Aquaculture 342–343 (2012) 112–116

Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Substitution of fishmeal with microbial floc meal and soy protein concentrate in diets for the pacific white shrimp *Litopenaeus vannamei*

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ARTICLE INFO

Article history:
Received 10 August 2011
Received in revised form 17 February 2012
Accepted 21 February 2012
Available online 1 March 2012

Keywords:
Shrimp
Fishmeal
Substitution
Floc meal
Soy protein concentrate

ABSTRACT

Nutritional values of microbial floc meal and soy protein concentrate were evaluated as alternative protein sources to fishmeal in diets for *Litopenaeus vannamei* juveniles. A feeding trial was conducted during 28 days to investigate the effects of partial and total replacement of fishmeal with microbial floc meal and soy protein concentrate on feed intake and growth of *L. vannamei*. Five diets were formulated to contain 38% crude protein and 17 kJ/g gross energy. Microbial floc meal was obtained from super-intensive shrimp farm effluent. A control diet (with fishmeal as the main protein source) was compared to four diets containing microbial floc meal and soy protein concentrate at 25%, 50%, 75% and 100% replacement levels and a commercial diet. This feeding trial was conducted in a recirculating aquaculture system, utilizing shrimps weighing 2.48 ± 0.29 g. The weight gain, final weight, feed conversion ratio, specific growth rate, protein efficiency ratio and survival were not significantly different among treatments, suggesting that fishmeal can be completely replaced with soy protein concentrate and microbial floc meal without adverse effects on *L. vannamei* performance. These results suggest that a mixture of soy protein concentrate and microbial floc meal can be utilised as a substitute for fishmeal in diets for *L. vannamei* juveniles.

1. Introduction

Fishmeal is considered an essential ingredient in marine shrimp diets because of its balanced amount of essential amino acids and fatty acids, vitamins, minerals and palatability (Suárez et al., 2009). Tacon and Metian (2008) report that the aquaculture industry consumed 68.2% of global fishmeal production in 2006; however, fishmeal production has remained relatively constant since 1985 at about 7 million tons per year (IFFO, 2006). The steady growth of aquaculture and consequent increase in demand for fishmeal has caused a significant increase in fishmeal prices in the last decade (Duarte et al., 2009; FAO, 2009). It has led to an increase in feed prices reducing the use of this ingredient in diets for animals (Naylor et al., 2009).

In spite of stabilization in the production of fishmeal, aquaculture production continues to grow year after year. In part, technological advances have allowed significant improvements in the feed efficiency of fish and shrimp (Tacon and Metian, 2008). Nevertheless, dependence on fishmeal remains high, affecting the profitability of aquaculture (FAO, 2006; Forster et al., 2003). Thus, the increasing demand for fishmeal, combined with overexploitation of fish stocks, has spurred a search for cheaper and sustainable protein ingredients to reduce or eliminate the use of fishmeal in aquaculture diets (Salze et al., 2010; Tacon et al., 2006).

Soybean meal is one of the ingredients used in the formulation of experimental diets for aquaculture (Alvarez et al., 2007; Amaya et al., 2007); however, antinutritional factors, low palatability and deficiencies in some amino acids (e.g., lysine and methionine) and essential fatty acids (e.g., eicosapentanoic acid and docosahexanoic acid) may limit its utilization (Davis and Arnold, 2000; Gatlin et al., 2007). On the other hand, ingredients derived from soy, such as soy protein concentrate (SPC), have benefits over soybean meal, such as a favorable amino acid profile, digestible protein and energy and better palatability (Cruz-Suárez et al., 2009; Gatlin et al., 2007). The use of SPC in diets for fish species has been reported (Deng et al., 2006; Salze et al., 2010), but for shrimp, the information is limited. According to Cruz-Suárez et al. (2009), SPC can be considered an excellent substitute for fishmeal in diets for shrimp, because during the manufacturing process, the majority of antinutritional factors are eliminated.

Another ingredient that has potential for use in shrimp feed is microbial floc meal-MFM (Kuhn et al., 2009, 2010). This product can be obtained from the effluent of super-intensive shrimp farms that use Biofloc Technology Systems (BFT). The BFT is a production system that has been used with great success in the shrimp industry. The microbial community growth in the pond taking up dissolved nitrogen and it can be used as food supplement (Avnimelech, 2007). Therefore,

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doi:10.1016/j.aquaculture.2012.02.023

BFT can reduce the protein content required in shrimp feed due to an improvement in feed conversion rates (Avnimelech, 1999). Thus, the conversion of rich effluents from bioflocs to MFM can offer the aquaculture industry an alternative source for the preparation of aquafeeds while minimizing potential environmental problems. According to Kuhn et al. (2010), the use of MFM as an ingredient in diets for shrimp, if implemented successfully, could be a sustainable alternative to the use of fishmeal.

This study aims to analyze the effects of the dietary replacement of fishmeal with SPC and MFM on the performance of *Litopenaeus vannamei* juveniles.

2. Materials and methods

2.1. Experimental design

The experiment was performed at the Marine Aquaculture Center — FURG over a 28-day period and was conducted in a recirculation aquaculture system, comprised of 15 tanks (40 L) coupled to a biological filter. The water was pumped into the tanks at a flow rate of 1.2 L/min.

The treatment consisted of five diets with 0, 25, 50, 75 and 100% replacement of fishmeal with MFM and SPC. A commercial feed commonly used for intensive production of this species at this rearing phase was also tested as a reference diet. According to the feed manufacturer, the diet protein content was 38% and 7.5% of lipids. Each diet was replicated three times, and replicates were distributed randomly among the tanks.

Juvenile shrimps with a mean weight of 2.48 ± 0.29 g were stocked at a density of 35 individuals/m^2. Feed was offered three times per day on food trays at 10% of shrimp biomass. The excess feed was removed from the trays one hour before the next feeding, placed on filter paper and dried in an oven at 50 °C for 24 h. Then filters were weighed to determine rates of food consumption. Fecal matter was siphoned daily.

2.2. Bioflocs as an ingredient for shrimp feed

The effluent of a super-intensive shrimp farm that utilizes BFT was pumped into a 15.000-L tank, and it was maintained for 24 h without aeration for biofloc sedimentation. The supernatant water was removed by siphoning, and the final product was spread on trays in layers of 3 cm and sun dried until the moisture content was below 20%. The dried samples were milled to 300 μm, stored in plastic bags and frozen at −18 °C.

2.3. Diet preparation

The composition of feed ingredients was analyzed at the Laboratory of Food Technology — FURG (Rio Grande, RS, Brazil) according to Association of the Official Analytical Chemists (AOAC) (1995) methodology (Table 1).

Table 1
Proximate matter of the main ingredients (g/100 g of dry weight) used to formulate the experimental diets for *L. vannamei*.

Ingredients	Crude protein	Crude fat	Ash	NFE[a]	Moisture
Brazilian Fishmeal	55.81	14.74	17.54	4.86	7.05
Microbial floc meal	23.39	0.3	36.6	18.63	21.08
Shrimp head meal	52.52	13.66	14.88	14.57	4.37
Soy protein concentrate	64.45	0.36	5.78	18.24	11.17
Soybean meal	51.52	1.85	6.57	28.48	11.58
Wheat meal	19.15	3.64	2.90	60.74	13.57

[a] Calculated value (Merrill and Watt, 1973): NFE = 100 − (crude protein + crude fat + ash + moisture).

The ingredients were triturated to obtain a particle size of 300 μm before diet preparation. The pre-weighed ingredients were mixed and the mixtures were pelleted using a meat grinder to form pellets of 3 mm in diameter. Pellets were dried at 60 °C for 24 h. Finished diets were stored in plastic bags at −18 °C until use.

Diets were formulated to contain approximately 38% crude protein, 8% fat and 17.0 kJ/g of gross energy. Diet compositions and their analysis are presented in Table 2. Diets contained the essential amino acid requirements for shrimp (Table 3) following Tacon (1989). Values for the amount of essential amino acids in each ingredient were obtained in Rostagno (2005), excepting the MFM and shrimp head meal were obtained from Ju et al. (2008) and Guilherme et al. (2007), respectively. Diets contained the essential amino acid requirements for shrimp following Tacon (1989), unless methionine which were added to fulfill the desired amount. The amino acid profiles of experimental diets were determined by the Laboratory of Mycotoxicological Analysis (Gehrke et al., 1987).

Upon termination of the 4-week growth trial, final weights of the remaining shrimp were obtained as shrimp weight per tank. Survival (final number of shrimp/initial number of shrimp) × 100; feed conversion ratio (feed/total weight gain); specific growth rate (100% × [ln final weight − ln initial weight]/trial duration) and protein efficiency ratio (total weight gain/total protein intake) for each dietary treatment were determined.

Dissolved oxygen and temperature were measured using a digital oxymeter (YSI 55, Yellow Springs, OH, USA). The pH was measured using a digital pH meter (pH 100 Ecosense YSI, Yellow Springs, OH, USA) and salinity with an optical refractometer (RTS-101, Atago

Table 2
Ingredients composition (g/100 g) and proximate matter (g/100 g dry weight, n = 3) of diets containing different levels of fishmeal replacement.

Ingredient	0	25	50	75	100	Com. feed
Microbial floc meal	0	3.5	7.0	10.5	14.0	
Soy protein concentrate[a]	0	7.0	14.0	21.0	28.0	
Brazilian fishmeal[b]	40.0	30.0	20.0	10.0	0	
Soybean meal	15.0	15.0	15.0	15.0	15.0	
Shrimp head meal	3.0	3.0	3.0	3.0	3.0	
Brewer's yeast	5.0	5.0	5.0	5.0	5.0	
Starch corn	16.85	14.80	12.75	10.70	8.65	
Wheat meal	16.0	16.0	16.0	16.0	16.0	
Fish oil[c]	0	1.5	3.0	4.5	6.0	
Soy lecithin	0.5	0.5	0.5	0.5	0.5	
Mineral/vitamin premix[d]	1.0	1.0	1.0	1.0	1.0	
Cholesterol[e]	0.5	0.5	0.5	0.5	0.5	
Dicalcium phosphate[f]	2.0	2.0	2.0	2.0	2.0	
DL-Methionine	0.15	0.2	0.25	0.3	0.35	
Proximate analysis[g]						
Crude protein	39	38	37	37	36	42
Crude fat	6.57	7.85	6.95	8.85	7.69	9.43
Ash	11.44	11.57	12.02	12.22	12.7	14.76
NFE[h]	41.31	36.79	38.53	35.88	38.85	23.02
Moisture	1.78	5.59	5.10	6.45	4.56	10.32
Gross energy [kJ/g][i]	16.8	16.9	17.1	17.2	17.3	14.5

[a] IMCOSOY®, Araucária, PR, Brazil.
[b] LEAL SANTOS, Rio Grande, RS, Brazil.
[c] IRPEL, Capela de Santana, RS, Brazil.
[d] Premix M. Cassab, SP, Brazil (Vitamin A (500.000 UI/kg), Vit. D3 (250.000 UI/kg), Vit. E (5.000 mg/kg), Vit. K3 (500 mg/kg), Vit. B1 (1.000 mg/kg), Vit. B2 (1.000 mg/kg), Vit. B6 (1.000 mg/kg), Vit. B12 (2.000 mcg/kg), Niacin (2.500 mg/kg), Calcium pantothenate (4.000 mg/kg), Folic acid (500 mg/kg), Biotin (10 mg/kg), Vit C (10.000 mg/kg), Choline (100.000 mg/kg), Inositol (1.000 mg/kg). Trace elements: Selenium (30 mg/kg), Iron (5.000 mg/kg), Copper (1.000 mg/kg), Manganese (5.000 mg/kg), Zinc (9.000 mg/kg), Cobalt (50 mg/kg), Iodine (200 mg/kg)).
[e] VETEC, Duque de Caxias, RJ, Brazil.
[f] VETEC, Duque de Caxias, RJ, Brazil.
[g] Values are means of three determinations.
[h] Calculated value (Merrill and Watt, 1973): NFE = 100 − (crude protein + crude fat + ash + moisture).
[i] Energy was calculated as 16.7, 16.7 and 37.7 kJ/g of protein, carbohydrate and lipids, respectively (Calculated from physiological fuel values).