Aquaculture 426–427 (2014) 120–125

Contents lists available at ScienceDirect

Aquaculture

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

Fishmeal substitution with *Arthrospira* (*Spirulina platensis*) in a practical diet for *Litopenaeus vannamei*: Effects on growth and immunological parameters

Joaquin Macias-Sancho [a,c], Luís Henrique Poersch [a,c], William Bauer [a,c], Luis Alberto Romano [a,b], Wilson Wasielesky [a,c], Marcelo Borges Tesser [a,d,*]

[a] Programa de Pós-Graduação em Aquicultura, Universidade Federal do Rio Grande (FURG), Rio Grande, Brazil
[b] Laboratório de Patologia e Imunologia de Organismos Aquáticos, Instituto de Oceanografia (IO), Universidade Federal do Rio Grande (FURG), Rio Grande, Brazil
[c] Laboratório de Carcinocultura, Instituto de Oceanografia (IO), Universidade Federal do Rio Grande (FURG), Rio Grande, Brazil
[d] Laboratório de Nutrição de Organismos Aquáticos, Instituto de Oceanografia (IO), Universidade Federal do Rio Grande (FURG), Rio Grande, Brazil

ARTICLE INFO

Article history:
Received 10 December 2013
Received in revised form 22 January 2014
Accepted 24 January 2014
Available online 7 February 2014

Keywords:
Apoptosis
Arthrospira
Fishmeal
Growth
Shrimp

ABSTRACT

The use of fishmeal (FM) and fish oil in aquafeeds is a major challenge in the development of aquaculture. The discovery of alternative ingredients for aquaculture feedstuffs will allow for the sustainable growth of this activity. The cyanobacteria *Arthrospira* (*Spirulina platensis*) contains high quality protein and also immune-stimulatory properties that have been tested in mammals, fish and crustaceans. The purpose of this work was to evaluate the growth and immunological parameters of the white shrimp *Litopenaeus vannamei* when it was fed experimental isonitrogenous diets (~35% protein), containing 0%, 25%, 50%, 75% and 100% cyanobacteria *A. platensis* replacing fishmeal for 50 days. At the end of the experiment, there were no significant differences ($p < 0.05$) in growth performance among the shrimp fed diets with up to 75% fishmeal replacement. The immunological parameters differed significantly in the percentage of hyaline and granular hemocytes in the shrimp fed the diets with at least 25% fishmeal replacement. The apoptotic index also showed highly significant differences, but only in those shrimp fed a diet consisting of 100% fishmeal replacement. The results showed that up to 75% of fishmeal could be replaced by *A. platensis* without affecting survival or creating a growth depression. Moreover, the smaller substitution level (25%) promoted an enhancement of the tested immunological parameters.

1. Introduction

Protein is the most expensive nutrient in practical diets for shrimp culture, and fishmeal (FM) constitutes the most commonly used protein source in the commercially manufactured feeds (Oujifard et al., 2012). The largest consumers of fishmeal in 2008 were shrimp with 27.2% of the total fishmeal used in aquafeeds, followed by marine fish with 18.8% and salmon with 13.7% (Tacon et al., 2011).

Although the aquaculture production sector has succeeded in reducing the use of fishmeal and fish oil in aquafeeds (Naylor et al., 2009), the demand for both fishmeal and fish oil is still rising because of the steady increase in aquaculture production (FAO, 2012). This scenario contributes to increased demand and prices for these products and concerns about over-fishing (Olsen and Hasan, 2012). To sustain the aquafeed industry, a large part of the nutritional research has been focused on the search for alternative protein sources (NRC, 2011).

Microalgae possess high protein contents and amino acid profiles comparable to those of other reference food proteins (Becker, 2007). Apart from the high protein content (60–70%), *Spirulina platensis* has been receiving attention as an animal food source due to its rich source of vitamins, minerals, essential fatty acids (γ-linolenic acid—GLA) and antioxidant pigments such as carotenoids (Belay et al., 1996; James et al., 2006). Studies have been conducted using *Spirulina* as a supplement in diets for fish and crustaceans (Cuzon et al., 1981; Jaime-Ceballos et al., 2007; James et al., 2006; Silva-Neto et al., 2012) and also as a partial substitution for fishmeal in tilapia, carp and sturgeon (Nandeesha et al., 2001; Olvera-Novoa et al., 1998; Palmegiano et al., 2005, 2008).

In addition to the high nutritional value, *Spirulina* serves as an effective immune modulator in mammals and fish (Hirahashi et al., 2002; Watanuki et al., 2006) and may have the potential for use as an antimicrobial agent in aquafeed, or perhaps may even be of pharmaceutical interest (Pradhan et al., 2012). The use of hot extracted *Spirulina* has been shown to enhance the innate immunity and increase the resistance against *Vibrio alginolyticus* infection in *L. vannamei* (Lin et al., 2010; Tayag et al., 2010).

The experiment reported here was designed to evaluate the effects of fishmeal substitution by *Arthrospira platensis* meal in the *Litopenaeus*

* Corresponding author at: Programa de Pós-Graduação em Aquicultura, Universidade Federal do Rio Grande (FURG), Av. Itália km 8, Rio Grande, RS, Cx.P. 474, CEP 96.200-970, Brazil. Tel./fax: +55 5332368042.
E-mail address: mbtesser@gmail.com (M.B. Tesser).

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http://dx.doi.org/10.1016/j.aquaculture.2014.01.028

vannamei diet on survival, growth performance and immune system function.

2. Materials and methods

2.1. Shrimp source and experimental design

Post larvae (PL 10) *L. vannamei* were obtained from Aquatec, RN, Brazil. They were maintained in 10,000 L tanks with a salinity of 34 and temperature 27 ºC, and fed with a commercial diet (Guabi® (Brazil)—38% crude protein) for 1 month until they reached the desired juvenile size.

The experiment lasted 50 days and was conducted in 15 plastic tanks of 310 L and 0.35 m^2 bottom area. Juvenile shrimp with a mean weight of 0.7 g ± 0.1 (±SD) were stocked at a density of 59 individuals/m^2. The water exchange rate was 33% of the total tank volume per day. The treatment consisted of five diets with 0%, 25%, 50%, 75% and 100% replacement of fishmeal with dried *A. platensis*. Each diet was replicated three times, and the replicates were distributed randomly among the tanks. The shrimp were fed three times per day at 8:00, 14:00 and 19:00 h. The amounts of feed offered were calculated based on an expected weight gain of 1.5 g per week and a feed conversion of 1.8:1 (Amaya et al., 2007). Fecal matter and the remainder of the uneaten food were siphoned out daily to maintain water quality. At the end of the experiment, all of the juveniles were weighed to determine the values of growth indicators and survival.

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 the salinity was measured with an optical refractometer (RTS-101, Atago U.S., Bellevue, WA, USA). All water parameters were measured daily. Water samples were taken weekly to measure the total concentrations of ammonia (UNESCO, 1983), nitrite (Bendschneider and Robinson, 1952) and nitrate (Aminot and Chaussepied, 1983). A photoperiod of 12:12 (light:dark) was maintained throughout the experimental period.

2.2. Diet preparation

A. platensis was reared in open photo-bioreactors under uncontrolled conditions at the facilities of the Laboratory of Biochemical Engineering, College of Chemistry and Food Engineering, Federal University of Rio Grande (FURG) (Morais et al., 2009).

The ingredient compositions of all feeds were analyzed at the Laboratory of Food Technology—Santa Maria Federal University (Santa Maria, RS, Brazil) according to the methodology of the Association of the Official Analytical Chemists (AOAC, 1995). The dried *A. platensis* and the fishmeal proximal composition are presented in Table 1.

The ingredients were triturated to obtain a particle size of 100 μm before diet preparation. The pre-weighed ingredients were mixed and the mixtures were pelleted using a meat grinder to form pellets. The pellets were dried at 60 °C for 24 h. Finished diets were stored in plastic bags at −18 °C until use. During the trial, pellet size was adjusted with the shrimp growth according to Jory et al. (2001).

Five diets were formulated to contain approximately 35% crude protein, 8% fat and 16.0 kJ/g of gross energy. Fishmeal was replaced at 0%, 25%, 50%, 75% and 100% by *A. platensis* meal. Fish oil and cellulose were adjusted to balance the diets. The diet compositions and their analysis are presented in Table 2. The amino acid profiles of the experimental diets were determined by the Laboratory of Mycotoxicological Analysis—Santa Maria Federal University (Santa Maria, RS, Brazil) according to Gehrke et al. (1987) and are presented in Table 3.

2.3. Evaluation of growth parameters and proximal muscle composition

At the end of the experimental trial, individual weights were obtained using a digital balance (1 mg precision) for the determination of the growth parameters for each dietary treatment.

Weight gain = (final wet weight − initial wet weight);
Weekly weight gain = [(final wet weight − initial wet weight) / weeks];
Feed conversion ratio = (dry feed intake / wet weight gain);
Specific growth rate = (100% × [ln final weight − ln initial weight] / trial duration);
Protein efficiency ratio = (wet weight gain / dry protein intake);
Survival = (final number of shrimp/initial number of shrimp) × 100.

Proximal tail muscle composition was determined for 5 shrimp per experimental unit at the end of the study. Prior to the analysis, the exoskeletons were removed and the samples were stored in −20 ºC. The amount of moisture was determined by drying the samples at 105 °C for 24 h. The ash was analyzed by combustion at 550 °C for 12 h.

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

Ingredients	Crude protein	Lipids	Ash	NFE[a]	Crude fiber
A. platensis	66.93	1.75	8.70	20.85	1.77
Fishmeal[b]	68.63	11.48	13.63	4.86	-

[a] Calculated value (Merrill and Watt, 1973). NFE = 100 − (crude protein + lipids + ash + moisture).
[b] Leal Santos, Rio Grande, RS, Brazil.

Table 2
Ingredient composition (g/100 g) and proximate analysis (g/100 g dry weight, $n = 3$) of experimental diets for *L. vannamei* containing different levels of fishmeal replacement.

Ingredients	0	25	50	75	100
Fishmeal [a]	40	30	20	10	0
Arthrospira platensis [b]	0	10	20	30	40
Soybean meal	5	5	5	5	5
Brewer's yeast	5	5	5	5	5
Corn starch	20	20	20	20	20
Wheat meal	15	15	15	15	15
Fish oil [c]	2.2	3.2	4.2	5.2	6.2
CMC [d]	2	2	2	2	2
Mineral/vitamin premix [e]	1	1	1	1	1
Cholesterol [f]	0.5	0.5	0.5	0.5	0.5
$Ca(H_2PO_4)$ [f]	2	2	2	2	2
Cellulose [e]	7.3	6.3	5.3	4.3	3.3
Proximate analyses					
Protein	36.6	35.8	33.9	33.4	33.8
NFE [g]	36.9	37.9	38.7	40.3	40.8
Lipids	8.3	8.3	6.4	6.8	8.3
Ash	8.9	8.9	8.4	8.2	7.1
Fiber	9.3	8.6	8.6	7.4	9.3
Carbohydrate [h]	46.2	46.4	45.2	47.7	50.1
Gross energy (kJ/g) [i]	15.5	15.7	15.9	16.1	16.2

[a] Leal Santos, Rio Grande, RS, Brazil.
[b] Laboratory of Biochemical Engineering, College of Chemistry and Food Engineering, Federal University of Rio Grande.
[c] Campestre, São Paulo, Brazil.
[d] CMC (Carboxymethylcellulose), Vetec, Rio de Janeiro, Brazil.
[e] Premix M. Cassab, São Paulo, Brazil (vitamin A (500,000 UI/kg), vitamin D3 (250,000 UI/kg), vitamin E (5000 mg/kg), vitamin K3 (500 mg/kg), vitamin B1 (1000 mg/kg), vitamin B2 (1000 mg/kg), vitamin B6 (1000 mg/kg), vitamin B12 (2000 mcg/kg), niacin (2500 mg/kg), calcium pantothenate (4000 mg/kg), folic acid (500 mg/kg), biotin (10 mg/kg), vitamin C (10,000 mg/kg), choline (100,000 mg/kg), and inositol (1000 mg/kg). Trace elements: selenium (30 mg/kg), iron (5000 mg/kg), copper (1000 mg/kg), manganese (5000 mg/kg), zinc (9000 mg/kg), cobalt (50 mg/kg), and iodine (200 mg/kg)).
[f] VETEC, Duque de Caxias, RJ, Brazil.
[g] Calculated value (Merrill and Watt, 1973). NFE = 100 − (crude protein + lipids + ash + moisture).
[h] Carbohydrate = NFE + fiber.
[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).