Aquaculture 380–383 (2013) 33–40

Contents lists available at SciVerse ScienceDirect

Aquaculture

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

Simultaneous estimation of the nutritional contribution of fish meal, soy protein isolate and corn gluten to the growth of Pacific white shrimp (*Litopenaeus vannamei*) using dual stable isotope analysis

Julián Gamboa-Delgado *, Mónica G. Rojas-Casas, Martha G. Nieto-López, Lucía Elizabeth Cruz-Suárez

Programa Maricultura, Departamento de Ecología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, A.P. F-67. San Nicolás de los Garza N.L. 66451, México

ARTICLE INFO

Article history:
Received 11 September 2012
Received in revised form 26 November 2012
Accepted 27 November 2012
Available online 4 December 2012

Keywords:
Stable isotopes
Nutrient contribution
Fish meal
Soy protein
Corn gluten
Litopenaeus vannamei

ABSTRACT

The nutritional contribution of the dietary nitrogen, carbon and total dry matter supplied by fish meal (FM), soy protein isolate (SP) and corn gluten (CG) to the growth of Pacific white shrimp *Litopenaeus vannamei* was assessed by means of isotopic analyses. As SP and CG are ingredients derived from plants having different photosynthetic pathways which imprint specific carbon isotope values to plant tissues, their isotopic values were contrasting. FM is isotopically different to these plant meals with regards to both, carbon and nitrogen. Such natural isotopic differences were used to design experimental diets having contrasting isotopic signatures. Seven isoproteic (36% crude protein), isoenergetic (4.7 kcal g^{-1}) diets were formulated; three diets consisted in isotopic controls manufactured with only one main ingredient supplying dietary nitrogen and carbon: 100% FM (diet 100F), 100% SP (diet 100S) and 100% CG (diet 100G). Four more diets were formulated with varying mixtures of these three ingredients, one included 33% of each ingredient on a dietary nitrogen basis (diet 33FSG) and the other three included a proportion 50:25:25 for each of the three ingredients (diets 50FSG, 50SGF and 50GFS). At the end of the bioassay there were no significant differences in growth rate in shrimps fed on the four mixed diets and diet 100F ($k = 0.215–0.224$). Growth rates were significantly lower ($k = 0.163–0.201$) in shrimps grown on diets containing only plant meals. Carbon and nitrogen stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) were measured in experimental diets and shrimp muscle tissue and results were incorporated into a three-source, two-isotope mixing model. The relative contributions of dietary nitrogen, carbon and total dry matter from FM, SP and CG to growth were statistically similar to the proportions established in most of the diets after correcting for the apparent digestibility coefficients of the ingredients. Dietary nitrogen available in diet 33FSG was incorporated in muscle tissue at proportions representing 24, 35 and 41% of the respective ingredients. Diet 50GSF contributed significantly higher amounts of dietary nitrogen from CG than from FM. When the level of dietary nitrogen derived from FM was increased in diet 50FSG, nutrient contributions were more comparable to the available dietary proportions as there was an incorporation of 44, 29 and 27% from FM, SP and CG, respectively. Nutritional contributions from SP were very consistent to the dietary proportions established in the experimental diets.

1. Introduction

Information gathered from traditional nutritional assays in conjunction with data from chemical analyses of diets and animal tissues provides valuable information to infer on the dietary performance of specific ingredients. Among these chemical analyses, the use of stable isotopes represents an additional tool for nutritional studies conducted on aquatic species. The integration of isotopic data into isotopic mixing models has made possible to convert the isotopic values of consumers and their different trophic elements to dietary contributions (Phillips, 2012). In the fields of ecology and nutrition, the isotopic techniques have provided an improved understanding of how organisms incorporate the elements they consume. In this context, it has been pointed out that animal tissue often does not reflect the bulk isotopic composition of the diet, but the isotopic composition of the dietary components from which the tissue was biosynthesized (Gannes et al., 1997; Newsome et al., 2011). In aquaculture nutrition, the natural isotope ratios of nitrogen and carbon ($^{15}N/^{14}N$ and $^{13}C/^{12}C$, respectively measured and reported in delta notation as $\delta^{15}N$ and $\delta^{13}C$) have been used as natural biomarkers to estimate dietary contributions in organisms fed either on different types of live food and inert diets, or raised on formulated diets having ingredients with contrasting isotopic signatures (Gamboa-Delgado and Le Vay, 2009a, 2009b; Gamboa-Delgado

* Corresponding author. Tel./fax: +52 81 8352 6380.
E-mail addresses: julian.gamboad@uanl.mx, jgam97@yahoo.com (J. Gamboa-Delgado).

0044-8486/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aquaculture.2012.11.028

et al., 2008; Jomori et al., 2008; Martínez-Rocha et al., in press; Matsuda et al., 2009).

Partial or total replacement of fish meal in aquaculture diets represents important advantages in economical and ecological terms. The progressively higher production of several aquaculture species is in turn exerting a higher demand for aquafeeds. Among these mass-produced marine animals, the Pacific white shrimp *Litopenaeus vannamei* has become the main shrimp species produced through aquaculture practices since 2003 (FAO, 2007). Hence, numerous nutritional studies conducted on this species have focused on testing different plant-derived meals and purified, isolated plant proteins as dietary ingredients to replace fish meal (*e.g.* Amaya et al., 2007; Harter et al., 2011; Liu et al., 2012; Oujifard et al., 2012). Different dietary resources found in the aquatic and terrestrial ecosystems frequently show distinct isotopic values due to the effect of characteristic nutrient flows and metabolic pathways. This natural isotopic labeling allows conducting studies aimed to elucidate the nutritional contribution of specific dietary sources to the growth of a consuming organism. Plants exhibit three different photosynthetic pathways (C3, C4 and CAM), which imprint different isotopic values to vegetal tissues. For example, soy is a C3 or Calvin cycle plant (called C3 because during photosynthesis, the first product of CO_2 fixation is a 3-carbon compound), while corn is a C4 or Hatch–Slack cycle plant (Leegood, 2002). The reaction kinetics of these photosynthetic pathways has a significant influence on the carbon isotopic values ($\delta^{13}C$) of each type of plant. C3 plants have a mean $\delta^{13}C$ value of −29‰, while C4 plants show a more isotopically-enriched, mean $\delta^{13}C$ value of -13‰ (Ehleringer and Cerling, 2001; O'Leary, 1988). In the case of $\delta^{15}N$ values, most plants have isotopic values ranging from 2 to 6‰; however, the nitrogen isotope values of most traditional crops are strongly influenced by the $\delta^{15}N$ values of the inorganic fertilizers used to grow them. As the isotopic mixing models are able to estimate dietary contributions at higher resolution when the nutrient sources are isotopically distinct (Phillips, 2012), the isotopic values of primary producers have been systematically manipulated using specific fertilizers (Gamboa-Delgado et al., 2011; Le Vay and Gamboa-Delgado, 2011). The present study employed the natural isotopic differences found in soy protein isolate, corn gluten and fish meal, to simultaneously assess the relative incorporation of dietary nitrogen, carbon and total dry matter supplied by these three sources to the muscle tissue of Pacific white shrimp. In addition, the nitrogen and carbon half times in muscle tissue of shrimps fed on the different experimental diets were estimated.

2. Material and methods

2.1. Experimental animals

Pacific white shrimp (*L. vannamei*) postlarvae were obtained from a commercial hatchery (Maricultura del Pacífico) located in Mazatlán, Mexico. After reception, animals were placed in 500 L tanks and acclimated for 20 d to a bioassay room under the following conditions: seawater temperature 30.2 ± 0.7 °C, salinity 35.4 ± 0.7 g l^{-1}, pH 8.4 ± 0.1 and saturated dissolved oxygen. Total ammonia nitrogen (0.09 ± 0.06 mg/L), nitrite (not detected), and nitrate (12.9 ± 4.6 mg/L) were monitored using a commercial kit (FasTest; Aquarium Systems, Sarrebourg, France). A photoperiod was set up to provide a light:dark ratio of 10:14h. During the acclimation period, shrimps were exclusively fed a crumbled commercial compound diet (35% protein, Grupo Costamar, Hermosillo, Mexico) that established a known isotopic baseline in shrimp tissue before the start of the experiment. It has been demonstrated that fast-growing postlarval Penaeid shrimps achieve isotopic equilibrium with their respective diets in 15 to 20 d (Gamboa-Delgado and Le Vay, 2009a; Gamboa-Delgado et al., 2011). The commercial diet was analyzed for nitrogen and carbon content and their respective isotopic values ($\delta^{15}N$ and $\delta^{13}C$) before the experimental feeding trial.

2.2. Experimental diets

Seven isonitrogenous (36% crude protein) and isoenergetic (4.7 kcal g^{-1}) experimental compound diets were formulated with different proportions of fish meal (FM), soy protein isolate (SP) and corn gluten (CG) (Table 1). The software Nutrion (Nutrion Software, Chapala, Mexico) was used to assist with the formulation of experimental diets. Diets were not manufactured to conduct an ingredient-substitution study; instead, they were formulated with ingredients having contrasting isotopic values to explore their nutritional contributions to shrimp growth as described below. Three diets were formulated with only one ingredient supplying dietary nitrogen: 100% FM (diet 100F), 100% SP (diet 100S) and 100% CG (diet 100G). These diets were used as isotopic controls to correct for the isotopic differences between diets and consumers (isotopic discrimination factors) after having reached dietary equilibrium. The other four diets were formulated with varying mixtures of FM, SP and CG, one included 33% of each ingredient on a dietary nitrogen basis (diet 33FSG) and the other three included a proportion of 50:25:25 for each of the respective three ingredients (diets 50FSG, 50SFG and 50GFS). Before manufacturing the diets, macronutrients were finely ground using a Pulvex 200 grinder fitted with a size #35 mesh. Micronutrients were weighed to the nearest mg, hand-mixed for 5 min and added to the macronutrients, which in turn were homogenized for 15 min using a commercial blender. Lecithin was dissolved in pre-weighed, warm fish oil and added to the mixture. The dough was extruded through a die plate having orifices of 1.4 mm in diameter. Strands were collected on wire trays and post-conditioned by 5 min autoclaving (18.5 psi, 125 °C) to reduce nutrient leaching rates. Diets containing plant meals as the only protein source were sprayed-coated with a hydrolyzed protein to improve palatability. Diets were dried in a convection oven for 8 min at 100 °C and stored at 4 °C. Proximal analyses of the experimental diets included moisture content (method AOAC 930.15), protein content (Dumas method, LECO) and lipid content (Soxhlet system HT-1045, method AOAC 996.06) (Tecator, 1983). The energy content of the ingredients was estimated using a semi-micro bomb calorimeter (Parr 1425 PIC, Illinois, USA).

2.3. Experimental design and rearing system

Shrimps having an initial mean wet weight of 162 ± 36 mg were distributed in 21, 60-L capacity tanks. Twenty animals were placed in triplicate tanks after conducting a pre-selection aimed to allocate animals with the same size distribution pattern in each unit. The experimental tanks having built-in air lifts are connected to a recirculation system holding artificial seawater (Fritz, Chemical Co., Texas, USA). Seawater was exchanged in every tank at a rate of 800% d^{-1} and it was treated by mechanical cartridge filters, UV filter, protein skimmers and a bubble bead biological filter. The experimental tank array is designed so that possible water quality variations affect all tanks simultaneously. Animals were fed the experimental compound diets at daily amounts representing 10 to 15% of the animal biomass. Feed was delivered in four rations at 8:00, 12:00, 16:00 and 20:00 h for 29 days. Before the first feeding ration, uneaten feed, feces and moults were siphoned out daily. Tank walls were periodically scrubbed off with a rough fiber to avoid any possible biofilm growth. The experimental time period and sampling points to collect muscle samples for isotopic analysis were defined according to the exponential rate of isotopic change previously observed in experiments using small-sized Penaeid shrimp (Gamboa-Delgado et al., 2011; Martínez-Rocha et al., in press). In order to verify isotopic values shifting in time to isotopic equilibrium, on experimental days 0, 2, 4, 8, 15 and 22, one shrimp was randomly collected from each replicate tank, killed in ice/water slurry and dissected to isolate the abdominal muscle. The exoskeleton and hind gut were removed, muscle tissue samples were rinsed with distilled water and stored in Eppendorf tubes at −80 °C until sample pretreatment. As