

## Research Article

# Exploring the contribution of dietary protein from poultry by-product meal and fish meal to the growth of catfish *Ictalurus punctatus* by means of nitrogen stable isotopes

Oscar Daniel García-Pérez<sup>1</sup>, Julio César Cruz-Valdez<sup>1</sup>, Carlos Ramírez-Martínez<sup>1</sup>  
David Villarreal-Cavazos<sup>2</sup> & Julián Gamboa-Delgado<sup>2</sup>

<sup>1</sup>Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Nuevo León  
Nuevo León, México

<sup>2</sup>Programa Maricultura, Departamento de Ecología, Facultad de Ciencias Biológicas  
Universidad Autónoma de Nuevo León, Nuevo León, México  
Corresponding author: Julián Gamboa-Delgado ([julian.gamboard@uanl.mx](mailto:julian.gamboard@uanl.mx))

**ABSTRACT.** The natural nitrogen stable isotope signatures ( $\delta^{15}\text{N}$ ) found in poultry by-product meal (PBM) and fish meal (FM) were used to estimate the relative contribution of dietary nitrogen supplied by both ingredients to the somatic growth of juvenile channel catfish *Ictalurus punctatus*. Six isonitrogenous and isoenergetic experimental diets were formulated using FM and PBM. Two of these diets consisted of isotopic controls having only one ingredient supplying dietary nitrogen, either FM or PBM. Four combined diets were formulated with varying proportions of these ingredients in order to supply high proportions of PBM (FM:PBM, 50:50, 35:65, 20:80 and 5:95) on a nitrogen basis. There were significant differences in mean final weight of fish at the end of the trial. Lower growth was observed as the dietary level of PBM increased. In order to determine the relative contributions of the dietary nitrogen supplied by FM and PBM to catfish growth, an isotopic mixing model was applied. Results indicated that the incorporation of dietary nitrogen supplied by PBM was equivalent to the dietary proportions. The dietary nitrogen available in combined diets containing 50, 65 and 80% of PBM was incorporated in fish bodies as 50, 62 and 81%, respectively. However, high incorporation of dietary nitrogen from PBM was not always reflected in higher growth rates. Results demonstrate the viable use of stable isotopes to determine the allocation of dietary nitrogen and indicate that practical diets for catfish can be formulated with levels of PBM as high as 65% without affecting growth and survival.

**Keywords:** channel catfish, *Ictalurus punctatus*, poultry by-product meal, fish meal, nitrogen stable isotopes, nutrient allocation.

## INTRODUCTION

In order to become a more profitable and sustainable industry, the aquaculture sector has promoted the use of alternative ingredients to be used in compound feeds. The latter is a response to the high dependency on high protein ingredients, such as fish meal, which greatly contributes to the high production costs of the aquaculture industry (Ulloa *et al.*, 2014). According to data reported by Tacon & Metian (2008), up to 68% of the global fishmeal production can be utilized for the manufacture of compound aquaculture feeds. This trend will continue to increase, leading to further overexploitation of marine species, including small pelagic fish, from which fish meal is mainly processed. Many studies have demonstrated that renderer products

derived from the production of domestic animals, offer a high potential to be used as fish meal substitutes in aquafeeds (NRC, 2011). Poultry by-product meal (PBM) is an ingredient obtained from the different production stages occurring in the poultry slaughtering process. It has been demonstrated that PBM represents a viable ingredient and alternative source of nutrients. PBM presents characteristics such as palatability, high protein content, high digestibility coefficients for protein and dry matter and similar energy content when compared to FM (Hernández *et al.*, 2014). Previous studies have considered that the PBM could not replace more than 50% of FM in diets for aquatic organisms (Gallagher & Degani, 1988; Fowler, 1991; Steffens, 1994). Nevertheless, results from recent studies have demonstrated that the replacement of FM by PBM has

reached 100% in diets for several species of fish, without causing significant differences in performance parameters (Yang *et al.*, 2006; Parés-Sierra *et al.*, 2014).

The channel catfish (*Ictalurus punctatus*) is a native freshwater species widely distributed in USA, Canada, and North-Eastern Mexico. This species has a wide demand and offers great potential for continued growth, the latter being reflected in an important increase in global production in the last 30 years from 100,000 to 500,000 ton per year (FAO, 2015). Catfish farming represents an important activity in Mexico and it is supported by a constant hatchling production throughout the year, which is a key factor to supply demand for catfish producers (Lara-Rivera *et al.*, 2015). Estimated protein requirements of channel catfish vary between 25 to 45%, depending on the growing conditions and the size of the organism (López *et al.*, 2002). Catfish diets thus require to be formulated with ingredients having good protein content and acceptable nutritional characteristics, in particular for the earlier life stages. On the other hand, there is a wide variety of nutritional techniques that have been used to evaluate new dietary formulations. Frequently applied methods include those estimating the palatability of nutrients and the apparent digestibility, the latter by means of inert tracers (Jia *et al.*, 2005; Venou *et al.*, 2009). Other evaluation methods are represented by the assessment of energy balances (Ye *et al.*, 2009) and the determination of nutritional conditions using molecular markers (Benedito-Palos *et al.*, 2014). All these techniques allow evaluating the nutritional performance of a diet or test ingredient. Relatively recent nutritional studies have included the use of stable isotopes to estimate the physiological utilization of nutrients provided by alternative ingredients. Studies applying such methodologies generate relevant information on the suitability of new feedstuffs for aquaculture nutrition. Diverse isotopic techniques have been adopted from ecological studies that employ the relative ratios of carbon stable isotopes ( $^{13}\text{C}/^{12}\text{C}$ , expressed as  $^{13}\text{C}$  values) and nitrogen stable isotopes ( $^{15}\text{N}/^{14}\text{N}$  expressed as  $^{15}\text{N}$  values) naturally present in trophic elements and consuming organisms to infer on the transference of nutrients. The analytical techniques applying stable isotopes represent valuable tools in animal nutrition and are employed under the principle that the tissue of organisms reflects the isotopic composition of the diet which was fed to them (Badillo *et al.*, 2014; Gamboa-Delgado *et al.*, 2016). In addition, isotopic analyses have been useful to distinguish between different sources of protein contributing to animal biomass. This information allows evaluating the nutritional quality of a specific feeding item (Asano *et*

*al.*, 2010; Redmond *et al.*, 2010). The contribution of different sources of dietary nutrients to animal growth can be estimated using mass-balance, isotopic mixing models (Phillips, 2012). However, few studies using stable isotopes to quantify the retention of nutrients derived from alternative sources have been conducted in fish. The main objective of this study was to explore an isotopic methodology to quantitatively estimate the physiological allocation of dietary nitrogen supplied by PBM and FM, ingredients that were included at different levels in experimental diets. The nitrogen residency times in whole catfish bodies were also estimated.

## MATERIALS AND METHODS

### Experimental design and rearing system

Juvenile channel catfish *I. punctatus* were obtained from a commercial hatchery located in Ramos Arizpe, Coahuila, Mexico (La Rosa, km 40, Saltillo-Torreón Highway). Fish were transported and then acclimated to glass tanks having the following water conditions: temperature  $29.9 \pm 0.7^\circ\text{C}$ , pH  $8.4 \pm 0.1$  and saturated dissolved oxygen. Total ammonia nitrogen ( $0.08 \pm 0.05 \text{ mg L}^{-1}$ ), nitrite (below detection limits), and nitrate ( $11.3 \pm 3.9 \text{ mg L}^{-1}$ ) were monitored using a colorimetric test kit. A natural photoperiod provided a 12:12 h light:dark ratio. During acclimation, fish were exclusively fed a commercial catfish diet (Api-Bagre 1, Malta Cleyton<sup>®</sup>) previously analyzed for nitrogen content and  $\delta^{15}\text{N}$  value. This diet (36% crude protein and 4.5% crude lipid) was supplied for 20 days in order to establish an isotopic baseline in catfish bodies before the start of the experiment. Twenty juvenile fish having the initial mean wet weight of  $131 \pm 18 \text{ mg}$  were selected and allocated to duplicate glass tanks conforming an array of five dietary treatments. Tanks were filled up to an operative volume of 120 L and each unit was individually fitted with biological filters and air supply. The full water volume was exchanged every third day.

### Experimental diets and sampling procedure

Six isonitrogenous (30.5% crude protein) and isoenergetic ( $3.9 \text{ kcal g}^{-1}$ ) experimental diets were formulated using fish meal (Sardine, 68% protein) and poultry by-product meal (pet food grade, 69% protein (Grupo Bachoco<sup>®</sup>, México). Experimental diets were formulated with ingredients having contrasting nitrogen isotopic values, which in turn facilitated the estimation of nutritional contributions to growth. Diet formulation was done using a spreadsheet. FM and PBM represented the only nitrogen sources and were used to formulate six experimental diets. Different proportions

of dietary nitrogen supplied by FM were substituted with high levels of PBM at 50, 65, 80, 95, and 100% (Table 1). Micronutrients were weighed to the nearest milligram and mixed and then added to the ground macronutrients. This mixture was homogenized for 15 min using a blender. Water was added until the dough was formed and it was extruded through a die plate (1.6 mm orifices). Strands were collected and dried overnight (40°C) in a convection oven. Once dried, strands were broken into smaller pieces using mortar and pestle. Each week, a sieve array was used to select feed particles of appropriate size for the growing fish. Bromatologic analyses of the preconditioning diet and experimental diets were conducted according to Gamboa-Delgado *et al.* (2014).

Experimental diets were delivered in excess at 9:00 and 13:00 h. Uneaten feed and feces were siphoned out daily before first feeding. Feeding rations were progressively adjusted in relation to observed fish weight gain and number of sampled animals. In order to estimate treatment-dependent growth rates, the individual wet weights of five animals per duplicate tank were registered throughout the feeding period. Fish were captured with a net, blotted dry and individually weighed using a digital balance. Experimental sampling points were defined according to the exponential rate of isotopic shift frequently observed in fast-growing aquatic animals. On days 0, 4, 8, 15, and 22, fish were randomly collected from every replicate tank. A group of sub-sampled fish for isotopic analysis was starved for 12 h in order to allow gut evacuation. Animals were sacrificed in ice/water slurry. On the final day (29) all animals were collected and a selection of five fish per replicate was used as a subsample for isotopic analysis. All samples were kept frozen at -80°C until pretreatment.

#### Sample pretreatment and stable isotope analyses

Collected fish were dehydrated at 60°C until samples reached a constant weight. Dry samples were manually ground to obtain a fine, homogenized powder. Diet and fish samples of 1 mg (yielding around 150 µg N) were packed in tin cups (Elemental Microanalysis Ltd., Okehampton, UK). Samples were analyzed at the Stable Isotope Facility (SIF) of the Department of Plant Sciences, 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). Repeated measurements of calibration standards (glutamic acid, bovine liver) indicated that instrument precision (SD) was 0.19‰ for  $\delta^{15}\text{N}$  values. Isotopic results are expressed in delta notation ( $\delta$ ), which is defined as per mill (‰) deviations

from the  $\delta^{15}\text{N}$  value of the standard reference material (atmospheric nitrogen). The term “discrimination factor” is used in this study to describe differences in isotopic values between fish and their respective diets after isotopic equilibrium was reached ( $\Delta^{15}\text{N}$ ).

#### Estimation of nutrient contribution and nitrogen residency time

The proportional dietary nitrogen contributions from FM and PBM to fish growth were estimated using a two-source, one-isotope mixing model (Phillips & Gregg, 2001). Fundamental assumptions required by the models were met or considered before application (*e.g.*, the isotopic equilibrium between diet and consumer, knowledge of elemental nitrogen content in dietary items). Estimation of isotopic discrimination factors ( $\Delta^{15}\text{N}$ ) is useful to integrate correction factors into the mass-balance isotopic mixing models. Such values were obtained from the isotopic differences between the  $\delta^{15}\text{N}$  values of catfish and those in diets containing 100% FM and 100% PBM. Once  $\delta^{15}\text{N}$  values were corrected (*i.e.*,  $\delta^{15}\text{N}$  values of fish fed control diets were obtained), isotopic values of fish fed combined diets were introduced into the mixing model to estimate the proportional assimilation of nitrogen supplied by both ingredients.  $\delta^{15}\text{N}$  values were measured in fish collected on different experimental days and from these values, an exponential model of isotopic change (Eq. 1, Hesslein *et al.*, 1993) was used to obtain an estimate of the metabolic nitrogen turnover rate in catfish bodies.

$$C_{\text{SAMPLE}} = C_n + (C_o - C_n)e^{-(k+m)t} \quad (1)$$

where  $C_{\text{SAMPLE}}$  is the isotope value in fish tissue at time  $t$ ,  $C_o$  is the isotope value of fish tissue in equilibrium with the initial diet,  $C_n$  is the isotope value reached when fish are in equilibrium with a new diet. The model allows calculating a quantitative coefficient to distinguish the isotopic change caused by growth ( $k$ ) or metabolic turnover ( $m$ ). The treatment-specific growth rate constant,  $k$ , was in turn estimated by fitting an exponential growth model to observed weight data,  $k = \log(\text{final weight}/\text{initial weight})/\text{time (d)}$ . From these data, parameter  $m$  was calculated using iterative nonlinear regression. Coefficients  $k$  and  $m$  can be substituted in the following equation  $t_{50} = \ln 2 / (m + k)$  to estimate the time necessary for half of the body nitrogen to be replaced by new nitrogen after an organism experiments a diet shift (half-time,  $t_{50}$ ) (MacAvoy *et al.*, 2005).

#### Statistical analysis

Nitrogen contents and  $\delta^{15}\text{N}$  values in FM and PBM were compared by means of Student's  $t$ -tests. Dietary effects on  $\delta^{15}\text{N}$  values of fish at different times and final

**Table 1.** Nutritional (g 1000 g diet<sup>-1</sup>, dry weight) and isotopic composition ( $\delta^{15}\text{N}$  ‰) of experimental diets fed to channel catfish *Ictalurus punctatus* to estimate the nutritional contribution of fish meal (F) and poultry by-product meal (P) to somatic growth. <sup>1</sup>Micronutrientes y Aditivos S.A. de C.V., México. <sup>2</sup>Bachoco, S.A. de C.V., México. <sup>3</sup>Ragaza Industrias Proteínas Naturales S.A. de C.V. Monterrey, Mexico. <sup>4</sup>Sigma-Aldrich. St. Louis, MO, USA. <sup>5</sup>Constant ingredients: Antifungal agent 0.5 g kg diet<sup>-1</sup>, antioxidant 0.5 g kg diet<sup>-1</sup>. Mineral and vitamin mixes were formulated following estimated requirements reported by NRC (2011).

Diet (g kg <sup>-1</sup> )	100F	50F:50P	35F:65P	20F:80P	5F:95P	100P
Fish meal <sup>1</sup>	473	236	165	94	24	0
Poultry by-product meal <sup>2</sup>	0	235	306	376	447	470
Wheat starch <sup>1</sup>	286	303	308	313	315	315
Fish oil <sup>3</sup>	59	41	36	31	25	24
CMC <sup>4</sup>	20	20	20	20	20	20
Celulose <sup>4</sup>	153	156	156	157	160	162
Constant ingredients <sup>5</sup>	9	9	9	9	9	9
Total	1000	1000	1000	1000	1000	1000
Proximal analysis						
Crude protein (g kg <sup>-1</sup> )	307	305	306	306	307	305
Lipids (g kg <sup>-1</sup> )	93	91	93	89	89	92
Gross energy (kcal g <sup>-1</sup> )	3.8	3.8	3.9	3.8	3.8	3.9
$\delta^{15}\text{N}$ (‰)	16.3	10.8	8.7	7.5	6.0	5.3

weight were analyzed by Kruskal-Wallis tests. When significant differences were detected, pair comparisons were done by Mann-Whitney tests. In order to detect statistical differences in the expected proportions of dietary nitrogen (contributed by FM and PBM) and the observed proportions of dietary nitrogen actually allocated in catfish bodies, Chi-square goodness of fit tests ( $\chi^2$ ) were applied. All tests were conducted using SPSS 17.0 software (SPSS Inc.) at a significance level of  $P < 0.05$ .

## RESULTS

### Dietary effects on growth

During the experimental feeding period, temperature, pH and dissolved oxygen concentration in the experimental tanks remained within the recommended optimal values for catfish *I. punctatus*. At the end of the 29-day bioassay, significant differences in final individual wet weight were observed, although the variability within treatments was high (Table 2). Higher weight gain was observed in a fish fed diet containing only FM ( $1.13 \pm 0.21$  g), followed by diets supplying FM and PBM proportions of 50:50 ( $1.00 \pm 0.16$  g) and 65:35 ( $0.92 \pm 0.14$  g). The individual mean weight of catfish decreased as a function of PBM dietary level. Final weights of animals fed diets 20F:80P, 5F:95P and 100P were statistically similar and ranged from 0.80 to 0.88 g.

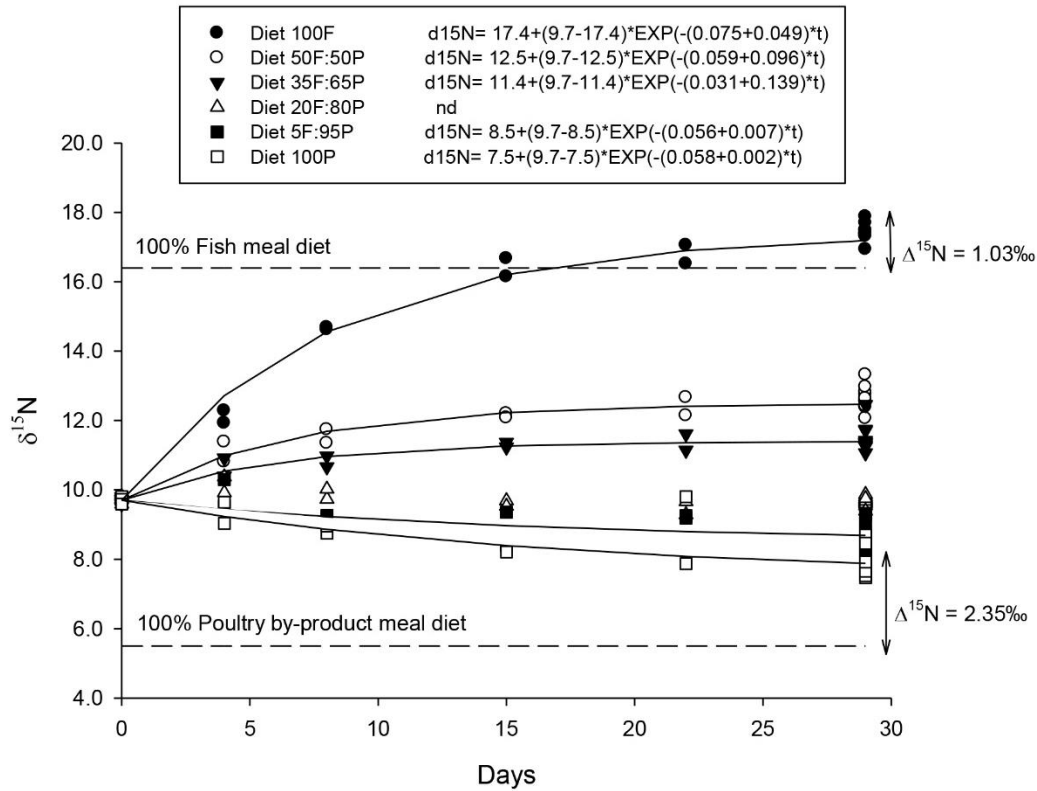
Overall fish survival rate was 88% and no statistical differences were detected among treatments.

**Table 2.** Final wet weight (FW), weight gain (WG), specific growth rate (SGR) of catfish *I. punctatus* reared under diets containing high proportions of poultry by-product meal (P) substituting fish meal (F). Different superscripts indicate significant differences for that particular column.

Diet	FW	WG (%)	SGR
100F	$1.13 \pm 0.21^a$	763	$7.4 \pm 2.1^a$
50F:50P	$1.00 \pm 0.16^{ab}$	664	$7.0 \pm 2.9^{ab}$
35F:65P	$0.92 \pm 0.14^{ab}$	605	$6.8 \pm 1.90^{ab}$
20F:80P	$0.88 \pm 0.22^b$	590	$6.7 \pm 1.85^b$
5F:95P	$0.87 \pm 0.23^b$	569	$6.7 \pm 1.89^b$
100P	$0.80 \pm 0.34^b$	493	$6.1 \pm 3.2^b$

### Isotopic influence of diets and nitrogen residency times in catfish bodies

The conditioning diet allowed establishing a basal isotopic value of  $\delta^{15}\text{N} = 9.7\text{‰}$  in fish whole bodies and after the start of the nutritional trial, there was a fast isotopic influence on fish tissue elicited by the respective experimental diets (Fig 1).  $\delta^{15}\text{N}$  values of FM and PBM were  $16.8 \pm 0.2$  and  $5.6 \pm 0.1\text{‰}$ , respectively. This isotopic difference in the main dietary ingredients caused contrasting isotopic shifts in catfish under different treatments, which allowed estimating the dietary contributions from both ingredients. The full isotopic equilibrium between fish bodies and the respective diets was reached between day 22 (Fig. 1). Isotopic discrimination factors ( $\Delta^{15}\text{N}$ ) between catfish and their different diets ranged from



**Figure 1.** Observed changes in nitrogen stable isotope values (‰) in whole bodies of channel catfish *Ictalurus punctatus* after a dietary shift from a conditioning diet to six experimental diets formulated with different proportions of fish meal and poultry by-product meal. Equations represent predicted values generated by an exponential model and are described by lines showing the best fit to observed data. Arrows indicate nitrogen isotopic discrimination factors between isotopic control diets and fish.  $n = 2$  individuals, 10 on final day.

**Table 3.** Estimated growth rates ( $k$ ), nitrogen metabolic turnover rates ( $m$ ) in whole bodies and nitrogen half times ( $t_{50}$ ) in catfish *I. punctatus* fed experimental diets having different levels of fish meal (F) and poultry by-product meal (P).  $\Delta^{15}\text{N}$  represents the isotopic difference between diets and muscle tissue after isotopic equilibrium was reached. \* $m$  values were estimated using iterative non-linear regression to fit expected values on observed values,  $R^2 = 0.73$  to 0.98.

Diet	$k$ ( $\text{d}^{-1}$ )	$m$ ( $\text{d}^{-1}$ )*	$t_{50}$ (d)	$\Delta^{15}\text{N}$ (‰)
100F	$0.075 \pm 0.014$	0.049	5.6	1.03
50F:50P	$0.071 \pm 0.012$	0.096	4.5	1.63
35F:65P	$0.067 \pm 0.010$	0.139	4.1	2.60
20F:80P	$0.065 \pm 0.011$	-	-	2.07
5F:95P	$0.065 \pm 0.017$	0.007	11.0	2.46
100P	$0.058 \pm 0.010$	0.002	11.6	2.35

1.03 to 2.60‰ (Table 3).  $\Delta^{15}\text{N}$  values increased as a function of the dietary inclusion of PBM ( $r = 0.87$ ). Estimated nitrogen turnover rates in fish fed the different diets showed a high variability (0.002-0.096  $\text{d}^{-1}$ , Table 3) and these values were not correlated to PBM dietary inclusion. The lowest turnover rate was determined in fish fed diet 100P. Nitrogen half times in

fish bodies ranged from 4.1 to 11.6 days. Values showed a tendency to increase from diet 100F to diet 100P.

#### Estimation of dietary nitrogen contributions

Isotopic analysis of ingredients and fish bodies collected over the experimental period and the inclu-

**Table 4.** Proportions of dietary nitrogen supplied from the fish meal (FM) and poultry by-product meal (PM) and their respective estimated contributions to the growth of channel catfish *I. punctatus* fed experimental diets having high levels of poultry meal (mean  $\pm$  CI, n = 10). Superscripts indicate absence of significant differences between expected and mean observed dietary contributions (Chi-square tests).

Diet/Ingredient Proportion	Expected	Observed in whole bodies		
		min	mean	max
50F:50P				
FM	48.9 <sup>a</sup>	46.7	49.9 <sup>a</sup>	53.2
PM	51.1	46.8	50.1	53.3
35F:65p				
FM	33.7 <sup>a</sup>	35.0	38.1 <sup>a</sup>	41.2
PM	66.3	58.8	61.9	65.0
20F:80P				
FM	20.2 <sup>a</sup>	16.0	18.9 <sup>a</sup>	22.1
PM	79.8	77.9	81.1	84.0
5F:95P				
FM	4.0 <sup>a</sup>	0.4	7.4 <sup>a</sup>	10.5
PM	96.0	89.5	92.6	95.6

sion of asymptotic values into the isotope mixing model indicated that the contributions of dietary nitrogen from FM and PBM to the growth of catfish were statistically similar to the expected contributions of dietary nitrogen established in the experimental diets (Tables 1-4). For example, fish receiving diets supplying 51.1 and 66.3% of dietary nitrogen from PBM, ended up having relative nitrogen allocations of 50.1 and 61.9%, respectively. As nitrogen contents in PBM and FM were similar (10.4%) it is assumed that the contribution of dry matter to growth from both sources was also equivalent.

## DISCUSSION

Observed significant differences in final fish weight among treatments indicated that PBM was suitable to replace up to 65% of dietary FM. These observations are comparable to results reported for other fish species supplied with diets having high levels of PBM. Riche (2015) recently demonstrated that diets with an inclusion level of 67% of PBM replacing FM, did not affect growth, feed intake, and body composition of Florida pompano, *Trachinotus carolinus*. Other fish species seem to be even more tolerant to this alternative ingredient. For example, Nengas *et al.* (1999) demonstrated that sea bream (*Sparus aurata*) could be grown with diets containing 75 and 100% of poultry meal as protein source without a significant decrease in weight gain. The lower growth rates observed in the present study were positively correlated with increasing dietary levels of PBM. This might be a consequence of the use of only two protein sources in the combined compound

diets, which was a requirement for the experimental design. Although the amino acid profiles of FM and PBM has been deemed as similar (Parés-Sierra *et al.* 2014), it is plausible that high concentrations of PBM do not yield enough essential fatty acids (Lochmann & Phillips, 1995), in particular for the early life stages of catfish. Poultry fat has little or no n-3 highly unsaturated fatty acids (HUFA), essential for many fish.

Due to their different origins, FM and PBM were very contrasting in terms of isotopic values ( $\delta^{15}\text{N}$ ). FM is manufactured from pelagic, marine fish species, while PBM is derived from farmed animals that in turn reflect the isotopic values of the supplied feed, generally formulated with plant-derived meals (soybean). The isotopic (and nutritional) transference of dietary nitrogen from FM and PBM was relatively fast and catfish reached isotopic equilibrium with their respective diets by day 22. This allows inferring that nutrients supplied by both main ingredients were ingested, digested and assimilated fast. The experimental diets elicited relatively fast growth and estimation from the exponential model of isotopic change (Hesslein *et al.*, 1993) indicated that most of the isotopic change that occurred in fish bodies was attributed to tissue accretion (parameter  $k$ ) and not mainly due to the metabolic turnover rate ( $m$ ) of nitrogen. The isotopic discrimination factors ( $\Delta^{15}\text{N}$ ) showed a tendency to increase from 1.03 between diet 100F and fish to 2.4 between diets 5F:95P and 100P and the respective fish. Some authors have suggested that high  $\Delta^{15}\text{N}$  values occur when animals are fed diets that show an incomplete biological value for the target species (Waddington & MacArthur, 2008). Although clear confirmation is yet to be shown, some studies have found a consistency of decreasing  $\Delta^{15}\text{N}$  values as a function of higher protein quantity and quality in crustaceans (Fantle *et al.*, 1999) and fish (McMahon *et al.*, 2015). It is believed that low  $\Delta^{15}\text{N}$  values simply indicate a more direct use of nutrients, while limiting nutrients might elicit higher metabolic cycling of nutrients, which increases the  $\Delta^{15}\text{N}$  values by virtue of an additional accumulation of heavy isotopes in tissues ( $^{15}\text{N}$ ) and even in the structural components of this tissue, as this tendency has been observed also in individual amino acids (Hoen *et al.*, 2014). It can be thus assumed that based on the growth parameters and relatively low  $\Delta^{15}\text{N}$  values determined in the present study, the supplied experimental diets did not elicit important nutritional restrictions.

Isotopic analysis of ingredients and fish bodies collected over the experimental period indicated that the contributions of dietary nitrogen from FM and PBM to the growth of catfish were statistically similar to the

expected contributions of dietary nitrogen available in the experimental diets (Tables 1, 4). For example, diets supplying 51.1 and 66.3% of dietary nitrogen from PBM, caused relative tissue allocations of 50.1 and 61.9% respectively. When dietary levels of PBM increased to 80 and 96%, the incorporated nitrogen levels in fish were still statistically similar (81 and 92.6%). An equivalent transference of nutrients has not always been observed in other studies applying isotopic techniques. The magnitude of transference depends on the nutritional quality or biological value of the dietary ingredients/food items available for the target organisms. For example, in marine fish larvae, it has been reported that the dietary carbon supplied by live feed (*Artemia*) is physiologically incorporated at significantly higher proportions than that supplied by inert feed (Gamboa-Delgado *et al.*, 2009). Filbrun & Culver (2014) applied isotopic measurements to demonstrate that, even in the presence of compound feed, channel catfish reared in ponds derived all or most of their nutrients from live prey during the first three weeks of culture. As mentioned above, the determination of high proportions of incorporated nutrients from specific ingredients does not always correlate with higher growth rates in an organism. The latter is partially explained by the different allocation of nutrients, either to growth or other metabolic uses. The different magnitudes of isotopic change in whole bodies or specific tissues are the result of both, tissue accretion and metabolic turnover rates.

## CONCLUSIONS

Results from the present study demonstrate that, in conjunction with growth data, isotopic measurements are used to infer on the transference of dietary nitrogen from specific ingredients supplied in experimental diets. Additionally, the study also demonstrates the suitability of poultry by-product meal as an alternative ingredient to replace up to 65% of dietary fish meal in formulated diets for juvenile channel catfish. In future studies, the analytical techniques relying on isotopic measurements might assist in elucidating which amino acids are preferentially transferred from additional alternative ingredients.

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