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Assessment of the relative contribution of dietary nitrogen from fish meal and biofloc meal to the growth of Pacific white shrimp (*Litopenaeus vannamei*)

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Abstract

The relative contribution of the dietary nitrogen supplied by fish meal and a biofloc meal to the growth of Pacific white shrimp was evaluated using stable isotope analysis. Bioflocculated material was obtained from an experimental tilapia culture system. Five formulated diets were supplied. Two of them consisted in isotopic controls having only fish meal or biofloc meal as protein source. Three mixed diets were formulated with varying proportions of these ingredients on a dietary nitrogen basis (75:25, 50:50 and 25:75). At the end of the trial, survival rates were similar (92–100%) but significant differences in mean final weight were observed and a negative correlation between the inclusion of biofloc meal and weight gain was evidenced. Mean final weight in shrimp fed on diet containing only fish meal was 2.8 g, while mean final weight of animals fed on diet containing 50% biofloc was 1.9 g. Isotopic mixing models indicated that all diets contributed higher proportions of dietary nitrogen from fish meal than from biofloc meal. Dietary nitrogen available in diets containing 25%, 50% and 75% of biofloc meal was incorporated in muscle tissue as 5%, 41% and 64% respectively. Diet supplying 25% of nitrogen from biofloc was the only mixed diet eliciting growth comparable to diet containing only fish meal. Lower growth and nitrogen deposition in shrimp fed on diets containing high proportions of biofloc meal were possibly associated to the use of only two protein sources and a restriction of essential amino acids.

Keywords: biofloc meal, fish meal, contribution to growth, *Litopenaeus vannamei*, nutritional tracers, stable isotopes

Introduction

Aquaculture production technologies have greatly diversified and novel methodologies have been implemented to promote higher production outputs. Among these production techniques, intensive, microbial-based systems have been applied to culture shrimp and other species. The nutritional basis of these systems relies on the formation and continuous availability of flocculated material mainly composed of microbial aggregates (biofloc). Depending on the culture type, bioflocs can also contain protozoans fungi, invertebrates and small organic and inorganic particles (Avnimelech 2007). These communities provide an additional food source for the farmed organisms and also maintain the water quality. Microorganisms such as bacteria, microalgae and yeast have been deemed as good sources of protein, vitamins, antioxidants and other compounds. At the end of the production cycles in biofloc-based systems, the remaining flocculated material represents one of the generated

byproducts having further nutritional applications (Valle, Dantas, Silva, Bezerra, Correia, Peixoto & Soares 2015).

Diverse isotopic techniques have been used as nutritional tools in studies conducted on aquatic species. Measurements of the stable isotope values of carbon and nitrogen at natural abundance levels in dietary sources and animal consumers have been applied to evaluate the transference of dietary nutrients into tissue. As animal tissue reflects the isotopic composition of the dietary components used to build up this tissue, collection and integration of these data into isotopic mixing models has allowed converting isotopic values into dietary contributions (Gannes, O'Brien & Martínez del Rio 1997; Newsome, Fogel, Kelly & Martínez Del Rio 2011; Phillips 2012). In aquaculture nutrition, these procedures have shed light on how larval and juvenile aquatic organisms incorporate the specific components of the supplied nutritional items (Gamboa-Delgado & Le Vay 2009; Duffy, Godwin, Nolan & Purvis 2011). The natural isotope ratios of nitrogen ($^{15}N/^{14}N$, reported in delta notation as $\delta^{15}N$) have been used as natural biomarkers to estimate the dietary contributions of protein and dry matter in organisms fed on experimental diets having ingredients with contrasting isotopic signatures (Gamboa-Delgado, Castañeda-Solis, Nieto-López & Cruz-Suárez 2014). Some studies have taken advantage of the atypical isotopic values conferred by specific fertilizers and culture media (methanol) to macroalgae and bacteria, respectively used as shrimp feed and dietary ingredient (Cam, Rollet, Mariotti & Guillaume 1991; Gamboa-Delgado, Peña-Rodríguez, Cruz-Suárez & Ricque 2011). The biofloccculated material is mainly composed of bacterial biomass and the high metabolic rate of these microbial communities has a very significant effect on the isotopic values. These dynamic isotopic values in live microbial biomass make difficult to estimate nutritional contributions to growth (Burford, Thompson, McIntosh, Bauman & Pearson 2004). In this context, the present study employed the natural isotopic differences measured in fish meal and dry, inactive biofloc meal to assess the relative incorporation of dietary nitrogen supplied by these two sources to the muscle tissue of Pacific white shrimp. In addition, the nitrogen half times in muscle tissue of shrimps fed on the different experimental diets were estimated.

Materials and methods

Experimental diets

Microbial aggregates from an experimental biofloc-based, tilapia rearing system (Universidad Autónoma de Sinaloa, Mazatlán, Mexico) were collected at the end of the culture period by filtering water through a series of cloth sieves. Most of the biofloc slurry was recovered and the material was immediately frozen, transported, freeze-dried and ground. Mean protein and ash contents of biofloc were 24% and 17% respectively. The latter was further analysed to determine its calcium and phosphorus content (3.2 and 0.9% respectively). Fish meal (prime Mexican sardine, 68% protein) was used as a second dietary source of protein. The amino acid profile of both ingredients was determined at the Agricultural Experiment Station of the University of Missouri (AOAC, 2006). A restriction of methionine and lysine was evident in the biofloc meal (Table 1). Proximal analyses were applied to both ingredients and five isonitrogenous (22% crude protein) and nearly isoenergetic (18.4 kJ g^{-1}) experimental compound diets were formulated (Table 2) by means of the software

Table 1 Amino acid composition (weight %) of fish meal and biofloc obtained from an experimental tilapia culture system

Amino acid	Fish meal	Biofloc
Taurine	0.77	0.54
Hydroxyproline	1.30	0.76
Aspartic Acid	9.50	12.76
Threonine	4.51	5.03
Serine	3.68	4.00
Glutamic Acid	12.65	12.97
Proline	4.70	5.24
Glycine	6.89	8.16
Alanine	6.52	7.62
Cysteine	0.91	1.89
Valine	5.41	6.49
Methionine	2.80	1.41
Isoleucine	4.53	4.38
Leucine	7.79	8.11
Tyrosine	3.65	3.62
Phenylalanine	4.33	5.14
Hydroxylysine	0.29	0.43
Ornithine	0.11	0.76
Lysine	8.60	4.49
Histidine	3.49	1.51
Arginine	6.43	4.11
Tryptophan	1.16	0.59

Table 2 Nutritional (g 1000 g^{-1} diet dry weight) and isotopic ($\delta^{15}N$, ‰) composition of formulated diets fed to *Litopenaeus vannamei* to estimate the nutritional contribution of fish meal and biofloc meal to shrimp muscle tissue

Ingredient/Diet	100F	75F/25B	50F/50B	25F/75B	100B
Fish meal*	271.7	203.5	136.0	68.0	0.0
Biofloc meal	0.0	204.9	407.7	612.0	843.1
Wheat starch†	610.9	454.4	310.6	152.9	0.0
Lecithin‡	34.9	34.9	35.4	40.0	40.0
Fish oil*	23.4	28.3	32.0	32.3	36.7
Cholesterol§	0.0	0.0	0.7	0.8	1.2
Disodium phosphate¶	30.0	30.0	33.5	50.0	50.0
Choline chloride*	2.0	2.0	2.0	2.0	2.0
Mineral premix*	2.5	2.5	2.5	2.5	2.5
Cellulose¶	0.0	15.0	15.0	15.0	0.0
Alginate¶	20.0	20.0	20.0	20.0	20.0
Vitamin premix*	2.5	2.5	2.5	2.5	2.5
Vitamin C*	1.0	1.0	1.0	1.0	1.0
Antioxidant*	0.5	0.5	0.5	0.5	0.5
Antifungic agent*	0.5	0.5	0.5	0.5	0.5
Total	1000	1000	1000	1000	1000
Proximal and isotopic analyses					
Crude protein (g kg^{-1})	216	211	218	220	217
Lipids (g kg^{-1})	81	83	83	82	85
Ash (g kg^{-1})	98	115	152	205	214
Gross energy (kJ g^{-1})	18.4	17.6	17.6	18.8	17.9
$\delta^{15}N$ (‰)	16.4	13.3	11.1	8.6	6.4

*Alimentos Costamar (Sonora, Mexico).
†Almidones y gluten S.A. (Monterrey, Mexico).
‡Ragaza Industrias Proteínas Naturales S.A. de C.V. (Monterrey, Mexico).
§Solvay Pharmaceuticals (Houston, TX, USA).
¶Sigma-Aldrich (St. Louis, MO, USA).

NUTRION® (Nutrion, Chapala, Mexico). Diets were not manufactured to conduct an ingredient-substitution study; instead, they were formulated with ingredients having contrasting nitrogen isotopic values ($\delta^{15}N$) in order to explore dietary contribution to shrimp growth. The experimental diets were adjusted considering the low protein content of the biofloc meal. Two diets were formulated either with fish meal or biofloc meal as the only ingredient supplying dietary nitrogen: diet 100F and diet 100B. These diets were used as isotopic controls to correct for the isotopic differences observed between diets and consumers (isotopic discrimination factors) after having reached equilibrium. The other three mixed diets were formulated with combinations of fish meal and biofloc meal to supply the following proportions of dietary protein: 25:75, 50:50 and 75:25. Before manufacturing the diets, macronutrients were finely ground. Micronutrients were weighed to the nearest mg, hand-mixed and added to the macronutrients. The mixture was homogenized in a blender. Lecithin was dissolved in warm fish oil and added to the mixture. Water was slowly added until the mixture formed dough which was extruded through a die plate (orifices of 1.4 mm). Diet strands were dried in a convection oven for 10 min at 100°C and stored at 4°C. Proximal analyses of the experimental diets included moisture content (method 930.15), protein content (Dumas method, direct combustion, LECO; AOAC 990.03) and lipid content (Soxhlet system HT-1045, method AOAC 996.06) (Tecator 1983). The energy content of the ingredients was determined in a semi-micro bomb calorimeter (Parr 1425; PIC, Moline, IL, USA).

Experimental animals and rearing system

Juvenile shrimp, *Litopenaeus vannamei* (high health certified) were obtained from a commercial hatchery (Acuacultura Mahr) located in Baja California Sur, Mexico. Animals were allocated to a 500 L tank and acclimated to local conditions: water temperature 28.9 ± 0.8 C, salinity 34.6 ± 0.7 g L^{-1}, pH 8.4 ± 0.1 and saturated

dissolved oxygen. Total ammonia nitrogen (0.07 ± 0.04 mg L^{-1}), nitrite (not detected) and nitrate (12.7 ± 4.9 mg L^{-1}) were monitored using a colorimetric kit (FasTest; Aquarium Systems, Sarrebourg, France). A natural photoperiod provided a 10:14 h light-dark ratio. Shrimps were exclusively fed on a crumbled commercial diet (35% crude protein, Grupo Costamar, Hermosillo, Mexico) supplied as 8% of the standing shrimp biomass and divided in three rations. This diet was previously analysed for nitrogen content and $\delta^{15}N$ value and it was supplied for 15 days to establish a known $\delta^{15}N$ baseline value in shrimp bodies before the start of the experiment. Twelve shrimps having initial mean wet weight of 0.59 ± 0.08 g were allocated to 10, 60-L capacity tanks individually fitted with air lifts. Artificial seawater (Fritz, Chemical, TX, USA) was exchanged at a rate of 800% d^{-1} in every unit. Recirculated water was treated by mechanical cartridge filters, UV filter, protein skimmers and a bubble bead biological filter. The experimental tank array (clear-water) is designed so that possible water quality variations affect all tanks simultaneously. The experimental diets were assigned to duplicate tanks and feed rations were delivered in excess at 6:00, 9:00, 12:00, 15:00 and 18:00 h. Uneaten feed, faeces and moults were siphoned out before first feeding. In order to avoid any possible biofilm growth contributing as food, tank walls were periodically scrubbed off. Feeding rations were adjusted in relation to observed growth, survival and number of animals after sampling. The experimental time period, sampling and weighing points were defined according to the exponential rate of isotopic shift previously observed in experiments using small shrimp (Gamboa-Delgado *et al.* 2014). On days 0, 4, 8, 15 and 22, one or two shrimps (depending on individual weight) were randomly collected from each replicate tank, killed in ice/water slurry, rinsed with distilled water and frozen. On day 29, all remaining animals were scarified. The exoskeleton and hind gut were removed from the abdominal segments and samples were kept in labelled vials at −80°C until pretreatment for isotopic analysis.

Sample pretreatment and stable isotope analyses

Samples of shrimp muscle tissue and compound diets were dehydrated at 50°C until constant weight in a convection oven, samples were manually ground using mortar and pestle. Muscle tissue samples were not lipid extracted as part of the pretreatment as shrimp muscle contains low lipid levels and it has been shown that $\delta^{15}N$ values in muscle tissue of decapod crustaceans experience minimal changes after solvent treatment (Stenroth, Holmqvist, Nyström, Berglund, Larsson & Granéli 2006; Bodin, Le Loc'h & Hily 2007). Diet and muscle tissue samples of 900–1100 μg were packed in tin cups (D1008; Elemental Microanalysis, Okehampton, UK) and organized in 96-well microplates. Samples were analysed at the Stable Isotope Facility of the Department of Plant Sciences, University of California, (Davis, CA, USA) using a Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon, Cheshire, UK). Repeated measurements of a calibration standard indicated that instrument precision (SD) was 0.12‰ for $\delta^{15}N$ values. Isotopic results are expressed in delta notation (δ), which is defined as *per mil* (‰) deviations from the $\delta^{15}N$ value of the standard reference material (atmospheric nitrogen). The term 'discrimination factor' ($\Delta^{15}N$) is used in the present study to describe differences in isotopic values between consuming organisms (whole body or specific tissue, in this case muscle) and their diets after having reached isotopic equilibrium.

Estimation of nutrient contribution and nitrogen turnover rates

The proportional dietary nitrogen contributions from fish meal and biofloc meal to shrimp growth were estimated using a two-source, one-isotope mixing model (Phillips & Gregg 2001). Model assumptions such as verification of isotopic equilibrium and corrections for $\Delta^{15}N$ were fulfilled (Post 2002; Martínez del Rio, Wolf, Carleton & Gannes 2009). Estimation of isotopic discrimination factors ($\Delta^{15}N$) is necessary to integrate correction factors into the mixing models. These values were obtained from the isotopic differences between shrimps fed exclusively on diets 100F and 100B. Corrected $\delta^{15}N$ values (i.e. isotopic values of animals fed on control diets and not those of the diets) were used to estimate the proportional incorporation (±95% truncated confidence intervals) of dietary nitrogen derived either from biofloc meal or fish meal. An exponential model of isotopic

change (Hesslein, Hallard & Ramlal 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 that resulted in the least absolute sum of the differences between calculated 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, Arneson & Bassett 2006).

$$t_{50} = \ln 2/m + k \quad (1)$$

Statistical analyses

Student's t-tests were used to compare nitrogen contents and $\delta^{15}N$ values in fish meal and biofloc meal. Dietary effects on $\delta^{15}N$ values of muscle tissue at different times, mean shrimp wet weight and survival were analysed by Kruskal–Wallis tests and ensuing pair comparisons by Mann–Whitney tests. In order to detect statistical differences in the expected proportions of dietary nitrogen (contributed by fish meal and biofloc meal) and the observed estimated proportions of nitrogen incorporated in shrimp muscle tissue, Chi-square goodness of fit tests (χ^2) were applied. All tests were done using SPSS 17.0 software (SPSS, Chicago, IL, USA) at a significance level of $P < 0.05$.

Results

Growth and survival rates

During the experimental feeding period, water quality parameters remained within the recommended optimal values for this species. Temperature, pH, salinity, dissolved oxygen and nitrogenous waste concentrations were maintained as the previously described conditions for the bioassay room. At the end of the experiment, shrimps reared under the five experimental treatments showed similar survival rates (overall survival 96 ± 6%) but significantly different mean final weights (Table 3). There was a strong negative correlation between the final wet weight of shrimp and the dietary inclusion level of biofloc meal ($r = -0.98$).

Isotopic shifts and discrimination factors

Due to their different origins and chemical characteristics, fish meal and biofloc meal showed significantly different $\delta^{15}N$ values (16.4 ± 0.2 and 6.4 ± 0.1‰ respectively). These significant isotopic differences allowed formulating diets having contrasting isotopic values that in turn elicited a wide range of nitrogen isotope changes in muscle tissue (Fig. 1), which in turn increased the resolution of the assessment of nutritional contributions and the estimation of nitrogen residency times in tissue. All experimental diets exerted a rapid influence on the isotopic values of shrimp tissue and animals in all treatments reached isotopic equilibrium with their respective feed around experimental day 22. $\Delta^{15}N$ values between animals and their respective diets were also significantly different (Table 4). $\Delta^{15}N$ values between muscle tissue of shrimps and diet 100F were small and negative (−0.7‰), while values observed in shrimp fed on diet 100B were significantly larger (3.8‰). $\delta^{15}N$ value of diet 100B resembled the $\delta^{15}N$ value of shrimp tissue at the beginning of the trial and exponential isotopic changes were not generated (Fig. 1). $\Delta^{15}N$ values between shrimp and diets containing both protein sources ranged from 2.1 to 3.5‰.

Table 3 Mean 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 biofloc meal. Mean values ± SD

Diet	FW (mg)	WG (%)	SGR	S (%)
100F	2.82 ± 0.84^{a}	379	5.40	96 ± 6^{a}
75F/25B	2.52 ± 0.68^{a}	328	5.01	96 ± 6^{a}
50F/50B	1.86 ± 0.36^{ab}	216	3.97	100 ± 0^{a}
25F/75	1.35 ± 0.36^{b}	129	2.86	92 ± 12^{a}
100B	0.57 ± 0.20^{c}	4	0.09	96 ± 6^{a}

Different superscripts indicate significant differences for that particular column.

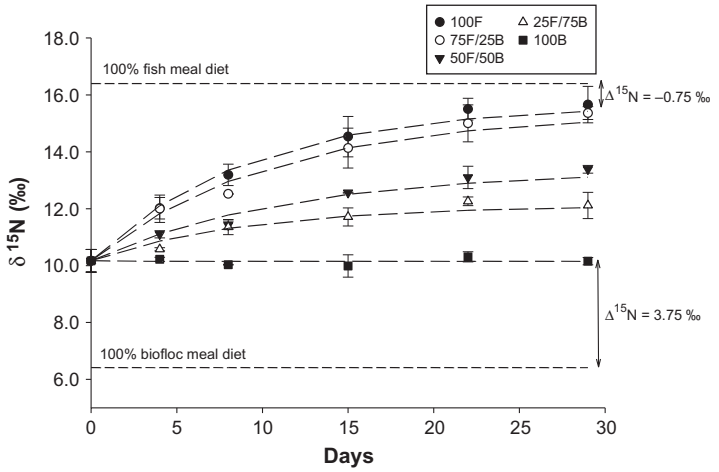

Figure 1 Changes in nitrogen stable isotope values (‰) in muscle tissue of Pacific white shrimp *Litopenaeus vannamei* following a shift from a commercial diet to five experimental diets containing different proportions of biofloc meal and fish meal. Lines represent predicted values generated by a model of isotopic change and show the best fit to observed data. Arrows indicate nitrogen isotopic discrimination factors between control diets and shrimps. $n = 2$ individuals, 12 on final point.

Table 4 Mean growth rates (k) and estimated half times (t_{50}) in muscle tissue of shrimp *L. vannamei* reared on diets having different dietary proportions of fish meal and biofloc meal

Diet	k (d^{-1})	t_{50} (d)	R^2	Δ^{15}N
100F	0.056 ± 0.010^{a}	6.4	99	−0.7
75F/25B	0.049 ± 0.009^{a}	7.1	98	2.1
50F/50B	0.039 ± 0.006^{ab}	7.9	97	2.3
25F/75B	0.027 ± 0.009^{b}	6.3	94	3.5
100B	0.009 ± 0.009^{c}	nd	–	3.7

Different superscripts indicate significant differences for that particular column. R^2 values indicate the degree of fitness of data generated by an exponential model of isotopic change and actual isotopic values measured in shrimp muscle tissue. Δ^{15}N represents the isotopic difference between diets and muscle tissue after isotopic equilibrium was reached. Mean values ± SD

Nitrogen turnover rates and nutritional contribution

Changes in δ^{15}N values in shrimp muscle followed an expected pattern characterized by an exponential trend caused by the isotopic values of the experimental diets. For most treatments, predicted isotopic values fitted well on the observed data. From each of these data groups, estimated parameters m and k indicated that nitrogen half times in tissue ranged from 6.3 days in shrimp fed diet 25F/75B to 7.9 days in shrimp fed on diet 50F/50B (Table 4). Isotopic changes observed over the experimental period and inclusion of asymptotic values into the isotopic mixing model indicated that, in all cases, contributions of dietary nitrogen from fish meal to shrimps growth were higher than expected contributions indicated by the respective proportions established in the diet formulations (Tables 2 and 5), although the difference in animals fed on diet 50F/50B was not significant ($\chi^2 = 3.0$, $P = 0.08$). The incorporation of dietary nitrogen from biofloc meal was lower than the corresponding dietary proportions established in the formulations.

Discussion

Growth and survival

Several microbial protein sources represent some of the emerging alternative feed ingredients for manufacturing and improving the highly demanded aquaculture feeds. A number of commercial enterprises have focused on the production of bacterial biomass in processes that employ agricultural byproducts as substrates (Glencross, Irvin, Arnold, Bourne & Preston 2014). The resulting products present good nutritional properties and have been proposed as fish meal replacements or feed additives (Valle *et al.* 2015). In the present study, although shrimp survival was statistically similar among dietary treatments, there were significant differences in shrimp growth fed on the different diets. Although reduced growth rate was expected due to the low dietary protein level imposed by the low protein content of this biofloc batch, comparison of treatments against the positive control (100F) indicated a further reduction in growth in dietary treatments containing increasingly higher levels of biofloc meal. Only diet 75F/25B elicited similar growth to diet containing

Table 5 Estimated relative proportions of dietary nitrogen available from fish meal and biofloc meal in experimental diets and observed proportions contributing to the growth of shrimp *L. vannamei* as indicated by a 2-source, 1-isotope mixing model (mean ± CI, $n = 12$)

	Contributions			
		Observed		
Diet	Expected	Min.	Mean	Max.
75F/25B				
Fish meal	75[a]	86.9	94.5[b]	100
Biofloc meal	25	0.0	5.5	13.1
50F/50B				
Fish meal	50[a]	53.9	58.7[a]	63.5
Biofloc meal	50	36.5	41.3	46.1
25F/75B				
Fish meal	25[a]	28.3	35.6[b]	43.0
Biofloc meal	75	57.0	64.4	71.7

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

only fish meal. The other experimental diets caused further decreases in shrimp final weight. The lower growth observed in shrimp fed on diets containing higher levels of biofloc meal can be attributed to a restriction of the amino acids methionine and lysine in this particular biofloc batch. The high ash content in dry biofloc and the probable presence of trace elements exerting toxicity effects (Tacon 1988) might have been factors contributing to lower growth in animals fed on diets containing higher biofloc levels. Bauer, Prentice-Hernandez, Tesser, Wasielesky and Poersch (2012) were able to replace 100% of fish meal with a mixture of biofloc meal and soy protein concentrate without detrimental effects for shrimp *L. vannamei*. In contrast, Anand, Kohli, Kumar, Sundaray, Roy, Venkateshwarlu, Sinha and Pailan (2014) conducted a study in which biofloc meal was included in diets for *Penaeus monodon* at low dietary levels (4, 8 and 12%) and based on growth and digestive enzymes data, authors recommend a dietary inclusion of biofloc of 4%, which is sixfold lower than the lowest dietary inclusion level tested in the present study.

Contrasting results have also been reported on the growth performance of postlarval and juvenile shrimps raised in clear-water or exposed to biofloc. Kim, Pang, Seo, Cho, Samocha and Jang (2014) reported higher growth, survival and immune activity in shrimps grown in biofloc-based systems; however, Emerenciano, Cuzon, Paredes and Gaxiola (2013) and Esparza-Leal, Pereira-Cardozo and Wasielesky (2015) did not observe improved growth in shrimps grown in biofloc-based systems as compared to clear-water systems. Valle *et al.* (2015) reported higher survival and weight gain in postlarval *L. vannamei* fed on diets containing biofloc and fish protein hydrolysate than shrimps fed on diet containing only fish meal. Contrasting reported results are possibly due to the characteristics and compositions of different types of biofloc, the substrates used to promote the microbial aggregates and the particular features of the supplied compound diets. Although the biofloc meal used in the present study was freeze-dried in order to preserve thermolabile nutrients, comparison of results with other studies allows inferring that some nutritional characteristics are lost in dry biofloc as compared to live biofloc, which is also permanently available for farmed animals growing under biofloc systems. Another important aspect to take into consideration is that the biofloc meal used in the present study was collected from a freshwater system containing tilapia, therefore the nutritional characteristics might be inferior to those in biofloc collected from shrimp farms. For example, Dantas, Valle, Brito, Calazans, Peixoto and Soares (2016) recently reported that up to 30% of fish meal in diets for *L. vannamei* was successfully replaced by a biofloc meal collected from shrimp tanks.

Isotopic shifts and discrimination factors

The $\delta^{15}N$ values of most experimental diets were fully reflected in shrimp muscle tissue in 22 days. The relatively short time needed to reach equilibrium in all dietary treatments was an indicator of fast ingestion, digestion and assimilation of the dietary components. Animals that consumed diets having more contrasting $\delta^{15}N$ values (e.g. 100F) in relation to the somatic isotopic values at the beginning of the experiment, required additional time to reach isotopic equilibrium. Isotopic discrimination factors ($\Delta^{15}N$) have 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 caused by nutrients that are incorporated fast and hence do not go through many metabolic modifications. In the present study, $\Delta^{15}N$ values between muscle tissue of shrimps and their respective diets,

significantly increased from −0.7 to 3.7‰ as a function of biofloc meal level. 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 strong tendency to increase ($r = 0.91$) as a function of biofloc meal dietary inclusion. Increased metabolic cycling of nutrients due to nutrient restriction is one of the tentative causes leading to higher $\Delta^{15}N$ values (Martínez del Rio & Wolf 2005). Previous studies conducted on *L. vannamei* have reported significantly higher $\Delta^{15}N$ values when animals are fed diets containing only plant proteins ($\Delta^{15}N = 7.4$‰ when fed on diets containing only pea meal) (Martínez-Rocha, Gamboa-Delgado, Nieto-López, Ricque-Marie & Cruz-Suárez 2013). Fantle, Dittel, Schwalm, Epifanio and Fogel (1999) reported higher $\Delta^{15}N$ values in crabs fed on diets having a restriction of essential amino acids. These results are consistent with observations in the present study as growth results and isotopic data indicate low nutritional performance of this biofloc batch. Average $\Delta^{15}N$ values reported in the literature are close to 3.0‰ (McCutchan, Lewis, Kendall & McGrath 2003; Caut, Angulo & Courchamp 2009) and are frequently applied to correct for $\Delta^{15}N$ values when estimating nutritional contributions in a wide range of animal taxa. However, as observed in the $\Delta^{15}N$ values elicited by the controls diets in the present study, this average might not be correct for different species/ages.

Nitrogen turnover rates and nutritional contributions

Although there was not a clear association between nitrogen half times and experimental diets, t_{50} values consistently increased from diet 100F to diet 50F/50B. Given that t_{50} values are estimated from the growth parameter k and the metabolic turnover rate m, t_{50} values provide an indicator of the nitrogen turnover rate in a specific tissue. Values observed in the present study are similar to those reported for juveniles of the same shrimp species fed on diets containing higher protein levels. For example, Gamboa-Delgado *et al.* (2014) reported a similar trend in shrimp fed on increasingly higher levels of poultry byproduct meal. It is considered that diets containing ideal nutritional profiles elicit higher growth rates and lower residency times of nutrients in tissue, which in turn are associated with higher metabolic rates (MacAvoy *et al.* 2006; Gamboa-Delgado & Le Vay 2009; Martínez-Rocha *et al.* 2013). Relatively higher t_{50} values are observed at higher dietary levels of plant or microbial protein. In the present study, the restrictive use of only two dietary sources and the low protein level in the diets, in conjunction with decreasing availability of methionine and lysine in diets containing higher levels of biofloc meal, was reflected in lower shrimp growth. The relative proportions of dietary nitrogen incorporated from both main ingredients were significantly different in two of the three mixed diets. Dietary nitrogen available in mixed diets containing 25%, 50% and 75% of biofloc meal was incorporated in muscle tissue at proportions of 5%, 41% and 64% respectively. These contributions indicated an uneven tissue allocation of dietary nitrogen derived from fish meal and biofloc meal. The allocation of dietary nitrogen from biofloc meal thus indicates that this dry ingredient supplied nutrients that were readily digested and assimilated, but at lower relative proportions than those supplied by fish meal. It is very likely that fresh biofloc might confer a higher nutritional value and digestibility than dry material. For example, previous studies applying isotopic methodologies have indicated a relatively high contribution of live biofloc to growth. Burford *et al.* (2004) evaluated in *L. vannamei* the retention of the dietary nitrogen supplied by live flocculated material and formulated feed. The authors concluded that, under intensive culture conditions, from 18 to 29% of the nitrogen retention in shrimp was derived from the natural biota, mainly composed of biofloc and Cardona, Lorgeoux, Geffroy, Richard, Saulnier, Gueguen, Guillou and Chim (2015) estimated that the nitrogen supplied by biofloc contributed up to 37% to the growth of shrimp *L. stylirostris*. The biofloc does not only represent a source of nitrogen for the farmed animals, it is a complex mixture of other compounds (carotenoids, phytosterols, amino sugars) that might promote growth. In fact, it has been shown that isolated biofloc fractions and whole dry biofloc promote similar growth in *L. vannamei* as compared to control diets (Ju, Forster, Conquest & Dominy 2008).

Conclusion

Isotopic analysis of ingredients, diets and shrimps indicated that the nitrogen isotope values in fish meal and dry biofloc were fully reflected by shrimp

in less than 22 days. Dietary nitrogen proportions were allocated into muscle tissue at significantly different proportions in two of the three mixed diets. Nutrients derived from fish meal were preferentially allocated into tissue. Increasing incorporations of dietary nitrogen from this biofloc batch were not correlated to higher growth. Results from growth parameters observed in the present study also indicate that the dietary inclusion of biofloc meal at levels above 25% of the available dietary nitrogen in low protein diets correlates with lower shrimp growth. Future studies applying isotopic methodologies will focus on evaluating the contribution to growth of dry biofloc obtained from shrimp culture systems and on testing commercially available products consisting in microbial biomass.

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