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Amino acid composition of Brazilian surubim fish (*Pseudoplatystoma coruscans*) fed diets with different levels and sources of fat

Paula Campos [a], Ricardo C. Martino [b,*], Luiz C. Trugo [a]

[a] *Laboratório de Bioquímica Nutricional e de Alimentos, Instituto de Química, Universidade Federal do Rio de Janeiro, Brazil*
[b] *Unidade de Tecnologia do Pescado, Fundação Instituto de Pesca do Estado do Rio de Janeiro, Brazil*

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Abstract

Surubim (*Pseudoplatystoma coruscans*) is a river fish from the Brazilian São Francisco basin which presents a great potential for commercialization. The amino acid composition of carcasses of surubim fed isoproteic diets with variable levels and sources of fat were determined. The increase of soybean oil at levels of 4%, 8% and 12% in the rations promoted a corresponding increase in nitrogen fixation in the fish carcass, reflected by higher amino acid contents. However, the application of 12% added amounts of pig lard, corn oil, linseed oil or soybean oil, as different sources of fat, did not promote much variation in the fish amino acid fixation. The determination of the essential amino acid composition in the fish carcasses and the A/E ratios of individual amino acids provided a pattern of amino acid requirements of surubim fish.

Keywords: Surubim fish; Amino acid composition; Amino acid requirements

1. Introduction

The efficiency of a fish diet is dependent on its balance in relation to the specific requirements of the fish species. Nutrient requirement differences exist between species, particularly in relation to the amino acid composition (Portz & Cyrino, 2003). Data for fish nutrient requirements from South America, including Brazil, are scarce and diets for farming of regional fish are elaborated using published information available for other species (Pezzato, 1995). This is what is observed for farming of surubim (*Pseudoplatystoma coruscans*), which is a river fish originally from the Brazilian São Francisco Basin (Petrere, 1995). Surubim has great commercial potential due to its size (it may reach 50 kg) and also due to its clear and delicate flesh which presents few inter-muscle bones (Sato, Cardoso, & Sallum, 1998). Surubim is being farmed in some places in Brazil but little information is available about its physiology and its nutritional requirements. Knowledge about the actual amino acid requirements of surubim would be very useful for an appropriate diet formulation and, as a consequence, a better growth performance, yield and higher nutritive value (Halver, 1976). Different approaches may be used to assess amino acid requirements of fish. The measurement of growth rate of the fish fed increasing levels of each amino acid (Sena & Trevor, 1995), the evaluation of the amino acid composition of the fish egg (Ketola, 1982) and the determination of the amino acid composition of the fish carcass, as an indication of the amino acid utilization (Sena & Trevor, 1995), are the usual procedures used. The latter is more practical and seems to give realistic information for formulation of adequate diets (Rob, 1999). In the present work, the amino acid composition was determined in surubim carcasses of fish submitted to isoproteic diets containing different levels and sources of fat. This approach may

* Corresponding author. Tel./fax: +55 21 2410 7002.
E-mail address: rmartino.rlk@terra.com.br (R.C. Martino).

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be useful for assessing the amino acid requirements of surubim fish and also for establishing the protein quality of its meat.

2. Materials and methods

2.1. Experimental fish

Three hundred fingerlings of surubim (*Pseudoplatystoma coruscans*) were purchased from a commercial fish farm (Projeto Pacu, Campo Grande, MS, Brazil) and maintained at the Nutrition Laboratory of the Animal Production Department of Escola Superior de Agricultura Luiz de Queiroz of São Paulo University. Prior to starting the feeding trial, fish were acclimatized to laboratory conditions for 12 days. During this period, they were distributed in two 1000 l plastic circular tanks, in a closed recirculation system and fed, twice a day, to satiation with a commercial fish diet, formulated with 40% of crude protein and 10% of crude lipid (Nutron Alimentos, Campinas, SP, Brazil).

2.2. Experimental design

Fish were selected according to their health condition and body weight and individually weighed at the start of the experiment. The initial body weight averaged 2.72 g (±0.2). Sample fish were not fed for 24 h prior to initiation of the feeding trials. Fish were stocked in 60 l net-cages (13 fish per cage) randomly distributed in the 1000 l plastic tanks (three net-cages per tank). The water temperature was maintained at 27 ± 1 °C by an electric heater. Fifteen fish from the stock were killed and kept frozen as control samples. During the 62-day feeding period, fish were fed a sinking pellet diet twice a day to apparent satiation. The plastic tank and the cages were cleaned every other day. Three replicate groups of fish were used for testing each diet. At the end of the experiment, starved fish were anesthetized with benzocain (0.5 g/l), individually weighed and sacrificed. Six fish carcasses from each group were minced and this pool was stored in plastic bags under nitrogen in a freezer at −20 °C for subsequent analysis. This same procedure was applied for all feeding trials.

2.3. Experimental diets

Prior to use, all feed ingredients were analyzed for their proximate composition and amino acid composition. The data obtained were used as a basis for making the diet formulation. Two experiments were carried out. In the first experiment, four isonitrogenous diets (1–4), with 46% crude protein, were prepared, containing approximately 6.0%, 10.0%, 14.0% and 18.0% (wet basis) crude lipid, respectively. Crude lipid was the result of lipid content in the basic diet plus the increasing additions of soybean oil. The objective of this experiment was to test the influence of fat level on the fixation of essential amino acids in the fish carcass. In the second experiment, four isonitrogenous and isoenergetic diets were prepared, in order to contain about 46% of crude protein and around 18% of crude lipid. The influence of the fat source on amino acid fixation was then studied. The fat sources were pig lard, corn oil, linseed oil and soybean oil. The first three sources were added to diets 5, 6 and 7, respectively. Soybean oil was added to diets 2, 3 and 4 at levels of 4%, 8% and 12%, respectively. The other sources were added at a level of 12%. The compositions of the experimental diets are given in Table 1. A mixture was prepared by mixing the ingredients in a mortar with distilled water until consistent. Then, 50% of the mixture was pelleted through a 1–2 mm sieve and the remainder through a 2–3 mm sieve. The pellets were then dried in a oven (Advantec, FC 610, Japan) with circulation air at 40 °C for 14 h. White fish meal (Anchoita, Chile), was the major ingredient in all diets. Cellulose (Sigma, USA) was used to replace soybean oil and to achieve the desired proximate composition of the diets. About 2% of a commercial mineral/vitamin premix (Nutron Alimentos, Brazil) was added to the diets. In order to prevent lipid rancidity, 0.02 g/100 g of butylated hydroxy toluene (BHT, Inspec Spain, Spain) was added to all diets.

2.4. Analytical procedures

2.4.1. Proximate analysis

The proximate analysis of the diets was carried out according to the procedures of the Association of Official Analytical Chemists (2000). Moisture was determined by drying the samples in an oven (Labostar – LG 122, Japan) at 110 °C to constant weight; crude ash was determined by incineration in a muffle furnace (Isuzu, Japan) at 600 °C for 3 h; crude fibre was determined by the acid detergent fibre (ADF) method; crude protein was determined by the Kjeldahl method ($N \times 6.25$) using an automatic Kjeldahl system (Buchi 430/323, Switzerland).

2.4.2. Calcium and phosphorous determination

Calcium was determined by titration of precipitated calcium oxalate from the dry ash by using a standard AOAC 927.02 Method (2000). Phosphorus was determined by the AOAC 964.06 Method (2000).

2.4.3. Lipid content

The lipid content was gravimetrically determined after extraction with chloroform/methanol (2:1, v/v), according to the method of Folch, Lees, and Stanley-Sloane (1957).

Table 1
Formulation and proximate composition of the experimental diets

Ingredients (%)	Diets						
	1	2	3	4	5	6	7
Fish meal	55	55	55	55	55	55	55
Corn gluten	8	8	8	8	8	8	8
Soybean meal	4	4	4	4	4	4	4
Wheat flour	14	14	14	14	14	14	14
Corn flour	5	5	5	5	5	5	5
Lard	–	–	–	–	12	–	–
Corn oil	–	–	–		–	12	–
Soybean oil	–	4	8	12	–	–	–
Linseed oil	–	–	–	–	–	–	12
Vit/min[a]	1.98	1.98	1.98	1.98	1.98	1.98	1.98
Cellulose	12	8	4	–	–	–	–
BHT[b]	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Proximate composition							
Moisture	6.7	7.4	7.1	7.2	6.9	6.0	8.0
Crude protein	46.7	46.4	46.3	45.7	45.8	45.4	45.5
Crude lipid	6.1	10.7	14.8	18.1	18.7	18.4	18.5
Crude ash	12.1	10.8	10.5	11.1	11.6	11.2	10.9
Crude fibre	11.7	9.6	5.2	1.2	1.2	1.2	1.2
NFE[c]	16.7	15.1	16.1	16.7	15.8	17.8	15.9
Calcium	3.4	3.1	3.2	3.3	3.4	3.3	3.5
Phosphorus	1.5	1.5	1.5	1.8	1.7	1.8	1.9

[a] Vitamin/Mineral mixture (Nutron Alimentos, Brazil) – units/kg of premix. Antioxidant – 0.60 g; Vit A – 1,000,000 IU; Vit D3 – 500,000 IU; Vit E – 20,000 IU; Vit K3 – 500 mg; thiamin – 1250 mg; riboflavin – 2500 mg; pyridoxine – 2485 mg; pantothenic acid – 5000 mg; niacin – 5000 mg; biotin – 125 mg; folic acid – 250 mg; cyanocobalamin – 3750 mg; ascorbic acid – 28,000 mg; cobalt (Co) – 25 mg; copper (Cu) – 2000 mg; iodine (I) – 100 mg; iron (Fe) – 13,820 mg; manganese (Mn) – 3750 mg; zinc (Zn) – 150 mg; selenium (Se) – 75 mg.
[b] Butylated hydroxy toluene (Inspec Spain, Spain).
[c] Nitrogen-free extract = 100 − (moisture + crude protein + crude lipid + crude ash + crude fibre).

2.4.4. Amino acid analyses

A pool of fish carcasses was obtained with six fish from each triplicate experiment and this was considered as the representative sample for amino acid analysis. The inter- and intra-assay coefficients of variation of these analyses was less than 4%. The amino acid analyses were performed in these pools according to the method of Spackman, Stein, and Moore (1958). Briefly, the equivalent of 25 mg of the sample protein was hydrolyzed under vacuum with 10 ml of 6 M HCl at 110 °C for 22 h. Amino acids were analyzed in a Diorex Dx300 apparatus by separation in an ion-exchange column and post-column reaction with ninhydrin, using, as reference, a standard solution of amino acids (Pierce, USA). Tryptophan was determined according to Spies (1967), after enzymatic hydrolysis of the sample with pronase at 40 °C for 24 h, followed by a colorimetric reaction with 4-dimethyl amino benzaldehyde (DAB) solution in sulfuric acid. The amount of tryptophan was calculated from a standard curve.

2.5. Statistical analyses

Data were statistically analyzed by ANOVA, with a post hoc Tukey test, to evaluate the effects of dietary lipid levels and sources on food conversion ratios (FCR) of fish. Values of $P < 0.05$ were considered as significant. Statistical analyses were performed using the SAS software, version 6.12 (SAS Institute, Inc., Cary, NC, USA).

3. Results and discussion

The composition of the diets and the proportion of ingredients used in the experiments are shown in Table 1. Table 2 shows the amino acid composition of the ingredients used in the diets.

The amino acid fixation in the fish carcass was determined in two groups of experiments. In one group, the amount of added fat varied from 0% to 12% (trials 1–4) and, in the other experiment, the amount of fat was fixed and the source varied (trials 5–7). In both experiments, the amount of protein was kept constant. Table 3 shows the results of amino acid fixation on the fish carcass in the two experiments (trials 1–7). Since the amino acid analyses were carried out in the pooled samples of each trial (as described in Section 2), no statistical analysis was performed.

In the first experiment, the amino acids showed a tendency to increase their contents in the carcass following the increase of total fat in the diet (Table 3), except for tryptophan, which presented somewhat irregular

Table 2
Amino acid composition of the ingredients and diet used in the experiments

Amino acid	Fish meal	Soybean meal	Corn gluten	Corn flour	Wheat flour	Diet
Arginine	3.77	2.92	1.46	0.23	0.40	4.46
Phe + Tyr	4.05	3.30	6.21	0.58	0.89	4.27
Histidine	2.64	1.07	0.90	0.17	0.21	3.05
Isoleucine	1.94	1.06	1.24	0.12	0.22	2.31
Leucine	4.32	2.77	8.88	0.86	0.74	5.71
Lysine	5.06	2.62	0.95	0.20	0.23	5.78
Met + Cys	1.96	0.83	1.51	0.19	0.29	2.20
Threonine	2.62	1.46	1.63	0.21	0.29	3.16
Tryptophan	0.52	0.56	0.22	0.05	0.08	0.61
Valine	2.32	1.12	1.64	0.20	0.32	2.87

Values are averages of duplicate determinations expressed on g/100 g dry basis.
Phe = Phenylalanine; Tyr = Tyrosine; Met = Methionine; Cys = Cystine.

Table 3
Amino acid composition of carcasses of surubim fish fed different diets

Amino acid	1	A/E[a]	2	A/E[a]	3	A/E[a]	4	A/E[a]	5	A/E[a]	6	A/E[a]	7	A/E[a]	A/E average
Arginine	0.46	142.9	0.67	178	0.76	144	0.90	134	1.02	132	0.87	130	0.84	133	132 (1.77)
Phe + Tyr	0.47	146.0	0.62	165	0.79	150	0.96	143	1.11	143	0.96	143	0.88	139	142 (2.10)
Histidine	0.16	49.7	0.22	58.5	0.34	64.4	0.29	43.2	0.35	45.2	0.30	44.8	0.32	50.6	46.0 (3.22)
Isoleucine	0.18	55.9	0.43	114	0.29	54.9	0.55	82.0	0.64	82.6	0.55	82.1	0.52	82.1	82.2 (0.27)
Leucine	0.50	155	0.30	89.8	0.85	161	1.03	154	1.18	152	1.03	154	0.96	152	153 (0.76)
Lysine	0.62	193	0.14	37.2	1.02	193	1.18	176	1.37	177	1.17	175	1.11	175	176 (1.11)
Met + Cys	0.11	34.2	0.38	101	0.23	43.6	0.43	64.1	0.53	68.4	0.45	67.2	0.44	69.5	66.6 (2.22)
Threonine	0.31	96.3	0.47	125	0.53	100	0.65	96.9	0.76	98.1	0.65	97.0	0.61	96.4	97.1 (0.72)
Tryhtophan	0.20	62.1	0.04	10.6	0.11	20.8	0.10	14.9	0.08	10.3	0.10	14.9	0.07	11.1	12.8 (2.44)
Valine	0.21	65.2	0.49	130	0.36	68.2	0.62	92.4	0.71	91.6	0.62	92.5	0.58	91.6	92.2 (0.49)
Total	3.22		3.76		5.28		6.71		7.75		6.70		6.33		

Trials 1–7 correspond to fish fed diets 1–7 as in Table 1.
Values are averages of duplicate determinations of pooled samples of each trial, expressed as g/100 g of fish carcasses, dry basis.
A/E average = average and standard deviation of A/E values of trials 4–7.
Phe = Phenylalanine; Tyr = Tyrosine; Met = Methionine; Cys = Cystine.
[a] A/E (each essential amino acid/total essential amino acid × 1000).

behaviour, although also showing a tendency to equilibrate in the carcasses obtained from higher fat diets. In general, the amino acid contents seemed to be higher in the carcass of fish fed the higher lipid diet (Table 3), which consistently also presented the best FCR ($P < 0.05$) (Fig. 1). This is in agreement with the results described for other species (Takeda, Shimeno, Hosakawa, & Kaysio, 1975) fed high lipid diets in which the FCRs appeared to be dependent on the fat level of the diets. In the second experiment, using fixed protein and fat levels and with variation of the source, there was no apparent effect on amino acid composition of the fish carcasses, and the results were in fact similar to those obtained with the higher fat diet used in experiment 1. These results indicate that 12% of fat addition to the diet is adequate for nitrogen fixation in the fish carcasses and that the source of lipids did not influence the amino acid utilisation.

The method used for assessing amino acid requirements of surubim in the present work was based on the analysis of the amino acid composition in the fish

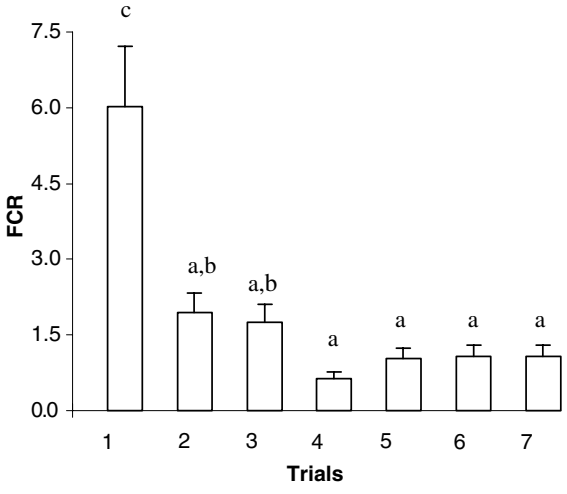

Fig. 1. Variation of food conversion ratios (FCR) of surubim fish fed diets with different levels and sources of lipids. Trials were carried out using diets 1–7 as shown in Table 1. No oil addition, 1; 4% addition of soybean oil, 2; 8% addition of soybean oil, 3; 12% addition of soybean oil, 4; 12% addition of pig lard, 5; 12% addition of corn oil, 6; 12% addition of linseed oil, 7.

carcass (Sena & Trevor, 1995). This method allows the establishment of the essential amino acid requirements of a particular species (Tacon, 1990) and basically the same results are found when this assessment is made by feeding increasing amounts of individual amino acids (Ogino, 1980). Consequently, the amino acid profile found in the fish carcass of the group fed diets with 12% of added lipid was considered to represent the adequate requirements of surubim. An additional parameter for assessing the amino acid requirements of fish is the ratio between individual amino acids and the sum of all essential amino acids (A/E ratio). A high correlation has been found between A/E ratios from amino acid composition of fish carcass and nutritional requirements determined by performance assays (Wilson & Poe, 1985). Table 3 shows the A/E ratios calculated for the different trials. The results show that, with the higher fat levels in the diets (diets 4–7), the A/E ratios for the essential amino acids become stable, with 12% of added fat being an adequate level for assessing the amino acid requirements for surubim fish. The same behaviour was observed for the FCRs, as shown in Fig. 1. FCR decreased with an insufficient level of fat, and increased with the increase of fat addition to the test diets, becoming stable when the fat level was 12%, independently of the fat source. It is apparent that considerable differences may be found for different fish species and that these differences must be considered when the diet is planned in order to achieve the best fish performance and a better nutritional quality for consumers.

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