Lat. Am. J. Aquat. Res., 43(5): 888-894, 2015
DOI: 10.3856/vol43-issue5-fulltext-8

Research Article

Estimation of the dietary essential amino acid requirements of colliroja *Astyanax fasciatus* by using the ideal protein concept

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ABSTRACT. Colliroja, *Astyanax fasciatus*, is a new aquaculture species, and information on its dietary essential amino acid requirements is lacking. The whole body composition of 120 farmed fish (16.2 ± 8.8 g) was determined to estimate the dietary essential amino acid requirement based on the ideal protein concept ((each essential amino acid/lysine) ×100), and the findings were correlated to the whole body essential amino acid content of Nile tilapia *Oreochromis niloticus*. The dietary essential amino acids, including cysteine and tyrosine, accounted for 5.46, 4.62, 1.16, 3.28, 5.63, 2.01, 2.59, 2.84, 4.66, 3.39, 0.65, and 3.51% of the total protein for lysine, arginine, histidine, isoleucine, leucine, methionine, methionine+tyrosine, phenylalanine, phenylalanine+tyrosine, threonine, tryptophan, and valine, respectively. There were positive linear and high correlations (r = 0.971) between the whole body amino acid profiles of colliroja and Nile tilapia. Thus, the whole body amino acid profile of colliroja might be used to estimate accurately the essential amino acid requirement.

Keywords: *Astyanax fasciatus*, amino acids, nutrition, protein, ideal protein concept, aquaculture.

Estimación de los requerimientos dietéticos de aminoácidos esenciales de colliroja, *Astyanax fasciatus*, basadas en el concepto de proteína ideal corporal

RESUMEN. Colirroja, *Astyanax fasciatus*, es una nueva especie de la acuicultura y no hay información sobre sus requerimentos de aminoácidos esenciales en la dieta. Se determinó la composición corporal de 120 peces de cultivo (16,2 ± 8,8 g) para estimar los requirimientos nutricionales de aminoácidos esenciales en la dieta, incluyendo cistina y tirosina, basada en el concepto de proteína ideal ((cada aminoácido essencial/lisina) x 100) y los resultados se correlacionaron con el perfil corporal de la tilapia del Nilo *Oreochromis niloticus*. El perfil dietético de aminoácidos esenciales explica el 5,46, 4,62, 1,16, 3,28, 5,63, 2,01, 2,59, 2,.84, 4,66, 3,39, 0.65 y 3,51% de la proteína, respectivamente para lisina, arginina, histidina, isoleucina, leucina, metionina, metionina+cistina, fenilalanina, fenilalanina+tirosina, treonina, triptófano y valina. Hubo una alta correlación lineal positiva (r = 0,971) entre el perfil corporal de aminoácidos de colirroja y el de tilapia del Nilo. Por lo tanto, el perfil corporal de los aminoácidos basado en el concepto de proteína ideal podría ser utilizado para estimar con precisión el requisito de aminoácidos esenciales de colirroja.

Palabras clave: *Astyanax fasciatus*, aminoácidos, nutrición, proteína, concepto de proteína ideal, acuicultura.

INTRODUCTION

Protein is the most costly portion of aquaculture feeds. Estimating the amino acid requirements of fish is important, because fish do not actually require dietary protein, but a rather well-balanced supply of dietary amino acids. The essential amino acid requirements have been established for only a few cultured fish spe-

Corresponding editor: Ronaldo Cavalli

cies (NRC, 2011). Individual amino acid requirements for fish have been determined by using time-consuming and costly dose-response feeding assays. As an alternative, the whole body composition of a species can be used to estimate simultaneously the requirements for all ten essential amino acids (Tacon, 1989). This technique has been currently used to estimate the essential amino acid requirements for fish (Portz & Cyrino, 2003; Gurure *et al*., 2007), because there is high correlation between the content of dietary amino acids required determined using dose-response experiments and that of amino acids in the whole body tissue (Wilson & Poe, 1985).

Since the amino acid requirements of a growing animal are known to be reflected by its amino acid composition (Mitchell, 1950), the ideal protein concept that uses the relationships of each essential amino acid profile in relation to lysine has been developed as a basis to formulate diets for fish (Furuya *et al*., 2004a; Kaushik & Seiliez, 2010; NRC, 2011). The advantage of this concept is that it can be adapted to various situations, provided the relationships between amino acids do not change for a given growth stage (Portz & Cyrino, 2003). For fish, the quantitative lysine requirement might vary, but not the ideal amino acid profile expressed relative to lysine. Given the dietary requirement for lysine, the requirement for the remaining essential amino acids can be predicted based on the relative ratio of amino acids in the whole body.

A. fasciatus (Cuvier, 1819) is an omnivorous freshwater fish; it is widely used as a game and food fish. Rapid growth, low dietary protein requirement, and mild white flesh attract consumers, rendering it an economically important fish. However, at present, the information on the dietary requirements for this fish species is not available. Thus, this study aimed to estimate the dietary essential amino acid requirement for colliroja by analyzing the whole body composition.

MATERIALS AND METHODS

Fish and management

The study was performed at an application unit of the Aquaculture Laboratory, Universidade Estadual de Ponta Grossa, Paraná, Brazil. Fish were obtained from a local fish farm (Piscicultura Águas Claras, Castro, Paraná, Brazil; 24º42'32"S, 50º02'37"W). In total, 120 cultivated mixed-sex fish (16.2 ± 8.8 g) were collected randomly in March (60 fish) and October (60 fish) of 2013, and fish collected during each month were divided in two groups (30 fish each) and considered as replicates. Specimens are deposited in the Núcleo de Pesquisa em Limnologia, Ictiologia e Aquicultura/ Coleção Ictiológica at the Universidade Estadual de Maringá (NUP 11864), Maringá, Paraná, Brazil.

The fish samples were obtained from two earth ponds of 300 m^2 each. While collecting colliroja, 30 Nile tilapias (15 fish during each collection) were collected (621 ± 37 g) from a 100 m^2 earth pond for whole body analysis of crude protein and amino acids. They were fed only naturally available feed and not artificial diets. Fish were caught using surface and bottom trawl nets (mesh size = 1 cm (colliroja) and 12 cm (Nile tilapia)), and placed in a 500 L indoor fiberglass tank aerated to maintain a dissolved oxygen of >5 and <6 $mg \cdot L^{-1}$. They were fasted for 24 h before the beginning of the experiment and euthanized by an overdose of benzocaine (3 g 10 L^{-1}). The total length (0.1 cm) and body weight (0.01 g) of colliroja were measured. Fish samples were stored in a freezer at -80ºC for subsequent analysis.

Calculation

The whole body profile of essential amino acids, including cysteine and tyrosine, was expressed relative to the lysine content according to equation (1): $EAA_P = EAA/L \times 100$, where EAA_P is the whole body essential amino acid profile, and L is the whole body lysine content. The dietary lysine requirement was estimated to be 5.46% of the protein; this represents the mean value of dietary lysine requirement of common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), and Nile tilapia (*Oreochromis niloticus*) reported by the NRC (2011). The essential amino acid profile in the diets for colliroja was estimated according to equation (2): $EAA_D = 5.46 \times (EAA_P/100)$, where EAA_P (% dietary protein) is the essential amino acid profile in the diet, 5.46 is the mean value of dietary lysine requirement (% dietary protein) of common carp, grass carp, and Nile tilapia (NRC, 2011), and EAA_P is the whole body essential amino acid profile determined using equation (1).

Analytical procedures

Pooled samples of fish were ground in a meat grinder and analyzed in duplicate for body composition of moisture and protein, as per the standard methods (AOAC, 2003). Moisture content was determined by drying the samples in an oven (TE-391-1; Tecnal, Piracicaba, SP, Brazil) at 105°C until a constant weight was reached. Nitrogen content was determined using a micro-Kjeldahl apparatus, and crude protein was estimated by multiplying the nitrogen content by 6.25. Amino acid content was determined after acidic and basic digestion for chromatographic and ionic change analyses (high-performance liquid chromatography) performed in sealed glass tubes under nitrogen atmos-

phere at 110°C. Cysteine and methionine contents were determined by hydrolysis after oxidation with performic acid. After hydrolysis, the solutions were vacuum-filtered, diluted to 0.25 M with 0.02 N HCl for adjusting the pH to 8.5, and filtered through a Millipore membrane (0.45 mm). Tryptophan analysis was performed after alkaline hydroxylation of the samples with lithium hydroxide. Free amino acids were separated using an auto-analyzer (Hitachi L-8500A; Tokyo, Japan).

Statistical analysis

The results are presented as means ± standard deviation of duplicate samples. Data of whole body amino acid profile of Nile tilapia and colliroja were subjected to one-way analysis of variance (ANOVA), and differences between treatment means were determined using *t*-test. The correlation among the dietary essential amino acid profiles determined based on the ideal protein concept of Nile tilapia and colliroja was expressed using linear regression analysis, and each essential amino acid mean was compared using *t*-test ($P < 0.05$) by using the SPSS Statistical Package (Version 15.0; SP Inc., Chicago, IL). The dietary amino acid requirement was recommended as percentage of protein found in the whole body composition of colliroja.

RESULTS

The results of the quantitative analysis of whole body essential and non-essential amino acids of colliroja are shown in Table 1.

Among the essential amino acids, the mean concentration of lysine in the whole body composition of colliroja was the highest, followed by those of leucine and arginine. The concentration of tryptophan was the lowest. The profiles of essential amino acids, including cysteine and tyrosine, of colliroja and Nile tilapia obtained on the basis of the ideal protein concept are shown in Table 2 and Figure 1.

There were no differences between whole body concentrations of arginine, histidine, isoleucine, methionine, methionine+cysteine, phenylalanine, phenylalanine+tyrosine, and tryptophan of Nile tilapia and colliroja. However, Nile tilapia showed higher ($P < 0.05$) values of methionine, threonine, and valine than those in colliroja.

Regression analysis showed high correlation ($r = 0.9710$) between whole body essential amino acid profiles of colliroja and Nile tilapia (Fig. 2); the regression could be described according to the following equation: $y = 8.4964+6.4956x$. The dietary essential amino acid profile recommended for colliroja is shown in Table 3.

Table 1. Whole body composition of essential and non-essential amino acids of colliroja *Astyanax fasciatus* (dry matter basis). Values are mean ± standard deviation of two replicate analyses.

Composition	%
Crude protein	42.44 ± 0.41
Arginine	2.74 ± 0.05
Phenilalanine	1.64 ± 0.03
Phenilalanine + tyrosine	2.82 ± 0.04
Histidine	0.76 ± 0.00
Isoleucine	1.64 ± 0.03
Leucine	2.89 ± 0.05
Lysine	3.15 ± 0.06
Methionine	1.04 ± 0.01
Methionine + cysteine	1.39 ± 0.01
Threonine	1.66 ± 0.02
Tryptophan	0.41 ± 0.00
Valine	1.92 ± 0.04
Glutamic acid	5.25 ± 0.07
Aspartic acid	3.64 ± 0.01
Alanine	2.69 ± 0.01
Cysteine	0.34 ± 0.00
Glycine	3.10 ± 0.01
Serine	1.68 ± 0.00
Tyrosine	1.18 ± 0.01

DISCUSSION

Among all essential amino acids, the proportion of lysine was high in colliroja; this is in agreement with the results obtained in carnivorous pikeperch, *Sander lucioperca* (Jarmolowicz & Zakęś, 2014), black bass, *Micropterus salmoides* (Portz & Cyrino, 2003), and omnivorous fish species (Abimorad & Castellani, 2011; Taşbozan *et al.*, 2013). According to the ideal protein concept, amino acid profile is expressed relative to lysine content, because lysine is usually the first limiting amino acid and exclusively used for body protein synthesis. In addition, lysine is one of the most studied amino acids in fish nutrition, and adequate crystalline lysine supplementation is positively related to the growth and feed efficiency of fish (Wu, 2013).

Despite the higher quantitative value of whole body lysine, the arginine to lysine ratio obtained for colliroja (0.87:1) approached 0.83:1 for Nile tilapia as revealed by a dose-response experiment (Santiago & Lovell, 1988) and 0.84:1 for channel catfish, *Ictalurus punctatus* (NRC, 2011). The lysine to arginine ratio might be evaluated in fish diets to avoid antagonisms, because impaired ratios of lysine or arginine can reduce fish growth and feed efficiency (Wu, 2013). Crystalline

Table 2. Whole body amino acid profile of colliroja *A. fasciatus* and Nile tilapia *Oreochromis niloticus* determined on the basis of the ideal protein concept. EEA/L: each essential amino acid (including cysteine and tyrosine) relative to lysine. Values are means ± standard deviation (n = 2), and values within the same row with different letters are significantly different ($P < 0.05$) by *t*-test.

Amino acid	EEA/L		
	Nile tilapia	Colirroja	*P*-value
Lysine	100.00 ± 0.00	100.00 ± 0.00	-
Arginine	84.76 ± 10.82[a]	87.13 ± 0.42[a]	0.628
Histidine	21.32 ± 1.43[a]	24.07 ± 0.37[a]	0.399
Isoleucine	60.16 ± 2.57 [a]	52.08 ± 0.23[a]	0.165
Leucine	103.39 ± 0.85[a]	92.01 ± 0.55[b]	0.013
Methionine	36.95 ± 0.03[a]	33.12 ± 0.31[b]	0.033
Methionine + cysteine	47.51 ± 1.04[a]	44.01 ± 0.42[a]	0.152
Phenylalanine	52.09 ± 1.99[a]	52.08 ± 0.34[a]	0.552
Phenylalanine + tyrosine	85.56 ± 4.64[a]	89.51 ± 0.70[a]	0.883
Threonine	62.15 ± 0.43[a]	52.60 ± 0.45[b]	0.040
Tryptophan	11.85 ± 1.08[a]	12.88 ± 0.13[a]	0.060
Valine	64.34 ± 0.41[a]	61.06 ± 0.17[b]	0.037

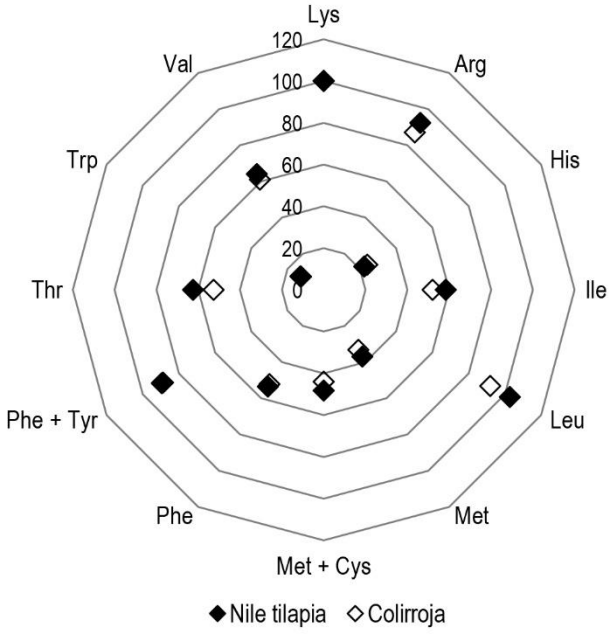

Figure 1. Whole body essential amino profile (including cysteine and tyrosine) of Nile tilapia *Oreochromis niloticus* and colliroja *A. fasciatus* determined on the basis of the ideal protein concept. Arg: arginine, His: histidine, Ile: Isoleucine, Leu: leucine, Met: methionine, Met+Cys: methionine+cysteine, Phe: phenylalanine, Phe+Tyr: phenylalanine+tyrosine, Thr: treonine, Trp: triptofano, and Val: valine.

amino acid supplementation based on the ideal protein profile is useful for estimating the dietary requirement of all essential amino acids from the analysis of only one amino acid, lysine (NRC, 2011).

Cysteine and tyrosine are considered semi-essential because they are only biosynthesized from methionine and phenylalanine, respectively, and their dietary requirement should be presented as the sum of methionine+cysteine (sulfur amino acids) and phenylalanine+tyrosine (aromatic amino acids; Wu, 2013). However, methionine is the most limiting amino acid in soybean meal, one of the most frequently used plant-protein in fish diets, and minimum dietary methionine is included during feed formulation to avoid methionine deficiency (Furuya *et al.*, 2004b). In contrast, phenylalanine is not considered a limiting amino acid in fish nutrition, and few studies have determined its dietary requirement.

There are contradicting results on methionine and total sulfur amino acid concentrations determined in dose-response experiments and values estimated from whole body amino acid composition of fish. The dietary amino acid requirements of colliroja were compared to those of Nile tilapia obtained from dose-response experiments and whole body analysis, considering the similarity between colliroja and Nile tilapia in terms of food habits (omnivorous) and taxonomic classification (Characidae family). Moreover, both are freshwater fish, and the complete dietary requirement of all essential amino acids is known for Nile tilapia (NRC, 2011). Santiago & Lovell (1988) described the dietary sulfur amino acid requirement of 0.90% for fingerlings of Nile tilapia fed purified diet, which included 0.75% of methionine and 0.25% of cysteine. However, Furuya *et al.* (2004b) suggested the requirement of 1.13% sulfur amino acids, including 0.54% of methionine. The proportion of methionine was higher in relation to cysteine (0.83:0.17) in the purified diets used by Santiago

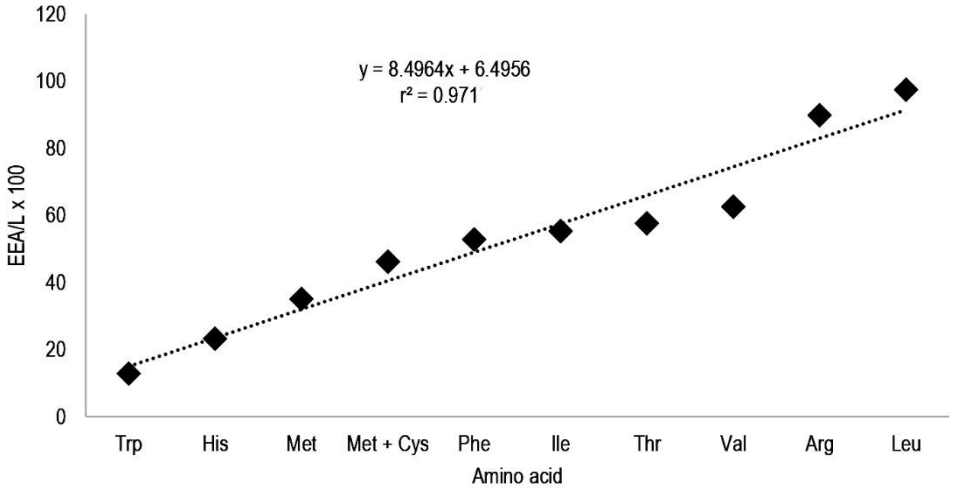

Figure 2. Linear correlation between Nile tilapia *Oreochromis niloticus* and colliroja *Astyanax fasciatus* whole body amino acid profile determined on the basis of the ideal protein concept (EEA/L × 100), considering the essential amino acids (EAAs) to lysine (L) ratios. Arg: arginine, His: histidine, Ile: Isoleucine, Leu: leucine, Met: methionine, Met+Cys: methionine+cysteine, Phe: phenylalanine, Phe+Tyr: phenylalanine+tyrosine, Thr: treonine, Trp: triptophan, and Val: valine.

Table 3. Dietary quantitative essential amino acid requirement (including cysteine and tyrosine) of colliroja *Astyanax fasciatus* considering the diets containing different levels of crude protein (as feed basis).

Amino acid	Dietary amino acid profile (% dietary protein)
Lysine	5.46
Arginine	4.62
Histidine	1.16
Isoleucine	3.28
Leucine	5.63
Methionine	2.01
Methionine + cysteine	2.59
Phenylalanine	2.84
Phenylalanine + tyrosine	4.66
Threonine	3.39
Tryptophan	0.65
Valine	3.51

& Lovell (1988), whereas Teixeira *et al.* (2008) revealed a methionine to cysteine ratio of 0.75:0.25 for Nile tilapia by analyzing the body composition. There is a discrepancy in these ratios because food used for the preparation of commercial diets for fish and other aquatic organisms possess higher proportions of cysteine relative to methionine, unlike that in purified casein, which is the main protein source used in fish nutrition dose-response studies.

Adequate dietary methionine supplementation is necessary for the maximum growth and health of fish. Methionine plays a role in protein synthesis and is important for physiological functions. Further, it is essential for normal growth of fish and is a donor of methyl groups required for methylation reactions via S-adenosylmethionine (Bender, 2003). S-adenosylmethionine is synthesized from methionine, which is then catalyzed by adenosyl triphosphate cyclase, allowing methyl group donation to various substrates, including nucleic acids, proteins, phospholipids, and biogenic amines. The S-adenosyl methionine generates compounds such as carnitine (Wu, 2013), cysteine, and choline (Kasper *et al.*, 2000), which are important compounds required for the metabolism of lipids and affect the growth performance and lipid deposition of Nile tilapia (Graciano *et al.*, 2010).

The complete quantitative essential amino acid requirements have been established for only a few fish species (NRC, 2011), and determining the amino acid requirements of the new aquacultural species colliroja is of economic importance. Dose-response experiments require large numbers of feeding trials to determine individual essential amino acid profiles. In addition, these trials are time-consuming and very costly. As a practical alternative, whole body essential amino acid profile has been extensively used to estimate simultaneously the requirements for all essential amino acids. This technique was used in the present study by considering that dietary amino acid profile is close to the pattern in the muscle tissues of fish (Mitchell, 1950; Fuller *et al.*, 1989). However, the whole body amino acid composition varies across fish species (Bicudo *et al.*, 2009). To date, Taşbozan *et al.* (2013) found large variations among whole body amino acid profiles of five different *Tilapia* species. Thus, estimating the dietary amino acid requirements for each fish species is

important for precisely formulating aquafeeds. However, the quantitative dietary requirement of fish is more precisely obtained by dose-response experiments compared to estimates from whole body amino acid profiles (NRC, 2011). Nonetheless, comparison of profiles obtained using the two methods in colliroja and Nile tilapia revealed that there is not much difference in the estimated values. In conclusion, the whole body composition of amino acids might be used to estimate the dietary requirements of essential amino acids, including cysteine and tyrosine, for new aquaculture fish species such as colliroja.

ACKNOWLEDGMENTS

We are grateful to Ajinomoto do Brasil Indústria e Comércio de Alimentos Ltda. - Animal Nutrition Division, for performing the amino acid analysis.

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Received: 6 August 2014; Accepted: 17 August 2015