meal with a combination of SBM and DDGS in diets for pond-cultured freshwater prawn.

## MATERIALS AND METHODS

### *Description, preparation, and stocking of ponds*

Less than 1 week prior to the anticipated stocking date, ponds were filled and treated with two applications of liquid fertilizer (10:34:0) at a rate of 9.0 kg phosphorus/ha to achieve an algal bloom. The water surface area of all experimental ponds was 0.02 ha and average water depth was approximately 1.1 m. Water for replacing evaporative losses and flushing ponds was obtained from a reservoir filled by rain runoff. Ponds were located at the Aquaculture Research Center, Kentucky State University, Frankfort, KY. One 7.6-cm air-lift pump was located in the deepest area of each pond to prevent thermal stratification.

Graded juvenile prawns were shipped by air from a commercial hatchery (Aquaculture of Texas, Weatherford, TX) on 3 June 1992. Shrimp were held overnight in two 3000-liter tanks partially filled with plastic netting to provide substrate. On the stocking date (4 June 1992), the mean stocking weight was determined from a sample of 45 prawns that were blotted free of surface water and individually weighed ( $\bar{x} \pm \text{s.d.} = 0.51 \pm 0.27$  g). Three replicate ponds were randomly assigned to each of the three experimental treatments, which consisted of diets containing different levels (15, 7.5, and 0%) of fish meal. Prawns were hand-counted and stocked in each pond at a density of 39,520/ha.

### *Samples*

A 3.2-mm square mesh seine was used to collect a sample of  $\geq 20$  individuals from each pond every 3 weeks during the growing season. Prawns composing the sample were counted, group-weighted (drained weight) to the nearest 0.1 g, and returned to the pond.

### *Feed and feeding rates*

Experimental diets were formulated to contain 32% crude protein. Ingredient composition of the control diet was similar to that of the diet utilized by D'Abramo et al. (1989) and Tidwell et al. (1993) and contained 15% fish meal. In the two experimental diets, fish meal was reduced to 7.5 and 0%.

TABLE 1

Ingredient and chemical composition of experimental diets containing different percentages of fish meal and fed to pond cultured freshwater prawns

Ingredient	Diet no.		
	1	2	3
Menhaden fish meal (67%)	15.00	7.50	0.00
Soybean meal (44%)	25.00	15.00	26.25
DDGS	0.00	40.00	40.00
Wheat flour	13.00	13.00	13.00
Meat and bone meal (54%)	8.00	8.00	8.00
Ground corn meal	28.75	5.25	0.00
Mineral mix <sup>1</sup>	0.10	0.10	0.10
Vitamin mix <sup>2</sup>	0.10	0.10	0.10
Choline chloride	0.05	0.05	0.05
Dicalcium phosphate	0.00	0.50	1.00
Cod liver oil	0.00	0.50	1.50
CMC <sup>3</sup>	5.00	5.00	5.00
Lignosulfonate binder	5.00	5.00	5.00
Analyzed composition (%) <sup>4</sup>			
Protein	33.70	32.40	33.00
Lipid	6.50	9.10	9.30
Moisture	11.20	11.60	10.06
Relative ingredient cost <sup>5</sup>	1.045	1.000	1.006

<sup>1</sup>Mineral mix contained: Mn, 10.0% (as MnSO<sub>4</sub>); Zn, 10.0% (as ZnSO<sub>4</sub>); Fe, 7.0% (as FeSO<sub>4</sub>); Cu, 0.7% (as CuSO<sub>4</sub>); I, 0.24% (as CaIO<sub>3</sub>); Co, 0.10% (as CoSO<sub>4</sub>).

<sup>2</sup>Vitamin mix contained: thiamine (B<sub>1</sub>), 1.01%; riboflavin (B<sub>2</sub>), 1.32%; pyridoxine (B<sub>6</sub>), 0.9%; nicotinic acid, 8.82%; folic acid, 0.22%; cyanocobalamin (B<sub>12</sub>), 0.001%; pantothenic acid, 3.53%; menadione (K), 0.2%; ascorbic acid (C), 22.1%; retinopalmitate (A), 4409 IU/kg; cholecalciferol (D<sub>3</sub>), 2 204 600 IU/kg;  $\alpha$ -tocopherol (EO), 66.2 IU/kg; ethoxyquin, 0.66%.

<sup>3</sup>CMC = carboxymethylcellulose, used in combination with lignosulfonate to further improve pellet stability.

<sup>4</sup>Dry weight basis.

<sup>5</sup>Ingredient costs based on prices quoted in *Feedstuffs* 1992, 64(3): 62.

DDGS was added at 40% of the formulation. The proportion of soybean meal varied to maintain the crude protein level at 32%. The DDGS used in the study was a homogeneous composite from seven distilleries as provided by the Distillers Feed Research Council, Ft. Wright, KY. Dietary ingredients were processed into 5-mm sinking pellets by a commercial feed mill (Farmers Feed Mill, Lexington, KY). Dietary protein levels were determined using macro-Kjeldahl, dietary fat by acid hydrolysis, and moisture by drying to constant weight in a convection oven at 95°C (AOAC, 1990). A sample (approximately 1000 g) of each diet was frozen for subsequent amino acid analysis. Ingredient composition, proximate analysis, and relative ingredient costs of the diets are provided in Table 1.

Two separate feedings, each consisting of one-half of the total daily ration, were distributed over the entire surface of each pond between 09.00 and 10.00 h and between 15.00 and 16.00 h. Prawns were fed a percentage of body weight based on a feeding schedule reported by D'Abramo et al. (1989). Feeding rates were adjusted weekly based on an assumed 3.0 feed conversion (D'Abramo et al., 1989). Every 3 weeks, biomass estimates were adjusted according to sample weights. Survival was assumed to be 100%.

#### *Water quality management*

Dissolved oxygen (DO) and temperature of all ponds were monitored twice daily (09.00 h and 15.30 h) by means of a YSI Model 57 oxygen meter (Yellow Springs Instruments, Yellow Springs, OH). When the DO level of any pond was predicted (graphically) to decline below 4.0 mg/l, nightly aeration was provided to that pond using an electric vertical pump aerator. Levels of total ammonia nitrogen (TAN) and nitrite in water samples collected from each pond at approximately 13.00 h were determined weekly according to the outlined procedures for a Hach DREL/5 spectrophotometer (Hach Co., Loveland, CO). The pH of each pond was determined daily at 13.00 h using an electronic pH meter (Omega Engineering, Inc., Stamford, CT). If afternoon pH was measured to be  $\geq 9.5$ , the pond was slowly flushed overnight with water from a reservoir pond.

#### *Harvest*

One day prior to harvest, water levels in ponds were lowered to approximately 0.9 m at the drain end. On 21 September 1992 each pond was seined 3 times using a 1.3-cm square mesh seine. Complete draining of the ponds followed. Remaining prawns were manually harvested from the pond bottom and purged in clean water. Total bulk weight and number of prawns from each pond were recorded. A sample of 50 prawns per pond was randomly collected and individually weighed. At the conclusion of the study, six prawns were randomly selected from a pool of harvested prawns and sacrificed. The abdominal muscle of these individuals was isolated, chopped into small pieces,

immediately frozen in liquid nitrogen ( $-196^{\circ}\text{C}$ ) and (stored ( $-40^{\circ}\text{C}$ ) until amino acid analysis. Eggs in late stages of development were removed from 12 randomly selected prawns, frozen, and stored, as described previously, for subsequent amino acid and fatty acid analysis.

### *Evaluation*

Growth performance and feed conversion were evaluated by final individual shrimp weight (g), yield (kg/ha), survival (%), and feed conversion ratio (FCR), which was calculated as:

$$\text{FCR} = \text{total dry feed fed (kg)} / \text{total wet weight gain (kg)}.$$

Amino acid compositions of diets were compared to amino acid composition of prawn muscle and eggs using the following indices:

$$A/E = \text{individual essential amino acid content} / \text{total essential amino acid content} \times 1000.$$

$$EAAI = \frac{aa_1}{AA_1} \times \frac{aa_2}{AA_2} \times \dots \times \frac{aa_n}{AA_n} 1/n$$

where *EAAI* is the *n*th root of the product of the ratios of essential amino acids in the test diet (*aa*) to the content of each of those amino acids in the reference tissue (*AA*) and *n* is the number of amino acids evaluated.

### *Statistical analysis*

Data were analyzed by ANOVA using the SAS ANOVA procedure (SAS, 1988). Duncan's multiple range test was used to compare treatment means. Percentage survival, FCR, and fatty acid and amino acid compositions were transformed to *arc sin* values prior to analysis (Zar, 1984).

## RESULTS AND DISCUSSION

### *Production*

For the duration of the study, means of recorded water quality values did not differ significantly ( $P > 0.05$ ) among treatments. Mean values ( $\pm$ s.e.) were: water temperature (AM),  $22.9 \pm 0.2^{\circ}\text{C}$ ; water temperature (PM),  $24.9 \pm 0.2^{\circ}\text{C}$ ; DO (AM),  $6.7 \pm 0.2$  mg/l; DO (PM),  $10.1 \pm 0.7$  mg/l; TAN,  $0.245 \pm 0.189$  mg/l; nitrite,  $0.041 \pm 0.040$  mg/l; and pH,  $8.6 \pm 0.4$ . Maximum recorded pH was 10.0. Minimum recorded temperature (AM) was  $18.4^{\circ}\text{C}$ .

Pond culture was terminated 110 days after stocking (Fig. 1). Mean weight of prawns fed diets containing 15, 7.5, and 0% fish meal averaged 43.9, 40.8,

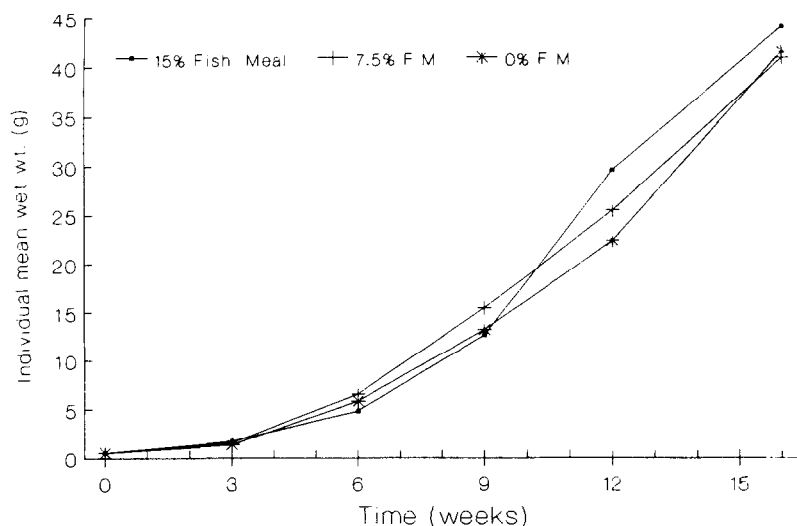


Fig. 1. Mean weight of prawn populations sampled at 3-week intervals.

TABLE 2

Individual weight, survival, yield, and feed conversion ratio (FCR) of prawn fed experimental diets with varying percentages of fish meal for 110 days<sup>1</sup>

	Diet no.		
	1	2	3
Individual wet weight (g)	43.9 ± 2.1	40.8 ± 2.5	41.4 ± 3.8
Survival (%)	80.8 ± 15.0	78.3 ± 12.9	75.3 ± 14.9
Yield kg/ha	1,291 ± 320	1,278 ± 132	1,233 ± 151
FCR <sup>2</sup>	2.51 ± 0.05	2.94 ± 0.25	3.11 ± 0.34

<sup>1</sup>Values are means ± s.e. of 3 replications. There were no significant differences ( $P > 0.05$ ) among treatments for any variable.

<sup>2</sup>FCR = total dry feed/total wet weight gain.

and 41.4 g, respectively (Table 2), and were not significantly different ( $P > 0.05$ ). Average weights were similar to or higher than those previously reported for prawns raised at the same stocking density (25–44 g) (D'Abramo et al., 1989) and only slightly lower than weights reported for low density (19,760/ha) prawn monoculture (50–60 g) under similar conditions of pond size, latitude, feed composition, and feeding rates (Tidwell et al., 1993).

Survival, feed conversion, and yield did not differ significantly ( $P \geq 0.05$ ) among treatments (Table 2). Survival in individual ponds ranged from 65% to 92.6%, averaging 78% overall. Tidwell et al. (1993) reported a similar range of survivals (62–91%), with an overall average of 75%. Feed conversions in

this study averaged 2.9 and were similar to an average of 3.1 reported by Tidwell et al. (1993) and lower than a 2-year average (4.1) reported by D'Abramo et al. (1989) for prawns stocked at 39,536/ha. Mean yield was 1268 kg/ha. overall and exceeded a 2-year average of 863 kg/ha reported by D'Abramo et al. (1989) for the same density. However, D'Abramo et al. (1989) used larger ponds and smaller prawns at stocking.

### Biochemical considerations

Weight gain is known to be affected by quantity and quality of dietary protein. Balazs and Ross (1976) reported that when soybean meal and fish meal were used as the major protein sources, the optimum dietary protein level for *M. rosenbergii* was 35%. Sick and Millikin (1983) reported that 25–30% di-

TABLE 3

Amino acid composition (% of total amino acids)<sup>1</sup> of the diets, prawn tail muscle, and prawn eggs, and essential amino acid index (EAAI) of the diets

Amino acid	Diet			Muscle <sup>2</sup>	Eggs <sup>2</sup>
	1	2	3		
Asp	12.23 ± 0.07 <sup>a</sup>	8.79 ± 0.05 <sup>b</sup>	8.92 ± 0.10 <sup>b</sup>	12.91 ± 0.59	10.14 ± 0.27
Thr	4.21 ± 0.11	4.12 ± 0.08	4.15 ± 0.05	4.21 ± 0.11	5.63 ± 0.29
Ser	4.93 ± 0.10	5.05 ± 0.16	5.14 ± 0.05	4.03 ± 0.12	6.31 ± 0.16
Glu	17.09 ± 0.60 <sup>b</sup>	19.43 ± 0.25 <sup>a</sup>	19.43 ± 0.50 <sup>a</sup>	15.72 ± 0.50	14.51 ± 0.37
Pro	6.07 ± 0.30 <sup>c</sup>	7.54 ± 0.00 <sup>a</sup>	7.26 ± 0.04 <sup>b</sup>	3.78 ± 0.24	4.54 ± 0.54
Gly	7.80 ± 0.20 <sup>a</sup>	6.30 ± 0.11 <sup>b</sup>	6.14 ± 0.07 <sup>b</sup>	5.85 ± 0.32	5.11 ± 0.08
Ala	6.49 ± 0.28 <sup>b</sup>	7.10 ± 0.09 <sup>ab</sup>	7.77 ± 0.60 <sup>a</sup>	5.21 ± 0.47	44.4 ± 0.30
Cys	1.29 ± 0.06	1.37 ± 0.03	1.32 ± 0.01	1.11 ± 0.07	1.25 ± 0.03
Val	5.10 ± 0.00	4.96 ± 0.18	4.94 ± 0.02	4.43 ± 0.09	5.88 ± 0.15
Met	2.18 ± 0.16	2.19 ± 0.06	2.01 ± 0.01	3.17 ± 0.06	3.32 ± 0.16
Ile	4.00 ± 0.00	4.01 ± 0.18	4.00 ± 0.00	4.32 ± 0.18	4.74 ± 0.18
Leu	7.35 ± 0.04	8.92 ± 0.08	8.76 ± 0.08	7.37 ± 0.12	7.72 ± 0.15
Tyr	2.08 ± 0.13	2.31 ± 0.21	2.38 ± 0.12	3.12 ± 0.05	3.42 ± 0.11
Phe	4.42 ± 0.01 <sup>b</sup>	4.63 ± 0.04 <sup>a</sup>	4.68 ± 0.03 <sup>a</sup>	3.88 ± 0.14	4.27 ± 0.15
His	2.83 ± 0.30	3.36 ± 0.11	3.20 ± 0.27	2.70 ± 0.09	3.77 ± 0.08
Lys	5.70 ± 0.08 <sup>a</sup>	4.67 ± 0.02 <sup>b</sup>	4.52 ± 0.05 <sup>b</sup>	8.57 ± 0.17	7.54 ± 0.26
Arg	6.18 ± 0.06 <sup>a</sup>	5.40 ± 0.07 <sup>b</sup>	5.47 ± 0.01 <sup>b</sup>	9.63 ± 0.17	7.43 ± 0.21
EAA index <sup>3</sup>					
Muscle	89.6	90.0	88.4		
Egg	82.0	82.3	80.8		

<sup>1</sup>Means of 2 replications (±s.e.) for diets and 3 replications for tissues. Diet means within a row having the same superscript were not significantly different ( $P > 0.05$ ).

<sup>2</sup>Muscle and egg values are for comparative purposes and are not included in statistical analysis.

<sup>3</sup> $EAAI = \frac{aa_1}{AA_1} \times \frac{aa_2}{AA_2} \times \dots \times \frac{aa_n}{AA_n} / n$  where EAAI is the  $n$ th root of the product of the ratios of essential amino acids in the test diet ( $aa$ ) to the content of each of those amino acids in the reference tissue ( $AA$ ) and  $n$  is the number of amino acids evaluated.

TABLE 4

A/E ratios<sup>1</sup> of experimental diets, eggs, and tail muscle of *M. rosenbergii*

Amino acid	Diet			Tail muscle <sup>2</sup>	Prawn eggs <sup>2</sup>
	1	2	3		
Arg	147.3 ± 0.4 <sup>a</sup>	121.5 ± 0.0 <sup>b</sup>	127.8 ± 5.3 <sup>b</sup>	199.4 ± 4.2	150.5 ± 8.5
His	67.4 ± 6.7	75.6 ± 1.6	74.7 ± 3.3	55.9 ± 1.9	76.1 ± 2.0
Ile	95.3 ± 0.6	90.2 ± 3.0	93.6 ± 3.8	89.5 ± 3.1	95.7 ± 1.9
Leu	175.1 ± 2.1 <sup>b</sup>	200.6 ± 0.9 <sup>a</sup>	204.7 ± 10.0 <sup>a</sup>	152.7 ± 3.0	155.7 ± 3.7
Lys	135.8 ± 2.9 <sup>a</sup>	103.0 ± 1.8 <sup>b</sup>	105.5 ± 3.1 <sup>b</sup>	177.4 ± 2.4	152.1 ± 7.0
Met	52.0 ± 3.3	49.3 ± 0.6	47.0 ± 2.3	65.7 ± 0.8	67.0 ± 0.8
Phe	105.2 ± 0.9	104.1 ± 0.6	109.4 ± 5.1	80.4 ± 2.4	86.0 ± 2.9
Thr	100.3 ± 3.0	92.7 ± 3.1	97.0 ± 5.0	87.3 ± 2.9	113.6 ± 6.4
Val	121.7 ± 0.9	111.5 ± 2.6	115.4 ± 4.2	91.8 ± 2.1	118.5 ± 2.1

<sup>1</sup>Means of 2 replications ( $\pm$  s.e.) for diets and 3 replications for tissues. Diet means within a row having the same superscript were not significantly different ( $P > 0.05$ ).

<sup>2</sup>Muscle and egg values are for comparative purposes and were not included in statistical analyses.

etary protein was sufficient for larger freshwater prawns (4–20 g). Feeds in this study contained 32% crude protein.

Determination of exact amino acid requirements in crustaceans is difficult. Purified diets containing crystalline amino acids are subject to considerable leaching due to slow feeding and shredding of feed pellets (Sick and Millikin, 1983). Arai (1981) suggested that the ratio of individual amino acid content to total essential amino acid content of the whole body ( $\times 1000$ ) (A/E ratios) could be used in formulating diets containing adequate levels and proportions of amino acids. Tacon and Cowey (1984) proposed an essential amino acid index (EAAI) to compare overall essential amino acid content in a diet to the amino acid patterns of the species being studied. EAAI is calculated as the  $n$ th root of the product of the ratios of each of the essential amino acids in the test diet to their contents in the reference tissue. Wilson and Poe (1985) compared the quantitative amino acid requirements determined for channel catfish to whole body and egg amino acid content and found a strong linear relationship. In the current study amino acid profiles of prawn tail muscle and eggs were determined.

Replacement of fish meal in diets caused significant ( $P < 0.05$ ) increases in concentrations of glutamine, proline, alanine, leucine, and phenylalanine (Table 3). Concentrations of aspartic acid, glycine, lysine, and arginine showed significant ( $P < 0.05$ ) decreases with fish meal replacement. EAAI for diets in this study ranged from 88 to 90 when compared to prawn muscle and from 81 to 82 when compared with prawn eggs (Table 3). Decreasing the fish meal content of the diet and substituting soybean meal and DDGS caused almost no change in EAAI. Piedad-Pascual (1990) compared EAAI ratios in



diets containing various levels of defatted soybean meal with the EAAI in tissue of the shrimp *Penaeus monodon*. EAAI ranged from 75 to 70, decreasing as the level of fish meal decreased, and SBM increased.

A/E ratios for muscle tissue of adult prawns in this study (Table 4) were similar to values determined for juveniles fed semi-purified diets (Reed and D'Abramo, 1989). Replacement of fish meal with SBM and DDGS caused a significant increase ( $P < 0.05$ ) in A/E ratio for leucine and a significant decrease ( $P < 0.05$ ) in A/E ratios for arginine and lysine in diets 2 and 3. A/E ratios of both tail muscle and prawn eggs, compared to the A/E ratios of feeds, would indicate that all three diets may be most limiting in arginine, lysine, and methionine. If requirements for these amino acids are low, natural food items present in the pond environment would likely provide sufficient levels,

TABLE 5

Fatty acid composition (% of total fatty acids)<sup>1</sup> of the experimental diets and prawn eggs

Fatty acid	Diet			Prawn eggs <sup>2</sup>
	1	2	3	
14:0	1.28 ± 0.02 <sup>a</sup>	1.21 ± 0.00 <sup>b</sup>	1.26 ± 0.01 <sup>a</sup>	1.23 ± 0.01
16:0	14.02 ± 0.16 <sup>b</sup>	15.72 ± 0.05 <sup>a</sup>	15.64 ± 0.04 <sup>a</sup>	17.85 ± 0.64
16:1 $n-7$	2.73 ± 0.01 <sup>a</sup>	1.83 ± 0.01 <sup>b</sup>	1.85 ± 0.01 <sup>b</sup>	3.84 ± 0.65
18:0	4.00 ± 0.03	3.86 ± 0.04	3.88 ± 0.05	6.79 ± 0.25
18:1 $n-9$	34.57 ± 0.05 <sup>a</sup>	25.20 ± 0.09 <sup>b</sup>	24.83 ± 0.02 <sup>c</sup>	26.82 ± 2.31
18:2 $n-6$	32.04 ± 0.38 <sup>c</sup>	41.96 ± 0.09 <sup>a</sup>	41.44 ± 0.09 <sup>b</sup>	24.79 ± 2.35
18:3 $n-3$	2.31 ± 0.02 <sup>a</sup>	1.93 ± 0.02 <sup>c</sup>	1.98 ± 0.02 <sup>b</sup>	1.51 ± 1.01
20:1 $n-9$	1.28 ± 0.01 <sup>b</sup>	1.28 ± 0.01 <sup>b</sup>	1.60 ± 0.01 <sup>a</sup>	0.82 ± 0.02
20:2 $n-6$	0.20 ± 0.01	—	—	1.52 ± 0.15
20:3 $n-6$	0.16 ± 0.00	—	—	0.58 ± 0.03
20:4 $n-6$	0.48 ± 0.02 <sup>a</sup>	0.24 ± 0.00 <sup>b</sup>	0.24 ± 0.00 <sup>b</sup>	2.10 ± 0.15
20:5 $n-3$	1.88 ± 0.01 <sup>a</sup>	1.75 ± 0.04 <sup>c</sup>	1.85 ± 0.01 <sup>b</sup>	3.16 ± 0.40
22:5 $n-3$	0.37 ± 0.00 <sup>a</sup>	0.25 ± 0.00 <sup>b</sup>	0.25 ± 0.00 <sup>b</sup>	0.41 ± 0.06
22:6 $n-3$	1.59 ± 0.01 <sup>a</sup>	1.30 ± 0.02 <sup>b</sup>	1.30 ± 0.01 <sup>b</sup>	1.55 ± 0.06
Other	1.42 ± 0.01	0.79 ± 0.01	0.72 ± 0.01	5.08 ± 0.72
Total	96.88 ± 0.05	96.48 ± 0.04	96.09 ± 0.01	95.99 ± 0.72
Saturates	20.73 ± 0.13 <sup>b</sup>	22.43 ± 0.05 <sup>a</sup>	22.40 ± 0.09 <sup>a</sup>	27.73 ± 0.65
Monoenes	39.37 ± 0.04 <sup>a</sup>	29.71 ± 0.10 <sup>c</sup>	30.17 ± 0.02 <sup>b</sup>	32.08 ± 2.97
Diene	32.33 ± 0.28 <sup>c</sup>	42.16 ± 0.06 <sup>a</sup>	41.63 ± 0.06 <sup>b</sup>	26.41 ± 2.49
Triene	2.46 ± 0.23 <sup>a</sup>	2.03 ± 0.01 <sup>c</sup>	2.08 ± 0.01 <sup>b</sup>	3.69 ± 1.07
PUFA	39.53 ± 0.23 <sup>b</sup>	48.21 ± 0.11 <sup>a</sup>	47.91 ± 0.06 <sup>a</sup>	36.17 ± 3.34
$n-3$	6.46 ± 0.03 <sup>a</sup>	5.72 ± 0.05 <sup>c</sup>	5.93 ± 0.01 <sup>b</sup>	7.06 ± 1.37
$n-6$	33.10 ± 0.27 <sup>b</sup>	42.67 ± 0.06 <sup>a</sup>	42.15 ± 0.06 <sup>a</sup>	29.06 ± 2.63
$n-3/n-6$	0.20 ± 0.01 <sup>a</sup>	0.13 ± 0.00 <sup>c</sup>	0.14 ± 0.00 <sup>b</sup>	0.24 ± 0.04
$n-6/n-3$	5.12 ± 0.09 <sup>c</sup>	7.46 ± 0.08 <sup>a</sup>	7.10 ± 0.02 <sup>b</sup>	4.28 ± 1.11

<sup>1</sup>Means of 2 replications ( $\pm$  s.e.) for diets and 3 replications for eggs. Diet means within a row having the same superscript are not significantly different ( $P > 0.05$ ).

<sup>2</sup>Egg values are for comparative purposes and were not included in the statistical analyses.

even at moderate to high prawn densities. In areas with a more extended growing season, or at much higher stocking densities, differences may be more pronounced.

Replacement of fish meal in diets caused significant ( $P < 0.05$ ) increases in several individual fatty acids (16:0, 18:2 $n$ -6, and 20:1 $n$ -9) and fatty acid categories (saturates, dienes, polyunsaturates, and  $n$ -6 fatty acids) (Table 5). Replacement of fish meal was accompanied by decreases in 16:1 $n$ -7, 18:1 $n$ -9, 18:3 $n$ -3, 20:4 $n$ -6, 20:5 $n$ -3, 22:5 $n$ -3, and 22:6 $n$ -3, and summary categories of monoenes, trienes,  $n$ -3 fatty acids, and  $n$ -3/ $n$ -6 fatty acid ratios.

Qualitative dietary polyunsaturated fatty acid requirements of crustaceans have been previously identified by a variety of methods, including lack of biosynthesis, growth enhancement by diet inclusion of high PUFA oils, inclusion of individual fatty acids in purified diets, and tissue analysis (D'Abramo and Sheen, 1993). High levels of individual or categories of fatty acids in tissues relative to dietary levels may indicate relative importance (Reigh and Stickney, 1989). Comparison of the fatty acid profiles showed that levels of four fatty acids (16:1 $n$ -7, 18:0, 20:4 $n$ -6, and 20:5 $n$ -3) in prawn eggs exceeded those in experimental diets. Reigh and Stickney (1989) demonstrated that palmitoleate (16:1 $n$ -7) and stearate (18:0) could be synthesized *de novo* by *M. rosenbergii*. They also reported that prawn could produce arachidonic acid (20:4 $n$ -6) by elongation and desaturation of linoleic acid (18:2 $n$ -6) but that tissue levels were not affected by fatty acid composition of the diet. In white shrimp (*Penaeus setiferus*) synthesis of 20:4 $n$ -6 does occur, but so inefficiently that arachidonic acid is considered a dietary essential (Lilly and Bottino, 1981). In this study levels of 20:4 $n$ -6 were 338–775% higher in eggs than in the experimental diets. D'Abramo and Sheen (1993) reported an approximately 250% increase in weight gain of juvenile prawns fed a purified diet supplemented with 0.15% 20:4 $n$ -6. In contrast with Reigh and Stickney (1989), D'Abramo and Sheen (1993) found that in polar and neutral fractions of tissue lipids, concentrations of 20:4 $n$ -6 increased with dietary supply, implying an important metabolic role.

Levels of eicosapentaenoic acid (EPA, 20:5 $n$ -3) were 68–81% higher in eggs than in the three diets. Reigh and Stickney (1989) found that the level of EPA in abdominal tissue of *M. rosenbergii* was 119% higher than the level in the feed, and stated that this indicated the importance of EPA as a structural component of muscle cell membranes. Docosahexaenoic acid (DHA; 22:6 $n$ -3) is considered by some to be the third essential, highly unsaturated fatty acid (HUFA) (with 20:4 $n$ -6 and 20:5 $n$ -3). In this study levels of DHA in eggs and the diets were similar. This is supported by the findings of Reigh and Stickney (1989).

Although replacement of fish meal caused significant changes in dietary concentrations of several fatty acids, these differences had little impact on growth and survival in this study. According to recent work by D'Abramo and

Sheen (1993) it appears that the nutritive value of 20:5 $n$ -3, 22:6 $n$ -3, and 20:4 $n$ -6 is similar in *M. rosenbergii*. The dietary requirement for polyunsaturated fatty acids can apparently be satisfied at levels as low as 0.075% (dry weight) of the diet, with no apparent discrimination between  $n$ -6 and  $n$ -3 families.

## CONCLUSIONS

In this study growth, survival, and yield of freshwater shrimp were unaffected by 50 or 100% replacement of dietary fish meal with SBM and DDGS. The cost effectiveness of substituting SBM and DDGS for fish meal will vary depending on location and local cost of ingredients. Areas with longer growing seasons and/or pond production systems that achieve final yields exceeding 1300 kg/ha. could possibly see negative effects on prawn growth as supplemental nutrition from natural biota is reduced. Additional reductions in ingredient costs combined with enhancement of secondary productivity in the pond system should be evaluated as a method to further reduce production costs.

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## REFERENCES

- Akiyama, D., 1988. Soybean meal utilization by marine shrimp. Paper presented at: AOCS World Congress on Vegetable Protein Utilization in Human Food and Animal Feedstuffs, Singapore, 2-7 October, 1988. American Soybean Association, 27 pp.
- AOAC (Association of Official Analytical Chemists), 1990. Official Methods of Analysis of the Association of Official Analytical Chemists, 15th edn. Association of Official Analytical Chemists, Inc., Arlington, VA, 1298 pp.
- Arai, S., 1981. A purified test diet for coho salmon, *Oncorhynchus kisutch*, fry. Bull. Jpn. Soc. Sci. Fish., 47: 547-550.
- Balazs, G.H. and Ross, E., 1976. Effect of protein source and level on growth and performance of the captive freshwater prawn, *Macrobrachium rosenbergii*. Aquaculture, 7: 200-213.
- Cowey, C.B., Pope, J.A., Adron, J.W. and Blair, A., 1971. Studies on the nutrition of marine flatfish: growth of plaice *Pleuronectes platess* on diets containing proteins derived from plants and other sources. Mar. Biol., 10: 145-153.

- D'Abramo, L.R. and Sheen, S.S., 1993. Polyunsaturated fatty acid nutrition in juvenile freshwater prawn *Macrobrachium rosenbergii*. *Aquaculture*, 115: 63–86.
- D'Abramo, L.R., Heinen, J.M., Robinette, H.R. and Collins, J.S., 1989. Production of the freshwater prawn *Macrobrachium rosenbergii* stocked as juveniles at different densities in temperature zone ponds. *J. World Aquacult. Soc.*, 20: 81–89.
- Jackson, A.J., Capper, B.S. and Matty, A.J., 1982. Evaluation of some plant proteins in complete diets for the tilapia *Sarotherodon mossambicus*. *Aquaculture*, 27: 97–109.
- Lilly, M.L. and Bottino, N.R., 1981. Identification of arachidonic acid in Gulf of Mexico shrimp and degree of biosynthesis in *Penaeus setiferus*. *Lipids*, 16: 871–875.
- Lovell, R.T., 1989. Nutrition and Feeding of Fish. Van Nostrand Reinhold, New York, 260 pp.
- Lovell, R.T., Prather, E.E., Tres-Dick, J. and Lin, C., 1974. Effects of addition of fish meal to all-plant feeds on the dietary protein needs of channel catfish in ponds. *Proc. Annu. Conf. Southeast. Assoc. Game Fish Comm.*, 28: 222–228.
- Piedad-Pascual, F., Cruz, E.M. and Shumalangcay, A., Jr., 1990. Supplemental feeding of *Penaeus monodon* juveniles with diets containing various levels of defatted soybean meal. *Aquaculture*, 89: 183–191.
- Reed, L. and D'Abramo, L.R., 1989. A standard reference diet for crustacean nutrition research. III. Effects on weight gain and amino acid composition of whole body and tail muscle of juvenile prawns *Macrobrachium rosenbergii*. *J. World Aquacult. Soc.*, 20: 107–113.
- Reigh, R.C. and Stickney, R.R., 1989. Effects of purified dietary fatty acids on the fatty acid composition of freshwater shrimp *Macrobrachium rosenbergii*. *Aquaculture*, 77: 157–174.
- SAS Institute, Inc., 1988. SAS User's Guide: Statistics. SAS Institute, Cary, NC, 1028 pp.
- Sick, L.V. and Millikin, M.R., 1983. Dietary and nutrient requirements for culture of the Asian prawn, *Macrobrachium rosenbergii*. In: J.P. McVey (Editor), *CRS Handbook of Mariculture*. CRS Press, Boca Raton, FL, pp. 381–411.
- Tacon, A. and Cowey, C.D., 1984. Protein and amino acid requirements. In: P. Tytler and P. Calow (Editors), *Fish Energetics: New Perspectives*. Croom Helm, London, pp. 155–183.
- Tidwell, J.H., Webster, C.D., Clark, J.A. and D'Abramo, L.R., 1993. Evaluation of distillers dried grains with solubles as an ingredient in diets for pond culture of the freshwater prawn *Macrobrachium rosenbergii*. *J. World Aquacult. Soc.*, 24: 66–70.
- Webster, C.D., Tidwell, J.H., Goodgame, L.S., Yancey, D.H. and Mackey, L., 1992. Use of soybean meal and distillers grains with solubles as partial or total replacement of fish meal in diets for channel catfish, *Ictalurus punctatus*. *Aquaculture*, 106: 301–309.
- Wilson, R.P. and Poe, W.E., 1985. Relationship of whole body and egg essential amino acid patterns to amino acid requirement patterns in channel catfish, *Ictalurus punctatus*. *Comp. Biochem. Physiol.*, 80B: 385–388.
- Zar, J.H., 1984. *Biostatistical Analysis*. Prentice Hall, Englewood Cliffs, NJ, 383 pp.