rccp Revista Colombiana de Ciencias Pecuarias

Whole-body and muscle amino acid composition of Plata pompano *(Trachinotus marginatus)* and prediction of dietary essential amino acid requirements¤

Composición de aminoácidos de la carcasa y del músculo del Pámpano (Trachinotus marginatus) y estimación de las necesidades por aminoácidos esenciales

Composição de aminoácidos da carcaça e do músculo do Pampo prateado (Trachinotus marginatus) e estimativa das necessidades por aminoácidos essenciais

Marcelo Borges Tesser[1], Oceanól, MSc, Dr; Eduardo Martins da Silva[1*], Biól, MSc; Luís A Sampaio[2], Oceanól, MSc, Dr.

[1]*Laboratório de Nutrição de Organismos Aquáticos, Instituto de Oceanografia (IO), Universidade Federal do Rio Grande – FURG, Rio Grande-RS, Brasil.*

[2]*Laboratório de Piscicultura Marinha e Estuarina, Instituto de Oceanografia (IO), Universidade Federal do Rio Grande – FURG, Rio Grande-RS, Brasil.*

(Received: September 9, 2013; accepted: June 9, 2014)

Summary

Background: knowing the essential amino acid (EAA) requirement values is fundamental to formulate good quality and cost-effective fish feeds. However, such requirements have been established for few fish species. The estimation of amino acid requirements based on amino acid composition of fish is a fast and reliable alternative. **Objective:** to determine whole-body and muscle amino acid composition of Plata pompano *(Trachinotus marginatus)* and estimate its EAA requirements. **Methods:** EAA requirements were estimated using A/E ratios [(Individual EAA ⁄ Total EAA) * 1000]. **Results:** hystidine, leucine, lysine and phenylalanine were present in higher concentrations in muscle tissue in comparison with the whole-body. On the other hand, arginine, isoleucine, methionine, tryptophan and valine concentration were not different between whole-body and muscle. A/E ratios for Plata pompano determined in the present study are similar to those reported in other fish species, although valine A⁄E ratios were slightly smaller. **Conclusion:** until dose-response experiments are conducted to precisely determine EAA requirements, the estimated EAA values using whole-body EAA—as proposed in this study—could be used to formulate diets for Plata pompano.

Keywords: *A ⁄E ratio, Carangidae, diet formulation, fish nutrition.*

¤ To cite this article: Tesser MB, da Silva EM, Sampaio LA. Whole-body and muscle amino acid composition of Plata pompano *(Trachinotus marginatus)* and prediction of dietary essential amino acid requirements. Rev Colomb Cienc Pecu 2014; 27:299-305.

* Corresponding author: Eduardo Martins da Silva. Laboratório de Nutrição de Organismos Aquáticos, Instituto de Oceanografia (IO), Universidade Federal do Rio Grande – FURG, Rio Grande-RS, Brasil. Rua do Hotel, nº2, Rio Grande – RS, Brasil. Email: edu.aquicultura@gmail.com

Resumen

Antecedentes: conocer los requerimientos de aminoácidos esenciales (EAA) es fundamental para la formulación de raciones rentables y de buena calidad para peces. Sin embargo, dichos requerimientos se han establecido solo para unas pocas especies de peces. La determinación de las necesidades de aminoácidos basada en la composición de aminoácidos de los peces es un método alternativo rápido y viable. **Objetivo:** determinar la composición de aminoácidos del cuerpo completo y del músculo del Pámpano *(Trachinotus marginatus)* y estimar sus necesidades de EAA. **Métodos:** el requerimiento de aminoácidos esenciales se calculó utilizando el índice A/E [(Individual EAA⁄Total EAA) * 1000]. **Resultados:** la histidina, leucina, fenilalanina y lisina estaban en una mayor concentración en el músculo que en el cuerpo. Por otro lado, arginina, isoleucina, metionina, triptófano y valina no mostraron ninguna diferencia significativa entre la composición del músculo y el cuerpo. Los valores de A/E para Pámpano determinados en este estudio son similares a los reportados para otras especies de peces, pero no los de valina, que fue levemente menor. **Conclusiones:** hasta que no sean realizados experimentos de dosis-respuesta para determinar con precisión los requerimientos de EAA, el cálculo de los requerimientos de aminoácidos esenciales a partir de la concentración de aminoácidos del cuerpo -como se propone en este estudio- puede ser utilizado en la formulación de dietas para pámpano.

Palabras clave: *Carangidae, formulación de dietas, nutrición de peces, razón A/E.*

Resumo

Antecedentes: estimar as necessidades por aminoácidos essenciais (AAE) é importante para formular dietas de boa qualidade e com bom custo-benefício. No entanto, poucas espécies de peixes possuem suas necessidades por (AAE) estabelecidas. A determinação das necessidades por (AAE) baseada na composição de aminoácidos do peixe é uma alternativa rápida e viável. **Objetivo:** determinar a composição de aminoácidos da carcaça e do músculo do Pampo prateado *(Trachinotus marginatus)* e estimar suas necessidades por (AAE). **Métodos:** as necessidades por AAE foi estimada usando o índice A/E [(AAE individual⁄AAE total) * 1000]. **Resultados:** histidina, leucina, lisina e fenilalanina estavam em maior concentração no músculo do que na carcaça. Por outro lado, arginina, isoleucina, metionina, triptofano e valina não apresentaram diferenças significativas entre a composição da carcaça e do músculo. O índice A/E determinado no presente estudo para o Pampo prateado é similar ao reportado para outras espécies de peixe, entretanto o valor do índice A/E para a valina foi ligeiramente menor. **Conclusão:** até que experimentos dose resposta sejam realizados para determinar com exatidão as necessidades por aminoácidos essenciais, os valores estimados para as necessidades por aminoácidos essenciais usando a composição da carcaça como proposto neste estudo pode ser usada na formulação de dietas para o Pampo prateado.

Palavras chave: *Carangidae, formulación de dietas, nutrição de peixes, razão A/E.*

Introduction

Although great research efforts have been conducted to establish protein requirements for different species, fish and other organisms do not have an actual protein requirement, but do require a well-balanced mixture of essential (EAA) and non-essential amino acids (NEAA) (Wilson, 2002). A diet providing the required EAA maximizes growth and feed utilization (Zhang *et al.,* 2008), reducing the need for crude protein (Ng and Hung, 1994).

A requirement estimate of EAA is an important input in the formulation of cost-effective, good-quality feeds for fish. According to the NRC (2011) EAA requirements have been established for few fish species. Generally, EAA requirements have been experimentally estimated by feeding diets containing graded levels of the particular amino acid to be examined (Ahmed, 2012; Khan, 2012). This methodology is considered costly and time consuming. On the other hand, measurement of whole-body EAA composition has been used to estimate dietary EAA requirements for several fish species (Meyer and Fracalossi, 2005; Bicudo and Cyrino, 2009; Hossain *et al.,* 2011), being an inexpensive and rapid alternative to dose–response studies. Arai (1981) introduced the concept of A/E ratios [(EAA⁄total EAA) * 1000] when formulating diets for coho salmon (*Oncorhynchus kisutch*). In his study, fish receiving diets supplemented with amino acids to simulate whole-body A/E ratio had improved growth and feed efficiency. Wilson and Poe (1985) found a strong correlation (r = 0.96) between body amino acid composition and EAA requirements of *Ictalurus punctatus*. Moreover, Wilson (2002) reported a good correlation between EAA requirements determined by the traditional method with those calculated from whole-body amino acid concentration for channel catfish.

Some *Trachinotus* species are considered appropriate for aquaculture in virtue of their quick adaptation to captivity, good tolerance to extreme environmental conditions and rapid growth (Jory *et al.,* 1985). Plata pompano (*Trachinotus marginatus*) is native to the southern Atlantic Ocean (Menezes and Figueiredo, 1980). Due to a great interest in its commercial production, several studies of this species are underway (Sampaio *et al.,* 2003; Costa *et al.,* 2008; Kütter *et al.,* 2012). However, to the best of our knowledge, essential amino acid requirements of Plata pompano have not ben reported. Thus, this study aimed to determine muscle and whole-body EAA composition of Plata pompano, and, accordingly, estimate its EAA requirements.

Materials and methods

Ethical considerations

The Ethics Committee for Animal Research of Universidade Federal do Rio Grande, Brazil (23116.001423/2014-59) approved this study.

Procedures

Plata pompano juveniles were captured using a beach seine net (8 mm mesh) at Rio Grande, Brazil (Southwestern Atlantic Coast, 32º17'S - 52º10'W) and stocked at a density of 0.1 juveniles/L in five 300 L tanks, where they were acclimated for two weeks prior to sampling. Fish were reared with filtered seawater (35 ppt) kept at 25 ºC, with a water exchange rate of 100% per day. Photoperiod was adjusted to 12 h dark and 12 h light. Animals were hand-fed four times per day with a commercial diet (NRD – 0.8/1.2, INVE – Salt Lake City, UT, USA) to apparent satiety.

A total of 30 fish (25.03 ± 7.29 g) were used for amino acid determinations. Prior to sampling, fish were fasted for 48 h to clean the digestive tract. Fish were euthanized with benzocaine (500 ppm). Pools from five fish were considered an analytical sample. Three samples of whole-body intact fish and three samples of muscle tissue (whole fish fillet) were stored at -80 °C until protein and amino acid analysis. All samples were dried at 105 ºC for 5 hours, ground and kept frozen until analysis. Muscle and whole-body protein were assayed according to the method by Hagen and Augustin (1989). Amino acids were dosed according to White *et al.* (1986).

The concentration of each specific amino acid was expressed relative to the total amino acid content of the sample. The A/E ratios of EAA composition for whole-body and muscle were calculated using the formula suggested by Arai (1981): A ⁄ E ratio = [(Individual EAA ⁄ Total EAA) * 1000]. The EAA requirement profile of Plata pompano was estimated based on a known lysine requirement of 4.8 g per 100 g protein for sea bass (*Dicentrarchus labrax)* (Tibaldi and Lanari, 1991) using the formula suggested by Kaushik, (1998): EAA requirement = (determined requirement for lysine * A ⁄ E ratio of individual amino acid) ⁄ A ⁄ E ratio for lysine. A similar procedure was conducted by Hossain *et al.* (2011) to estimate EAA requirements of silver promfet (*Pampus argenteus)* using the lysine requirement of Asian sea bass (*Lates calcarifer).*

Data were compared with the reported EAA values and A/E ratios for other carnivorous marine species. The whole-body and muscle amino acid composition were compared by paired-comparison t-test at 5% significance level. Plata pompano A/E ratios were subjected to linear regression analysis to determine their relationships with A/E ratios for other carnivorous marine species.

Results

Whole-body and muscle amino acid composition of *T. marginatus* is presented in Table 1. Hystidine, leucine, lysine and threonine were present in higher concentrations ($p<0.05$) in the muscle tissue than in the whole-body. On the other hand, arginine, isoleucine, methionine, phenylalanine, tryptophan and valine did not present significant differences ($p>0.05$) between whole-body and muscle composition.

Table 1. Whole-body and muscle amino acid (aa) composition (g in 100 g protein) of *Trachinotus marginatus* and other carnivorous fish.

	Pleuronectes ferruginea A	*Hippoglossus hippoglossus* A	*Paralichthys olivaceus* A	*Salmo salar* B	Other teleosts C	*Trachinotus marginatus**	
						Whole-body	**Muscle**
EAA							
Arg	6.79±0.03	6.85±0.02	6.75±0.03	6.61±0.03	6.16±0.98	6.63±0.36	6.26±0.11
His	2.45±0.02	2.88±0.02	2.36±0.01	3.02±0.08	2.47±0.63	2.13±0.10b	2.39±0.05a
Ile	4.11±0.11	4.36±0.03	3.91±0.02	4.41±0.03	4.29±0.92	2.94±0.26	3.31±0.23
Leu	7.57±0.04	7.82±0.03	7.59±0.03	7.72±0.03	7.20±0.70	7.06±0.38b	8.31±0.08a
Lys	8.56±0.04	8.85±0.04	9.15±0.04	9.28±0.30	7.38±0.89	7.93±0.45b	9.87±0.23a
Met	2.28±0.08	2.83±0.05	2.92±0.00	1.83±0.03	2.75±0.45	3.02±0.43	3.09±0.05
Phe	3.98±0.01	4.63±0.02	4.55±0.02	4.36±0.03	4.10±0.47	3.59±0.26	3.83±0.19
Thr	4.43±0.05	4.62±0.01	4.49±0.02	4.95±0.02	4.39±0.54	4.00±0.03b	4.24±0.03a
Trp	1.32±0.03	1.07±0.03	1.06±0.01	0.93±0.01	ND	1.04±0.19	1.15±0.17
Val	5.63±0.33	5.24±0.10	4.57±0.01	5.09±0.02	4.73±0.53	3.43±0.40	3.71±0.18
NEAA							
Ala	6.42±0.03	6.00±0.02	6.39 ± 0.02	6.52±0.05	6.17±0.82	6.77± 0.25	6.30±0.24
Asp	9.87± 0.09	10.02±0.02	10.24 ± 0.02	9.92±0.11	9.19±0.85	9.04±0.19	10.19±0.07
Cys	1.22±0.04	0.87±0.02	0.97 ± 0.02	0.95±0.05	1.00±0.30	0.97±0.07	0.43±0.06
Glu	14.58±0.08	14.42±0.03	15.18 ± 0.03	14.31±0.01	14.29±2.49	13.28±0.45	14.28±0.11
Gly	8.40±0.14	6.65±0.06	6.54 ± 0.11	7.41±0.17	6.81±1.69	11.07±1.15	7.53±0.09
Pro	4.58±0.08	4.68±0.04	4.73 ± 0.04	4.64±0.01	4.37±1.13	6.98±0.78	5.34±0.19
Ser	4.62±0.07	4.48±0.03	4.69 ± 0.02	4.61±0.03	4.15±0.47	4.73±0.14	4.50±0.04
Tau	0.65±0.04	0.90±0.03	0.58 ± 0.06	ND	ND	2.45±0.06	2.08±0.21
Tyr	2.53±0.08	2.82±0.03	3.31 ± 0.02	3.50±0.01	3.02±0.62	2.99±0.12	3.21±0.02

*Data for individual amino acids are expressed as mean ± SD, n=3, (g/100 g protein). A Data obtained from whole-body tissue (Kim and Lall, 2000); B Data obtained from whole-body tissue (Wilson and Cowey, 1985); C Mean data from several teleosts compiled by Mambrini and Kaushik (1995). ND: not determined.

The A/E ratios for whole-body and muscle of Plata pompano determined in the present study are similar to those reported for other marine fish species, although A/E values for valine were slightly smaller (Table 2). A high correlation was observed between whole-body A/E ratios of Plata pompano with A/E ratios for yellowtail flounder ($y = 14.945 + 0.8508 * x$, $r^2 = 0.93$, $p<0.001$), halibut ($y = 12.2677 + 0.877 * x$, $r^2 = 0.93$, $p<0,001$) and Japanese flounder ($y = 3.1809 + 0.9687 * x$, $r^2 = 0.96$, $p<0.001$). The estimated amino acid requirements for Plata pompano are presented in Table 3.

Discussion

The differences found in whole-body and muscle amino acid composition were also reported for common carp *(Cyprinus carpio)* and lambari *(Astyanax altiparanae;* Buchtová *et al.,* 2007; Abimorad and Castellani, 2011). Whole-body and muscle amino acid contents of Plata pompano were compared to values reported to other fish (Table 1) and the variations detected among amino acid content of Plata pompano and yellowtail flounder *(Pleuronectes ferruginea)*, halibut *(Hippoglossus hippoglossus)*,

Table 2. A/E ratios of whole-body and muscle tissue for *Trachinotus marginatus* and other carnivorous fish species.

	Pleuronectes ferruginea [A]	*Hippoglossus hippoglossus* [A]	*Paralichthys olivaceus* [A]	*Salmo salar* [B]	*Trachinotus marginatus*	
					Whole-body	**Muscle tissue**
Arg	133.4	129.5	130.7	125.5	144.95 *	125.73
His	48.2	54.5	45.7	57.3	46.57	48.00
Ile	80.8	82.5	75.6	83.8	64.28	66.48
Leu	148.8	148.0	147.1	146.6	154.35 *	166.9
Lys	168.3	167.6	177.6	176.2	173.37 *	198.23
Met + Cys[c]	69.0	70.1	75.3	52.8	84.23 *	70.69
Phe + Tyr[d]	128.0	141.0	152.4	149.3	144.07	141.1
Thr	87.1	87.4	87.0	94.0	87.45	85.16
Trp	26.0	20.2	20.6	17.6	22.74	23.10
Val	110.6	99.2	88.5	96.7	74.99	74.51

*Significant difference of A/E ratio between whole-body and muscle tissue. [A]Kim and Lall (2000) whole-body ratio; [B]Calculated from Wilson and Cowey (1985); [c]Methionine + cystine, [d]Phenylalanine + tyrosine.

Table 3. Dietary amino acid requirement estimates (g/100g protein) for selected species and of *Trachinotus marginatus*.

	Trachinotus marginatus [A]		*Paralichthys olivaceus* [C]	*Pagrus major* [C]	*Salmo salar* [D]
EAA	**Whole-body**	**Muscle tissue**			
Arg	4.01	3.04	3.4	3.5	4.0
His	1.29	1.16	1.3	1.4	1.5
Ile	1.78	1.61	2.0	2.2	ND
Leu	4.27	4.04	3.9	4.2	ND
Lys[B]	4.80	4.80	4.6	4.4	5.3
Met + Cys	2.33	1.71	1.9	2.2	3.4
Phe + Tyr	3.99	3.42	3.8	4.1	5.6
Thr	2.42	2.06	2.3	1.8	2.7
Trp	0.63	0.56	0.5	0.6	0.7
Val	2.08	1.80	2.5	2.5	3.1

[A]Amino acid requirement estimates through the methodology proposed by Kaushik (1998). [B]Attributed lysine requirement (Tibaldi and Lanari, 1991). [C]Dietary amino acid requirement estimates based on lysine requirement and whole-body amino acid composition (Forster and Ogata, 1998). [D]Dietary amino acid requirement estimates in a growth experiment for lysine and calculated requirement of other AAE for whole-body (Rollin *et al.,* 2003). ND: not determined.

Japanese flounder *(Paralichthys olivaceus)* and Atlantic salmon *(Salmo salar)* were considered small. Wilson and Cowey (1985) have already shown no differences in whole-body amino acid composition among salmon species, and Mambrini and Kaushik (1995) also suggested that whole-body amino acid composition should not be significantly different for most teleosts.

A/E ratios for Plata pompano are similar to those reported for other fish species. Moreover, a high correlation between whole-body A/E ratios of Plata pompano with the A/E ratios for yellowtail flounder, halibut, and Japanese flounder was observed, showing a preserved amino acid profile between salt water species. A significant correlation between whole-body A/E ratios of different species was also determined by Gatlin (1987) and Hossain *et al.* (2011). According to Wilson and Cowey (1985), the occurrence of similar A/E ratio among different fish species suggests that amino acid requirements expressed as percentage of dietary protein are similar, and this may also apply for the species shown in Table 2.

Since lysine is normally the first limiting amino acid in most feedstuffs, the requirements for the other indispensable amino acids are expressed in relation to the lysine requirement based on the ideal protein concept (NRC, 2011). In the present study, Plata pompano EAA requirement profiles were estimated using the lysine requirement for sea bass (*Dicentrarchus labrax*) determined through a dose-response experiment (Tibaldi and Lanari, 1991), since no lysine requirement has been established for Plata pompano. The estimated amino acid requirements for Plata pompano are presented in Table 3 and a considerable homogeneity in the amino acid requirement estimated from whole-body for Plata pompano and Japanese flounder, red sea bream, and Atlantic salmon were encountered. In agreement with this study, Meyer and Fracalossi (2005) found similar amino acid requirements among omnivorous freshwater fish.

According to the NRC (2011) most fish species fed high quality diets deposit between 25-55% of the total amino acid intake. Accordingly, protein deposition is one of the determinants of amino acid requirement by fish. Although this concept does not account for the amino acids used for maintenance of metabolic demands (Gurure *et al.,* 2007), the body amino acid composition might be a reasonable starting point when attempting to define dietary EAA requirements. Furthermore, several authors have demonstrated that diets formulated on amino acid patterns similar to body tissues result in improved growth and feed efficiency (Arai 1981; Brown 1995; Small and Soares Jr, 1998). In conclusion, until dose-response experiments are conducted to determine EAA requirements more precisely, the estimated EAA values using whole-body or muscle EAA, as proposed in this study, could be used when formulating diets for Plata pompano.

Acknowledgments

E.M. Silva is a graduate student in Aquaculture at FURG and is supported by the Brazilian Ministry of Fisheries and Aquaculture (MPA) and CAPES. L.A. Sampaio is a research fellow at CNPq (# 308013/2009-3).

Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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