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Comparing the assimilation of dietary nitrogen supplied by animal-, plant- and microbial-derived ingredients in Pacific white shrimp *Litopenaeus vannamei*: A stable isotope study

Julián Gamboa-Delgado*, Martha G. Nieto-López, Maribel Maldonado-Muñiz, David Villarreal-Cavazos, Mireya Tapia-Salazar, Lucía Elizabeth Cruz-Suárez

Programa Maricultura, Departamento de Ecología, Facultad de Ciencias Biológicas Universidad Autónoma de Nuevo León, UANL. San Nicolás de los Garza, Nuevo León, Mexico

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ABSTRACT

In order to become a more profitable and sustainable industry, the aquaculture sector is constantly exploring alternative nutrient sources. In the present study, the natural stable isotope signatures of different animal-, plant- and microbial-derived ingredients were determined to evaluate the assimilation of dietary nitrogen in Pacific white shrimp. Experimental diets were manufactured to replace fish meal and squid meal with microbial- and plant- derived ingredients (48 and 66 % dietary inclusion) and were also used as additives (4%). Ingredients were also used as additives to study their effects on growth performance and assimilation. Corn gluten, soy protein isolate, *Arthrospira* (*Spirulina*) biomass and a bacterial meal (ProFloc™) were used to formulate six, isotopic control diets containing one source of dietary nitrogen, while six combined diets had varying proportions of ingredients. At the end of the trial, survival rates were similar among treatments (88 ± 9%) but there were significant differences in mean final weight. Microbial and animal proteins promoted similar final weight when combined (1080 − 1537 mg), while plant ingredients and *Arthrospira* caused lower growth when used alone (420 − 970 mg). Isotopic values indicated significant differences in the assimilation proportions of dietary nitrogen, mainly attributed to the ingredients' different amino acid profiles. The dietary nitrogen contained in microbial-derived ingredients was assimilated at similar, or even higher proportions than fish meal and squid meal. Diet formulated with 33 % fish meal, *Arthrospira* and corn gluten, contributed 31, 36 and 33 % of dietary nitrogen to muscle growth, respectively. The second 33 % combination supplied 42, 34 and 24 % from squid meal, bacterial meal and soy protein. When ingredients were used at 4%, additive levels, they also contributed structural nitrogen to shrimp muscle tissue despite low dietary inclusions. Results demonstrated the viable use of stable isotopes to evaluate the assimilation of dietary nitrogen supplied by emerging alternative ingredients.

1. Introduction

In 2015, fish provided about 3.2 billion people with 20 % of their average per capita intake of animal protein (FAO, 2018). Over the last 40 years, the aquaculture industry has developed into one of the most important economic sectors and it estimated that on the following years, aquaculture production will exceed landings from industrial fisheries. The growth rate of aquaculture has, in turn, encouraged the development of other subsidiary activities that support it, such as the production of feeds and additives. During the period from 1995 to 2015, the production of farmed aquatic species increased from 12 to 51 million tonnes, largely through intensification of production methods for shrimp, tilapia, carp and salmonids (Hasan, 2017). Today, 66 % of total global aquaculture production (excluding macroalgae) requires using exogenous feed (FAO, 2018). In order to manufacture these aquaculture feeds, fish meal is used as one of the main ingredients and it has been estimated that around 70 % of the global production of fish meal can be utilized for such end (Tacon and Metian, 2008). Even small pelagic fish are currently under pressure as they are captured and used to manufacture fish meal, hence exacerbating the overexploitation of marine species. As animal-derived proteins greatly contribute to the production costs of aquaculture, this industrial sector has welcomed the use of alternative ingredients for compound feeds. Plant proteins can be economically viable and nutritionally suitable for aquatic organisms

* Corresponding author.
E-mail addresses: julian.gamboad@uanl.mx, jgam97@yahoo.com (J. Gamboa-Delgado).

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when used as partial replacement for fish meal. However, they might be unsuitable for carnivorous species (Romarheim et al., 2011) and, on the other hand, the limited availability of water and arable land will impose future restrictions to satisfy the growing demand of plant meals (Malcorps et al., 2019). In view of this, there has been growing interest in exploring alternative nutrients, such as those derived from different types of microorganisms. The term "single cell protein" refers to the biomass extracted from microorganisms or to specific, isolated products. There are clear advantages in the use of microorganisms to produce biomass, and these include fast growth rates, adaptability to different culture media and efficient use of space. On the other hand, the nutritional properties of many types of microbial biomass have been reported as highly suitable when used as fish meal replacements in diets for terrestrial and aquatic animals. These positive impacts range from growth promoting effects to improvements in survival, pigmentation, immune status and reproductive performance (Gamboa-Delgado and Márquez-Reyes, 2018). Therefore, efforts to escalate production of microalgae, bacteria and yeasts are focusing on innovative, energetically-efficient methods (Acién et al., 2012; Duong et al., 2015). Some of these, patented processes, recycle agricultural and industrial residues to sustainably generate microbial biomass (Lee and Kim, 2011; WEF, 2015). Different types of microbial biomass (or their purified products) have been deemed as promising nutrient sources for animal nutrition.

The Pacific white shrimp (*Litopenaeus vannamei*) is the dominant shrimp species produced through aquaculture practices, and farm production has surpassed production derived from wild catches (FAO, 2007). In addition to its economic importance, this species represents an excellent invertebrate model organism not only for its availability and domestication, but also for its physiological characteristics such as omnivore habits, salinity tolerance and fast growth rates. Assessment of the suitability of new aquaculture feed ingredients is typically conducted through bioassays that compare physiological and chemical parameters. Observations on the ingestion-digestion-assimilation processes are helpful to further characterize specific feed stuffs. The biological assimilation can be defined as the absorption of nutrients in the gastrointestinal tract, followed by a chemical modification and a final physiological use (metabolism, tissue biosynthesis). Indirect methods to measure assimilation include the use of indigestible markers in food and faeces (Goodman-Lowe et al., 1999) and the assessment of energy loss as excretory products (Drazen et al., 2007). Recent studies have applied measurements of stable isotopes (at natural abundance levels) to draw inferences on the physiological allocation of nutrients available in alternative ingredients. Due to their natural abundance in organic matter, carbon and nitrogen have been used to determine their stable-isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$, respectively expressed as delta notation, $\delta^{13}C$ and $\delta^{15}N$). Such values can be used as dietary markers and represent a powerful complementary tool in nutritional studies. Organisms acquire different isotopic signatures from their specific environments and feeding items (wild, farmed). In the specific case of aquaculture systems, organisms are grown in confined environments where they receive few feeding items. Such systems are thus very amenable to conduct nutritional studies that employ isotopic techniques. The changing ratio of stable isotopes in tissues of animals kept under controlled conditions can be used to determine the contribution of dietary sources to growth (Phillips, 2012). By integrating such isotopic changes with growth and time data, the carbon and nitrogen turnover rates can also be estimated (deVries et al., 2015). The main aim of the present study was to use the Pacific white shrimp as a model organism to compare the relative assimilation proportions of the dietary nitrogen derived from animal, plant and microbial sources. Additionally, the nitrogen turnover rates and isotopic discrimination factors between experimental diets and muscle tissue were evaluated to denote different nutritional conditions.

2. Materials and methods

2.1. Experimental design and rearing system

Pacific white shrimp (*Litopenaeus vannamei*) postlarvae were obtained from a commercial hatchery (AcuaMar, Baja California Sur, Mexico). After transport, animals were acclimated to bioassay room conditions (seawater temperature 29.2 ± 1.5 °C, salinity 32.4 ± 0.6 g l^{-1}, pH 8.3 ± 0.1 and saturated dissolved oxygen). These parameters and the nitrogenous compounds levels were maintained within recommended ranges for Penaeid shrimps throughout the bioassay. Shrimps were offered a commercial compound diet (37 % crude protein, Grupo Costamar, Hermosillo, Mexico) previously analyzed for elemental and isotopic values ($\delta^{15}N$ and $\delta^{13}C$). After 15 days, this diet allow establishing reference, known isotopic values in shrimp before the start of the experiment. At the beginning of the bioassay, twenty shrimps (initial mean wet weight 360 ± 36 mg) were placed in triplicate, 60-L glass fiber tanks. Care was taken to distribute animals with the same size distribution pattern in each unit. Individual tanks were fitted with air-water lifts and were interconnected to allow circulation of artificial seawater (Instant Ocean® Blacksburg, VA, USA). Seawater flowed through cartridge and UV filters, protein skimmers and a bubble bead biological filter.

2.2. Experimental diets and sampling procedure

Twelve isonitrogenous (35 % crude protein) and isoenergetic (4.7 kcal/g) experimental diets were formulated with six main ingredients. Fish meal (sardine) and squid meal (*Dosidicus gigas*) represented two, well referenced sources of animal-derived protein. Corn gluten and soy protein isolate were used as plant ingredients due to its proven nutritional performance when used in diets for this species. Microbial ingredients consisted in *Arthrospira* (*Spirulina*) biomass collected from intensive commercial cultures (Pronat, Iztapalapa, Mexico) and a bacterial-derived meal produced using a patented technology that up cycles nutrients from food and beverage wastewater treatment plants (ProFloc™, Nutrinsic Corporation, Boulder, CO). This ingredient has a high protein content (> 60 %) and favorable amino acid profile. It has also been shown that it provides high digestibility and palatability to terrestrial and aquatic animals (Wen, 2018).The production process modifies the biological conditions in wastewater systems to favour the growth of specific, protein producing bacteria. The produced biomass is harvested, concentrated, dried and sterilized to produce a single cell protein intended for animal feeds (WEF, 2015). From the proximal analysis data of individual ingredients, the experimental diets were formulated using the software Nutrion® (Nutrion, Chapala, Mexico).

Experimental diets were formulated using ingredients having contrasting isotopic values for carbon and nitrogen. Specific isotopic values of ingredients are defined by their origin (marine, terrestrial) and/or metabolism (photosynthesis type). Such selection allowed estimating the nutritional contributions of dietary nitrogen to shrimp muscle growth. Six diets were formulated with only one ingredients supplying protein (Diets 1–6) and represented isotopic controls. Six more diets were manufactured with different proportions of dietary nitrogen supplied by animal-, plant- and microbial derived biomass. Diet 7 was formulated with equivalent proportions (33 % of dietary nitrogen) of fish meal, *Arthrospira* (*Spirulina*) and corn gluten, while diet 8 contained squid meal, bacterial meal and soy protein isolate (33 %). Diets 9–12 were manufactured with low dietary inclusions (on a dietary nitrogen basis) of microbial and plant-derived ingredients (4 %) and high proportions (96 %) of animal/plant or animal/microbial protein combinations. Micronutrients were weighed and mixed and then added to the ground (< 200 um) macronutrients. The resulting mixture was homogenized for 15 min using a blender. Water was slowly added to form dough and it was pressed through a die plate (1.6 mm orifices) to form strands. Strands were collected and dried overnight (40 °C) in a

convection oven and were finally crushed to form small pellets.

Proximal analyses of the experimental diets were conducted as described in Gamboa-Delgado et al. (2016). Experimental diets were delivered in excess at 8:00, 12:00, 16:00 and 20:00 h for 21 days. Uneaten feed and faeces debris were siphoned out daily before first feeding. Feeding rations were progressively adjusted in relation to observed weight gain and number of sampled animals. Experimental sampling points were defined according to the exponential rate of isotopic shift frequently observed in fast growing Decapod crustaceans (Gamboa-Delgado et al., 2011). The individual wet weight of all animals was registered throughout the feeding period in order to estimate treatment-dependent specific growth rates (SGR). On experimental days 0, 4, 8 and 15, one or two shrimps (depending on size) were collected from every replicate to measure isotopic changes. At the end of the feeding trial, animals were euthanized in ice/water slurry, weighed and dissected to extract muscle tissue. All samples were kept frozen at −80 °C until analysis.

2.3. Sample pretreatment, amino acid and stable isotope analyses

Shrimp muscle and ingredient samples were dehydrated in a convection oven (60 °C until constant weight). Dry samples were manually ground to obtain a fine, homogenized powder. The amino acid profile of the main ingredients was determined at the Agricultural Experiment Station of the University of Missouri (Association of Official Analytical Chemist (AOAC, 2006). For the stable isotope analysis, diet and shrimp samples of 1 mg were packed in tin cups (Elemental Microanalysis Ltd., Okehampton, UK). Dual (carbon and nitrogen) analysis were done at the Stable Isotope Facility, University of California, (Davis, CA, USA) using a Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., UK). Glutamic acid, nylon and bovine liver were used as internal calibration standards, while enriched L-glutamic acid (USGS-41, $\delta^{15}N_{AIR}$ = 47.6‰, $\delta^{13}C_{VPDB}$ = 37.6‰) was used as international standard to calibrate internal standards. Instrument precision (SD) was 0.09‰ for $\delta^{15}N$ values and 0.13‰ for $\delta^{13}C$ values. Isotopic results are expressed in delta notation (δ), which is defined as *per mill* (‰) deviations from the isotopic values of standard reference materials (N in air and C in belemnite). The latter reference material is highly enriched in ^{13}C, therefore, comparison with most organic samples (depleted in ^{13}C) results in negative values. The term "discrimination factor" is used in this study to describe differences in isotopic values between shrimp muscle and their respective diets/ingredients ($\Delta^{15}N$ and $\Delta^{13}C$) after isotopic equilibrium was reached or approached (± 0.5‰).

2.4. Estimation of dietary nitrogen contribution

The relative assimilation proportions of dietary nitrogen (protein) supplied by the three types of ingredients was estimated using a mass-balance, elemental concentration-dependent, isotope mixing model (Phillips and Koch, 2002). Fundamental assumptions required by the models (Martínez del Rio et al., 2009a, 2009b; Post, 2002) were met or considered for the calculations (*e.g.* isotopic equilibrium between diet and consumer, knowledge of elemental concentrations in experimental ingredients). Isotopic discrimination factors were obtained from the isotopic differences observed between control diets and control shrimps, integration of such isotopic corrections into the mixing models tends to increase the precision of results (Phillips, 2012). An indicator of treatment-specific variability of the nutritional contributions was obtained by introducing into the model isotopic values measured in individual animals (not mean values).

2.5. Nitrogen residency times in shrimp bodies

$\delta^{15}N$ and $\delta^{13}C$ values were determined at different times of the experimental period and were introduced into an exponential model (Eq. 1) (Hesslein et al., 1993) that allows separating the isotopic change caused by growth (k) or metabolic turnover (m).

$$C_{sample} = Cn + (Co - Cn)e^{-(k+m)t} \quad (1)$$

where C_{sample} is the isotope value in shrimp tissue at time t, Co is the isotope value of shrimp tissue in equilibrium with the initial (basal) diet, Cn is the isotope value reached when animals are in equilibrium with the new diet(s). The growth rate, k, was estimated by fitting an exponential growth model to observed weight data, k = log(final weight/initial weight)/time (d). Using growth, time and isotope data, parameter m was calculated by means of iterative nonlinear regression. Coefficients k and m provide an indicator of the time period necessary for half of the constituent nitrogen to be replaced in muscle tissue after animals consume a new diet (half time, t_{50}) (Eq. 2) (MacAvoy et al., 2005).

$$t_{50} = In2/m + k \quad (2)$$

where $In2$ represents the natural logarithm of 2.

2.6. Statistical analysis

Final mean weight and survival, and dietary effects on isotopic values in shrimp muscle were analyzed by one-way ANOVA followed by pair-wise comparisons after normal distribution and homoscedasticity were confirmed. In order to compare the expected proportions of dietary nitrogen (available in the different diets) and the observed proportions of dietary nitrogen actually assimilated in shrimp muscle tissue, Chi-square goodness of fit tests (X^2) were applied. All tests were conducted using SPSS 17.0 software (SPSS Inc.) at a significance level of $P < 0.05$.

3. Results

3.1. Dietary effects on specific growth and survival rates

During the experimental feeding period, water quality parameters in the experimental tank array remained within the recommended optimal values for this species. At the end of the feeding trial, no significant differences were observed in survival rates (p = 0.078), but there were significant differences in the treatment-dependent specific growth rates (p < 0.001).

While most diets elicited similar, high SGR, the control diets containing corn gluten and *Arthrospira* caused significantly lower weight. SGR were higher in animals fed diets having combined ingredients, except those fed diet containing 48 % fish meal, 48 % corn gluten and 4% *Arthrospira*. The bacterial meal, as a sole ingredient, elicited similar SGR as most of the treatments, which was also the case when used as additive (4%) or at high animal protein replacement levels (33–48 %). Diets containing corn gluten and soy protein at additive levels also promoted SGR similar to those observed in animals fed on diets containing fish meal or squid meal.

3.2. Isotopic influence of diets and nitrogen residency times in muscle tissue

The conditioning diet allowed establishing basal isotopic values of $\delta^{15}N$ = 9.7‰ and $\delta^{13}C$ = -20.5‰ in shrimp muscle tissue. Over the first few days of the nutritional trial, there was a fast isotopic influence on shrimp muscle tissue exerted by the respective experimental diets (Fig. 1a–d). The different $\delta^{15}N$ and $\delta^{13}C$ values of experimental ingredients caused fast and contrasting isotopic shifts on the shrimps under different treatments. Such differences conferred better resolution to the isotopic mixing model and allowed estimating dietary contributions from the three types of ingredients. Isotopic equilibrium between shrimp bodies and the respective diets was reached between days 15 and 21 (Fig. 1). When isotopic trends did not fully reached steady state

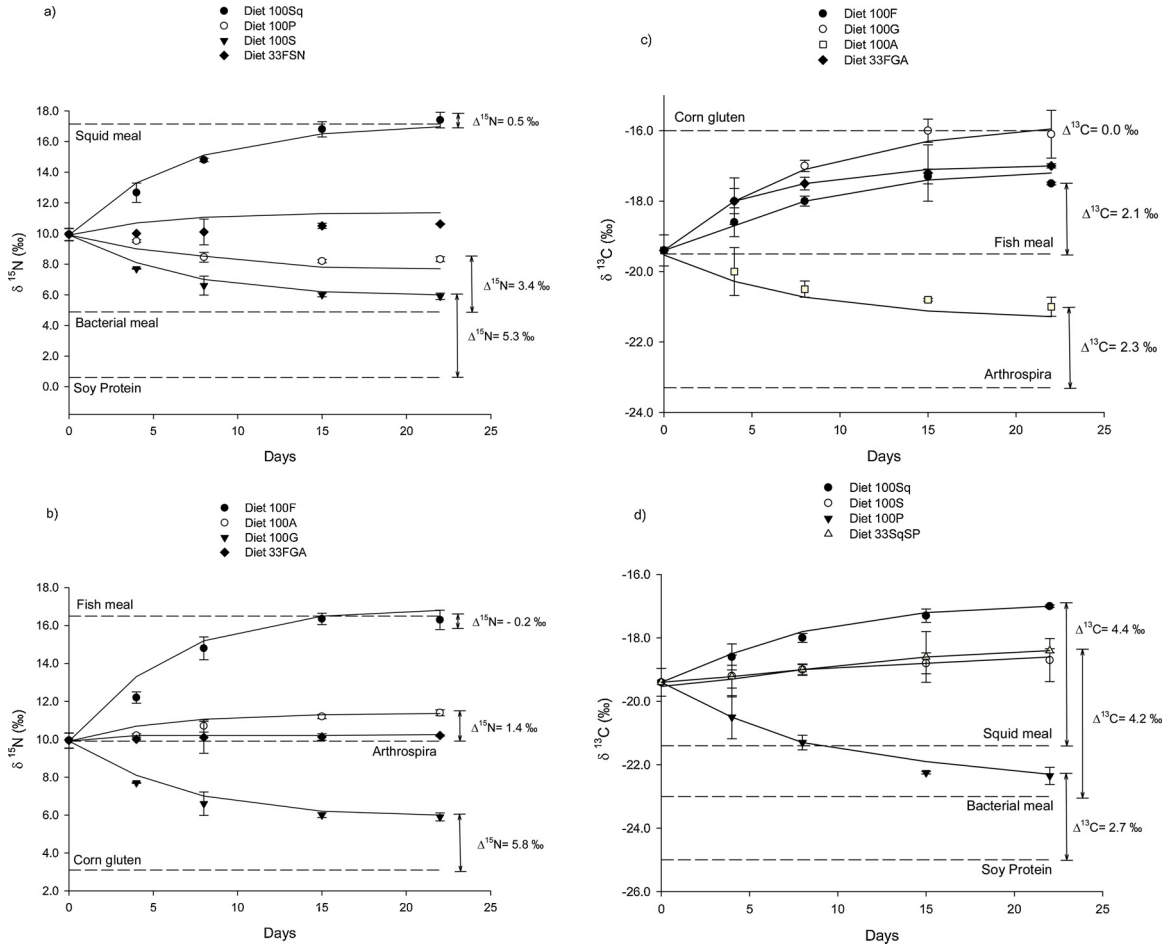

Fig. 1. Changes in nitrogen (1a and 1b) and carbon (1c and 1d) stable isotope values (‰) in shrimp muscle tissue after a dietary shift from a conditioning diet to six control diets and two combined diets having similar proportions of dietary nitrogen (33 %) derived from animals, plants and microorganisms. Lines represent predicted values and show the best fit to observed data. Arrows indicate isotopic discrimination factors between control diets and shrimps. n = 3 individuals, 9 on final day. Values of diets containing ingredients at additive level are not shown as they strongly resembled the isotopic values of main ingredients.

(*e.g.* $\delta^{15}N$ values in diet 100Sq), asymptotic values generated by non-linear regression were used to estimate nutritional contributions. Isotopic discrimination factors between shrimp muscle and their different diets ranged from -0.2–6.0 ‰ for nitrogen ($\Delta^{15}N$) and from 0.0–4.4‰ for carbon ($\Delta^{13}C$).

Higher $\Delta^{15}N$ were observed between shrimps and diets containing plant proteins (5.1 and 6.0‰), followed by lower values caused by the microbial sources (1.4 and 3.4‰) and very small for animal-derived meals (-0,2 and 0.3‰). Such trend was not observed for $\Delta^{13}C$ values. Estimated nitrogen turnover rates in shrimps under the different treatments showed a high variability (0.020 - 0.062 d^{-1}, Table 4). These values were not correlated to type of ingredient or combination used. The lowest nitrogen turnover rate was determined in muscle tissue of shrimp fed diet 100 P (bacterial meal). It was not possible to estimate the nitrogen turnover rate for diets having 33 % combinations because the dietary shift from basal diet to these diets did not elicit exponential trends in the isotopic changes. Nitrogen half times (t_{50}) in shrimp muscle ranged from 6.3–10.0 days. Higher values corresponded to animals fed on diet 100 G and 4AFG, containing 100 % and 48 % of corn gluten, respectively. The former diet caused the lowest observed SGR. Animal-derived ingredients, the bacterial meal, and most combined diets elicited low residency times in tissue (nitrogen), which is associated to high growth rates. In general, carbon half times were shorter than those determined for nitrogen, and ranged from 3.3–4.6 days (Table 1)

3.3. Estimation of relative assimilation proportions

Isotopic data from ingredients and shrimp samples and the ensuing integration into the isotope mixing model indicated that, in most cases, the contribution of assimilated dietary nitrogen was not statistically similar to the proportions of dietary nitrogen established in the experimental diets (Table 2 and Fig. 2). Diet 33SqSP, formulated with 33 % of dietary nitrogen from squid meal, soy protein and bacterial meal, supplied 42, 24 and 34 % to growth. In contrast, similar dietary nitrogen contributions were observed in animals fed diet 33FGA, which supplied 31, 33 and 36 % of dietary nitrogen from fish meal, corn gluten and *Arthrospira*. When ingredients from plant or microbial origin were used at additive levels in diets containing combinations of fish meal and plant proteins or fish meal and microbial ingredients, it was observed that such low dietary inclusions levels still promoted assimilation proportions that were higher than the established 4 % dietary values. Relative assimilation proportions were 10.5 % for corn gluten, 11.6 % for *Arthrospira*, and 9.5 % for the bacterial meal, as compared to the inclusion level established in the diets (4% on a dietary nitrogen basis). The only exception was the soy protein isolate, which supplied a slightly lower proportions of dietary nitrogen to growth (3.6 %) when use as additive, while the other ingredients in this combination, fish meal and bacterial meal, supplied 44 and 53 %, respectively (Table 3)

Table 1
Proximate, isotopic and essential amino acid composition (gr 100 gr protein^{-1}) of ingredients derived from animals, plants and microorganisms. Experimental diets were formulated to compare specific growth rates, survival and assimilation proportions using stable isotope values as biomarkers. n = 3.

	Fish meal	Squid meal	Corn gluten	Soy Protein	*Arthrospira*	Bacterial meal
Crude protein (gr kg^{-1})	720	689	704	885	594	686
Lipids (gr kg^{-1})	86	65	44	1	22	29
Ash (gr kg^{-1})	83	85	29	67	73	210
Fiber (gr kg^{-1})	10.0	6.7	15.0	31.0	12.0	nd
NFE (gr kg^{-1})	101	154	208	16	299	75*
Moisture (%)	5.9	6.3	10.0	7.8	7.9	9.0
δ^{15}N (‰)	16.5	17.1	3.1	0.6	9.9	4.8
δ^{13}C (‰)	−19.5	−21.4	−16.0	−25.0	−23.3	−23.0

Aminoacid							Recomm. for Penaeid shrimp**
Arginine	6.43	3.86	3.21	7.67	7.20	5.34	4.68
Isoleucine	4.53	6.42	4.06	4.80	5.94	4.54	2.37
Phenylalanine	4.33	3.83	6.27	5.42	4.80	4.57	2.71
Histidine	3.49	4.40	2.01	2.67	1.61	2.24	1.87
Leucine	7.79	6.06	15.99	8.13	9.29	7.64	3.99
Lysine	8.60	9.16	1.80	6.32	4.91	5.08	4.93
Methionine	2.80	2.47	2.08	1.26	2.30	2.07	2.37
Threonine	4.51	4.58	3.25	3.90	5.09	5.28	3.30
Tryptophan	1.16	2.0	0.45	–	0.47	1.28	0.49
Valine	5.41	6.3	4.58	4.8	6.72	6.73	3.20

* NFE (Nitrogen free extract) includes fiber for this ingredient. **Average essential amino acid levels recommended for Penaeid shrimps using lysine as a 100 % reference (NRC, 2011). Estimated lysine requirement has been reported as 4.93 % of dietary protein (Xie et al., 2012).

4. Discussion

4.1. Shrimp specific growth and survival rates

Observed tendencies in final shrimp weight among treatments indicated that most dietary treatments performed as well as those formulated with fish meal or squid meal. As experimental diets were isonitrogenous and isocaloric, lower specific growth rates promoted by diets containing corn gluten or *Arthrospira* biomass might be attributed to less favorable amino acid profiles than those found in animal-derived ingredients. In the present study, all combined diets caused high SGR in shrimps, except diet 4AFG, which contained 48 % fish meal, 48 % gluten and 4 % *Arthrospira* biomass. Lower levels or lysine, methionine and arginine are present in corn gluten than values recommended for this species (NRC, 2011). On the other hand, *Arthrospira* biomass contains lower histidine levels than those reported as optimal. *Arthrospira* has been reported as an effective fish meal replacement for aquatic animals (Gamboa-Delgado and Márquez-Reyes, 2018) due to its good proximal profile and the fact that *Arthrospira* cells walls do not contain cellulose and it is easily digested (Dillon et al., 1995). In contrast, other species of microalgae require cell wall disruption to improve nutrient bioavailability (Agboola et al., 2019; Teuling et al., 2019). The bacterial

Table 2
Nutritional and isotopic composition of formulated diets fed to Pacific white shrimp (*Litopenaeus vannamei*) to estimate the nutritional contribution of fish meal (F), squid meal (Sq), corn gluten (C), soy protein isolate (S), *Arthrospira* (*Spirulina*) biomass (A) and a bacterial-derived product (ProFloc™) (P) to shrimp muscle tissue.

Ingredient / Diet	100F	100Sq	100 G	100S	100A	100 P	33FGA	33SqSP	4GFA	4AFG	4SFP	4PFS
Fish meal[a]	556.2	0.0	0.0	0.0	0.0	0.0	157.0	0.0	228.0	228.0	228.7	228.0
Squid meal[a]	0.0	505.5	0.0	0.0	0.0	0.0	0.0	168	0.0	0.0	0.0	0.0
Corn gluten[b]	0.0	0.0	600.2	0.0	0.0	0.0	183	0.0	22.1	265	0.0	0.0
Soy protein isolate[c]	0.0	0.0	0.0	449.5	0.0	0.0	0.0	137.0	0.0	0.0	16.5	198.0
Arthrospira (*Spirulina*)[d]	0.0	0.0	0.0	0.0	583.9	0.0	210.0	0.0	306.0	25.5	0.0	0.0
Bacterial meal (ProFloc™)[e]	0.0	0.0	0.0	0.0	0.0	530.3	0.0	176.0	0.0	0.0	254.0	21.2
Wheat starch[f]	348.8	350.3	253.5	390.7	247.1	331.2	297.2	363.2	323.9	369.9	375.5	408.8
Lecithin[g]	35.0	35.0	54.5	54.1	30.0	34.5	43.2	41.1	42.3	39.1	35.4	46.3
Fish oil[a]	24.0	21.8	10.4	34.6	47.5	20.0	23.1	26.0	23.5	22.5	20.7	25.0
Disodium phosphate[h]	0.0	21.1	39.0	20.2	35.0	20.2	25.5	21.6	19.6	15.0	5.1	6.6
Calcium chloride[h]	0	0	11	11	11	11	5	5	5	5	5	5
Cellulose[h]	9.0	38.0	3.0	11.7	17.4	23.9	28.8	33.9	3.0	3.0	32.1	34.1
Cholesterol[g]	0.0	1.3	1.5	1.2	1.1	1.9	0.2	1.31	0.0	0.0	0.0	0.0
Constant ingredients[i]	27	27	27	27	27	27	27	27	27	27	27	27
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Proximal and isotopic analysis												
Crude protein (g kg^{-1})	362	358	365	356	363	356	349	360	355	351	362	365
Lipids (g kg^{-1})	91	87	93	94	88	90	93	89	89	92	94	88
Ash (g kg^{-1})	92	115	80	72	88	147	97	119	105	84	124	109
NFE + Fiber (g kg^{-1})	455	440	462	478	461	407	461	432	451	473	420	438
Gross energy (Kcal g^{-1})	4.6	4.8	4.6	4.8	4.7	4.5	4.5	4.8	4.5	4.8	4.7	4.5
δ^{15}N (‰)	16.5	17.1	3.1	0.6	9.9	4.8	9.5	12.1	12.9	9.8	11.8	9.4
δ^{13}C (‰)	−19.5	−21.4	−16.6	−25.1	−23.1	−23.0	−21.5	−22.8	−22.8	−20.1	−22.4	−23.3

[a] Alimentos Costamar (Sonora, Mexico). [b] Trow Nutrition International (Putten, The Netherlands). [c] American Soybean Association (St. Louis, MO, USA). [d] ProNat (Iztapalapa, Mexico) [e] Nutrinsic Corporation (Glendale, CO, USA). [f] Almidones y gluten S.A. (Monterrey, Mexico). [g] Solvay Pharmaceuticals (Houston, TX, USA). [h] Sigma-Aldrich (St. Louis, MO, USA). [i] Constant ingredients: Alginate 20 kg diet^{-1}. Mineral and vitamin mixes, each 2.5 kg diet^{-1} were formulated according to estimated requirements reported by NRC (2011)., Vitamin C 1 kg diet^{-1}, Antifungic agent 0.5 g kg diet^{-1}, antioxidant 0.5 g kg diet^{-1}.

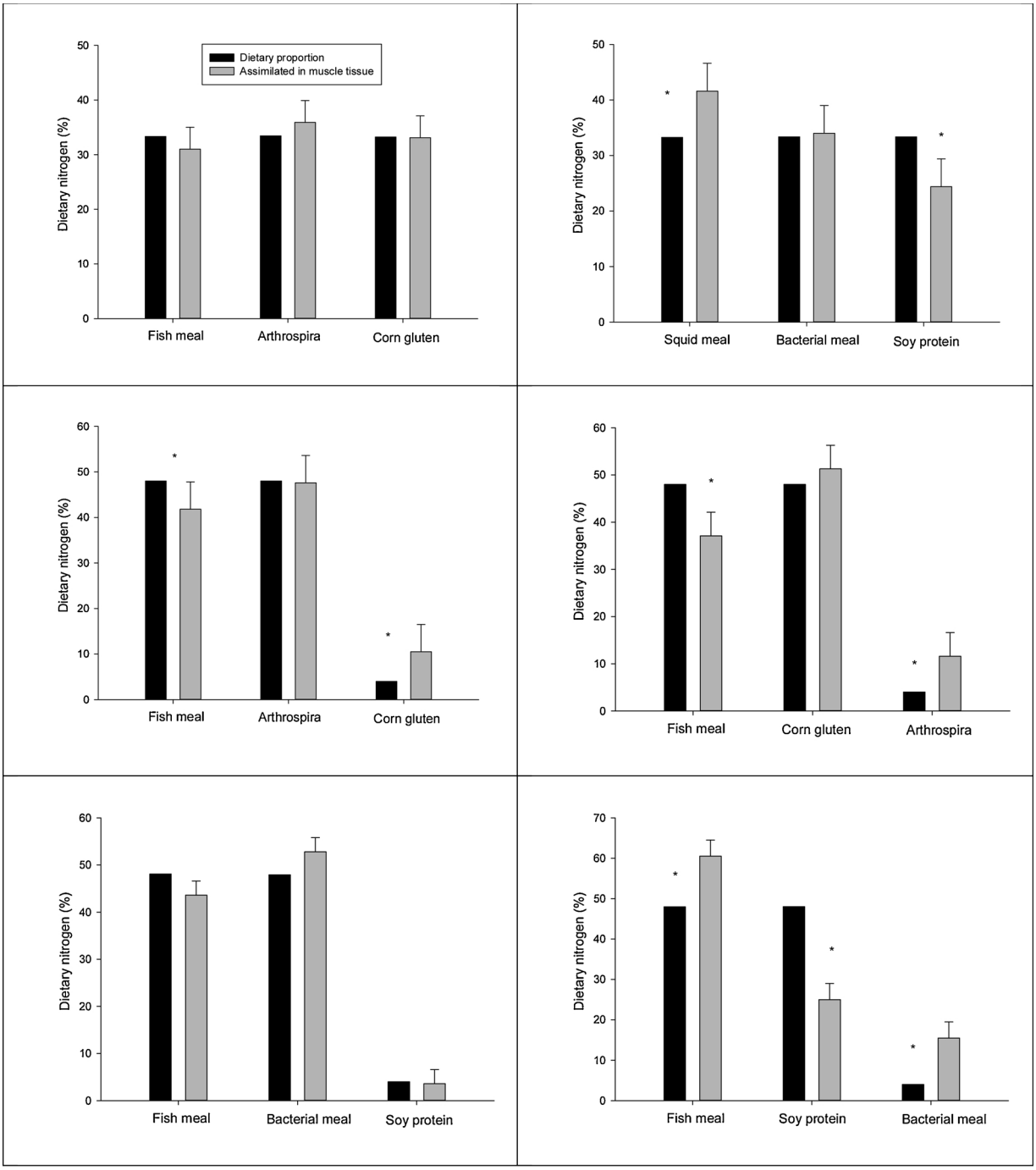

Fig. 2. Estimated relative proportions of dietary nitrogen supplied from animal- plant- and microbial-derived ingredients and their respective assimilated proportions in muscle tissue of Pacific white shrimp *L. vannamei* (mean values ± SD, n = 9 per diet). Columns with asterisk denote significant differences (Chi-square tests, $p < 0.05$) between dietary (expected) and assimilated (observed) proportions.

meal performed well as dietary additive and as a squid meal replacement. Although the amino acid profile of the bacterial meal indicates lower levels of histidine and lysine than fish meal and squid meal, such levels are still higher than those recommended for Penaeid shrimp. Previous studies have applied other types of bacterial products as ingredients in diets for terrestrial and aquatic animals. For example, it has been shown that the biomass obtained from the methanotroph bacteria *Methylococcus capsulatus* is a promising nutritive source of protein due to its amino acid composition, crude protein digestibility (79–85 %), and animal production parameters observed in poultry, pigs and Atlantic salmon (Øverland et al., 2010). The amino acid profile of the latter bacterial meal, and that used in the present study resemble that of fishmeal. Such similarity further suggests that bacterial-derived ingredients can indeed be used as future fishmeal replacements for aquaculture diets.

4.2. Isotopic influence of diets and nitrogen residency times in muscle tissue

Previous studies have shown that $\delta^{15}N$ and $\delta^{13}C$ values in muscle tissue reflect well those measured in whole shrimp bodies (Gamboa-Delgado et al., 2011). Muscle tissue was selected for analysis because it represents the main nitrogen reservoir and as it contains very low lipids levels, hence, lipid extraction was not required to standardize comparisons. Additionally, muscle is a metabolically active tissue and it has

Table 3
Final wet weight (FW, mg), weight gain (WG, %), specific growth rate (SGR) and survival rate (S,%) of Pacific white shrimp L. *vannamei* reared on diets having different dietary proportions of fish meal, squid meal, plant proteins and microbial-derived ingredients. Initial mean wet weight, 360 mg Mean values ± S.D.

Diet	FW	WG	SGR	S
100F	1295 ± 167^{a}	260 ± 32^{a}	6.10 ± 0.51^{a}	86 ± 5^{a}
100Sq	1312 ± 189^{a}	264 ± 29^{a}	6.16 ± 0.54^{a}	91 ± 7^{a}
100 G	420 ± 119^{c}	17 ± 5^{c}	0.73 ± 0.14^{c}	83 ± 12^{a}
100S	1080 ± 101^{ab}	200 ± 19^{ab}	5.23 ± 0.34^{ab}	88 ± 6^{a}
100A	970 ± 147^{b}	169 ± 12^{b}	4.72 ± 0.53^{b}	87 ± 5^{a}
100 P	1248 ± 133^{a}	247 ± 26^{a}	5.92 ± 0.47^{a}	92 ± 6^{a}
33FGA	1284 ± 202^{a}	257 ± 33^{a}	6.06 ± 0.36^{a}	88 ± 4^{a}
33SqSP	1427 ± 215^{a}	296 ± 29^{a}	6.56 ± 0.51^{a}	89 ± 12^{a}
4GFA	1273 ± 188^{a}	254 ± 30^{a}	6.02 ± 0.47^{a}	93 ± 8^{a}
4AFG	1033 ± 165^{ab}	187 ± 27^{ab}	5.02 ± 0.45^{ab}	85 ± 14^{a}
4SFP	1537 ± 231^{a}	327 ± 42^{a}	6.91 ± 0.65^{a}	92 ± 8^{a}
4PFS	1350 ± 208^{a}	275 ± 38^{a}	6.29 ± 0.56^{a}	87 ± 11^{a}
p-value	< 0.001	< 0.001	< 0.001	0.078

Different superscripts indicate significant differences ($p < 0.05$) for that particular column.

a high turnover rate as compared to other tissues. The latter characteristic is ideal to track isotopic changes caused by diet. Due to their different origins, ingredients had very contrasting isotopic values. For example, fish meal and squid meal are isotopically enriched as these are manufactured from pelagic, marine species that occupy intermediate trophic positions in the food webs. As the heavier isotopes (^{15}N, ^{13}C) tend to accumulate in tissues due to metabolic discrimination, animals show higher isotopic values than plants and microorganisms (De Niro and Epstein, 1978). The isotopic values of plants and their products are strongly influenced by photosynthesis type (carbon) (O'Leary, 1988) and the use of inorganic fertilizers (nitrogen). Similarly, the isotopic values of microbial biomass (microalgae, bacteria and yeasts) are highly dependent on culture media characteristics and the intrinsic, metabolic type of each microorganism. The isotopic and nutritional transferences of dietary nitrogen from the experimental ingredients were relatively fast and shrimps reached isotopic equilibrium with their respective diets. Muscle tissue clearly reflected the isotopic values of the main ingredients after experimental day 15; therefore, it can be argued that nutrients supplied by the main ingredients were ingested, digested and assimilated fast. The exponential model of isotopic change indicated that most the isotopic change observed in muscle tissue (nitrogen) was attributed to tissue accretion (50–90%, parameter *k*) and to a lesser extent, by the metabolic turnover rates (10–50%, parameter *m*). The only exception was observed in shrimp fed diet 100 G, where the low SGR caused a higher attribution of *m* to isotopic change (90 %), As elemental carbon turns over faster than nitrogen, the opposite trend was observed for carbon (*i.e.* isotopic changes in tissue were mainly attributed to metabolic turnover rate). Combining parameters *k* and *m* allowed estimating residency times for nitrogen and carbon in muscle tissue (t_{50}). Estimated t_{50} for nitrogen ranged from 6.3–10.0 days and from 3.3–4.6 days for carbon. Such rather short values are consistent with data reported in similar studies where postlarvae of this species have been fed optimal diets (Gamboa-Delgado et al., 2014, 2016). It is considered that low t_{50} in juvenile organisms, indicate high SGR, which is frequently associated to diets having good biological value and thus promoting a reduction in the turnover of proteins (higher anabolism than catabolism) (Mente et al., 2017).

The isotopic discrimination factors for nitrogen ($\Delta^{15}N$) showed a tendency to increase from animals fed on animal derived proteins to those fed on microbial and plant proteins (5.1‰ for soy protein and 6.0‰ for corn gluten). The latter values were significantly higher than the assumed, literature $\Delta^{15}N$ values (3–4 ‰) (Minagawa and Wada, 1984) commonly adopted by ecologists and used to infer trophic interactions. The use of species-specific and tissue-specific $\Delta^{15}N$ values is important to increase the isotope mixing models' precision (Martínez del Rio et al., 2009a, 2009b). Corrected $\delta^{15}N$ values imply the use of isotopic values of organisms fed on specific diets and not the isotopic value of the diets *per se*. In previous studies on isotope dynamics, some authors have suggested that high $\Delta^{15}N$ values are observed in animals fed nutritionally incomplete diets. Recent studies conducted on terrestrial and aquatic organisms have also reported decreasing $\Delta^{15}N$ values as a function of higher dietary protein quantity and quality (Caut et al., 2009). The overall assumption from these studies is that lower $\Delta^{15}N$ values indicate a more direct physiological use of nutrients. In contrast, nutrient restrictions might elicit higher metabolic cycling of nutrients, thereby increasing the $\Delta^{15}N$ values due to additional accumulation of heavier isotopes in tissues. In the present study, it can be stated that among all the supplied experimental diets, only diet containing corn gluten elicit important nutritional restrictions (three limited essential amino acids) as indicated by lower SGR, higher t_{50} and $\Delta^{15}N$ values. It also interesting to underline the observation that $\Delta^{15}N$ values showed a tendency to increase from ingredients having near optimal amino acids profiles to plant ingredients having amino acid restrictions. Discrimination factors for carbon ($\Delta^{13}C$) observed in this study were higher (2.2–4.4 ‰) than the average value reported in the literature (1.0‰). The only exception was observed between muscle tissue and corn gluten (0.0‰). Although we do not have a certain explanation for such isotopic enrichments, they might be attributed to the $\delta^{13}C$ values of

Table 4
Mean specific growth rates (*k*, d^{-1}) and estimated half times (d) of nitrogen and carbon in muscle tissue of Pacific white shrimp L. *vannamei* reared under diets having different dietary proportions of animal- plant- and microbial-derived ingredients. $\Delta^{15}N$ and $\Delta^{13}C$ (‰) represent the isotopic difference between dietary ingredients and muscle tissue values (isotopic discrimination factors).

Diet	Nitrogen		Carbon				
	k	*m*	half time	$\Delta^{15}N$	*m*	half time	$\Delta^{13}C$
100F	0.061 ± 0.005^{a}	0.023 ± 0.004^{a}	8.3 ± 0.6^{a}	−0.2	0.126 ± 0.014^{a}	3.7 ± 0.9^{a}	2.1
100Sq	0.062 ± 0.005^{a}	0.032 ± 0.007^{b}	7.4 ± 1.0^{a}	0.3	0.119 ± 0.027^{b}	3.8 ± 1.1^{b}	4.4
100 G	0.007 ± 0.001^{c}	0.062 ± 0.006^{c}	10.0 ± 1.3^{b}	6.0	0.149 ± 0.016^{c}	4.4 ± 0.4^{a}	0.0
100S	0.052 ± 0.003^{ab}	0.038 ± 0.003^{a}	7.7 ± 0.8^{a}	5.1	0.157 ± 0.011^{a}	3.3 ± 0.5^{a}	2.7
100A	0.047 ± 0.005^{a}	0.040 ± 0.004^{a}	8.0 ± 0.5^{a}	1.4	0.106 ± 0.009^{a}	4.5 ± 0.7^{a}	2.3
100 P	0.059 ± 0.005^{a}	0.051 ± 0.005^{a}	6.3 ± 1.0^{a}	3.4	0.091 ± 0.030^{a}	4.6 ± 1.0^{a}	4.2
33FA	0.060 ± 0.004^{ab}	n.d.	n.d.	0.7	0.119 ± 0.018^{ab}	3.9 ± 0.5^{a}	3.4
33SqP	0.066 ± 0.005^{a}	n.d.	n.d.	0.5	0.126 ± 0.022^{a}	3.6 ± 0.9^{a}	4.0
4GFA	0.060 ± 0.007^{a}	0.040 ± 0.007^{b}	6.9 ± 1.1^{a}	3.1	0.111 ± 0.032^{b}	4.0 ± 1.1^{a}	2.1
4AFG	0.050 ± 0.004^{b}	0.020 ± 0.006^{c}	9.9 ± 0.7^{b}	1.9	0.099 ± 0.019^{c}	4.6 ± 0.6^{a}	2.6
4SFP	0.069 ± 0.006^{a}	0.027 ± 0.003^{a}	7.2 ± 1.1^{a}	0.3	0.127 ± 0.021^{a}	3.5 ± 1.2^{a}	4.4
4PFS	0.063 ± 0.006^{a}	0.044 ± 0.004^{a}	6.5 ± 0.7^{a}	4.6	0.136 ± 0.038^{a}	3.5 ± 0.7^{a}	2.2

Different superscripts indicate significant differences for that particular column. R^2 values ranged from 0.69 to 0.97 and indicate the degree of fitness between data generated by the exponential model and actual isotopic values measured in shrimp muscle tissue.

individual amino acids comprising the different protein types. It has been reported that both, essential and non-essential dietary amino acids, show isotopic enrichment after deposition in tissues (Whiteman et al., 2019).

4.3. Estimation of relative assimilation proportions

The dietary nitrogen available in the experimental diets was, in most cases, assimilated fast and in significantly different proportions than those established in the dietary formulations. It has been previously reported that most ingredients used in the present study show high apparent digestibility coefficients for crude protein when fed to white shrimp (78–96 %) (Cruz-Suárez et al., 2009; Terrazas et al., 2010). It can be stated that both types of microbial biomass (conformed by single cells and their products) offer a higher surface-area-to-volume ratio for the enzymatic attack than the relatively larger ground particles obtained from animal- and plant meals. The latter might play an important role in improving the digestion and ensuing assimilation of the microbial biomass. Moreover, as the transference and uptake of nutrients depends on the bioavailability and biological value of food items, it is very likely that the unequal nutritional contributions might be further explained by the differences in the ingredients' amino acid profiles. Although animal-derived proteins contain higher levels of methionine and lysine than plant-derived ingredients (Galili and Amir, 2013), it is noteworthy that corn gluten contains 100 % more leucine than levels available in fish meal and squid meal. It has been shown that this branch-chained amino acid is the only dietary amino acid that stimulates the protein synthesis, hence decreasing the degradation of muscle tissue (Etzel, 2004). Corn gluten also contained the highest level of phenylalanine among the tested ingredients, which might have contributed to increased assimilation of dietary nitrogen from this source. Although corn gluten caused lower SGR when used as the only protein source, results indicate that this plant-derived ingredient provides a good complementary effect when used in combination with other ingredients. After comparing expected and observed dietary nitrogen contributions for diet FGA, having a 33 % proportion of fish meal, *Arthrospira* and corn gluten, it was observed that ingredients supplied similar amounts of nitrogen. On the other hand, the second 33 % combination (squid meal, bacterial meal and soy protein; diet SqSP), promoted different assimilation proportions. Relative assimilation proportions were higher for squid meal (42 %) and lower for soy protein (24 %). Similarly, the less favorable amino acid profile of soy protein (restriction of methionine and arginine) might have led to lower assimilation, while squid meal and the bacterial meal, despite their high ash contents, contributed to higher assimilation. The bacterial meal contained higher levels of arginine, threonine and phenylalanine than squid meal and its essential amino acid profile exceeds the dietary suggested values for Penaeid shrimp (NRC, 2011). When used at additive levels, the *Arthrospira* biomass, the corn gluten and the bacterial meal, supplied higher proportions of dietary nitrogen than their respective 4 % dietary level. Results are consistent with previous results reporting a detectable assimilation of dietary nitrogen (1.6–5.8 %) even when ingredients are added at 5% levels in diets for shrimp, in the referred study, the biomass of two marine microalgae (*Grammatophora* and *Schizochytrium*) (Pacheco-Vega et al., 2018). A finer isotopic analysis might indicate the origin and fate of different dietary amino acids since it has been demonstrated through compound specific isotopic analysis (CSIA) that the isotopic values ($\delta^{15}N$ and $\delta^{13}C$) of individual amino acids (within the same protein) can be significantly different (Boecklen et al., 2011). Therefore, future studies offer the possibility of using such natural isotopic labeling to explore the transfer of individual, dietary amino acids contributed by animal, plant and microbial proteins.

5. Conclusions

After comparing the nutritional performance of selected animal-plant- and microbial-derived ingredients, it was found that *Arthrospira* biomass and the bacterial meal elicited high SGR when used in combination with other ingredients. The microbial ingredients also promoted a physiological allocation of nutrients in proportions that were similar or higher than those observed in shrimps fed on diets formulated with plant- and animal-derived ingredients. Isotopic measurements greatly assisted in evaluating the nutritional performance of the experimental ingredients. The present study also provides species- and tissue-specific isotopic discrimination factors, which can be used in future studies aimed to determine nutritional contributions. Although the use of many types of microbial biomass as ingredients is still economically prohibitive, improved production techniques are being developed to enhance the nutritional value of microbial products and to explore alternative substrates to lower production costs and increase profitability. It is thus forecast that in the near future, the microbial biomass will be increasingly used as a sustainable feed ingredient.

Declaration of Competing Interest

No conflict of interest has been reported by the co-authors.

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References

Acién, F.G., Fernández, J.M., Magán, J.J., Molina, E., 2012. Production cost of a real microalgae production plant and strategies to reduce it. Antibiot. Chemother. 30, 1344–1353.

Agboola, J.O., Teuling, E., Wierenga, P.A., Gruppen, H., Schrama, J.W., 2019. Cell wall disruption: an effective strategy to improve the nutritive quality of microalgae in African catfish (*Clarias gariepinus*). Aquacult. Nutr. 25 (783), 797.

Association of Official Analytical Chemist (AOAC), 2006. Official Methods of Analysis of AOAC. International. Method 982.30 E (a,b,c), chp. 45.3.05. Association of the Official Analytical Chemist. USA, AOAC International, Gaithersburg, Maryland.

Boecklen, W.J., Yarnes, C., Cook, B.A., James, A.C., 2011. On the use of stable isotopes in trophic ecology. Annu. Rev. Ecol. Evol. S. 42, 411–440.

Caut, S., Angulo, E., Courchamp, F., 2009. Variation in discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$): the effect of diet isotopic values and applications for diet reconstruction. J. Appl. Ecol. 46, 443–453.

Cruz-Suárez, L.E., Tapia-Salazar, M., Villarreal-Cavazos, D., Beltran-Rocha, J., Nieto-López, M.G., Lemme, A., Ricque-Marie, D., 2009. Apparent dry matter, energy, protein and amino acid digestibility of four soybean ingredients in white shrimp *Litopenaeus vannamei* juveniles. Aquaculture 292, 87–94.

De Niro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Act. 42, 495–506.

deVries, M.S., Martínez del Rio, C., Tunstall, T.S., Dawson, T.E., 2015. Isotopic incorporation rates and discrimination factors in mantis shrimp crustaceans. PLoS One 10, e0122334.

Dillon, J.C., Phuc, A.P., Dubacq, J.P., 1995. Nutritional value of the alga Spirulina. World Rev. Nutr. Diet. 77, 32–46.

Drazen, J.C., Reisenbichler, K.R., Robison, K.H., 2007. A comparison of absorption and assimilation efficiencies between four species of shallow- and deep-living fishes. Mar. Biol. 151, 1551–1558.

Duong, V.T., Ahmed, F., Thomas-Hall, S.R., Nowak, K., Schenk, P.M., 2015. High protein- and high lipid-producing microalgae from outback Australia as potential feedstock for animal feed and biodiesel. Front. Bioeng. Biot. 3, 53.

Etzel, M.R., 2004. Manufacture and use of dairy protein fractions. J. Nutr. 134, 996S–1002S.

FAO, 2007. The State of the World Fisheries and Aquaculture (SOFIA) 2006. FAO Fisheries and Aquaculture Department, Rome, Italy 180 pp.

FAO, 2018. In: The State of World Fisheries and Aquaculture 2018 - Meeting the Sustainable Development Goals. Rome. Licence. CC BY-NC-SA 3.0 IGO.

Galili, G., Amir, R., 2013. Fortifying plants with the essential amino acids lysine and methionine to improve nutritional quality. Plant Biotechnol. J. 11, 211–222.

Gamboa-Delgado, J., Márquez-Reyes, J.M., 2018. Potential of microbial-derived nutrients for aquaculture development. Rev. Aquacult. 10, 224–246.

Gamboa-Delgado, J., Peña-Rodríguez, A., Ricque-Marie, D., Cruz-Suárez, L.E., 2011. Assessment of nutrient allocation and metabolic turnover rate in Pacific white shrimp *Litopenaeus vannamei* co-fed live macroalgae *Ulva clathrata* and inert feed: dual stable isotope analysis. J. Shellfish Res. 30, 969–978.

Gamboa-Delgado, J., Castañeda-Solis, J.D., Nieto-López, M.G., Cruz-Suárez, L.E., 2014. Isotopic evaluation of the nutritional contribution of poultry by-product meal and fish meal to the growth of white shrimp, *Litopenaeus vannamei*. J. World Aquacul. Soc. 45, 430–438.

Gamboa-Delgado, J., Fernández-Díaz, B., Nieto-López, M.G., Cruz-Suárez, L.E., 2016. Nutritional contribution of torula yeast and fish meal to the growth of shrimp *Litopenaeus vannamei* as indicated by natural nitrogen stable isotopes. Aquaculture 453, 116–121.

Goodman-Lowe, G.D., Carpenter, J.R., Atkinson, S., 1999. Assimilation efficiency of prey in the Hawaiian monk seal (*Monachus schauinslandi*). Can. J. Zool. 77, 653–660.

Hasan, M.R., 2017. Feeding Global Aquaculture Growth 56. FAO Aquaculture Newsletter, pp. ii–iii.

Hesslein, R.H., Hallard, K.A., Ramlal, P., 1993. Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$. Can. J. Fish. Aquat. Sci. 50, 2071–2076.

Lee, B.-K., Kim, J.K., 2011. Production of *Candida utilis* on molasses in different culture types. J. Aquac. Eng. Fish. Res. 25, 111–124.

MacAvoy, S.E., Macko, S.A., Arneson, L.S., 2005. Growth versus metabolic tissue replacement in mouse tissues determined by stable carbon and nitrogen isotope analysis. Can. J. Zool. 83, 631–641.

Malcorps, W., Kok, B., van't Land, M., Fritz, M., van Doren, D., Servin, K., van der Heijden, P., Palmer, R., Auchterlonie, N.A., Rietkerk, M., Santos, M.J., Davies, S.J., 2019. The sustainability conundrum of fishmeal substitution by plant ingredients in shrimp feeds. Sustainability 11, 1212.

Martínez del Rio, C., Wolf, N., Carleton, S.A., Gannes, L.Z., 2009a. Isotopic ecology ten years after a call for more laboratory experiments. Biol. Rev. 84, 91–111.

Martínez del Rio, C., Wolf, N., Carleton, S.A., Gannes, L.Z., 2009b. Isotopic ecology ten years after a call for more laboratory experiments. Biol Rev. 84, 91–111.

Mente, E., Coutteau, P., Houlihan, D.F., Davidson, I., Sorgeloos, P., 2017. Protein turnover, amino acid profile and amino acid flux in juvenile shrimp *Litopenaeus vannamei*: effects of dietary protein source. J. Exp. Biol. 205, 3107–3122.

Minagawa, M., Wada, E., 1984. Stepwise enrichment of N-15 along food chains – further evidence and the relation between delta-N-15 and animal age. Geochim. Cosmochim. Act. 48, 1135–1140.

NRC, 2011. National Research Council. Nutrient Requirements of Fish and Shrimp. National Academies Press, Washington, DC 376 pp.

O'Leary, M.H., 1988. Carbon isotopes in photosynthesis. Bioscience 38, 328–336.

Øverland, M., Tauson, A.-H., Shearer, K., Skrede, A., 2010. Evaluation of methane- utilising bacteria products as feed ingredients for monogastric animals. Arch. Anim. Nutr. 64, 171–189.

Pacheco-Vega, J.M., Gamboa-Delgado, J., Alvarado-Ibarra, A.G., Nieto-López, M.G., Tapia-Salazar, M., Cruz-Suárez, L.E., 2018. Nutritional contribution of fish meal and microalgal biomass produced from two endemic microalgae to the growth of shrimp *Penaeus vannamei*. Lat. Am. J. Aquat. Res. 46, 53–62.

Phillips, D.L., 2012. Converting isotope values to diet composition: the use of mixing models. J. Mammal. 93, 342–352.

Phillips, D.L., Koch, P.L., 2002. Incorporating concentration dependence in stable isotope mixing models. Oecologia 130, 114–125.

Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83, 703–718.

Romarheim, O.H., Øverland, M., Mydland, L.T., Skrede, A., Landsverk, T., 2011. Bacteria grown on natural gas prevent soybean meal-induced enteritis in Atlantic salmon. J. Nutr. 141, 124–130.

Tacon, A.G., Metian, M., 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. Aquaculture 285, 146–158.

Terrazas, M., Civera, R., Ibarra, L., Goytortúa, E., 2010. Coeficientes de utilización digestiva aparente de materia seca, proteína y aminoácidos esenciales de ingredientes terrestres para el camarón del Pacífico *Litopenaeus vannamei* (Decapoda: penaeidae). Rev. Biol. Trop. 58, 1561–1576.

Teuling, E., Wierenga, P.A., Agboola, J.O., Gruppen, H., Schrama, J.W., 2019. Cell wall disruption increases bioavailability of *Nannochloropsis gaditana* nutrients for juvenile Nile tilapia (*Oreochromis niloticus*). Aquaculture 499, 269–282.

WEF, 2015. Water environment federation. highlights: turning beer waste into animal feed. J. Fulcher. http://news.wef.org/turning-beer-waste-into-animal-feed/.

Wen, J., 2018. Effect of Amino Acids and Vitamin D3 on Performance and Biological Responses in Poultry. PhD Thesis. Virginia Tech. 193 pp.

Whiteman, J.P., Elliott Smith, E.A., Besser, A.C., Newsome, S.D., 2019. A guide to using compound-specific stable isotope analysis to study the fates of molecules in organisms and ecosystems. Diversity 11, 8.

Xie, F.J., Zeng, W.P., Zhou, Q.C., Wang, H.L., Wang, T., Zheng, C.Q., Wang, Y.L., 2012. Dietary lysine requirement of juvenile Pacific white shrimp, Litopenaeus vannamei. Aquaculture 358–359, 116–121.