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Short Communication

Evaluation of methods to estimate the essential amino acids requirements of fish from the muscle amino acid profile

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ABSTRACT. Many methods to estimate amino acid requirement based on amino acid profile of fish have been proposed. This study evaluates the methodology proposed by Meyer & Fracalossi (2005) and by Tacon (1989) to estimate amino acids requirement of fish, which do exempt knowledge on previous nutritional requirement of reference amino acid. Data on amino acid requirement of pacu, *Piaractus mesopotamicus*, were used to validate de accuracy of those methods. Meyer & Fracalossi's and Tacon's methodology estimated the lysine requirement of pacu, respectively, at 13 and 23% above requirement determined using dose-response method. The values estimated by both methods lie within the range of requirements determined for other omnivorous fish species, the Meyer & Fracalossi (2005) method showing better accuracy.

Keywords: *Piaractus mesopotamicus,* fish nutrition, ideal protein, nutritional requirements, methodology.

Evaluación de métodos para estimar los requerimientos de aminoácidos esenciales en peces a partir del perfil de aminoácidos de músculo

RESUMEN. Se han propuesto muchos métodos para estimar requerimientos de aminoácidos basados en el perfil de aminoácidos de peces. Este estudio valida la metodología propuesta por Meyer & Fracalossi (2005) y por Tacon (1989) para estimar los requerimientos de aminoácidos en peces, que exime del conocimiento previo sobre el requerimiento nutricional de aminoácidos de referencia. Se utilizaron datos sobre los requerimientos de aminoácidos de pacú, *Piaractus mesopotamicus*, para validar la precisión de los métodos analizados. Las metodologías de Meyer & Fracalossi (2005) y de Tacon (1989) estimaron el requerimiento de lisina de pacú, respectivamente, en 13 y 23% por sobre el requerimiento determinado utilizando el método dosis-respuesta. Los valores estimados, en ambos casos, se encuentran dentro del rango de valores de los requerimientos determinados para otras especies de peces omnívoros, presentando el método de Meyer & Fracalossi (2005) una mejor precisión.

Palabras clave: *Piaractus mesopotamicus*, nutrición de peces, proteina ideal, requerimientos nutricionales, metodología.

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Dietary protein may add up to 70% of production costs of intensive fish farming systems. However, fish has no nutritional requirement for protein *per se*; fish require a quantitatively and qualitatively balanced mix of essential amino acids (EAA). Diets with inappropriate EAA profile not only hamper performance and health of farmed fish and quality of fisheries products, but also worsen environmental impact of fish farming operations (Li *et al.,* 2009; Conceição *et al.,* 2012).

Aquaculture diets must be complete and balanced, supplying adequate levels of EAA for fish optimal growth and health. Dietary requirement of EAA are usually determined by time-consuming, expensive dose-response trials using semi-purified test diets. After considerations of Ogino (1980), the "ideal protein" concept, originally designed for studies on nutritional requirements of swine, has been widely used to estimate nutritional requirement of EAA by fish. The ideal protein concept state that even though requirement of individual amino acids may vary at different stages of development, the ratio between essential and non-essential amino acids (A/E ratio) remains constant.

Data on dietary requirements of essential amino acids are available for a relatively small number of fish (NRC, 2011). Experimentally, it has been determined for several species the dietary requirement for one or more essential amino acids, such as jundiá, *Rhamdia quelen* (Montes-Girao & Fracalossi, 2006) and pacu, *Piaractus mesopotamicus* (Bicudo *et al.*, 2009), for which the lysine requirement have been determined experimentally.

Because body amino acid profile and EAA requirements of fish present high correlation (Wilson & Cowey, 1985; Peres & Oliva-Teles, 2008), EAA contents of fish whole carcass or muscle have been used to estimate the EAA requirement of several fish, native species included (Bicudo & Cyrino, 2009; Abimorad & Castellani, 2011). However, methodology developed and used for that purpose, *e.g.*, Arai (1981), the widely used method described by Tacon (1989), and a method described more recently by Meyer & Fracalossi (2005), have diverse design and approaches, so estimated values can differ. This work compares these methods in the aim to define their accuracy and appropriateness to estimate EAA requirement of fish.

Pacu was used as a biological model so as to confront the lysine requirement of the species experimentally determined by Bicudo *et al.* (2009) with the requirements on lysine and other EAA as determined by Tacon (1989) and Meyer & Fracalossi (2005). The A/E ratio in the muscle of pacu was estimated based on results of Machado & Sgarbieri (1991), and Abimorad *et al.* (2008), using the method proposed by Kaushik (1998): A/E = 1000 × (% EAA on muscle/[Σ EAA + cystine + tyrosine on muscle]). Requirements in EAA, except lysine, were estimated following the formula recommended by Arai (1981): AA requirement ratio = (A/E of essential amino acid × (lysine requirement (%))/(A/E ratio of lysine in the muscle) using lysine requirements determined by Bicudo *et al.* (2009).

For comparison purposes, requirement on EAA of pacu were thus estimated:

(i) as proposed by Tacon (1989), where the requirement for SEA (EAAreq) is calculated as: AAEexig = [(% CP diet × Q × Z)/10,000], were CP is dietary crude requirement (27%; Bicudo *et al.* 2010); Q = EAA + cystine + tyrosine (%), which corresponds to 35% of the species' CP requirement; and Z is the body contents of the AA being estimated, expressed as % of ΣAAE + cis + tir; estimated values were expressed as percentage of protein; and as proposed by Meyer & Fracalossi (2005), in which: EAAreq = (contents of a specific AA in fish muscle tissue × average sum of the total EAA requirement of channel catfish, Nile tilapia and common carp) ÷ total EAA contents on the muscle of the target species.

The A/E ratio has been the most widely used method to estimate amino acids requirements of fish. However, its application depends on the determination of requirements of a reference amino acid, usually lysine, through dose-response trials (Peres & Oliva-Teles, 2008). Lysine is the reference amino acid because it is metabolized only for growth purposes, that is, accretion of body protein, it can be determined through relatively simple laboratory methods, and requirements on lysine has already been determined for a large number of species (NRC, 2011). However, running dose-response trials are time consuming, requires using purified or semi-purified diets, and therefore expensive.

The smallest absolute requirement was estimated for histidine, varying from 1.14 to 1.61% of dietary protein, as opposed to requirements on lysine, estimated on 5.79 and 6.64% protein by methods of Meyer & Fracalossi (2005) and Tacon (1989), respectively (Table 1); those values are, respectively, 23 and 41% higher than values determined by the A/E ratio technique (Arai, 1981), lysine requirement determined for pacu by Bicudo *et al.* (2009) on dose-response assays included.

The key differences between the two methods were recorded when estimating lysine, leucine, phenylalanine + tyrosine and arginine requirements (Fig. 1).

Using the method proposed by Tacon (1989), Teixeira *et al.* (2008) estimated the requirement on EAA of Nile tilapia to exceed on 28% requirements experimentally determined for the species. Meyer & Fracalossi (2005) also overestimated by 13% the lysine requirement of catfish in comparison to values determined experimentally by Montes-Girao & Fracalossi (2006). The sum of essential amino acids plus cystine and tyrosine for pacu was lower (51.38%) than that recorded for the jundiá (53.46%) by Meyer & Fracalossi (2005) because contents on tryptophan were not determined. This fact explains why differences on estimates of lysine requirement for pacu (23%) are larger than that of jundiá (13%) when compared to the species EAAs requirements determined in dose-response experiments. It also should be noticed that Bicudo *et al.* (2009) and Montes-Girao & Fracalossi (2006) operated quadratic

Table 1. Comparison between estimated amino acids requirements of pacu, *Piaractus mesopotamicus*, as based on muscle amino acid profile values.

	Dietary requirement (% protein)			EAA contents[1]	Methods		
					Arai (1981)	Meyer & Fracalossi (2005)	Tacon (1989)
Amino acid	*I. punctatus*	*O. niloticus*	*C. carpio*		*P. mesopotamicus*		
Arginine	4.30	4.20	4.30	6.63	3.19	3.94	4.51
Phenylalanine + tyrosine	5.30	5.54	4.40	7.88	3.78	4.68	5.37
Histidine	1.50	1.72	2.10	2.37	1.14	1.41	1.61
Isoleucine	2.60	3.11	2.50	4.36	2.09	2.59	2.97
Leucine	3.50	3.39	3.30	8.58	4.12	5.09	5.84
Lysine	5.10	5.12	5.70	9.75	4.692	5.79	6.64
Methionine + cystine	2.30	3.22	2.10	3.25	1.57	1.93	2.21
Trheonine	2.00	3.75	3.90	4.31	2.07	2.56	2.93
Tryprophan	0.50	1.00	3.60	nd	nd	nd	nd
Valine	3.00	2.80	0,80	4.27	2.05	2.53	2.90
∑TEAA +Cys+Tyr-Try	29.60	32.85	29.10	51.38			
Average		30.52					

[1]Average values from Machado & Sgarbieri (1991) and Abimorad *et al.* (2008).
[2]As determined through dose-response trials by Bicudo *et al.* (2009).

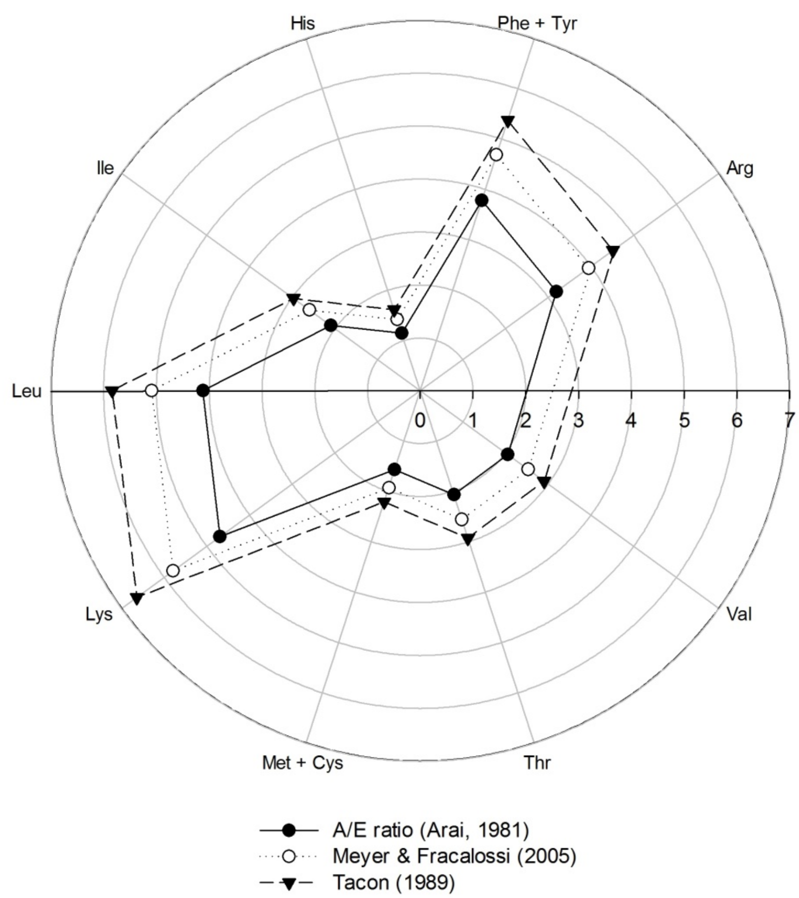

Figure 1. Graphical comparison of methods for estimating essential amino acids (EAA) requirements from muscle amino acids profile of pacu, *Piaractus mesopotamicus*. Arg: arginine, Val: valine, Thr: threonine, Met + Cys: methionine + cysteine, Lys: lysine, Leu: leucine, Ile: isoleucine, His: histidine, Phe + Tyr: phenylalanine + tyrosine.

polynomial models to determine EAA requirements of pacu and jundiá, respectively, a technique that usually yields higher estimated values than those determined by the broken line model (Dairiki *et al.,* 2007). For the other amino acids, Tacon's (1989) method also resulted in values exceeding those estimated by Meyer & Fracalossi (2005) method.

Body amino acid profile of phylogenetically related species or even species with similar feeding habits are not similar, so determining species-specific dietary amino acid requirements are an absolute need (Akiyama *et al.,* 1997; Bicudo & Cyrino, 2009). As a matter of fact, Rollin *et al.* (2003) registered that even though body amino acid profile and fish nutritional requirements are correlated, grounding estimates of dietary amino acids requirements solely on body amino acid profile lead to overestimating the requirement on branched chain and aromatic amino acids, and underestimate the sulfur amino acids requirements of the Atlantic salmon, *Salmo salar*. However, nutritional EAA requirements of fish estimated in this study range within the commonly registered values, except for requirements on leucine, estimated to be higher than the average values recorded for most fish species (Wilson, 2002; NRC, 2011). Using the amino acid profile to estimate the nutritional requirements of fish can thus be justified only when data from dose-response trials are not available (Bicudo & Cyrino, 2009).

Limitations of methodologies to estimate EAA requirements of fish from data on body amino acid profile have been demonstrated in many species (Rollin *et al.,* 2003; Peres & Oliva- Teles, 2007). The efficiency of utilization of amino acids, *i.e.*, deposition of EAA in the carcass, particularly in the muscle tissue, is an important factor in the assessment of EAA requirements, but there is a virtual lack of results on the subject, posing serious obstacles to studies on the modeling of amino acid requirements (Kim *et al.,* 2012).

The method of Tacon (1989) rounds the sum of the requirement on EAAs plus cystine and tyrosine at 35% of the requirement on protein, regardless of the species or stage of development; the utilization of a fixe value usually is more likely the cause of under or overestimating the requirements on amino acids. The method of Meyer & Fracalossi (2005) relies in the average value of EAA requirements of three omnivorous species, a potentially limiting factor to base estimates of EAA requirements of carnivorous fish, which do have higher protein requirements than omnivores (NRC 2011). However, hence dietary amino acids requirement can be expressed differently (*e.g.*, % diet; % dietary protein; kcal g^{-1} DE), there are also different "opinions" on the most appropriate way to express EAA requirements (Cowey, 1994; Wilson, 2002). Possibly, to the express amino acids requirements as a percentage of dietary protein, all methods minimize the effects associated with differences in the feeding habits of the studied species. However, once again, the scarcity of studies on EAA requirement of neotropical carnivorous fish is a limiting factor for the demonstration of this hypothesis.

Despite the apparently limited accuracy, the method described by Meyer & Fracalossi (2005) seems to be better suited for estimating EAA requirements from body amino acids profile of fish, and it is safe to state that body amino acid profile of fish is an usable tool to base estimating EAAs requirements of fish by researchers and the industry alike, at least until information on dose-response experiments are available. Using such a tool is further justified considering the large number of species already used in fish farming, especially in neotropical latitudes, and the immediate need to develop balanced, efficiently used and environmentally friendly diets which can elicit maximizing protein synthesis and concomitant reduction of excretion of nitrogen to the aquatic environment.

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