



Requirement for digestible lysine in Nile tilapias (*Oreochromis niloticus*) with live weight between 500 and 600 g

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ABSTRACT. The present study was carried out with the objective of determining the requirements for digestible lysine in Nile tilapia during the 500 to 600 g live weight stage. Isocaloric and isonitrogenous diets were tested with increasing amounts of digestible lysine. L-lysine HCl (78%) were used to replace glutamic acid at increasing levels, resulting in treatments of 9.3, 12.3, 15.3, 18.3 and 21.3 g kg⁻¹ of digestible lysine. Three hundred Nile tilapias with an average weight of 519 ± 27.23 g were used and distributed among 25 tanks. The physical and chemical parameters of the water were pH, dissolved oxygen, salinity, conductivity and temperature. The mortality rate was registered daily. Two slaughters were performed at 28 and 50 days after the beginning of the experiment. It was estimated that an amount of 13.1 g kg⁻¹ of digestible lysine was ideal for obtaining higher WG. Fish slaughtered after 50 days, the digestible lysine requirements were determined to be 14.5 g kg⁻¹ for the lowest carcass humidity and 14.6 g kg⁻¹ for the greatest carcass ethereal extract. Nile tilapias with live weight between 500 and 600 grams require 13.1 g kg⁻¹ of digestible lysine in the diet for greater WG (90.53 g) and better FCR (2.55).

Keywords: Amino acids; fillet yield; performance; specific growth rate.

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Introduction

Tilapia production shows a continuous increase, where this species is the second major produced in world aquaculture (Food and Agriculture Organization [FAO], 2020). Factors such as climate adaptability, precocity, rusticity, fillet yield, the possibility of super-intensive growth in tanks, consumer market acceptance (Bromage, 1995), possibility of being fed with artificial feed from early developmental stages (Meurer, Hayashi, Soares, & Boscolo, 2000) and genetic improvements put tilapia in prominence. For tilapia to express its genetic potential, it is necessary to determinate the species' specific nutritional requirements (Furuya, 2010), which would also allow reduction of nutrients excretion to the environment, making its cultivation more sustainable.

Among the nutritional requirements that must be studied are the amino acids, which are fundamental in the organic processes of the fish. The amino acid lysine composes a large proportion of the Nile tilapia body, representing 3.39% of the dry weight (Teixeira et al., 2008). This is an essential amino acid present in large amounts in the muscular tissue, and the adequate use of this amino acid may result in increased weight gain and improved feed conversion (Furuya, 2013). In addition, lysine is very important for studies on ideal protein, which is currently a very relevant concept, since it allows the amount of proteins in diet to be reduced without compromising yield, and decreases nitrogen excretion in the water resulting in a lower risk of eutrophication. The processing used in the production of fish diet and the amino acid composition of the ingredients used affect the lysine digestibility coefficient (Furuya, Santos, Silva, Furuya, & Sakaguti, 2006).

The digestible lysine requirements listed in the Brazilian Tables for Tilapia Nutrition (Furuya, 2010) differ between developmental phases for Nile tilapia; 22.00, 15.30 and 13.80 g kg⁻¹ are required in the sexual reversion phase, from post-reversion until a live weight of 100 g, and in fish with live weight greater than 100

g, respectively. According to the National Research Council (NRC, 2011), the digestible lysine requirement for the Nile tilapia diet is 16.00 g kg⁻¹ regardless of fish weight, which highlights the need for more studies that allow the expression of nutritional requirement for different ranges of body weight.

There are only few studies that use fish with live weight greater than 100 g (Furuya et al., 2013; Michelato et al., 2013; NRC, 2011) with that in mind, this study had the purpose to determine the digestible lysine requirements for Nile tilapia of live weight between 500 and 600 g.

Material and methods

Research on the animals was conducted according to the Institutional Committee on Animal Use 23083 008011/2014-74. The experiment was conducted in Seropédica, Rio de Janeiro State, Brazil at 22°45'59,1"S 43°41'48,5"W.

Three hundred Nile tilapias with an average weight of 519 ± 27.23 g and sexually reverted during the larval stage were acquired. The fish were distributed among 25 water tanks of 1000 L each, and all of the fish stored in each tank constituted an experimental unit.

Before the beginning of the experimental period, the fish were allowed to adapt to the experimental conditions for 14 days. Experimental diets were supplied daily in amounts corresponding to 3% of the tilapias' live weight in grams, divided over three feeding times, 9 a.m., 1 p.m. and 5 p.m.

The water's physical and chemical parameters such as pH, dissolved oxygen, salinity, conductivity and temperature were monitored daily using a commercial digital multi-parameter AK88 from AKSO, whereas toxic ammonia was monitored weekly. The mortality rate was registered daily.

The water renewal was done daily on the basis of 20% of the total volume, after removal of the waste. All tanks were covered with a grid to prevent any fish from jumping out of the tank. The tanks did not have a water heating system.

A complete randomized design was used, with five treatments and five repetitions. Twelve and six fish were placed in each water tank in the first experimental period of 28 days and second experimental period of 22 days, respectively, for a total of 50 days. Since the number of fish in the second phase was lower, only carcass and fillet characteristics were evaluated.

The experimental diets consisted of isocaloric and isonitrogenous diets with increasing amounts of digestible lysine. The basal experimental diet (Table 1) was supplemented with increasing amounts of L-lysine HCl (78%), which was added at the expense of L-glutamate (99%), in quantities 0.00, 3.88, 7.76, 11.64 and 15.52 g kg⁻¹, resulting in treatments of 9.32, 12.3, 15.3, 18.3 and 21.3 g kg⁻¹ of digestible lysine. The differences between the purities of the amino acid sources used were corrected with the use of starch in different concentrations in the diets. L-glutamic acid was chosen to be used in this research, because it is non-essential amino acid and widely used in research with the objective of studying amino acid requirements in fish, as used by Fontagné-Dicharry, Alami-Durante, Aragão, Kaushik, and Geurden (2017) and Peachey, Scott, and Gatlin III (2018).

The nutrient contents of the basal experimental diet were according to Furuya (2010), except for total and digestible lysine. The chlorine and sodium requirements were obtained from the NRC (2011). For methionine + cysteine, the contents used were estimated by Michelato et al. (2013), who worked with tilapia weighing between 550 and 700 g. Chemical analyses of the ingredients and diets were performed in the Nutritional Science Laboratory in the Animal Nutrition and Pasturage – IZ - UFRRJ.

The following parameters were evaluated: Weight gain (WG), feed intake (FI), digestible lysine consumption (DLC), feed conversion ratio (FCR), protein efficiency for gain (PEG), lysine efficiency for gain (LEG), specific growth rate (SGR), protein deposition rate (PDR), nitrogen retention efficiency (NRE), daily fat deposition rate (DFDR), protein retention efficiency (PRE), and survival rate (SR).

For obtaining the variables WG all fish were weighed at the beginning and at the end of the experiment, being that WG was obtained by the difference between the final weight and the initial weight. For the initial weight, a plastic box and a plastic mesh were used on a digital scale (0.001 g) to weight each experimental unit. For the final weight the fish were individually weighed.

For the FI, tilapias received the rations until the apparent satiety, when they did not rise in the surface of the water to capture the feed. At the end of the experiment the values of added ration and leftover values were considered. In case of mortality, leftovers were immediately weighed to correct consumption. For the calculation of DLC the FI was multiplied by the percentage of digestible lysine in the diet. The PEG and LEG were calculated by dividing WG by consumption of crude protein or DLC, respectively.

Table 1. Ingredient and nutrient compositions of basal diets used in experiments.

Ingredients	(g kg ⁻¹ as fed)
Corn (7,64% PB) ¹	571.72
Soybean meal (45,36% PB) ¹	233.14
Corn gluten feed (60% PB) ¹	163.81
Dicalcium phosphate	17.02
Glutamic acid	15.00
Mineral and vitamin feed ²	14.00
Limestone	8.59
Common salt	6.35
Starch	5.00
Soybean oil	5.00
L-Threonine	4.00
DL-Methionine	2.72
L- Tryptophan	2.44
Kaolin	1.00
BHT	0.20
L-Lysine HCl	0.00
Total	1000.00
Nutritional composition	(g kg ⁻¹ as fed)
Crude protein	259.2
Digestible protein	246.9
Digestible energy (Kcal kg ⁻¹)	3041.20
Raw fiber	23.07
Calcium	8.20
Chlorine	4.31
Available phosphorus	4.50
Sodium	2.70
Digestible lysine	9.32
Total Lysine ³	9.45
Digestible arginine	11.41
Digestible threonine	11.10
Digestible tryptophan	3.80
Digestible methionine	6.21
Digestible methionine + cystine	9.00

¹Value determined in the food science laboratory of the Animal Science Institute of the UFRRJ. 2Vitamin A (min.) 860,000 IU kg⁻¹; vitamin D3 (min.) 240.00 IU kg⁻¹; vitamin E (min.) 10,500 IU kg⁻¹; vitamin K3 (min.) 1,400 mg kg⁻¹; vitamin B1 (min.) 2,100 mg kg⁻¹; vitamin B2 (min.) 2.150 mg kg⁻¹; vitamin B6 (min.) 2,100 mg kg⁻¹; vitamin B12 (min.) 2,200 mg kg⁻¹; vitamin C (min.) 25 g kg⁻¹; niacin (min.) 10 g kg⁻¹; calcium pantothenate (min.) 5,600 mg kg⁻¹; folic acid (min.) 580 mg kg⁻¹; biotin (min.) 17 mg kg⁻¹; choline chloride (min.) 60 g kg⁻¹; inositol (min.) 3.570 mg kg⁻¹; copper (min.) 1.800 mg kg⁻¹; manganese (min.) 5,000 mg kg⁻¹; zinc (min.) 8,000 mg kg⁻¹; iodine (min.) 90 mg kg⁻¹; selenium (min.) 36 mg kg⁻¹; cobalt (min.) 55 mg kg⁻¹; Analyzed amino acid.

For the SGR the following formula was used: $SGR = (\log FW - \log IW) / EP$. In which: logFW = logarithm of final weight(g); logIW = logarithm of initial weight(g); EP = experimental period (days).

For the other variables, it was necessary to slaughter the animals, which proceeded as follows: at the beginning of the experimental period, 30 fish from the same batch were used to determine the chemical composition of the initial carcass. At the end of the experiment, the fish were fasted for 24 hours and weighed on a digital scale (0.001 g) and euthanized with 300 mg L⁻¹ Eugenol (Vidal et al., 2008). The fish for chemical analysis of the carcass composition were autoclaved the whole body at 1 atm at 120°C for 30 minutes and then dried in a forced ventilation oven at 55°C for 72 hours and ground until homogeneous samples were obtained. Subsequently, the analyses of dry matter, nitrogen and extra ethereal were performed according to the methodology recommended by Association of Official Agricultural Chemists (AOAC, 1995).

The PDR (g) was calculated according to the formula: $PDR = (Pfc - Pic) / EP$. In which: Pfc = protein in the final carcass(g); Pic = protein in the initial carcass(g); EP = experimental period (days). The NRE and DFDR was obtained in a similar way, using the nitrogen and fat content in the initial and final carcass, for the respective parameter. The PRE was calculated by the ratio between PDR and DLC. The hepatosomatic index (HSI) was calculated by the liver weight in relation to the body weight.

Data were interpreted by variance analysis at 5% probability with the use of the software SISVAR – Variance Analysis System – version 5.4 (Ferreira, 2010). The statistical assumptions of homogeneity and normality were attended. The effects on the relationships with increasing amounts of lysine were analyzed by quadratic or linear regression models, depending on the best adjustment determined for each variable.

Results and discussion

The average physical and chemical parameters of the water were $24.89 \pm 1.07^\circ\text{C}$ for temperature, $3.66 \pm 0.59 \text{ mg L}^{-1}$ for dissolved oxygen, 7.13 ± 0.27 for pH, $611.22 \pm 68.55 \mu\text{S cm}^{-1}$ for conductivity and 0.29 ppt for salinity. These values are within acceptable ranges for raising Nile tilapia (Kubitza, 1998).

The weight gain parameter (Table 2) exhibited a quadratic response ($p < 0.05$) due to increasing amounts of digestible lysine, and the digestible lysine requirement for maximal weight gain was estimated to be 13.10 g kg^{-1} . For food conversion, the response was quadratic ($p < 0.05$); the digestible lysine requirement was estimated to be 13.10 g kg^{-1} for the best FCR. A requirement of 13.30 g kg^{-1} of digestible lysine was estimated ($p < 0.05$) for a specific growth rate (SGR) of 0.25% ($R^2=0.7454 - y = -0.2142x^2+0.5716x -0.1279$).

Table 2. Performance, specific growth rate (SGR), protein efficiency for gain (PEG), lysine efficiency for gain (LEG) of 500 to 600 g Nile tilapia fed increasing amounts of digestible lysine.

Variable	Lysine Amounts (g kg^{-1})					¹ Reg.	² CV
	9.30	12.30	15.30	18.30	21.30		
Final weight (g)	608.6	611.8	599.52	566.24	580.56	NS	12.55
Weight gain (g)	72.35	107.63	82.52	59.57	46.89	Q ³	10.68
Feed intake (g)	235.25	219.5	232.2	225	214	NS	4.07
Feed conversion ratio (g)	3.277	2.057	2.824	3.8	4.662	Q ⁴	13.17
SGR	0.197	0.3	0.23	0.173	0.131	Q ⁵	11.4
PEG	1.19	1.9	1.37	1.02	0.84	Q ⁶	11.81
LEG	33.11	39.98	23.22	14.47	10.23	L ⁷	12.87

¹Regression. NS = not significant; Q = significant quadratic effect; L = significant linear effect, all at 5% significance; ²Coefficient of variation; ³ $y = -71.857x^2 + 187.81x - 32.192$; $R^2 = 74.01$; ⁴ $y = 3.3586x^2 - 8.8169x + 8.337$; $R^2 = 87.21$; ⁵ $y = -0.2142x^2 + 0.5716x - 0.1279$; $R^2 = 74.50$; ⁶ $y = -1.2183x^2 + 3.2206x - 0.5878$; $R^2 = 64.54$; ⁷ $y = -23.643x + 60.434$; $R^2 = 82.09$

The value of digestible lysine found in this research for the variable weight gain was less than the requirement presented by the NRC (2011) of 16.0 g kg^{-1} where it is highlighted that the requirements may vary with the stage of development and physiological condition of the fish. The lysine content determined in this study for the best weight gain was less than the value of 13.80 g kg^{-1} set by the Brazilian table for Tilapia Nutrition (Furuya, 2010); however, the table recommends this value for fish of any weight above 100 grams. The lysine content in the diet obtained in the present study was similar to the requirement of 13.10 g kg^{-1} determined by Furuya et al. (2013) for Nile tilapia of 87 to 226 grams.

The requirement observed for better FCR was similar to the value estimated for the greatest weight gain. The observed FCR results were mostly due to the weight gain variations, since no significant response to FI was observed. The estimated lysine contents in this study were lower than the ones estimated by Furuya et al. (2012) for a better FCR of 16.40 g kg^{-1} of digestible lysine. However, Furuya et al. (2013) noted a requirement of 10.30 g kg^{-1} of digestible lysine for a better FCR when working with fish of initial live weight of 87 g and slaughtered at approximately 226 g.

Higher protein efficiency for weight gain was estimated to be 13.20 g kg^{-1} of digestible lysine ($R^2=0.6454 - y = -1.2183x^2+3.2206x -0.5878$). For LEG, a linear decrease was observed ($R^2=0.8209 - y = -23.643x + 60.434$).

Values for SGR determined by the present study may be related to the age and the size of the fish, since the growth rate was lower than that of fish with a live weight below 100 g. The results are lower than those found by Bomfim et al. (2010), who observed an average value of 7.9% for SGR; the same authors stated that fish in the initial phase show elevated SGR due to muscular tissue deposition. The low value of PEG observed (1.54 g g^{-1}) could be related to a lower deposition of muscular tissue in fish in the termination phase.

After the first euthanasia, there was no significant difference in carcass and fillet yield (Table 3). Regarding fillet quality, there were no significant differences in fillet humidity, crude protein, ethereal extract, ashes, PDR, DFDR or PRE for both slaughters (Table 4).

In contrast, for fish that were slaughtered 50 days after the beginning of the experiment, it was estimated that a digestible lysine amount of 1.45 g kg^{-1} would result in the lower carcass humidity with 69.67% ($p < 0.05$). A digestible lysine content of 1.46 g kg^{-1} for the ethereal extract of the carcass ($p < 0.05$) was estimated, to obtain the maximum of 7.40% of EE. A mortality rate of 12.15% was observed.

After the first euthanasia, the increase in weight gain observed as the lysine level increased did not affect the carcass and fillet yields at 28 or 50 days of feeding. This result is partly because the gain was uniform throughout the entire body of the fish. Other authors have not observed differences in carcass yield (Furuya, Botaro, Neves,

Silva, & Hayashi, 2004; Furuya et al., 2013; Rampe et al., 2014). Many studies in the literature have not evaluated carcass yield, which make comparisons more difficult. This parameter should be considered in the studies, regardless of fish size, due to its relevance for determination of fish nutritional requirements.

Table 3. Yield, humidity, protein, ethereal extract (EE) and ashes of carcass and fillet and hepatosomatic index (HSI) of Nile tilapia from 500 to 600 g, at 28 days of experiment, fed increasing amounts of digestible lysine.

Variable (%)	Lysine amount (g kg ⁻¹)					¹ Req	² CV
	9.30	12.30	15.30	18.30	21.30		
Carcass yield	83.87	84.48	83.39	84.8	85.23	NS	2.0
Carcass humidity	69.65	68.71	68.19	68.6	69.94	NS	5.95
Carcass protein	16.21	17.33	16.64	16.69	16.39	NS	4.5
Carcass EE	7.23	8.09	8.87	7.92	7.91	NS	20.01
Carcass ashes	6.65	5.34	5.17	6.46	4.68	NS	12.38
Fillet yield	30.39	30.74	30.98	30.95	30.56	NS	3.46
Fillet humidity	79.27	79.04	78.93	79.1	78.45	NS	1.65
Fillet protein	18.15	19.25	18.46	18.48	18.95	NS	5.39
Fillet EE	1.66	1.3	1.92	1.9	1.77	NS	27.62
Fillet ashes	1.16	1.14	1.16	1.13	1.14	NS	4.99
HSI	2.37	2.08	2.23	2.27	1.99	NS	13

¹Regression. NS = not significant; Q = significant quadratic effect; L = significant linear effect, all at 5% significance; ²Coefficient of variation.

Table 4. Yield, humidity, protein, ethereal extract (EE) and ashes of carcass and fillet and hepatosomatic index (HSI), of Nile tilapia from 550 to 600 g, at 50 days of experiment, fed increasing amounts of digestible lysine.

Variable (%)	Lysine amount (g kg ⁻¹)					¹ Reg.	² CV
	9.30	12.30	15.30	18.30	21.30		
Carcass yield	83.67	85.21	82.55	81.87	84.43	NS	2.56
Carcass humidity	70.68	70.38	69.29	70.31	71.69	³ Q	1.66
Carcass protein	16.92	16.17	16.82	16.8	15.95	NS	4.96
Carcass lipid	6.36	7.60	7.42	6.98	5.49	⁴ Q	15.15
Carcass ashes	5.42	5.59	5.63	5.42	5.27	NS	13.18
Fillet yield	30.22	30.32	29.57	30.31	30.97	NS	4.85
Fillet humidity	79.95	79.51	80.38	79.32	79.79	NS	0.47
Fillet protein	18.13	18.09	17.85	17.78	18.56	NS	4.39
Fillet lipid	0.93	0.89	0.58	0.84	0.25	NS	36.12
Fillet ashes	1.18	1.12	1.14	1.16	1.17	NS	4.84
HSI	2.46	2.45	3.04	2.72	2.03	NS	23.03

¹Regression at 5% significance: NS = not significant; Q = significant quadratic effect; ²Coefficient of Variation; ³y = 4.2712x² - 12.467x + 78.765; R² = 85.35; ⁴y = -3.9886x² + 11.658x - 1,1199; R² = 97.26

There were no significant differences in the fillet quality parameters, which is in agreement with Michelato et al. (2013) while determining the methionine + cysteine requirements for tilapia slaughtered at 700 g, they did not observe significant differences in fillet parameters.

It was possible to estimate the lysine level requirement of 1.45 g kg⁻¹ for the lower carcass humidity (69.67%). Lowering water activity can increase shelf life of fish (Abbas, Saleh, Mohamed & Lasekan, 2009).

The rationed lysine levels influenced carcass humidity and ethereal extract. Since there was no difference in fillet humidity and ethereal extract, those differences observed in the carcass could be due to filleting residues and fish skin. Tilapia filleting residues have high fat contents, derived mostly from the offal (Boscolo, Hayashi, Feiden, Meurer, & Signor, 2008).

After two weeks from the beginning of the experiment, agonistic behavior among conflicts between the fish were observed; this may be because the fish were not separated by size in the water tanks as the fish were growing, a management practice that, according to Volpato and Fernandes (1994), avoids fish developing faster and showing hierarchical dominance behavior. Carvalho, Camargo, and Zanatta (2010) showed that fish separated during the juvenile phase achieved almost twice the size of those that were not separated at this phase. In this study, it was observed that the fish closer to the monk had darker coloration and stayed in the superficial portion of the tank, with their caudal fin practically out of the water. According to Volpato, Frioli, and Carrieri (1989), this behavior indicates that these fish are submissive; these fish more frequently occupy the superior portion of the water column and acquire darker coloration after antagonistic encounters.

Conclusion

Nile tilapia in the live weight range of 500 to 600 grams require 13.1 g kg⁻¹ of digestible lysine for maximum weight gain and better feed conversion, a value which corresponds to 5.31% of the digestible protein in the diet and 0.431 McCall of digestible energy.

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