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Digestible lysine requirement of Nile tilapia fingerlings fed arginine-to-lysine-balanced diets[1]

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ABSTRACT - This study was conducted to determine the digestible lysine requirements of Nile tilapia fingerlings. Fish (n = 300; average initial weight = 1.44 g) were distributed 15 300-L aquariums, in a completely randomized design with five treatments and three replicates, and fed extruded diets containing 11.3, 13.7, 16.1, 18.4 or 20.8 g/kg of digestible lysine. The arginine:lysine ratio was maintained at 1.3:1. All fish were fed diets containing 281 g/kg of digestible protein and 3,372 kcal digestible energy/kg, hand-fed until apparent satiation. There was no effect of the dietary lysine levels on survival rate, or protein and ash body rates. With increasing levels of lysine in the diet, a quadratic effect on weight gain, feed conversion, protein efficiency ratio, protein deposition rate, deposition rate of fat, body moisture and body lipids was observed, where the best values of the variables were estimated at 15.96, 16.4, 14.35, 15.21, 15.87, 15.21 and 16.29 g/kg of lysine, respectively. The digestible lysine requirement of Nile tilapia fingerlings is 15.21 g/kg (5.41 g/100 g of digestible protein), in diets balanced for the arginine:lysine ratio.

Key Words: amino acids, fish, growth, protein deposition

Introduction

Aquaculture is one of the fastest growing sectors of the global livestock production (FAO, 2010). Approximately 40% of fish from aquaculture is originated from tilapia production, where the Brazilian production was 133,000 t in 2009 (Scorvo-Filho et al., 2010).

Advancements in genetic improvement of tilapia have contributed to increased production, especially in fish raised in cages. To express their genetic potential, it is important to determine nutritional requirements, considering fish strain and stage of growth.

Lysine is an essential amino acid present in high proportion in fish muscle tissue, involved in growth and maintenance of positive nitrogen balance, also used in “cross-linking” protein, especially collagen (UNM, 2006). Moreover, it plays an important role in the synthesis of carnitine, which is important for the transport of long-chain fatty acids into the mitochondrion for energy generation (Walton et al., 1984). Dietary lysine supplementation is related to advantages on weight gain feed conversion, nitrogen retention and reduction in body lipid contents (Berge et al., 1998; Furuya et al., 2006; Marcouli et al., 2006).

The higher values of lysine requirement in diets for tilapia described by the NRC (2011), in relation to the value previously recommended (NRC, 1993), is related not only to fish species and physiological stage of growth, but to several factors, such as the composition of the diets, feed management and statistical analysis used.

Santiago & Lovell (1988) estimated dietary requirement of 14.3 g/kg lysine (5.1 g/100 g crude protein) for Nile tilapia fingerlings. In Brazil, Bomfim et al. (2010) estimated requirement of 15.4 g/kg of digestible lysine (5.5 g/100 g of digestible protein) for Nile tilapia fingerlings, near the value previously found by Furuya et al. (2006), who estimated requirement of 14.3 g/kg of digestible lysine (5.4 g/100 g of digestible protein) for the same fish species.

The antagonism of lysine on arginine is widely known (Jones, 1964; Jones et al., 1967) due to the importance of arginine in nitrogen excretion, especially in diets with excess essential amino acids (Snetsinger & Scott, 1961). In experimental and practical conditions, isolated lysine supplementation may result in antagonism with arginine because it changes the relationship between these amino acids.

Although many studies have been conducted in order to determine the lysine requirements for different species

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and life stages, there is little information on the requirements of digestible lysine of fish, especially considering the relationship between arginine and lysine. The present study was carried out to determine the digestible lysine requirement of Nile tilapia fingerling in diets corrected for the arginine:lysine ratio.

Material and Methods

The experiment was conducted at the Universidade Estadual de Maringá - Fish Culture "Estação de Piscicultura de Floriano" -UEM/CODAPAR, using fingerlings (initial average weight = 1.44 g) for 45 days, from December to January 2009.

Three hundred and thirty fish were distributed in 300-L plastic aquaria in a completely randomized design with five treatments and three repetitions. Fish were fed diets containing 281 g/kg of digestible protein and 3,372 kcal/kg of digestible energy to meet the requirements for tilapia (Furuya et al., 2005), and 11.3, 13.7, 16.1; 18.4 or 20.8 g/kg of digestible lysine (as fed basis), and arginine was supplemented in order to keep the arginine:lysine ratio close to 1.13:1 of the diet (Table 1).

Aquaria were kept in recirculating system with central biofilter and daily renewal of 10%. Data of water temperature (°C), dissolved oxygen (mg/L) and pH from each aquarium were collected weekly, twice a day, at 8:30 and 17:00 h, using digital oximeter (oxygen and temperature) and portable digital pH-meter, respectively. During the experimental period, values of 27.1±1.45 °C, 5.24±0.6 mg/L and 7.12 ± 0.11 were obtained for temperature, dissolved oxygen and pH, respectively.

All diets were balanced to meet the requirements of digestible protein and digestible energy described by Furuya (2010) for tilapia, according to the values of digestible energy, protein and digestible amino acids and available

Table 1 - Composition of diets (g/kg) and calculated composition (as fed basis)

	Digestible lysine (g/kg)				
	11.3	13.7	16.1	18.4	20.8
Corn meal	340.00	343.80	345.60	348.50	351.30
Wheat bran	130.00	130.00	130.00	130.00	130.00
Concentrate protein of soy	150.00	150.00	150.00	150.00	150.00
Corn gluten meal 60	200.00	200.00	200.00	200.00	200.00
Blood meal ("Spray-dried")	40.00	40.00	40.00	40.00	40.00
Dicalcim phosphate	35.00	35.00	35.00	35.00	35.00
Soybean oil	37.00	37.50	38.00	38.50	39.00
Glutamic acid	20.00	15.00	10.00	5.00	0.00
L-alanine	26.00	22.00	18.00	14.00	10.00
L-lysine	0.00	2.00	6.00	9.00	12.00
L-arginine	0.00	2.70	5.40	8.00	10.70
L-threonine	2.00	2.00	2.00	2.00	2.00
DL-methionine	2.30	2.30	2.30	2.30	2.30
Mineral and vitamin mix[1]	10.00	10.00	10.00	10.00	10.00
Vitamin C[2]	0.50	0.50	0.50	0.50	0.50
Antioxidant[3]	0.20	0.20	0.20	0.20	0.20
Antifungal[4]	2.00	2.00	2.00	2.00	2.00
Salt	5.00	5.00	5.00	5.00	5.00
	Calculated composition[5]				
Digestible energy (kcal/kg)	3.372.00	3.372.00	3.372.00	3.372.00	3.372.00
Digestible protein (g/kg)	281.00	281.00	281.00	281.00	281.00
Ether extract (g/kg)	51.00	51.60	52.10	52.70	53.20
Crude fiber (g/kg)	13.50	13.50	13.60	13.60	13.60
Calcium (g/kg)	9.40	9.40	9.40	9.40	9.40
Available phosphorus (g/kg)	7.00	7.00	7.00	7.00	7.00
Methionine + cystine (g/kg)	10.50	10.50	10.50	10.50	10.50
Lysine (g/kg)	11.30	13.70	16.10	18.40	20.80
Arginine (g/kg)	12.80	15.50	18.20	20.80	23.50
Threonine (g/kg)	10.20	10.20	10.20	10.20	10.20
Tryptophan (g/kg)	2.20	2.20	2.20	2.20	2.20

[1] Mineral and vitamin mix (Rovimix - DSM®): composition per kg: vit. A - 1.000 UI; vit. D3 - 312 UI; vit. E - 18.750 mg; vit. K3 - 1,250 mg; vit. B1 - 2,500 mg; vit. B2 - 2,500 mg; vit. B6 - 1,875 mg; vit. B12 - 3.75 mg; folic acid - 750 mg; pantothenic acid - 6.250 mg; nicotinic acid - 12.500 mg; vitamin C - 31.250 mg; biotin - 125 mg; choline - 50.000 mg; copper - 625 mg; iron - 6,250 mg; manganese -1,875 mg; cobalt - 12.5 mg; iodine - 62.5 mg; zinc - 6250 mg; selenium - 12.5 mg; inositol - 12.500 mg.
[2] Vitamin C - 3,500 mg/kg of vitamin C.
[3] Banox®. Composition: BHA. BHT. Propyl gallate and calcium carbonate - Alltech Brazil Agroindustrial Ltda.
[4] Mold Zap Aquativa®. Composition: dipropionate of ammonia, acetic acid, benzoic acid and ascorbic acid - Alltech do Brasil Agroindustrial Ltda.
[5] Furuya et al. (2001c). Pezzato et al. (2002), Guimarães et al. (2008a,b) and Gonçalves et al. (2009).

phosphorus determined by Furuya et al. (2001), Pezzato et al. (2002), Guimarães et al. (2008a, b) and Gonçalves et al. (2009).

After weighing and mixing all ingredients, the mixture was extruded in a single screw extruder in order to obtain granules with an average diameter of 2.5 mm at the Universidade Estadual do Oeste do Paraná – UNIOESTE, in Toledo, Paraná, Brazil. The experimental diets were dried in forced-ventilation oven at 52 °C for 12 h, disintegrated and sieved, selecting pellets according to fish size.

Fish were hand-fed three times a day: at 8, 12 and 18 h, until apparent satiation. At the beginning of the experiment, all fish were weighed (0.01 g) and at the the end of the experimental period, all fish were slaughtered by overdose of benzocaine (1 g/10 L of water), counted and weighed to determine data of weight gain, feed conversion, protein efficiency ratio and survival. Ten fish from each experimental unit were randomly collected for body composition analysis.

Two hundred fish were collected at the beginning of the experiment for calculation of body protein deposition ratio (PDR) and fat deposition ratio (FDR) using, respectively, the equations: PDR (CPf - CPi)/CP, where CPf is the final amount of body crude protein (mg), CPi is the initial amount of body crude protein (mg) and CP is the amount of crude protein consumed (mg); and FDR = (BFf - BFi)/FC, where, BFf is the final amount of body fat (mg), BFi is the initial amount of body fat (mg), and FC is the amount of fat consumed (mg). The protein efficiency ratio was calculated according to the expression described by Jauncey & Ross (1982).

Analyses of moisture, crude protein, ether extract and body ashes were performed at Laboratório de Análises de Alimentos - Department of Animal Science, Universidade Estadual de Maringá according to Silva & Queiroz (2002).

A completely randomized design with five treatments and three replications was used, and the experimental unit consisted of a 300-L aquarium containing 22 fish. All data were submitted to analysis of variance and polynomial regression using the SAS package (Statistical Analysis System, version 9.2).

Results and Discussion

An increasing dietary lysine level from 11.3 to 20.8 g/kg resulted in quadratic effect (P<0.05) on weight gain, feed conversion, protein efficiency ratio, protein deposition ratio and fat deposition ratio, and the best values of variables were estimated with 15.96, 16.4, 14.35, 15.21 and 15.87 g/kg of digestible lysine, respectively (Table 2, Figures 1 and 2).

In this study, the estimated value of lysine for maximum weight gain (15.96 g/kg diet) was higher than that described by Santiago & Lovell (1988), who estimated lysine requirement of 14.3 g/kg of the diet for Nile tilapia fingerlings, and lower than that obtained by Murthy & Varghese (1997) and Fagbenro et al. (1998), of 22.4 and 22.9 g/kg of lysine for common carp (*Cyprinus carpio*) juvenile and channel catfish (*Ictalurus punctatus*), respectively, fed diets formulated based on total lysine values, where the dietary lysine requirements were determined in relation to the proportion of the dietary crude protein values.

Bomfim et al. (2010) observed linear increase of lysine levels on the weight gain of Nile tilapia fingerlings fed diets containing 9.5, 11.0, 12.5, 14.0, 15.5 or 17.0 g/kg of lysine, which differs from the result obtained in this study, where the higher weight gain was obtained by fish fed the diet containing 15.96 g/kg of digestible lysine.

A lower dietary lysine requirement in relation to dietary protein value is mainly observed in diets for carnivorous fish fed high dietary protein values (Bomfim et al., 2010), as observed in the study carried out with red sea bream (*Pagrus major*) juvenile by Forster & Ogata (1998), who estimated value of 17.3 g/kg lysine (3.3 g/100 g crude protein) for maximum weight gain of fish fed diets containing 480 g/kg crude protein, confirming the previous result found by Tibaldi & Lanari (1991), which determined requirement of 21.7 g/kg lysine (4.34 g/100 g crude protein) for sea bass (*Dicentrarchus labrax*) fingerlings.

Keembiyehetty & Gatlin (1992) also observed lower proportion of lysine in relation to the dietary crude protein, where they estimated requirement of 1.14 g/kg of total

Table 2 - Mean values of performance of Nile tilapia fingerlings fed diets containing growing levels of digestible lysine

Variable	Digestible lysine (g/kg)					
	11.3	13.7	16.1	18.4	20.8	CV (%)
Initial weight (g)	1.44	1.44	1.44	1.44	1.44	-
Final weight (g)	5.27	5.86	5.78	5.35	5.13	5.90
Feed conversion ratio[1]	1.16	1.06	1.08	1.28	1.32	9.80
Protein efficiency ratio[1]	2.57	2.77	2.71	2.29	2.26	9.30
Fat deposition ratio (mg/day)[1]	6.94	8.98	8.89	7.86	6.99	12.43

CV - coefficient of variation.

[1] Quadratic effect (P<0.05): feed conversion ratio: $y = 2.250 – 0.164x + 0.005x^2$; $R^2 = 0.81$; protein efficiency ratio: $y = 0.703 + 0.287x – 0.010x^2$; $R^2 = 0.77$; fat deposition ratio: $y = - 12.420 + 2.698x – 0.085x^2$; $R^2 = 0.87$.

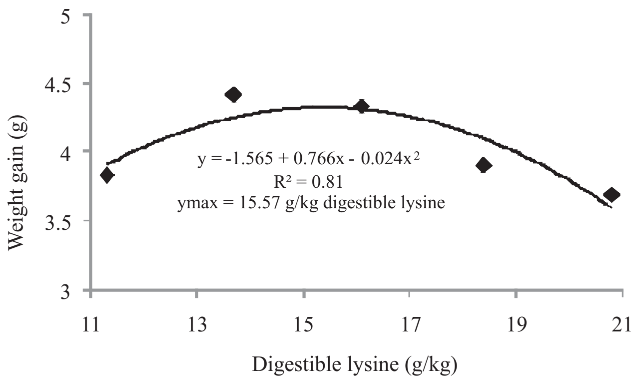

Figure 1 - Weight gain of Nile tilapia fingerlings fed diets containing growing levels of digestible lysine.

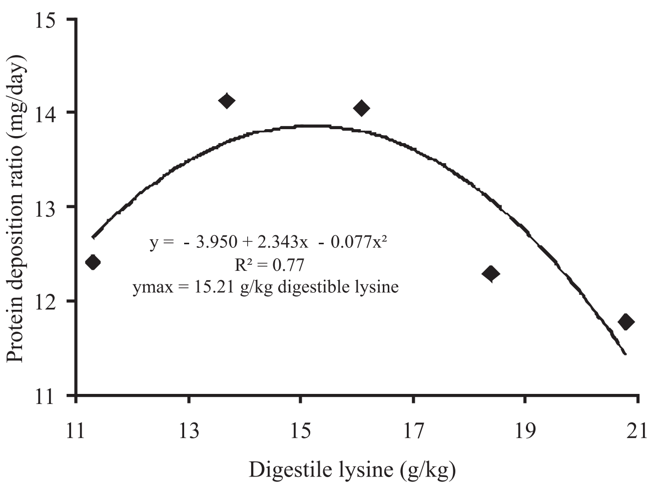

Figure 1 - Protein deposition ratio of Nile tilapia fingerlings fed diets containing growing levels of digestible lysine.

lysine (4.03 g/100 g crude protein) for juvenile striped bass (*Morone chrysops* × *M. saxatilis*), which was also observed for rainbow trout (*Onchorhynchus mykiss*) by Kim et al. (1992), who determined requirement of 13 g/kg dietary lysine (3.71 g/100 g of crude protein), and by Berge et al. (1998), in a study conducted with juvenile Atlantic salmon (*Salmo salar*) fingerling, who observed maximum weight gain of fish fed diet containing 18 g/kg of lysine (3.6 g/100 g crude protein).

The comparison of data based on values of dietary lysine in relation to dietary protein is relative, if considering the differences in protein levels, energy and other nutrients, as well as the presence of antinutritional factors, among others. Moreover, in the present study, the proportion of lysine was obtained based on the values of digestible lysine and digestible protein, while other authors obtained this relationship based on values of crude lysine and crude protein content using purified diets. However, the apparent digestibility of amino acids from purified ingredients is high, and considered by many authors as 100% digestible.

Fish fed increasing dietary lysine showed quadratic effect ($P<0.05$) on the protein deposition ratio, in which the highest value of this variable was estimated with fish fed diets containing 15.21 g/kg of lysine.

The estimated values of protein deposition ratio and fat deposition ratio obtained in this study were lower than those obtained by Berge et al. (1998), in a study conducted with Atlantic salmon (*Salmo salar*) juvenile, which determined requirement of 16 g/kg of lysine in the diet.

Besides the difference between species, for protein retention, it is notable that the dietary lysine estimated in this study can be influenced by the dietary amino acids balance, particularly arginine, by the importance of the relationship between lysine and arginine. In previous studies conducted in Brazil to determine the dietary lysine requirement for Nile tilapia, Furuya et al. (2006) and Bomfim et al. (2010) concluded that the best performance results were obtained by fish fed diets containing arginine:lysine ratios of 0.87 and 0.77, respectively. Thus, in the present research, as the ratio between the amino acids in the diets was kept constant, it is possible that the balance of the arginine:lysine ratio influenced the dietary lysine requirement for this variable.

No effect ($P>0.05$) of dietary lysine levels on body crude protein and ash were observed. The increasing levels of lysine in the diet resulted in quadratic effect ($P<0.05$) on body moisture and ether extract (Table 3), and the lowest values of the variables were estimated with 16.21 and 16:29 g/kg lysine, respectively.

Table 3 - Mean values of body composition of Nile tilapia fingerlings fed diets containing growing levels of digestible lysine

	Digestible lysine (g/kg)					
Variable (g/kg)	11.3	13.7	16.1	18.4	20.8	CV (%)
Moisture[1]	752.3	742.0	741.7	746.4	748.8	0.6
Crude protein	145.8	144.2	145.5	142.5	144.1	0.9
Ether extract[1]	73.5	81.7	82.2	80.1	75.9	4.8
Ash	35.2	37.0	35.9	35.4	35.4	2.1

CV - coefficient of variation.
[1] Quadratic effec ($P<0.05$): moisture: $y = 842.500 – 12.420x + 0.383x^2$; $R^2 = 0.82$; ether extract: $y = - 8.722 + 11.240x – 0.345x^2$; $R^2 = 0.94$.

Unlike the the results found in this study, Zarate & Lovell (1997) observed a linear increase in the content of body protein of four channel fingerlings fed diets supplemented with lysine (14.2, 21.4 or 28.5 g/kg diet). Comparing the performance of juvenile rainbow trout fed diets containing 8.9 (control) or 6.19 g/kg lysine (supplemented with lysine), Rodehutscord et al. (2000) found higher body protein content in fish fed diets supplemented with lysine.

The result of body fat obtained in this study differs from those obtained by Davies et al. (1997), Berge et al. (1998) and Ahmed & Khan (2004) for juvenile rainbow trout, Atlantic salmon and carp (*Cirrhinus mrigal*), respectively, where they observed a reduction in body lipid content in fish fed diets supplemented with lysine.

The proper relationship between amino acids is important to increase the use of dietary protein. Although arginine supplementation is not a common practice in commercial diets for fish, antagonisms can occur in diets supplemented with lysine. In a study conducted with birds, Jones et al. (1967) determined that lysine did not interfere in the digestion or absorption of arginine in the intestinal level, but is related to the competition between them during renal reabsorption, as confirmed by intravenous infusion of lysine, which resulted in an increase in the plasmatic concentration of lysine, and excretion and inhibiting arginine reabsorption.

In this study, although the best weight gain was obtained by fish fed diet containing 15.96 g/kg lysine, the highest rate of body protein deposition was obtained by fish fed the diet containing 15.21 g/kg of lysine, indicating more efficient use of dietary protein fraction, which is important for the sustainability of aquaculture.

It is important to determine the dietary lysine requirement for Nile tilapia for the development of diets with adequate amino acid balance, and to avoid possible antagonism between amino acids, in order to increase fish growth, dietary protein utilization and body composition.

Conclusions

Nile tilapia fingerlings require diets containing 15.21 g/kg of digestible lysine (5.41 g lysine/100 g of digestible protein), in diets balanced for the arginine-to-lysine ratio.

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