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Aquaculture

Dietary lysine requirement of adult lambari (*Astyanax altiparanae*) (Garutti and Britski, 2000)

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ABSTRACT - A 90-day feeding trial was conducted to estimate the dietary lysine requirement of adult lambari (*Astyanax altiparanae*), based on growth performance, whole-body composition, muscle development, and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities. Six isoproteic (345.0 g kg^{-1}) and isoenergetic (19.51 MJ kg^{-1}) diets were formulated, and crystalline L-lysine was added to obtain diets with lysine concentrations of 12.13, 13.31, 15.36, 18.79, 19.92, and 23.02 g kg^{-1} dry diet. Female adult lambari (n = 480; weight of 4.96±0.02 g) were distributed into 24 (70 L) aquaria and fed the experimental diets six times daily. Fish fed 12.13 to 19.92 g kg^{-1} lysine showed increased weight gain and percent weight gain, and fish from those treatments also showed improvement in final carcass quality by a decrease in whole-body lipid content. Fish fed 12.13 g kg^{-1} lysine showed lower ALT and AST activities in blood serum when compared with fish fed the highest lysine levels. No differences were observed in muscle growth in fish fed graded lysine levels. According to the broken-line model analysis of weight gain and dietary lysine levels, the dietary lysine requirement of adult lambari is estimated at 18.72 g kg^{-1} (5.41% of dietary protein).

Key Words: aminoacid, carcass quality, fish nutrition, lysine, nutritional requirement

Introduction

Protein is a major and the most expensive component of formulated aquafeeds (Wilson, 2002; NRC, 2011). To maximize dietary protein utilization, the amino acid profile must meet dietary requirements (Khan and Abidi, 2011) according to fish species and growth stage (Peres and Oliva-Teles, 2008). Lysine is the first-limiting essential amino acid in plant protein sources commonly used in fish diets (El-Sayed, 1999; Cao et al., 2012), especially in corn and corn byproduct ingredients. In addition, it is found in high concentrations in the whole body of many fish species (Kim and Lall, 2000; Michelato et al., 2016).

In aquaculture, environmental concerns have also stimulated the search for nutritional strategies to improve nitrogen use, minimizing its excretion into the environment. Excessive nitrogen in fish farm effluents stimulates eutrophication of water bodies and reduces water quality. Thus, the manipulation of feed formulation through the estimation of essential amino acid requirements must be considered, not only to maximize the efficiency of protein utilization but also to reduce nitrogenous waste (Talbot and Hole, 1994; Cho and Bureau, 1997).

Lambari, *Astyanax altiparanae*, was recently established as an aquaculture species in Latin America. This fish is produced for food purposes and used as live bait; moreover, it has potential to be marketed as a canned product (Dutra et al., 2012). Its small size, high reproductive rate and fry production without hormonal induction (Porto-Foresti et al., 2010) allows its use as an experimental model for other large-sized species (Gonçalves et al. 2014). Females grow faster than males, with slaughter weight of 5 to 10 g.

A few studies have estimated the dietary requirements for lambaris: the crude protein requirement is about 320 to 380 g kg^{-1} (Cotan et al., 2006), and the dietary essential amino acid requirement was estimated based on the whole-body composition and the ideal protein concept (Abimorad and Castellani, 2011; Furuya et al., 2015). However, individual amino acid requirements determined by using

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dose-response feeding assays have not been published to date. Therefore, the objective of this study was to estimate the dietary lysine requirement for female adult lambari based on the dose-response model, by evaluating the growth performance, whole-body composition, muscle development, and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities.

Material and Methods

This experiment was approved by the local Ethics Committee for Animal Use (Protocol nº 18/2013) and was performed in Viçosa, Minas Gerais, Brazil (20°45'20" S latitude, 42°52'40" W longitude, and 649 m altitude,).

Six isoproteic (345.0 g kg^{-1}) and isoenergetic (19.51 MJ kg^{-1}) experimental diets were formulated, with graded levels of crystalline L-lysine HCl based on corn gluten meal and soybean meal as protein ingredients (Table 1). The final levels of dietary lysine confirmed by amino acid analysis were: 12.13, 13.31, 15.36, 18.79, 19.92, and 23.02 g kg^{-1} of dry diet (Table 2). Macro ingredients were ground in a hammer mill (TRF-400 Trapp, Jaraguá do Sul, SC, Brazil) with 0.5-mm screens; all ingredients were mixed in a "V" mixer (MA200, Marconi, Piracicaba, SP, Brazil); the diets were extruded with a 3-mm Ex-Micro® extruder (ExTeec Company, Ribeirão Preto, SP, Brazil), and then dried for 12 h by oven-drying at 45 °C.

A completely randomized design with six treatments and four replicates was used. Female adult lambari weighing 4.96±0.02 g (mean weight ± SD) were weighed on a precision scale (model MB45 Toledo® 0.01 g, São Bernardo do Campo, SP, Brazil) and randomly distributed into 24 fiberglass aquaria (70 L of water) at 20 fish per aquarium. The aquaria were maintained in a recirculation system (2 to 3 L min^{-1}) with a mechanical and biological filter and covered with nylon nets to prevent fish from escaping.

Throughout the experimental period, water temperature was maintained at 27±1 °C by using a digital thermostat (Coel Tlj29, São Paulo, SP, Brazil). The other water parameters were measured with a multi-parameter meter (model HI 9828, Hanna Instruments, Barueri, SP, Brazil); average dissolved oxygen was 6.50±1.00 mg L^{-1}, pH was 7.30±0.50, and total ammonia was 0.02±0.00 mg L^{-1}. The photoperiod was adjusted to 12 h by fluorescent lamps (60 W). The fish were hand-fed to apparent satiation, six times daily (8:00, 10:00, 12:00, 14:00, 16:00, and 18:00 h) for 90 days.

At the end of the experimental period, after 24 h of starvation, all fish from each aquarium were counted and weighed to evaluate growth performance data, survival rate, weight gain, percent weight gain, feed efficiency, and protein efficiency rate and euthanized with a lethal dose of anesthetic (400 mg clove oil L^{-1}). Whole body, carcass, gonads, viscera, and liver weights were recorded to determine carcass yield and gonadosomatic, viscerosomatic,

Table 1 - Formulations of the experimental diets

Ingredient	Level of dietary lysine (g kg^{-1})					
	12.13	13.31	15.36	18.79	19.92	23.02
Soybean meal 45%	213.00	213.00	213.00	213.00	213.00	213.00
Corn gluten meal 60%	228.50	228.50	228.50	228.50	228.50	228.50
Corn meal	214.00	211.40	208.80	206.20	203.60	201.00
Wheat bran	244.00	244.00	244.00	244.00	244.00	244.00
Broken rice	48.80	48.80	48.80	48.80	48.80	48.80
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	30.00	30.00	30.00	30.00	30.00	30.00
L-lysine[1]	0.00	2.60	5.20	7.80	10.40	13.00
L-tryptophan[1]	3.00	3.00	3.00	3.00	3.00	3.00
L-arginine[1]	0.20	0.20	0.20	0.20	0.20	0.20
DL-methionine[1]	1.70	1.70	1.70	1.70	1.70	1.70
Vitamin C[2]	0.60	0.60	0.60	0.60	0.60	0.60
Salt	5.00	5.00	5.00	5.00	5.00	5.00
Mineral and vitamin mix[3]	8.00	8.00	8.00	8.00	8.00	8.00
Antioxidant[4]	0.20	0.20	0.20	0.20	0.20	0.20
Antifungic[5]	1.00	1.00	1.00	1.00	1.00	1.00

[1] L-lysine, L-tryptophan, L-arginine (Ajinomoto, São Paulo, SP, Brazil); DL-methionine (MCassab, São Paulo, SP, Brazil).
[2] Vitamin C (Saint Charbel, Viçosa, MG, Brazil).
[3] Assurance levels per kilogram of product: vitamin A, 1,200,000 IU; vitamin D3, 200,000 IU; vitamin E, 12,000 mg; vitamin K3, 2,400 mg; vitamin B1, 4,800 mg; vitamin B2, 4,800 mg; vitamin B6, 4,000 mg; vitamin B12, 4,800 mg; folic acid, 1,200 mg; calcium pantothenate, 12,000 mg; vitamin C, 48,000 mg; biotin, 48 mg; choline, 65,000 mg; niacin, 24,000 mg; Fe, 10,000 mg; Cu, 6,000 mg; Mg, 4,000 mg; Zn, 6,000 mg; I, 20 mg; Co, 2 mg; Se, 20 mg (Guabi Animal Nutrition, Brazil).
[4] Butylated hydroxytoluene (BHT) (ISOFAR Ind., Brazil).
[5] Mold Zap Aquatica® (Alltech Agroindustrial Ltda, São Paulo, Brazil).

and hepatosomatic index. The viscerosomatic index included weights of stomach, intestine, pyloric cecum, gonads, heart, liver, gallbladder, and swim bladder. The carcass was defined as the fish without scales and viscera. Protein productive value was also determined from the crude protein value of the animal.

For whole-body composition analysis, fish were previously lyophilized (Labconco, Kansas City, MO, USA) and ground in a ball mill (Marconi MA923, Piracicaba, SP, Brazil). Crude protein (N% × 6.25) was determined by the Kjeldahl method (Quimis, Diadema, SP, Brazil), total lipids were determined by ether extraction in a Soxtherm 2000 extractor (Gerhardt, New Orleans, LA, USA), and mineral matter was determined by combusting dry samples in a muffle furnace (TE-1100-1P, Tecnal, Piracicaba, SP, Brazil) at 550 °C for 6 h, in accordance with the Association of Official Analytical Chemists (AOAC, 1995). Gross energy content was determined by adiabatic bomb calorimetry (Parr 1266, Parr Instruments Co., Moline, IL, USA). All analyses were performed in triplicate.

The amino acid profiles of experimental diets were analyzed by Ajinomoto do Brasil Indústria e Comércio de Alimentos – Animal Nutrition Division (São Paulo, Brazil) by hydrolyzing 0.3 mg of sample in 1 mL of 6N HCl for 22 h. The obtained sample was diluted in 0.02N HCl and injected into an automatic amino acid analyzer (Hitachi L-888, Tokyo, Japan). Hydrolysis recovery was performed in 4N methanesulfonic acid for analysis of tryptophan and in performic acid for sulfur amino acids.

For muscle fiber frequency analysis, white muscle samples were collected from the epaxial region of eight fish from each treatment, fixed in 10% buffered formaldehyde solution for 24 h and transferred to 70% alcohol. Subsequently, muscles were dehydrated in an increasing ethanol series and embedded in glycol methacrylate (Historesin®, Leica, São Paulo, SP, Brazil). Transverse 3 μm fiber sections were obtained with a microtome and stained with toluidine blue. These fiber cross-sections were photo-documented under a light microscope (Olympus® BX53, São Paulo, SP, Brazil) with an attached camera (Olympus® DP73, São Paulo, SP, Brazil). Fiber cross-section diameter (μm) was estimated by measuring 200 white muscle fibers from each animal per group, using the smallest diameter method (Dubowitz and Brooke, 1973). White muscle fibers were grouped into three diameter classes (<20, 20-50, and >50 μm) (Almeida

Table 2 - Chemical composition and amino acid profiles (g kg^{-1}) of the experimental diets, based on dry matter

Chemical composition	Level of dietary lysine (g kg^{-1})					
	12.13	13.31	15.36	18.79	19.92	23.02
Dry matter (g kg^{-1})[1]	941.70	939.90	945.80	942.30	938.80	943.00
Gross energy (MJ kg^{-1})[1]	19.45	19.30	19.41	19.54	19.68	19.72
Crude protein (g kg^{-1})[1]	339.60	346.31	349.86	349.78	342.46	346.87
Gross fiber (g kg^{-1})[1]	26.76	27.56	26.45	26.96	27.69	26.52
Total lipid (g kg^{-1})[1]	135.98	133.11	133.78	132.11	132.80	134.66
Mineral matter (g kg^{-1})[1]	66.35	67.28	65.48	67.05	65.38	65.10
Essential amino acid[2]						
Lysine	12.13	13.31	15.36	18.79	19.92	23.02
Methionine	6.91	6.82	6.98	7.15	7.21	7.32
Threonine	12.15	11.60	11.71	12.46	12.43	12.64
Tryptophan	5.83	5.83	5.77	6.02	5.85	5.63
Arginine	16.84	15.55	15.93	17.04	16.61	16.69
Phenylalanine	18.47	16.78	17.03	17.99	18.16	18.49
Histidine	7.68	7.23	7.16	7.64	7.59	7.75
Isoleucine	12.92	11.86	12.05	12.97	12.98	12.96
Leucine	38.11	34.93	35.82	37.40	38.18	38.80
Valine	14.64	13.46	13.81	14.66	14.81	14.92
Non-essential amino acid[2]						
Alanine	21.97	19.45	20.41	21.47	21.56	21.60
Aspartic acid	25.46	24.44	24.89	26.51	26.15	26.76
Glutamic acid	67.55	62.20	63.64	66.89	66.57	68.09
Cystine	5.29	4.77	5.03	5.27	5.14	5.26
Glycine	12.01	11.01	10.85	11.99	11.56	11.83
Serine	16.12	15.30	15.71	16.42	16.61	16.96
Tyrosine	13.76	12.53	12.52	13.38	13.79	13.56

[1] Values determined at the Animal Nutrition Laboratory of the Department of Animal Science at the State University of Maringá, Paraná, Brazil.
[2] Values determined at the laboratory of Ajinomoto do Brasil Indústria e Comércio de Alimentos Ltda, São Paulo, Brazil.

et al., 2008). Muscle fiber frequency was expressed as the number of fibers from each diameter class relative to the total number of fibers measured.

For enzymatic activity analysis, blood was collected with a hypodermic syringe from the caudal vein. One milliliter of serum was separated by centrifugation (10,000 rpm for 15 min at 4 °C) and stored at −80 °C. Aspartate aminotransferase and ALT activities in serum were measured by using diagnostic reagent kits purchased from Bioclin® (Quibasa - Química Básica, Belo Horizonte, MG, Brazil) according to the manufacturer's instructions, in an automatic biochemical analyzer (Alizè, Lisabio, France). All analyses were performed in duplicate.

Statistical analysis of the data was subjected to the Lilliefors test, to verify the normality of errors, and to the Bartlett test, to verify the homogeneity of variances. Afterwards, one-way analysis of variance (ANOVA) at 5% significance was performed. The optimum dietary lysine requirements were estimated with a broken-line model ($Y = a + bx$) or second-order polynomial model ($Y = ax^2 + bx + c$); depending on the sum of squares about regression (SSR) and coefficient of determination (R^2) for the two models, the one with the smaller values of SSR and higher R^2 was chosen as the best fitting model. All statistical analyses were performed with SPSS (SPSS Inc., Chicago, IL, USA, version 23.0).

Results

Survival, feed efficiency, and carcass yield as well as viscerosomatic, gonadosomatic, and hepatosomatic index of adult lambari were unaffected by the dietary lysine levels (P>0.05). The same was observed for protein efficiency ratio and protein productive value (Table 3). No specific external signs or pathological symptoms were observed during the experimental period.

The lysine levels influenced (P<0.05) weight gain and percent weight gain of fish (Table 3). The R^2 values for weight gain were 0.97 and 0.65 for broken-line and second-order polynomial models, respectively. Similarly, for percent weight gain, these values were 0.98 and 0.67 for broken-line and second-order polynomial models, respectively. The SSR for weight gain and percent weight gain was also better for broken-line model. Based on the calculated R^2 and SSR, the broken-line model with the best fit in the two cases was chosen. According to broken-line regression analysis on weight gain ($Y = 0.561 + 0.093x$) (Figure 1) and percent weight gain ($Y = 11.062 + 1.897x$),

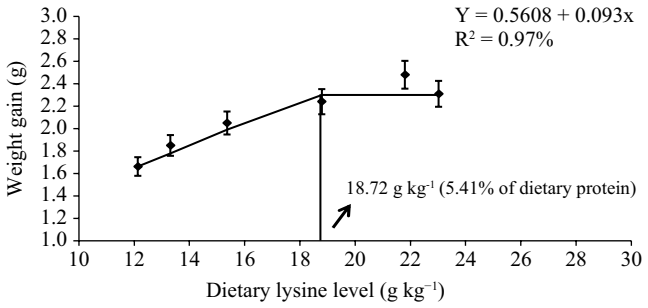

Figure 1 - Optimal dietary lysine requirement of finishing lambari (*Astyanax altiparanae*) based on broken-line model of weight gain versus dietary lysine level.

Table 3 - Growth performance of finishing lambari (*Astyanax altiparanae*) fed diets containing levels of dietary lysine

Parameter	Level of dietary lysine (g kg^{-1})						P-value
	12.13	13.31	15.36	18.79	19.92	23.02	
WG (g)[1]	1.66±0.09	1.85±0.27	2.00±0.41	2.24±0.07	2.48±0.26	2.31±0.14	0.0006
PWG (%)[2]	33.37±1.21	37.13±3.51	40.42±1.26	45.35±1.48	49.76±3.45	46.80±2.30	0.0003
FE[3]	0.36±0.05	0.31±0.01	0.31±0.05	0.37±0.01	0.34±0.02	0.34±0.02	0.1824
PER[4]	0.80±0.10	0.86±0.05	0.90±0.14	1.02±0.06	0.96±0.10	0.90±0.07	0.4095
PPV (%)[5]	21.39±2.99	24.12±1.04	22.59±1.99	26.98±1.11	24.41±1.18	23.40±1.75	0.4517
CY (%)[6]	73.06±1.55	72.52±2.25	73.05±1.24	72.23±2.40	74.43±2.96	73.31±1.34	0.7514
VSI (%)[7]	20.51±1.29	20.63±1.91	20.36±1.37	20.53±1.03	20.06±1.74	19.95±1.32	0.9801
GSI (%)[8]	15.94±1.19	15.22±1.91	15.09±0.43	15.90±0.68	15.89±1.78	15.63±1.47	0.9298
HSI (%)[9]	0.79±0.10	0.90±0.20	0.92±0.06	0.84±0.13	0.81±0.10	0.85±0.06	0.6929
SR (%)[10]	75.44±24.31	85.97±8.04	96.49±6.08	91.23±3.04	87.72±3.04	92.32±9.14	0.3695

Values are presented as mean±SD (n = 4).
[1] Weight gain = Final mean biomass − Initial mean biomass.
[2] Percent weight gain = (Final weight − Initial weight)/Initial weight × 100.
[3] Feed efficiency = Wet weight gain/Dry feed intake.
[4] Protein efficiency ratio = Wet weight gain/Dry protein intake.
[5] Protein productive value = Fish protein gain/Dry protein intake × 100.
[6] Carcass yield = Eviscerated fish weight/Whole fish weight × 100.
[7] Viscerosomatic index = Viscera weight/Whole body weight × 100.
[8] Gonadosomatic index = Gonad weight/Whole body weight × 100.
[9] Hepatosomatic index = Liver weight/Whole body weight × 100.
[10] Survival rate = Final number of fish/Initial number of fish × 100.

the optimum dietary lysine levels were estimated at 18.72 g kg^{-1} (5.41% of crude protein) and 18.83 g kg^{-1} (5.44% of dietary protein), respectively.

The lysine intake of 1.89 mg day^{-1} per animal was the amount required for maximum weight gain ($Y = -0.4898x^2 + 1.8749x + 0.627$; $R^2 = 0.67$) of adult lambari.

Whole-body moisture, crude protein, mineral matter, and gross energy content were not affected by the dietary lysine level (P>0.05) (Table 4). A quadratic relationship between dietary lysine level and whole-body total lipid content was observed ($Y = 0.0437272x^2 – 1.63695x + 24.9696$; $R^2 = 0.61$), and the optimum dietary lysine level was estimated at 18.76 g kg^{-1} dry diet (5.42% of dietary protein).

Adult lambari had muscle fibers distributed in the three classes of diameters established. Dietary lysine level did not influence (P>0.05) fiber frequency in any of the three classes of diameters established (Table 5).

Serum activities of AST and ALT of adult lambari were influenced (P<0.05) by dietary lysine levels. According to broken-line regression analysis, fish fed 15.23 g kg^{-1} lysine of dry diet had higher AST values ($Y = -273.486 + 51.697x$; $R^2 = 0.79$), and the highest activities of ALT ($Y = -20.513 + 3.730x$; $R^2 = 0.86$) were estimated in fish fed 21.77 g kg^{-1} lysine of dry diet. Fish fed 12.13 g kg^{-1} lysine of dry diet had the lowest concentrations of AST and ALT (Table 6).

Discussion

Weight gain and percent weight gain was affected by the supplementation of dietary lysine. Based on these parameters, the requirements of dietary lysine for adult lambari were estimated at 18.72 g kg^{-1} (5.41% of dietary protein) and 18.83 g kg^{-1} (5.44% of dietary protein), respectively. Based on the requirements of dietary lysine as a percentage of dietary protein, the values found in this study are within the expected range for other fish species, from 3.32 to 6.61% of dietary protein (Wilson, 2003), as well as for most species described in the NRC (2011). A higher dietary lysine requirement of 5.86% of dietary protein was estimated for adult lambari, based on the muscle amino acid composition and essential amino acid requirements of other omnivorous fish species (Abimorad and Castellani, 2011). Furuya et al. (2015) determined that the dietary lysine requirement for another species of adult lambari (*Astyanax fasciatus*) was 5.46% of the dietary protein. This represents the mean dietary lysine

Table 4 - Whole-body composition (% wet matter) of finishing lambari (*Astyanax altiparanae*) fed diets containing levels of dietary lysine

Composition	Level of dietary lysine (g kg^{-1})						P-value
	12.13	13.31	15.36	18.79	19.92	23.02	
Moisture	67.47±1.53	68.00±0.48	68.89±0.38	68.90±0.70	68.57±0.50	68.35±1.81	0.4772
Gross energy (MJ kg^{-1})	6.59±0.33	6.62±0.16	6.60±0.13	6.49±0.28	6.51±0.53	6.64±0.35	0.7835
Crude protein	20.24±0.67	20.38±0.28	19.59±0.26	20.01±0.76	19.91±0.14	20.21±1.35	0.2942
Total lipid	11.52±1.06	10.92±0.52	10.27±0.28	9.48±0.55	9.80±0.39	10.47±0.58	0.0229
Mineral matter	4.15±0.29	4.51±0.12	4.45±0.22	4.29±0.18	4.47±0.46	4.40±0.12	0.3044

Values are presented as mean±SD (n = 4).

Table 5 - Muscle fiber frequency into three diameter classes (<20, 20-50, and >50 µm) of finishing lambari (*Astyanax altiparanae*) fed diets containing levels of dietary lysine

Diameter (µm)	Level of dietary lysine (g kg^{-1})						P-value
	12.13	13.31	15.36	18.79	19.92	23.02	
<20	0.69±1.00	0.50±0.53	0.17±0.26	1.13±0.86	0.75±1.25	0.44±0.61	0.4720
20-50	34.00±11.83	28.71±9.70	28.58±10.08	29.50±12.34	27.69±9.43	26.76±6.90	0.8179
>50	65.31±12.47	70.79±9.92	71.33±10.27	69.38±13.65	71.56±11.29	73.00±6.26	0.8234

Values are presented as mean±SD (n = 4).
Muscle fiber frequency expressed in %.

Table 6 - Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in serum (U L^{-1}) of finishing lambari (*Astyanax altiparanae*) fed diets containing levels of dietary lysine

Enzyme	Level of dietary lysine (g kg^{-1})						P-value
	12.13	13.31	15.36	18.79	19.92	23.02	
AST	374.33±62.13	382.00±41.15	532.50±53.03	525.33±41.36	501.00±46.49	513.00±12.73	0.0008
ALT	25.00±9.85	29.33±13.87	35.33±1.53	52.67±6.11	51.67±4.16	60.67±4.62	0.0003

Values are presented as mean±SD (n = 4).

requirement value of common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), and Nile tilapia (*Oreochromis niloticus*), as reported by the NRC (2011), and is similar to the requirement estimated for adult lambari in this study. Methods to determine amino acid requirements based on whole-body amino acid composition and amino acid requirements of other fish species are used as guidelines for establishing indispensable amino acid patterns to formulate diets for fish species whose dietary essential amino acid requirements are unknown (Tibaldi and Kaushik, 2005). However, dose-response experiments with an increasing supply of amino acid is the most accepted method for determining dietary amino acid requirements (Cowey, 1995; Zhou et al., 2010).

There are few studies that estimate the dietary requirements for adult fish, because most adult fish species produced have a high final weight and conducting dose-response experiments with large fish is difficult and costly. Therefore, research with lambari can be advantageous because, due to its small size, lambari can be used as a model for studies of larger fish species (Gonçalves et al., 2014). In the present study, it was possible to determine the dietary lysine requirement for adult fish in the stationary growth rate phase, when growth performance is lower compared to fish in the fast growth rate phase. Adult lambari fed diets containing levels of dietary lysine presented the optimum percent weight gain estimated at 46.48%. Medium-sized Atlantic salmon (*Salmo salar*), with an average initial weight of 376.16±46.33 g, presented a percent weight gain of 18.18% for fish fed the lower dietary lysine level (5.9 g kg^{-1}) and 60.80% for fish fed the higher level (26.0 g kg^{-1}) (Berge et al., 1998). Similarly, finishing Nile tilapia with an initial weight of 117.90±0.67 g and fed increasing dietary lysine levels presented an average percent weight gain of 66.17% (Furuya et al., 2004). In another dietary lysine requirement experiment for adult Nile tilapia with an initial weight of 274.89±1.90 g, conducted in an outdoor net cage system and with low stocking density, the average percent weight gain was 101.11% (Michelato et al., 2016). Animals in the finishing phase or adults close to reaching their maximum size do not have the high growth rates of the younger stages.

The lysine requirement varies according to animal species and age (Forster and Ogata, 1998), feeding practices and rearing conditions (Forster and Ogata, 1998; Mai et al., 2006), digestibility of energy content and nutrient diets (Rodehutscord et al., 2000), and the model used to analyze the dose-response relationship (Mai et al., 2006). In this study, all the fish used were adult females and, in that life stage, the fish have much greater energy requirements for maturation of the gonads, especially females. Muscle growth slows down during the advanced stages of gonad development (Tveiten et al., 1998), as reported in salmonids during sexual maturation (Aksnes et al., 1986; Tveiten et al., 1998). This suppression or reduction of muscle growth is associated with high plasma concentrations of sex steroids and may also, at least in part, be mediated through stimulation of autolytic enzyme activity in the muscle tissue (Ando et al., 1986; Tveiten et al., 1998). For female adult lambari, lysine supplementation can prevent a decrease in growth, by increasing their weight even through advanced stages of gonadal development.

Supplementation of lysine can reduce whole-body lipid content of fish (Mai et al., 2006; Zhang et al., 2008; Carter and Hauler, 2011) and increase whole-body protein content (Wang et al., 2005; Abboudi et al., 2006; Lin et al., 2012), improving the final quality of carcass. This relationship could be attributed to the use of balanced diets, which reduces the catabolism of amino acids and improves protein synthesis, reducing the use of dietary protein for lipid formation (Rodehutscord et al., 2000; Tantikitti and Chimsung, 2001; Encarnação et al., 2004). Lysine is also a precursor of carnitine, which facilitates the removal of short-chain organic acids from mitochondria, thereby freeing intramitochondrial co-enzyme A to participate in β-oxidation, avoiding accumulation of whole-body lipids (Ozório et al., 2001). Burtle and Liu (1994) reported that whole-body lipids of channel catfish (*Ictalurus punctatus*) were reduced with carnitine supplementation, lysine supplementation, or both. In the present study, the dietary lysine level estimated to achieve lower whole-body lipids was 18.76 g kg^{-1} of dry diet, a value similar to the dietary lysine requirement determined based on weight gain. Some authors observed a reduction in whole-body lipids in fish fed optimal levels of lysine, even without changes in whole-body protein (Luo et al., 2006; Sánchez-Lozano et al., 2009; Deng et al., 2011).

In this study, fish showed muscle growth predominantly by hypertrophy of the cells. Most fish species present unlimited muscle growth, through active growth processes of hyperplasia and hypertrophy (Stickland, 1983). However, in some small fish species, such as zebrafish (*Danio rerio*), muscle growth is pre-determined, with low hyperplastic growth occurring after the juvenile phases (Biga and Goetz, 2006). Lambari is a small species; thus, the hyperplastic growth period is shorter, and muscle growth occurs mainly by hypertrophy (Veggetti et al., 1993; Koumans and Akster, 1995). On the other hand, in species capable of attaining large size,

hyperplasia continues to be an important contributor to muscle growth into the adult phase (Zimmerman and Lowery, 1999). Adult Nile tilapia present muscle growth characterized by different fiber diameters (mosaic pattern), and the frequency of <20 μm diameter muscle fibers confirmed that the hyperplasia growth process in white muscle occurs throughout all life stages (Michelato et al., 2016). Increasing the number of fibers during early life stages is associated with meat production and high growth rates (Rowlerson and Veggetti, 2001). Well-balanced diets for fish larvae promote increased growth rates associated with a higher contribution of hyperplasia, which in turn, promote an increase in the body size of adult fish (Galloway et al., 1999; Ostaszewska et al., 2008; Michelato et al., 2016). For adult lambari, the dietary lysine levels evaluated were not sufficient to reflect differences in frequency of diameter classes of muscle fibers. However, adult lambari have a low capacity to increase their size; therefore, promoting methods to increase hyperplasia in larvae of this species can possibly increase growth rates of adult fish.

The increased ALT and AST serum levels of fish that received the highest levels of dietary lysine suggest an increase in the catabolism of amino acids, since ALT and AST are the most important enzymes involved in deamination and transamination reactions (Cowey and Walton, 1989). The excess of amino acid provided cannot be stored by fish and is converted into energetic compounds (Stone et al., 2003). The same was demonstrated in fingerling black sea bream (*Acanthopagrus schlegelii*) fed diets with different lysine and arginine levels, which had lower plasma ALT activity when they received the control diet (Zhou et al., 2011). Increased levels of ALT activity were also observed in the sera of fry, fingerling, and juvenile Nile tilapia when the dietary protein level was between 25 and 45% (Abdel-Tawwab et al., 2010). The authors attributed the increase in ALT activity to the use of excess hydrocarbons from amino acids to supply energetic demands.

The appropriate lysine supplementation resulted in increased weight gain and decreased whole-body lipids, wherein the best results were obtained in fish fed diets with a ratio of arginine:lysine of 0.91:1.0. Besides the lysine requirement, the arginine:lysine ratio should be considered to prevent antagonism between these amino acids (Wu, 2013). Considering that the growth of lambari females results in animals with greater whole-body lipids, lysine supplementation constitutes a nutritional tool to bring the fish to market.

Conclusions

According to the broken-line model analysis of weight gain and dietary lysine levels, the dietary lysine requirement for adult lambari is 18.72 g kg^{-1} (5.41% of dietary protein).

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