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Isotopic Evaluation of the Nutritional Contribution of Poultry By-product Meal and Fish Meal to the Growth of Pacific White Shrimp, Litopenaeus vannamei

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Abstract

The nutritional contribution of the dietary nitrogen supplied by poultry by-product meal (PBM) and fish meal (FM) to the somatic growth of Pacific white shrimp, Litopenaeus vannamei was assessed by means of stable isotope analysis. Seven experimental diets were formulated with different proportions of PBM replacing FM. Mixed diets were formulated to replace 0, 35, 50, 65, 80, 95 and 100 % of FM with PBM, on a dietary nitrogen basis. At the end of the experiment, there were no significant differences in survival among dietary treatments (89±5 %); however, significant differences in final wet weights were observed. Diets having FM replacement levels of 35 and 50% with PBM, promoted mean final weights (708-789 mg) similar to those observed in shrimps fed on diet containing 100% FM (874 mg). Shrimp final mean weight significantly decreased as a function of PBM inclusion (r= -0.98) due to the use of only two dietary nitrogen sources and by possible nutritional restrictions as PBM levels increased. The relative proportions of dietary nitrogen supplied by PBM and FM were incorporated in muscle tissue at proportions that were statistically similar to those established in the dietary formulations.

Introduction

The industrial activities developed by different agribusinesses frequently generate important amounts of by-products as a result of their intrinsic manufacturing processes. Different uses and nutritional applications for these by-products have been tested and implemented. The poultry industry generates several by-products throughout its different production processes and among these; diverse poultry by-product meals (PBM) have been used as ingredients in diets for aquatic animals (Chi et al. 2009; Rossi and Davis 2012; Saadiah et al. 2011). As the aquaculture industry develops and production methods intensify, the demand for aquaculture feeds and ingredients has also increased. Due to its nutritional properties, fish meal (FM) has been one the main ingredients used for aquaculture feeds, however, the manufacture of FM has derived in ecological and economic concerns (Phillips 2005; Tacon and Metian 2008). It has been demonstrated that PBM is a viable alternative to other protein sources such as fish meal (FM). PBM contains high protein levels (60 to 80%) and presents high apparent digestibility coefficients (80-90% for dry matter and protein) when consumed by aquatic species such as tilapia and Pacific white shrimp, Litopenaeus vannamei (Cruz-Suárez et al. 2007; Zhou et al. 2004).

The use of stable isotopes in aquaculture nutrition has allowed exploring the contribution of available nutrients to the growth of larval and juvenile organisms (Gamboa-Delgado et al. 2008, 2013; Jomori et al. 2008; Martínez-Rocha et al. 2013). In addition to several growth parameters used to assess the suitability and nutritional performance of a specific ingredient, isotopic measurements can provide valuable information on nutrient assimilation. Poultry feed is mainly manufactured from grains having low nitrogen isotope values ($\delta^{15}N$), signatures that are in turn defined by the fertilizers used to grow these crops (Rogers 2009). In contrast, $\delta^{15}N$ values in fish meals are higher, as FM is mainly manufactured from small pelagic fish belonging to high trophic

levels in the marine ecosystems (Serrano et al. 2007). The objective of the present study was to employ the natural and contrasting isotopic signatures of nitrogen in poultry by-product meal and fish meal in order to estimate the relative incorporation of the dietary nitrogen supplied by both ingredients to the growth of Pacific white shrimp.

Material and Methods

Experimental Diets

Seven isonitrogenous (39% crude protein) and isoenergetic (4.7 kcal/gr) experimental diets were formulated using fish meal (prime Mexican sardine, 68% protein) and poultry by-product meal (pet food grade, Valley proteins/Carolina By-Products, 69% protein). Experimental diets were not manufactured to conduct an ingredient substitution study; instead, they were formulated with ingredients having contrasting isotopic values in order to explore their nutritional contributions to shrimp growth as described below. Diet formulation was assisted by means of the software Nutrion® (Nutrion, Chapala, Mexico). Both meals represented the only nitrogen sources and were used to formulate five mixed experimental diets in which different proportions of fish meal (FM) were substituted with high levels of poultry by-product meal (PBM) at 0, 35, 50, 65, 80, 95, and 100%. Substitutions were done based on available dietary nitrogen (Table 1). Diets containing only one source of protein were used as negative (100% PBM) and positive (100% FM) controls in order to estimate and correct isotopic discrimination factors. Micronutrients were weighed to the nearest mg and hand-mixed for 5 min before its addition to the finely ground macronutrients.

TABLE 1. Nutritional (g/1000 g diet, dry weight) and isotopic ($\delta^{15}N$ ‰) composition of seven formulated diets fed to Litopenaeus vannamei to estimate the nutritional contribution of fish meal (F) and poultry by product meal (P) to shrimp growth.

Diet (g/kg)	100F	65F:35P	50F:50P	35F:65P	20F:80P	5F:95P	100P
Fish meal[1]	556	361	277	194	111	28	0
Poultry by product meal[2]	0	194	276	360	443	526	553
Wheat starch[3]	337	350	357	362	368	371	371
Fish oil[1]	23.4	13.1	9.9	6.5	3.2	0.0	0.0
Lecithin[4]	35.5	33.0	31.0	29.5	27.5	25.0	23.6
Alginate[5]	20	20	20	20	20	20	20
Celullose[5]	19.0	18.8	18.7	18.6	18.5	18.3	18.3
Disodium phosphate[5]	0.0	0.0	0.0	0.0	0.0	2.6	4.6
Constant ingredients[6]	9	9	9	9	9	9	9
Total	1000	1000	1000	1000	1000	1000	1000
Proximal analysis							
Crude protein (g/kg)	384	389	386	394	394	392	393
Lipids (g/kg)	76	83	88	73	78	80	83
Gross energy (Kcal/g)	4.7	4.7	4.6	4.7	4.7	4.8	4.7
$\delta^{15}N$ (‰)	16.4	12.2	11.0	8.9	7.6	6.1	5.5

[1]Alimentos Costamar (Sonora, Mexico).
[2]National Renderers Association (Alexandria, VA, USA).
[3]Almidones y gluten S.A. (Monterrey, Mexico).
[4]Ragaza Industrias Proteínas Naturales S.A. de C.V. (Monterrey, Mexico).
[5]Sigma-Aldrich (St. Louis, MO, USA).
[6]Constant ingredients (g/kg diet): Mineral mix 2.5, Vitamin mix 2.5, Vitamin C 1, Choline chloride 2, Antifungic agent 0.5, Antioxidant 0.5.

The mixture was homogenized for 15 min using a commercial blender. Lecithin was dissolved in warm fish oil and was slowly added to the mixture. Distilled water was added until the dough was formed. The paste was then extruded through a die plate having orifices of 1.6 mm in diameter. Strands were collected and placed into wire trays to be post-conditioned by 5 min autoclaving (18.5 psi, 126 C). Diets were dried in a convection oven for 8 min at 100 C and stored at 4 C until used. Bromatologic analyses of a pre-conditioning diet and experimental diets, included protein content (Dumas method, LECO), lipid content (Soxhlet system HT-1045, method AOAC 996.06) (Tecator 1983), fibre content (AOAC 962.09B), moisture content (AOAC 930.15) and ash content (AOAC 942.05). The nitrogen-free extract was estimated as the difference of the latter assays subtracted from 100.

Experimental Design and Rearing System

Postlarval shrimp, L. vannamei were obtained from a commercial hatchery (Maricultura del Pacífico) located in Sinaloa, Mexico. After reception, animals were placed in 500 L tanks and acclimated to bioassay room conditions: water temperature 29.9 ±0.7 C, salinity 33.4 ±0.7 g/L, pH 8.4 ±0.1 and saturated dissolved oxygen. Total ammonia nitrogen (0.08 ±0.05 mg/L), nitrite (not detected), and nitrate (11.3 ±3.9 mg/L) were monitored using a commercial kit (FasTest; Aquarium Systems, Sarrebourg, France). A photoperiod was set up as to provide a 10:14h light-dark ratio. Shrimps were exclusively fed on a crumbled commercial diet (35% crude protein, Grupo Costamar, Mexico) previously analyzed for nitrogen content and $\delta^{15}N$ value. In order to establish a known isotopic baseline in shrimp tissue before the start of the experiment, this diet was supplied for 20 days. It has been demonstrated that fast-growing postlarval Penaeid shrimps achieve isotopic equilibrium with their diets over such time period (Al-Maslamani 2006; Gamboa-

Delgado and Le Vay 2009). Before the nutritional assay, animals were selected in order to distribute animals having the same size distribution pattern in each triplicate tank. Twenty shrimps having initial mean wet weight of 167 ±29 mg were allocated to 21, 60-L capacity tanks individually fitted with air lifts. Artificial seawater (Fritz, Chemical Co., Texas, USA) was exchanged at a rate of 800%/d in every unit. Outflowing seawater was treated by recirculation through mechanical cartridge filters, UV filter, protein skimmers and a bubble bead biological filter. The experimental diets were delivered in excess to every triplicate tank at 0800, 1400 and 1800 h. Uneaten feed, feces and moults were siphoned out daily before first feeding. Tank walls were periodically scrubbed off in order to avoid any possible biofilm growth contributing as food. Feeding rations were progressively adjusted in relation to observed survival and number of sampled animals. The individual wet weights of five animals per replicate were registered. The experimental time period and sampling points were defined according to the exponential rate of isotopic shift previously observed in experiments using small-sized Penaeid shrimp (Gamboa Delgado et al. 2011, 2013). On days 0, 2, 4, 8, 15, 22 and 29, one or two shrimps (depending on individual dry weight) were randomly collected from every replicate tank, killed in ice/water slurry, rinsed with distilled water and dissected to extract abdominal muscle. The exoskeleton and hind gut were removed from the abdominal segments and samples were kept in labeled vials at −80 C until isotopic analysis.

Sample Pretreatment and Stable Isotope Analyses

Samples of shrimp muscle tissue were dehydrated at 60 C until samples reached constant weight and were then manually ground using mortar and pestle to obtain a fine powder. Diet and muscle tissue samples of 900 to 1100 µg (yielding from 40 to 150 µg N) were packed in tin cups

(D1008 Elemental Microanalysis Ltd., UK) and organized in 96-well microplates. Samples were analyzed at the Stable Isotope Facility of the Department of Plant Sciences, University of California, (Davis, CA, USA) using a PDZ Europa Scientific elemental analyzer coupled to a PDZ Europa Hydra 20/20 stable isotope ratio mass spectrometer (Crewe, UK). Repeated measurements of a calibration standard indicated that instrument precision (SD) was 0.13 ‰ for $\delta^{15}N$ values. Isotopic results are expressed in delta notation (δ), which is defined as part per thousand (‰) deviations from the $\delta^{15}N$ value of the standard reference material (atmospheric nitrogen, $\delta^{15}N$= 0.0 ‰, 0.36% ^{15}N). The term "discrimination factor" is used in the present study to describe differences in isotopic values between a consuming organism (whole body or specific tissue, in this case muscle) and its diet after having reached isotopic equilibrium ($\Delta^{15}N$).

Estimation of Nutrient Contribution and Nitrogen Residency Time

The proportional dietary nitrogen contributions from FM and PBM to shrimp growth were estimated using a two-source, one-isotope mixing model (Phillips and Gregg 2001). Estimation of isotopic discrimination factors ($\Delta^{15}N$) is necessary to integrate correction factors into the model. Values were obtained from the isotopic differences between shrimps fed exclusively on diets 100% FM and 100% PBM. Corrected $\delta^{15}N$ values (*i.e.* signatures of animals fed control diets) and $\delta^{15}N$ values measured in shrimps fed mixed diets were introduced into the model to estimate the proportional dietary nitrogen incorporation of PBM and FM and their truncated 95% confidence intervals. $\delta^{15}N$ values were monitored through the experimental period (from the initial dietary shift from the basal diet to the experimental feeding regimes) and from these values an exponential model of isotopic change (Hesslein et al. 1993) was used to obtain an estimate of the metabolic nitrogen turnover rate in shrimp muscle tissue. The model provides a quantitative coefficient that allows distinguishing the isotopic change that is due to growth (*k*) or metabolic turnover (*m*). For

nitrogen turnover rate assessments, the treatment-specific growth rate constant, *k*, was estimated by fitting an exponential growth model to observed weight data, $k = log$(final weight/initial weight)/time(d), while parameter *m* was obtained using iterative non-linear regression. The best estimate of *m* was the value generating the least absolute sum of the differences between estimated and observed isotopic values. Coefficients *k* and *m* provide an indicator of the time period necessary for half of the muscle nitrogen to be replaced by new nitrogen after animals consume a new diet (half time, t_{50}) (MacAvoy et al. 2006).

$$t_{50} = In2 / m+k \quad (1)$$

Statistical Analysis

Student's t-tests were used to compare nitrogen contents and $\delta^{15}N$ values in FM and PBM. Dietary effects on $\delta^{15}N$ values of muscle tissue at different times, mean shrimp wet weight and survival were analyzed by one way ANOVA after normal distribution and data homoscedasticity were verified. Tukey's pair wise comparisons were used to detect treatments significantly differing from each other. In order to detect statistical differences in the expected proportions of dietary nitrogen (contributed by FM and PBM meal) and the observed proportions of nitrogen incorporated in shrimp muscle tissue, Chi-square goodness of fit tests (χ^2) were applied. Parameters required by the Hesslein model were estimated by iterative non -linear regression. All tests were done using SPSS 17.0 software (SPSS Inc.) at a significance level of $P<0.05$.

Results

Growth and Survival Rates

During the experimental feeding period, temperature, pH, salinity and dissolved oxygen concentration in the experimental tanks remained within the recommended optimal values for *L. vannamei*. At the end of the 29-day experimental trial, overall shrimp survival rates were high (89 ± 5%) and not statistically different among dietary treatments (Table 2). However, there were significant differences in final individual wet weight. Individual mean weight gain was negatively correlated to PBM dietary inclusion (r = -0.98). Diet 100F promoted statistically similar growth rates as diets 65F:35P and 50F:50P, although the variability was high. Final weights of animals fed on diets 50F:50P and 35F:65P were statistically similar, but the PBM inclusion level in the latter diet caused significantly lower weight gain than diets 100F, 65F:35P and 50F:50P (Table 2).

TABLE 2. Final wet weight (FW), weight gain (WG), specific growth rate (SGR) and survival rate (S) of Pacific white shrimp, L. vannamei reared under diets having different dietary proportions of fish meal and poultry byproduct meal as the only nitrogen sources.

Diet	FW (mg)	WG (%)	SGR	S (%)
100F	874 ± 290^{a}	421	5.69 ± 1.13^{a}	83 ± 6^{a}
65F:35P	789 ± 25^{ab}	370	5.34 ± 1.12^{ab}	88 ± 3^{a}
50F:50P	708 ± 290^{abc}	323	4.97 ± 1.50^{abc}	92 ± 8^{a}
35F:65P	630 ± 189^{bcd}	277	4.58 ± 1.03^{bcd}	87 ± 6^{a}
20F:80P	591 ± 221^{cd}	249	4.31 ± 1.31^{bcd}	90 ± 5^{a}
5F:95P	542 ± 212^{cd}	221	4.02 ± 1.35^{cd}	93 ± 3^{a}
100P	480 ± 188^{d}	188	3.64 ± 1.27^{d}	88 ± 8^{a}

Different superscripts indicate significant differences for that particular column.

Isotopic Shifts and Discrimination Factors

Before the nutritional trial, the conditioning diet imprinted a basal isotopic value of $\delta^{15}N$= 10.2 ‰ in shrimp tissue. After starting the experiment, there was a fast isotopic influence on shrimp tissue elicited by the respective experimental diets (Fig 1). $\delta^{15}N$ values in FM and PBM were very contrasting (16.8 ± 0.2 and 5.6 ± 0.1 ‰, respectively) and as these were the only nitrogen sources used to formulate the experimental diets, the diets showed a range of $\delta^{15}N$ values from 5.5 to 16.4 ‰. The isotopic shifts observed in shrimp muscle tissue facilitated the estimation of dietary contributions from the two main ingredients using an isotopic mixing model. The different dietary $\delta^{15}N$ values were reflected in shrimp muscle tissue in as little as 8 d, although full isotopic equilibrium between animals and their respective diets was reached between days 22 and 29 (Fig. 1). $\Delta^{15}N$ values between shrimps and their respective diets were different and ranged from -0.12 to 3.01 ‰ (Table 3). $\Delta^{15}N$ values between muscle tissue of shrimps and diet 100F were small and negative (-0.12 ‰), while values observed in shrimp fed diet 100P were significantly larger (3.01 ‰). $\Delta^{15}N$ values were significantly correlated (r= 0.93) to PBM inclusion level in diets.

Nitrogen Turnover Rates and Residency Time

Nitrogen turnover rates in muscle tissue of shrimp fed the different diets showed a high variability and were not correlated to PBM dietary inclusion (0.014-0.078 d^{-1}, Table 3). The lowest value was determined in muscle tissue of shrimp fed on diet 50F:50P. Nitrogen half times in muscle tissue of shrimp fed the different diets ranged from 4.9 to 8.3 days (Table 3). Values consistently increased from diet 100F to diet 35F:65P; however, there was not a clear association between nitrogen half times and experimental diets

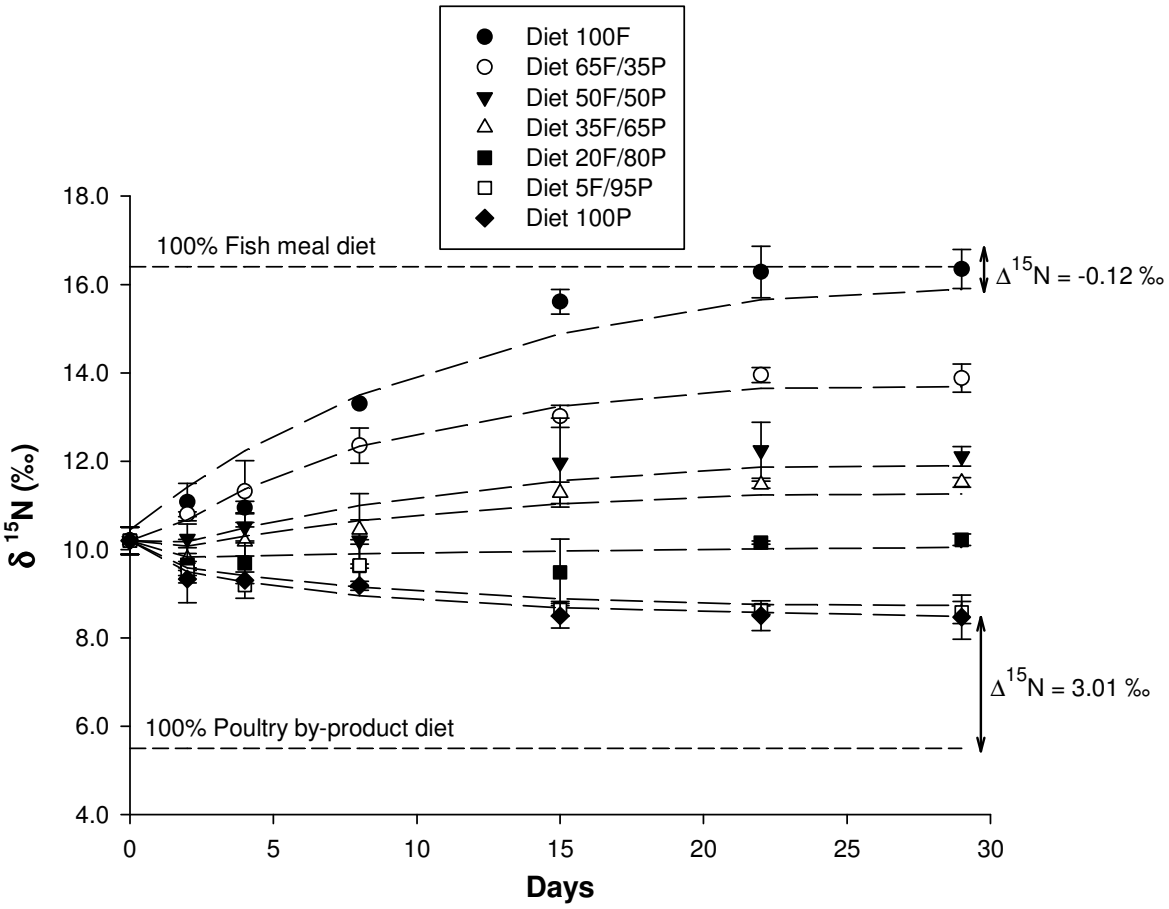

FIGURE. 1. Changes in nitrogen stable isotope values (‰) in muscle tissue of white shrimp, L. vannamei reared on experimental diets containing different proportions of poultry by-product meal and fish meal. Lines represent predicted values generated by the Hesslein et al. (1993) model and show the best fit to observed data. Arrows indicate isotopic discrimination factors between control diets and shrimps.

TABLE 3. Growth rates (*k*), estimated nitrogen metabolic turnover rates (*m*) and half times (t_{50}) in muscle tissue of Pacific white shrimp, L. vannamei reared on diets having different levels of fish meal (F) and poultry by-product meal (P). $\Delta^{15}N$ represents the isotopic difference between diets and muscle tissue after isotopic equilibrium was reached.

Diet	*k* (/d)	*m* (/d)	t_{50} (d)	$\Delta^{15}N$ (‰)
100F	0.057 ± 0.011	0.086	4.9	-0.12
65F:35P	0.053 ± 0.012	0.067	5.8	1.75
50F:50P	0.050 ± 0.015	0.035	8.3	1.25
35F:65P	0.046 ± 0.010	0.045	7.7	2.57
20F:80P	0.043 ± 0.013	-	-	2.56
5F:95P	0.040 ± 0.014	0.062	6.9	2.53
100P	0.036 ± 0.013	0.098	5.2	3.01

**m* values were estimated using iterative non-linear regression to fit expected values on observed values, r^2= 0.70 to 0.98. Different superscripts indicate significant differences at $P<0.05$.

TABLE 4. Estimated relative proportions of dietary nitrogen supplied from fish meal (FM) and poultry by-product meal (PBM) contributing to growth of L. vannamei (mean ± CI, n = 9).

Diet	Expected	Observed in muscle tissue		
		min.	mean	max.
65F:35P				
FM	64.8[a]	68.8	71.0 [a]	73.3
PBM	35.2	26.7	29.0	31.2
50F:50P				
FM	49.9[a]	46.5	48.4[a]	50.2
PBM	50.1	49.7	51.6	53.5
35F:65P				
FM	34.9[a]	36.0	37.7[a]	39.5
PBM	65.1	60.5	62.3	64.0
20F:80P				
FM	19.9[a]	19.5	21.4[a]	23.2
PBM	80.1	76.8	78.6	80.5
5F:95P				
FM	4.9[a]	0	1.5[a]	3.6
PBM	95.1	96.4	98.5	100

Superscripts indicate significant differences between expected and mean observed dietary contributions.

Changes in $\delta^{15}N$ values in shrimp tissue observed over the experimental period and inclusion of asymptotic values into the two-source, one-isotope isotopic mixing model indicated that the contributions of dietary nitrogen from PBM and FM to the growth of shrimps, were similar to the expected contributions indicated by the respective proportions of dietary nitrogen available in the dietary formulation (Tables 1 and 4). χ^2 goodness of fit tests did not detect significant differences in the contributions of PBM and FM in any of the five mixed experimental diets. As the nitrogen content in both protein sources was similar, no further corrections were applied to account for nitrogen concentration; therefore it is assumed that the contribution of dry matter from both sources was also similar.

Discussion

Growth and Survival Rates

Shrimp survival was statistically similar among dietary treatments; however, there were significant differences in shrimp growth at the end of the experiment. The lower growth rate observed in dietary treatments containing higher levels of PBM could be related to a decrease in the availability of specific fatty acids and/or amino acids caused by increasing PBM dietary levels. For example, previous studies have reported that a decrease in the availability of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) occurs as dietary levels of FM are replaced by PBM (Parés-Sierra et al. 2012). Although successful high dietary substitution of FM using PBM (up to 70-80%) has been demonstrated for L. vannamei (Cruz-Suárez et al. 2007; Shuyan et al. 2009), in the present study, animals fed on diet containing 65% PBM, showed significantly lower weight gain. A plausible explanation for this observation is the use of only two dietary ingredients as

protein sources, in comparison to up to six sources employed in previous studies. The use of two ingredients was an essential requirement for the experimental design of this study, as additional nitrogen sources confound the interpretation of results. Another possible explanation for the observed lower growth is the presence of associative effects (non-additivity) between ingredients. Under this effect, the digestibility of a mixture of ingredients might be greater or smaller than the mean digestibility of the individual feedstuffs composing the mixture (Mould 1988). For example associative effects have been reported for the digestibility of nutrients in ducks (Hong et al. 2002) and aquatic organisms such as abalone (Sales and Britz 2002) and crayfish (Brown et al. 1989).

Isotopic Shifts and Discrimination Factors

The isotopic value of most experimental diets was fully reflected in shrimp muscle tissue in less than 25 days. Animals consuming diets having more contrasting $\delta^{15}N$ values (*e.g.* 100F) in relation to somatic isotopic values at the beginning of the experiment, required additional time to reach isotopic equilibrium. The relatively short time needed to reach equilibrium in all treatments was an indicator of fast ingestion, digestion and assimilation of the dietary components. The isotopic difference between diet and animal consumer ($\Delta^{15}N$, isotopic discrimination factors) has been proposed as an indicator of the dietary quality of a trophic element for an animal consumer. It has been considered that small $\Delta^{15}N$ values are related to diets whose nutrients are incorporated fast, hence nutrients do not go through many metabolic modifications. In the present study, $\Delta^{15}N$ values between muscle tissue of shrimps and diet 100F were negative and very small (-0.12 ‰). Negative values indicate that a slightly higher proportion of light dietary nitrogen (^{14}N) was incorporated in muscle tissue in relation to heavy nitrogen. $\Delta^{15}N$ values showed a tendency to increase (r= 0.76) as a function of PBM dietary inclusion. Metabolic cycling of nutrients is one of

the tentative causes leading to higher $\Delta^{15}N$ values. In the present study, formulation of the experimental diets indicated a small decrease in the availability of lysine as PBM levels increased. The latter observation might have partially accounted to the increasing $\Delta^{15}N$ values (2.53-3.01 ‰) observed in diets containing more than 65% PBM. . Previous studies conducted on the same species have reported higher $\Delta^{15}N$ values when animals are fed diets containing only plant proteins ($\Delta^{15}N$ = 6.6 ‰ when fed soy protein isolate and $\Delta^{15}N$ = 7.4 ‰ when fed on pea meal) (Gamboa-Delgado and Le Vay 2009; Martínez-Rocha et al. 2013). In contrast, average $\Delta^{15}N$ values reported in the literature are ~3.0 ‰ (McCutchan et al. 2003, Caut et al. 2009), which highlights the need for additional studies to determine isotopic discrimination factors elicited by species, age/stage and specific nutritional conditions.

Nitrogen Residency Time in Muscle Tissue

Although there was not a clear association between nitrogen half times and experimental diets, t_{50} values consistently increased from diet 100F to diet 35F/65P. As t_{50} values are estimated from the growth parameter *k* and metabolic turnover rate (*m*), they provide an indicator of the nitrogen turnover rate in a specific tissue. Previous studies have indicated similar results as those observed in the present study. It is considered that diets containing only FM elicit higher growth rates and lower residency times of nutrients in tissue, which in turn are associated to higher metabolic rates (MacAvoy et al. 2006; Gamboa-Delgado and Le Vay 2009; Martínez-Rocha et al. 2013).

Nutritional Contributions from Poultry By-product Meal and Fish Meal to Shrimp Growth

The relative nutritional contributions of dietary nitrogen from both main ingredients to growth were similar to the dietary proportions available in the experimental diets. These contributions indicated an equivalent transference of dietary nitrogen (amino acids and protein) and dry matter from FM and PBM. For example, previous studies have shown that the assimilation of dietary nitrogen from plant proteins is significantly lower than assimilation of dietary FM contained in experimental shrimp diets (Gamboa-Delgado and Le Vay, 2009). In contrast to results from the latter study, when live macroalgae was co-fed with an inert diet to *L. vannmei*, significantly higher proportions of dietary carbon and nitrogen from the macroalgae were incorporated in shrimp probably due to its higher digestibility and constant availability (Gamboa-Delgado et al. 2011). Although nutritional contributions derived from PBM and FM were similar in the present study, growth rates were not equivalent among dietary treatments. As discussed above, this might be explained by the restrictive use of only two dietary nitrogen sources and by associative effects between the two main ingredients used to formulate the experimental diets. As it has been previously reported that a decrease in the availability of HUFAs occurs as dietary levels of FM are replaced by PBM (Parés-Sierra et al. 2012), the restriction of DHA and EPA might have also contributed to the lower growth rates observed at higher FM substitution levels (>80% PBM).

Conclusions

Isotopic analysis of ingredients, diets and animal consumers indicated that the dietary nitrogen proportions of PBM and FM were fully incorporated in less than 29 days and at statistically similar proportions in muscle tissue. Such similar contributions indicate that a

proportional assimilation of dietary nitrogen from both main ingredients occurs as a function of the established dietary inclusion level. As PBM and FM have similar dietary nitrogen levels, it can be also concluded that their contribution to growth in terms of total dry matter is equivalent. Results from growth parameters observed in the present study also indicate that high dietary inclusion of PBM correlates with lower shrimp growth rates in diets containing FM and PBM as the only protein (nitrogen) sources. Reduction in the availability of HUFA in diets containing high proportions of PBM might account to the latter observation. Future studies hold the potential to determine the allocation of specific amino acids supplied by PBM and FM by means of analytical separation and isotopic analysis of amino acids contained in both ingredients.

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