Aquaculture 308 (2010) 166–173

Contents lists available at ScienceDirect

Aquaculture

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

Apparent digestibility of dry matter, protein, and essential amino acid in marine feedstuffs for juvenile whiteleg shrimp *Litopenaeus vannamei*

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ARTICLE INFO

Article history:
Received 17 December 2009
Received in revised form 25 August 2010
Accepted 25 August 2010

Keywords:
Essential amino acids
Digestibility
Marine feedstuffs
Shrimp

ABSTRACT

Apparent protein and amino acid digestibility coefficients of feedstuffs are needed for more accurate, environmentally friendly, and economical feed formulations for shrimp. Coefficients of digestibility of nine feedstuffs of marine origin were measured, in terms of apparent dry matter, protein, and essential amino acid, for juvenile whiteleg shrimp, *Litopenaeus vannamei*, using 1% chromic oxide as the inert marker. The tested ingredients included four commercial fish meals (FMs) from different sources, batches, or species designated as A, B, C and D, fish soluble protein concentrate (FSPC), squid (*Loligo gahi*) meal (SM), shrimp (*Litopenaeus vannamei*) head meal (SHM), red crab (*Pleuroncodes planipes*) meal (RCM), and Catarina scallop (*Argopecten ventricosus*) by-product meal (CSBM). A reference diet was formulated and produced along with the experimental diets which included 30% of each ingredient and 70% of the reference diet. Apparent dry matter and protein (APD) digestibility coefficients varied very much among feedstuffs, from 46% to 102% and from 64% to 99%, respectively. APD for the fish soluble protein concentrate, squid and shrimp head meals were excellent (over 90%), Catarina scallop meal and FMA (sardine 66% CP) showed high protein digestibility (over 84%), while red crab meal (77%), FMB (sardine 70% CP; APD 71%) and FMD (tuna 60% CP; APD 70%) had low digestibility. The lowest dry matter (46%) and protein (63%) digestibilities were recorded for FMC (sardine 70% CP). Apparent amino acid digestibility (AAAD) coefficients were also variable among feedstuffs, and there was a reasonable, but not total, correspondence to protein digestibility. The most digestible feed ingredients for whiteleg shrimp were: fish soluble protein concentrate, squid meal, shrimp head meal, Catarina scallop by-product meal, and fish meal A (sardine 66% CP) showing that these ingredients are good sources of available protein and amino acids for juvenile whiteleg shrimp.

1. Introduction

Fish meal has been the most important feedstuff used as a source of protein in aquaculture feeds because of its essential amino acid composition and palatability (Davis et al., 2004). Demand for protein ingredients in aquaculture is expected to exceed supply in the next decade. The growth of the aquaculture industry will also raise the price of feedstuffs (New and Wijkstom, 2002). The current trend is to decrease fish meal in aquafeeds (Lemos et al., 2009) and this has generated research for finding alternative protein sources (Goytortúa-Bores et al., 2006; Cruz-Suárez et al., 2007, 2009; Yang et al., 2009). Protein and amino acids are expensive nutrients in feed; an oversupply of crude protein is the main cause of nitrogen contamination of pond water and environmental pollution (Primavera, 2005). Reducing protein content of feed, while satisfying amino acid requirements of shrimp can reduce production costs and nitrogen pollution. Generally, shrimp diets are formulated in terms of crude protein (CP) and amino acids content without considering the bioavailability of these ingredients. The quality of protein sources is expressed as the amount of essential amino acids in the CP. This information is important, but is not sufficient for optimizing formulations because digestive utilization of amino acids is always lower than the analyzed amount (Sibbald, 1987). Data on amino acid digestive utilization coefficients is one of the most important factors in preparing adequate shrimp feeds and there is an increasing interest in defining feedstuff quality using as criterion the coefficients of amino acid digestibility (Cruz-Suárez et al., 2009; Lemos et al., 2009; Yang et al., 2009). Methionine is the first limiting amino acid in most shrimp diets (Forster and Dominy, 2006); thus, knowledge of its availability, as well as the contents of other essential amino acids in feedstuffs is an important basis for determining nutritional quality. Digestibility studies of amino acids are particularly important for feed that includes marine animal ingredients because bioavailability of nutrients is affected by the temperature used during processing and manufacturing of ingredients (Parsons, 1991; Smith et al., 2000; Terrazas-Fierro et al., 2005). There is information on the digestibility of proteins and amino acids from practical feedstuffs for Pacific whiteleg shrimp; hence, creating a

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doi:10.1016/j.aquaculture.2010.08.021

database of digestibility of amino acids could provide more useful, accurate, and flexible information for low cost formulation processes. However, different factors affect utilization of feedstuffs, such as the raw material, recipient species, whole fish or scraps, freshness, processing methods, and storage conditions of the meal. The purpose of this study was to determine *in vivo* apparent digestibility of dry matter, protein, and essential amino acid in marine animal feedstuffs used in diets for whiteleg shrimp *Litopenaeus vannamei*.

2. Materials and methods

2.1. Feedstuffs

A digestibility trial was conducted to determine the apparent digestibility coefficients of dry matter (ADMD), protein (APD), and amino acids (AAAD) for nine feed ingredients. These included four commercial fish meals (FMs) from different sources, batches, or species; FMA (sardine meal, batch 2005, San Carlos, Mexico); FMB (sardine meal, batch 2006, Ensenada, Mexico); FMC (sardine meal, batch 2006, San Carlos, Mexico); FMD (tuna by-product meal, batch 2006), fish soluble protein concentrate (FSPC); squid meal (SM); shrimp head meal (SHM); red crab (*Pleuroncodes planipes*) meal (RCM); and Catarina scallop (*Argopecten ventricosus*) by-product meal (CSBM). The last two were prepared in our laboratory as described by Goytortúa-Bores et al. (2006).

2.2. Formulation and diet preparation

A reference diet (see Table 1) was formulated to contain ~37% crude protein and ~9% lipids. This diet contained 1% chromic oxide (Cr_2O_3) as an inert marker and 2% alginate as a binder. Nine experimental diets were prepared, 30% (wet basis) of the composition of these diets contained test ingredients and 70% was the reference diet. Table 2 lists the proximate composition and amino acid content of the diets. Prior to preparing the diets, the feed ingredients were pulverized and sieved (250 μm). The dry ingredients of each diet were mixed thoroughly in a food mixer before soybean lecithin and fish oil was added. After the oil was dispersed, water was added (approximately 40% of the total "as is" ingredient weight) and mixed. The resulting mixture was pelleted with a meat grinder and a 2-mm die, as described by Civera and Guillaume (1989). The pellets were dried to a moisture content of less than 10% in a forced-air oven at 45 °C and stored at 4 °C until used.

Table 1
Ingredient composition (g per 100 g of diet) of the reference diet used for the digestibility trial.

Ingredients	
Fish meal (sardine, 66.2% CP)[1]	33.60
Wheat meal[2]	30.11
Soybean meal[3]	20.00
Fish oil (sardine)[4]	4.00
Corn starch[5]	3.50
Alginate (binder)[6]	2.00
Soy lecithin[7]	2.00
Vitamin premix[8]	1.80
Sodium phosphate dibasic, [9]	1.20
Chromic oxide[10]	1.00
Mineral premix[11]	0.50
Choline chloride (62% active agent)[12]	0.20
Vitamin C (35% active agent)[13]	0.09
Butyl-hydroxy-toluene (BHT) [14]	0.004

[1] Monterrey sardine meal, batch 2005 (Conservera San Carlos, Puerto San Carlos, B.C.S., Mexico).
[2] Harinera Parayas, Guadalajara, Jalisco, Mexico.
[3] AGYDSA, Guadalajara, Jalisco, Mexico.
[4] Conservera San Carlos, B.C.S., Mexico.
[5] S-4126. Sigma, St. Louis, USA.
[6] A-0503-1. Sigma, St. Louis, USA.
[7] Rey Sol, La Paz, B.C.S., Mexico.
[8] Vitamin premix (IU or mg kg^{-1} diet): A acetate, 15000 IU; D3, 7500 IU; E, 400; K_3, 20; choline chloride (99%) 400; ascorbic acid, 300; thiamine HCl, 150; riboflavin, 10; pyridoxine HCl, 50; pantothenic acid, 100; niacin, 300; biotin, 1; inositol, 500; folic acid, 20; cyanocobalamin, 0.1.
[9] S-0876. Sigma, St. Louis, USA.
[10] IMPEX 12233101162. Monterrey, N.L., Mexico.
[11] Mineral premix (mg 100 g^{-1} diet): Na_2HPO_4, 2,370; $MgSO_4 \cdot 7H_2O$, 500; $ZnSO_4 \cdot 7H_2O$, 90; KCl, 500; $MnCl_2 \cdot 4H_2O$, 23.4; $CuCl_2 \cdot 2H_2O$, 5; KI, 5; $CoCl_2 \cdot 6H_2O$, 2.5.
[12] ICN101386. Biomedicals, Aurora, Ohio, USA.
[13] Stay-C, ROCHE, Mexico City.
[14] ICN 101162. Biomedicals, Aurora, Ohio, USA.

Samples of the test ingredients and test diets were finely ground and sieved before chemical analysis. The proximate composition of the samples was determined in triplicate according to published standards (AOAC, 2005). Dry matter was calculated by gravimetric analysis following oven-drying at 100 °C for 24 h. Ash content was determined gravimetrically by combustion in a furnace at 550 °C for 6 h (Method 942.05; AOAC, 2005). The Kjeldahl method was used to determine crude protein content. Crude fiber and ether extract were analyzed according to published standards (AOAC, 2005). Gross energy of ingredients and diets were determined with an adiabatic calorimeter (Parr Instruments, model 1261, Moline, IL). Pellet stability was estimated by dry matter weight loss of pellets in seawater (40‰) at 27 °C according to the method used by Obaldo et al. (2002). Samples of the pellets (2 g; 2-mm diameter and approximately 1-cm long) were gently shaken in seawater for 1 h at 100 rpm in an orbital shaker (Model OR200, Daigger & Co., Vernon Hills, IL).

2.3. In vivo digestibility

Juvenile whiteleg shrimp were obtained from a commercial farm and transported to the laboratory. The shrimp were fed a commercial feed (35% crude protein) without chromic oxide and maintained in three cylindrical 500-l plastic tanks until used in the digestibility bioassay. Four juvenile shrimp (15–19 g each) were stocked in each 60-l rectangular tanks (58 × 48 × 25 cm) within an open culture system. Three tanks were randomly assigned for each diet. Each tank was equipped with a 250-W submersible heater and an airlift pump. Seawater was filtered (5 μm), UV-sterilized, and maintained at 27 ± 0.5 °C, salinity of 40‰, and 5.0 ± 0.3 mg ml^{-1} dissolved oxygen. Daily water exchange was ~80% in all tanks.

Total daily feed was initially set at 7% of the biomass in each tank, distributed by hand in four rations (09:00, 13:00, 15:00 and 18:00 h). Rations were adjusted, so that shrimp were fed to slight excess at each feeding. Shrimp were acclimated to the experimental diets containing chromic oxide for 7 days before initial collection of feces. Unconsumed feed, exuviae, overnight feces, and dead shrimp were removed from the tanks every day. Feces were collected three times daily (10:00, 14:00 and 16:00 h), approximately one hour after each feeding by gently siphoning fecal strands with a Pasteur pipette. Feces were gently rinsed with distilled water, transferred to 30-ml conic tubes, and frozen at −20 °C. When feces from all tanks were collected after the first feeding, a second round of feces collection was performed, for a total collection time approximating 3 h (~1.5 h for each collection round). Daily samples of frozen fecal material corresponding to each tank were pooled, freeze-dried, ground, thoroughly mixed, and kept frozen at −80 °C until analyzed. Sample collections were accumulated until 1.5 g dry weight of fecal material (~15 g wet feces) from each tank had been collected.

Triplicate samples of feces analyzed were analyzed for nitrogen (micro-Kjeldahl method) and for amino acids. Defatted samples were hydrolyzed at 110 °C for 24 h in 6 N HCl and prepared for amino acid assay according to method 994.12 (AOAC, 2005). Amino acid assays were performed by reversed-phase HPLC (Wu et al., 1997) using a Model 1100 HPLC (Hewlett Packard, Santa Clara, CA) equipped with a fluorescence detector with a 350-nm excitation filter and a 455-nm emission filter. To avoid partial loss of cysteine and methionine (Wathelet, 1999) samples of ingredients, diets and feces were