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Complete List of Authors:	Diógenes, Alexandre; Universidade Estadual Paulista (UNESP), Centro de Aquicultura (CAUNESP) Fernandes, João Batista; Universidade Estadual Paulista (UNESP), Centro de Aquicultura (CAUNESP); UNESP, Centro de Aqüicultura-CAUNESP Dorigam, Juliano Cesar; Universidade Estadual Paulista (UNESP), Departamento de Zootecnia Sakomura, Nilva; Universidade Estadual Paulista (UNESP), Departamento de Zootecnia Rodrigues, Fabio Henrique; Universidade Estadual Paulista (UNESP), Centro de Aquicultura (CAUNESP) Lima, Bruno Tadeu; Universidade Estadual Paulista (UNESP), Centro de Aquicultura (CAUNESP) Gonçalves, Fernando Henrique; Universidade Estadual Paulista (UNESP), Centro de Aquicultura (CAUNESP)

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TITLE PAGE

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Establishing the essential amino acid requirement in juveniles of Nile tilapia (*Oreochromis niloticus*) by the deletion method

Alexandre Firmino Diógenes[1*], João Batista Kochenborger Fernandes[1], Juliano Cesar De Paula Dorigam[2], Nilva Kazue Sakomura[2], Fabio Henrique Fernandes Rodrigues[1], Bruno Tadeu Marotta Lima[1], Fernando Henrique Agostinho dos Santos Barbosa Gonçalves[1]

[1]Centro de Aquicultura da Universidade Estadual Paulista (CAUNESP) – Via de Acesso Paulo Donato Castellane, s/n, 14884-900, Jaboticabal, SP, Brazil.

[2] Departamento de Zootecnia da Universidade Estadual Paulista – Via de Acesso Paulo Donato Castellane, s/n, 14884-900, Jaboticabal, SP, Brazil.

*Corresponding author: João Batista Kochenborger Fernandes

e-mail: jbatista@caunesp.unesp.br

Centro de Aquicultura – UNESP/Jaboticabal – CAUNESP, Via de Acesso Paulo Donato Castellane, s/n14884-900 Jaboticabal – SP

(16)3203-2110 extension 205; (16)3209-7477 extension 8123

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ABSTRACT

One of the strategies used to improve fish production is lowering the feed costs and the environmental impact by reducing dietary protein content. Using the deletion method, we determined the optimal amino acid (AA) ratio for Nile tilapia (*Oreochromis niloticus*) (body weight 20g). Eleven experimental diets and four replications, taken two at a time, distributed in a randomized block design were used. For this trial, a balanced diet (BD) was formulated. Ten other diets were formulated by the deletion method, in which the BD diet was diluted to result in a reduction of 45% of all nutrients. Fish were fed three times a day for 57 days. Groups of fish at the beginning and at the end of the experiment were euthanized for further determination of the nitrogen (N) carcass composition. The optimal ratio of each AA was derived by dividing the requirement for each AA by the requirements for lysine. The essential AA ratios, expressed relative to lysine (=100), were: methionine 64, threonine 93, tryptophan 24, arginine 125, histidine 34, isoleucine 57, leucine 96, valine 76, and phenylalanine 101. Our findings might be used to design strategies aimed at reducing the production costs of *Oreochromis niloticus.*

Introduction

Aquaculture has been continuously challenged to improve economic profits while maintaining environmental sustainability. One of the strategies used to improve fish production is lowering the feed costs and the environmental impact by reducing dietary protein content. The possibility of reducing the dietary protein content was demonstrated by using the ideal protein concept to balance amino acid (AA) content in fish diets (Wilson & Poe, 1985; Boisen et al., 2000). Once the ideal AA profile has been determined, the requirement for a single AA can be determined experimentally for a given field situation, with the requirements for all other AAs being calculated from the ideal ratios (Furuya & Furuya, 2010).

A number of techniques have been employed to easily estimate the quantitative AA requirement for fish. These techniques are based on the analysis of essential amino acids (EAA) found in the body (Furuya & Furuya, 2010) and later used as reference for tilapia nutrition (Furuya, 2004). However, results from previous studies done in tilapia show that the AA profile in the body does not always correctly indicate the dietary nutrient requirements (Wilson, 2002; Wilson, 2003). Therefore, it is recommended that dose-response experiments be performed to determine the optimal AA profile in a fish feed (Furuya & Furuya, 2010) in order to prevent deficiencies of EAA. However, dose-response experiments are time-consuming and expensive, and require that multiple assays be performed for each AA (Rollin *et al.*, 2003).

The deletion method has been used as an alternative to determine the optimal AA ratio. This method has been used for growing pigs (Fuller *et al.*, 1989; Wang & Fuller, 1989) and later adapted for fish (Rollin *et al.*, 2003), specifically Atlantic salmon (*Salmo salar L.*). This method relies on the assumption that the reduction of a non-limiting AA has no effect on nitrogen (N) retention. However, if a single AA is limiting in the diet, the rate of body N retention is directly related to the reduction in levels of that individual AA. The changes in N retention are a function of reduction of each EAA, which in turn is used to calculate a dietary AA profile where all EAA are limiting.

Validated by Rollin *et al.* (2003), this method has been shown to be fast and accurate, and can be used to assess the EAA requirements of different species of fish and their varying requirements that also depend on their growth rates.

Nile tilapia (*Oreochromis niloticus*) is one of the most cultured species in Brazil and in many other countries, especially those with tropical and subtropical climates. Several studies have been conducted in order to determine the requirements for lysine in each creation phase of Nile tilapia. However, little information is available on other AA requirements and on their relationship to lysine, which is used as reference (Furuya & Furuya, 2010). Using the deletion method, we determined the optimal AA ratio for juvenile Nile tilapia. Our findings might be used to design strategies aimed at reducing the production costs of *Oreochromis niloticus.*

Materials and Methods

Experimental design

The study was conducted at the Aquaculture Center of the Universidade Estadual Paulista (CAUNESP), located in Jaboticabal, São Paulo, Brazil, to determine the EAA ratio for juveniles of Nile tilapia. The experimental design consisted of eleven experimental diets with four replications, taken two at a time, distributed in a randomized block.

Experimental diets

For this trial a balanced diet (BD) was formulated to meet the adequate nutritional requirements recommended in the Brazilian Table of Tilapia nutrition (Vidal *et al*., 2012; Furuya *et al*., 2004). The other diets were formulated by the deletion technique, in which the BD diet was diluted with corn starch resulting in a reduction of 45% of all nutrients. Except for the AA to be tested, the other AAs were supplemented with crystalline AA at the same concentration as the BD diet (100%), as well as the other nutrients. The composition and the nutritional content of the experimental diets are presented in Tables 1 and 2.

[Table 1 here]

[Table 2 here]

The experimental diets were kept isonitrogenous by supplementation with L-Alanine and L-Glutamate. The experimental diets formulated based on the deletion technique ensured that all the nutrients were in the same concentration as the positive control diet, with the exception of the AA to be tested. Thus, the deletion technique of the diets resulted in a reduction of the AAs in the same proportion while maintaining the optimum relationship between them.

Animals and feeding

The juveniles of the Nile tilapia (GIFT strain), with an initial weight of 20 g ± 0.9 g, were sexually inverted. The fish were stocked in masonry tanks of 1500L and kept in observation for two weeks and a number of specimens were evaluated at the CAUNESP Pathology Laboratory prior to taking any preventive measures.

The fish were weighed and distributed into 22 concrete tanks at a proportion of 34 fish per tank, and submitted to the trial where the fish received the experimental diets over the course of 57 days. During the experiment, feed intake was recorded. The fish were hand-fed slowly to prevent waste, three times a day (8:00, 12:00, and 16:00 hours) until apparent satiation, judged by observation of feeding behaviors.

Environmental control

The closed recirculating system used in this study was equipped with a biological filter and heater, and the water quality was monitored. To ensure that the physical-chemical water quality was appropriate for Nile tilapia, daily before the first and after the last feeding, the parameters of quality such as pH, temperature, conductivity, and oxygen were measured.

The total alkalinity of the water was monitored weekly and determined by titration according to the method of Golterman *et al.* (1978). In the same way, the concentration of ammonia, nitrate, and nitrite was determined by the colorimetric method and reading in the spectrophotometer, according Koroleff (1976), Golterman *et al*. (1978), and Mackereth *et al.* (1978), respectively.

The environment conditions were maintained at safe levels, as outlined by Boyd (1990).

Data collection

The fish had their initial and final weights measured in a digital balance. The body weight gain data were calculated by the difference between the final body weight and the initial body weight. The feed intake values were calculated based on individual daily intake, but the calculations of feed conversion and feed efficiency were based on total intake in relation to the number of fish in each repetition. All these values were corrected using the number of fish alive in each replicate. The mortalities were registered daily and this information was used to calculate the values of viability, defined by the arcsine of the root square of mortality in percentage. However, values were expressed as percentages in this work.

For determination of whole-body nitrogen composition, the comparative slaughter technique was used. Initially, a group of ten fish were randomly collected and euthanized by anesthetizing with a benzocaine solution and water and subsequently were identified and stored (-20 ^{o}C) for future analysis. All fish were bulk weighed every fifteen days and at the end of the experiment following the norms of the ethics committee for animal use; three fish from each experiment unit were randomly selected after a fast period of 24 hours to eliminate food residues from the digestive tract. After this procedure, they were anesthetized with benzocaine diluted in water and subsequently weighed and measured.

Chemical analysis

For the analysis of body composition, the fish were processed in an autoclave for 30 minutes (at 127^{o}C at 1 atm of pressure) and ground in an industrial blender. Afterwards, the carcass samples were dried for 72 hours at 55 °C in a forced air oven. The samples were weighed to quantify the dry matter content and were then ground in a Micro Mill (A11 Basic, IKA®). The total nitrogen content of the diets and fish carcasses was analyzed in a nitrogen distiller (kjeltecTM 8400 Foss®) using the Kjeldahl method (Method No. 2001.11) according to AOAC (1995). A factor of 6.25 was used in the conversion of the nitrogen value to crude protein (CP). The total AA content of the ingredients in the experimental diets was analyzed by Ajinomoto Ltd. using high performance liquid chromatography (HPLC).

The analyzed nitrogen content of whole-body composition was used to determine the nitrogen deposition using the following equation proposed by Rollin *et al*. (2003):

$$\text{N-Deposition} = \frac{(W_f \text{ x } N_f - W_i \text{ x } N_i)}{\frac{1}{2}\left(\left(\frac{W_f}{1000}\right)^{0.75} + \left(\frac{W_i}{1000}\right)^{0.75}\right) \text{ x } \Delta t}$$

where: W_f and W_i are the mean final and initial body weights (g), Δt is the duration of the feeding period (d), and N_f and N_i are the mean N contents of the whole-body fish at the end and at the beginning of the experimental period (g/g), respectively. From the relation of N deposition obtained in the AA deletion experiment it was possible to determine an optimal dietary AA pattern.

In practice, Rollin *et al*. (2003) proposed to calculate the EAA requirement values (gkg^{-1} DM) for a given EAA as follows:

$$\text{requirement} = (EAA)_{BD} \text{ x } \left(2\text{-DEL-} \left(\frac{ND_{EAA}}{ND_{BD}}\right)\right)$$

where: $(EAA)_{BD}$ is the concentration of the considered EAA in the BD (g/kg DM), DEL is the deletion rate of the EAA in the deficient diet compared with the BD, ND_{EAA} is the N deposition (mg $N/BW_{kg}^{0.75}$/d) corresponding to the EAA diet, and ND_{BD} is the N deposition observed on the BD (mg $N/BW_{kg}^{0.75}$/d). This method is based on the assumption that N retention is a linear function of dietary essential AA content when a particular AA is limiting. An optimal balance between the EAA was derived by dividing the estimated requirement for each EAA by the estimated requirements for lysine (base lysine = 100).

Statistical analysis

The statistics were performed using the R statistical package (version 3.0.1) and significant differences between deficient treatments and the balanced diet treatment responses were tested using the Dunnett's test; values of $P<0.05$ were deemed statistically significant.

Results

The experimental diets were accepted well by the fish and no pathological signs were observed during the trial. The mortality rate was very low and according to what is observed with regular diets (Furuya *et al*., 2004). Therefore, the deficient diets used in this study do not seem to have had any effect on the mortality rate (Table 3).

Responses to each AA-deficient diet on growth performance and feed efficiency are presented in Table 3. A higher body weight gain and feed efficiency were observed in the balanced diet group (BD). In all cases, lower levels of AA reduced significantly ($P<0.05$) the growth performance, and the extent of this decrease depended on which AA was presented in lower levels. In this study, lower levels of threonine, leucine, valine, and methionine+cystine led to the lowest body weight gain whereas phenylalanine+tyrosine, isoleucine, and lysine led to the highest body weight gain. Except for lysine, arginine, histidine, isoleucine, and phenylalanine+tyrosine, the feed efficiency was significantly ($P< 0.05$) reduced in presence of lower levels of AA.

Amino acid-deficient diets significantly ($P<0.05$) reduced daily nitrogen deposition, except for those lower in phenylalanine+tyrosine. Similar to growth performance, deletion of threonine promoted the greatest reduction in nitrogen deposition, followed by methionine and valine (Table 4).

[Table 4 here]

At the beginning of the trial there were no differences in whole-body nitrogen composition among experimental animals (Table 4). However, whole-body nitrogen composition was significantly (P<0.05) lower in fish fed diets deficient in threonine, methionine+cysteine, valine, and leucine, if compared to those deficient in lysine, isoleucine, or phenylalanine+tyrosine or the balanced diets.

The relationship between nitrogen deposition and the AA intake for the balanced and the amino acid-deficient diets is presented in Figure 1.

[Figure 1 here]

We estimated how much AA could be removed from the control diet, based on data shown in Table 4 and considering that a linear response between nitrogen deposition and AA intake occurs when feeding an amino acid-deficient diet.

Based on Table 4 and assuming that each AA is equally limiting, the ideal dietary AA profile relative to lysine (=100%) was estimated to be: methionine+cystine 64, threonine 93, tryptophan 24, arginine 125, valine 76, isoleucine 57, leucine 96, histidine 34, and phenylalanine+tyrosine 101.

Expressed as the dietary protein content g/16 g N, the optimal balance was estimated as: lysine 5.01, methionine+cystine 3.19, threonine 4.67, tryptophan 1.19, arginine 6.27, valine 3.78, isoleucine 2.84, leucine 4.83, histidine 1.72, and phenylalanine+tyrosine 5.03.

Discussion

The present study shows that the deletion method was able to successfully determine the optimal AA ratio for Nile tilapia.

Fish fed diets deficient in methionine, threonine, leucine, and valine had a lower body weight gain, if compared to those fed a balanced diet (Table 3). These data may be related to a low intake of these diets (Table 3), which leads to low feed efficiency. Consequently, the amino acid deletion in these diets affected nitrogen body deposition of these AAs. Peres and Oliva-Teles (2009) observed the same behavior while adopting the deletion method when studying juveniles of gilthead sea bream (*Sparus aurata*), in which threonine deletion led to higher reduction in growth and nitrogen retention, followed by reductions in growth associated to methionine deletion.

Rollin *et al.* (2003) found that methionine is the most limiting AA in Atlantic salmon (*Salmo salar L.*), followed by phenylalanine. In the present study, methionine was limiting whereas phenylalanine was not (Table 4). This is not a surprising finding considering that different species have unique AA requirements. In this study, threonine was the most limiting AA in the diets. As for methionine+cysteine, the ideal AA ratio found was similar to that estimated by Rollin *et al.* (2003) and established as 64% (Table 4). However, phenylalanine deletion of 37% was not enough to turn this AA limiting in the diet, but the ideal phenylalanine:lysine ratio, equivalent to 101%, was quite similar to the 105% value found by Rollin *et al.* (2003) for Atlantic salmon. The ideal 34% values for histidine and 96% for leucine are close to those found by Peres and Oliva-Teles (2009) (36.8 and 92.7%, respectively).

No morphological signs of deficiency were observed, such as scoliosis, fin erosion, and cataracts in Nile tilapia fed deficient diets, whereas other authors have observed these signs of deficiencies while feeding rainbow trout (*Salmo gairdneri*) (Poston & Rumsey, 1983; Ketola, 1983) deficient diets. The requirement for tryptophan was very high (0.37%), with a tryptophan:lysine ratio of 24%.

Analysis of the growth data in Nile tilapia (*Oreochormis niloticus*) by Santiago and Lovell (1988) revealed a break point in the growth response curve at 1.43% of dietary lysine. These authors indicated that increasing lysine beyond this level would not provide significant additional growth. On the other hand, the lysine requirement calculated in this study was 1.56% and showed that juveniles of Nile tilapia need a higher level of lysine for proper growth. Liebert and Benkendorff (2007) obtained a value similar to that found in the present study for Nile tilapia, in the initial phase, finding a requirement for 1.55% of dietary lysine.

For valine and leucine, we observed a higher estimate for their requirements: 1.18 and 1.50%, respectively. Values estimated by Santiago and Lovell (1988) in Nile tilapia were 0.78 and 0.95%, respectively. This higher recommendation found in our study might reflect a higher reduction in weight gain and the nitrogen deposition observed in these diets. As for the isoleucine requirement, it was found to be 0.89%, which is similar to that estimated by Santiago and Lovell (1988) (0.87%). Therefore, no leucine–isoleucine interaction was observed in the Nile tilapia, otherwise an increase in the isoleucine requirement would have led to an excess of leucine (Chance *et al.*, 1964).

The break point analysis made by Santiago and Lovell (1988) in the growth response curve in Nile tilapia indicates that 1.18% dietary arginine provided optimum growth. However, the arginine requirement determined in this study (1.96%) was higher than the recommendation by those authors, and thus a higher arginine:lysine ratio of 125% was found in our study. In this study, the arginine requirement value was above the expected value initially planned. It is possible that this greater amount of arginine may have caused a reduction in growth rate. Future studies should investigate this issue more deeply.

This experiment followed the method described by Rollin *et al.* (2003) and Peres and Oliva-Teles (2009) in which fish were fed either a balanced or other diets with levels of AA limitation varying between 37 and 47%. Despite the differences in values found in this study, if compared to values found in other studies (Santiago and Lovell, 1988), the deletion method is accepted as a fast and efficient method to estimate the ideal ratio of EAA in a given species (Boisen, 2003; Baker, 2004). Additionally, this method requires the use of crystalline AA in the formulation of diets.

The nutritional value of crystalline AA compared to protein-bound AA is still controversial for the nutrition of aquatic organisms (Dabrowski & Guderley, 2002), including the Nile tilapia. Some authors claim that the efficiency of utilization of the crystalline EAA is lower (Dabrowski & Guderley, 2002), which may impact estimates of the ideal ratio of EAA. However, the good growth performance observed in this study supports the use of diets containing crystalline AA. This point of view is supported by the observation made by Rollin *et al.* (2003) who found no differences in performance between diets with the same composition, formulated with protein of animal origin and those mixed with crystalline AA in Atlantic salmon.

The use of different sources of protein is a good indicator of the ideal ratio of the EAA in the diets of fish, and in most studies the AA profile of the body protein is used as reference. A good correlation between the ideal profile of EAA in the diet and the ideal profile of EAA in the animal body protein is expected in fast-growing animals, where a large proportion of EAA are used for protein deposition, while only a small proportion of the AA are used for maintenance (Peres & Oliva-Teles, 2009). Nevertheless, some differences in the EAA requirements for growth and maintenance are expected in fish, as has been shown with arginine (Fournier *et al*., 2002).

However, in this study, we assumed that all EAA are used with similar efficiencies, and that the requirements of EAA for maintenance are similar. Nevertheless, the EAA requirements for maintenance may differ among fish (Mambrini & Seudre, 1995; Mambrini & Kaushik, 1995; Rodehutscord *et al.*, 1997; Fournier *et al.*, 2002). Thus, the EAA requirements for maintenance in fish can differ from the EAA profile necessary for protein deposition in mammals (Said & Hegsted, 1970; Fuller *et al.*, 1989). EAA maintenance is determined according to the age of the animal, and although fish never stop growing, their growth rate becomes slower with time. Thus, the deletion method requires further studies for a more precise estimate of the ideal AA ratio in Nile tilapia.

Because of the great variation in daily protein consumption by fish, caused by variations in size, feeding practices, and diet formulations, it is highly recommended that AA requirements be reported as a percentage of the dietary protein, rather than as a percentage of the diet (Santiago & Lovell, 1988). Thus, the recommendations made by this study, and which are close to those observed in the literature, are found in Table 5.

[Table 5 here]

In conclusion, knowing the optimal amino acid profile may help in formulating diets with high nutritional density and which respect the proper balancing of AA and energy:protein ratio while producing a low pollution effect. These diets may be successfully used in intensive farming systems with tropical fish, as a strategy to increase sustainability without causing economic losses.

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Figure Legends

Figure 1. Effect of deleting 45% of each essential amino acid from the balanced diet on nitrogen retention of Nile tilapia juveniles

Table1. Composition of the amino acid-deficient diets used to determine the optimum balance among essential amino acids in Nile tilapia juveniles (*Oreochromis niloticus*)

Ingredients (g kg^{-1})	D1 BD	D2 Lys	D3 M+C	D4 Thr	D5 Trp	D6 Arg	D7 His	D8 Ile	D9 Leu	D10 P+T	D11 Val
Balanced diet (BD)	-	550	550	550	550	550	550	550	550	550	550
Corn	350	-	-	-	-	-	-	-	-	-	-
Corn starch	90	156	156	155	154	148	153	155	155	157	155
Fish meal	160	-	-	-	-	-	-	-	-	-	-
Soybean meal	55	-	-	-	-	-	-	-	-	-	-
Soybean oil	52	35	35	35	35	35	35	35	35	35	35
Gelatin	30	14	14	14	14	14	14	14	14	14	14
Cellulose	44	28	28	28	28	28	28	28	28	28	28
CMC*	20	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Glucose	20	45	45	45	45	45	45	45	45	45	45
Dicalcium phosphate	-	16	16	16	16	16	16	16	16	16	16
Supplement**	10.0	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Antioxidant***	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Potassium chloride	-	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2
Limestone	-	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Amino acids (g kg^{-1})											
L-Alanine	102	80	76	78	75	86	77	77	77	78	77
L-Glutamate	20	20	20	20	20	20	20	20	20	20	20
L-Lysine HCl	9.1	0	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2
L-Phenylalanine	8.2	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	0	7.5
L-Arginine	5.1	6.1	6.1	6.1	6.1	0	6.1	6.1	6.1	6.1	6.1
L-Threonine	7.4	5.4	5.4	0	5.4	5.4	5.4	5.4	5.4	5.4	5.4
L-Leucine	0.2	4.6	4.6	4.6	4.6	4.6	4.6	4.6	0	4.6	4.6
L-Isoleucine	4.4	4.2	4.2	4.2	4.2	4.2	4.2	0	4.2	4.2	4.2
DL-Methionine	5.1	4.2	0	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2
L-Valine	2.2	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	0
L-Histidine	2.3	2.4	2.4	2.4	2.4	2.4	0	2.4	2.4	2.4	2.4
L-Tryptophan	2.2	1.4	1.4	1.4	0	1.4	1.4	1.4	1.4	1.4	1.4

*Carboxymethylcellulose

**Vitamin and mineral supplement (content g/kg)

***Butylated hydroxytoluene

D: Experimental diets

Table 2. Nutritional composition of the experimental diets used to determine the optimum balance among essential amino acids in Nile tilapia juveniles (*Oreochromis niloticus*)

Nutrient* (g kg^{-1})	D1 BD	D2 Lys	D3 M+C	D4 Thr	D5 Trp	D6 Arg	D7 His	D8 Ile	D9 Leu	D10 P+T	D11 Val
Crude protein**	311	318	320	322	320	323	323	322	327	331	330
Digestible protein	268	268	268	268	268	268	268	268	268	268	268
Calcium	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Energy (Mkcal kg^{-1})	2.95	2.79	2.79	2.80	2.79	2.79	2.79	2.79	2.79	2.80	2.79
Crude fiber	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00
Available phosphorus	5.10	5.10	5.10	5.10	5.10	5.10	5.10	5.10	5.10	5.10	5.10
Fat	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00
Potassium	3.90	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sodium	1.00	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Amino acids (g kg^{-1})**											
Arginine	19.10	19.20	18.60	16.90	20.40	12.10	15.60	13.80	13.60	15.00	15.00
Phe+tyr	14.40	15.80	15.10	13.60	15.90	14.10	16.40	14.70	14.70	9.10	16.00
Histidine	5.57	5.21	5.35	4.63	5.62	5.54	3.37	5.47	4.92	5.45	5.62
Isoleucine	10.10	9.80	9.30	8.90	10.40	9.70	10.30	5.60	9.50	10.30	10.20
Leucine	12.70	12.20	11.60	11.00	12.70	11.80	12.60	11.20	7.90	12.50	12.30
Lysine	17.50	10.70	16.70	15.60	18.00	16.60	17.20	15.90	16.30	17.80	17.80
Met + cys	8.36	8.57	4.72	6.96	9.17	8.09	8.21	7.79	7.54	8.48	8.41
Threonine	11.90	11.50	11.30	6.60	12.80	11.70	12.10	1.11	11.60	11.70	11.70
Tryptophan	3.60	3.10	3.10	3.10	1.90	3.10	3.10	3.00	3.00	3.00	3.00
Valine	9.30	9.00	8.60	8.20	9.50	8.90	9.90	8.40	8.50	9.00	5.70

BD: Balanced diet
* Calculated.
** Analyzed crude protein and digestible content of the amino acids in the diets
D: Experimental diets

Table 3. Initial and final body weight (BW), body weight gain (BWG), feed intake (FI), feed efficiency (FE), and mortality of Nile tilapia juveniles (*Oreochromis niloticus*) given diets with reduction of individual amino acids for 57 d (Mean values with their standard errors)

Diets	BW (g)		BWG ($g\ d^{-1}$)	FI ($g\ d^{-1}$)	FE ($g\ g^{-1}$)	Mortality (%)
	Initial	Final				
BD*	20.2±1.02	70.2^{a}±19.50	0.88^{a}±0.35	1.01^{a}±0.04	0.85^{a}±0.13	1.25±0.09
Lys	20.3±0.78	56.3^{ab}±19.30	0.63^{ab}±0.35	0.91^{ab}±0.02	0.72^{a}±0.15	0.12±0.02
Met+Cys	20.2±0.63	34.7^{d}±4.450	0.25^{d}±0.09	0.82^{b}±0.04	0.48^{b}±0.07	0.15±0.06
Thr	20.7±0.80	34.7^{d}±2.39	0.25^{d}±0.05	0.84^{b}±0.02	0.48^{b}±0.05	0.15±0.04
Trp	20.6±1.18	41.4^{cd}±8.74	0.36^{cd}±0.16	0.84^{b}±0.03	0.54^{b}±0.08	0.18±0.06
Arg	20.3±0.60	51.7^{bc}±17.20	0.55^{bc}±0.31	0.87^{b}±0.02	0.73^{a}±0.09	0.10±0.07
His	20.6±0.52	52.9^{bc}±19.40	0.57^{bc}±0.35	0.89^{b}±0.04	0.72^{a}±0.13	0.07±0.08
Ile	20.4±0.54	57.9^{ab}±16.70	0.66^{ab}±0.30	0.92^{ab}±0.04	0.74^{a}±0.06	0.12±0.09
Leu	20.4±0.70	37.2^{d}±5.01	0.30^{d}±0.10	0.83^{b}±0.03	0.48^{b}±0.11	0.18±0.14
Phe+Tyr	20.0±0.96	62.5^{ab}±14.50	0.75^{ab}±0.26	1.00^{a}±0.05	0.78^{a}±0.10	0.06±0.07
Val	20.3±0.95	36.6^{d}±4.15	0.29^{d}±0.08	0.84^{b}±0.03	0.53^{b}±0.07	0.10±0.08

Values in the same column followed by different letters differ significantly ($P<0.05$) by Student's t test.

Balanced diet*

Table 4. Initial and final nitrogen (N) composition of the body, nitrogen composition and deposition, estimated requirements according the equation and the respective ideal ratio (IAAR) of each amino acid in Nile tilapia juveniles (*Oreochromis niloticus*) fed diets with individual limiting amino acids for 57 days (Mean values with their standard errors)

Diets	Body N composition (g)[§]		N Deposition[Ⱡ] ($g\ BW^{0.75}\ kg^{-1}\ d^{-1}$)	Requirements ($g\ kg^{-1}$)	IAAR (%)
	Initial	Final			
BD	2.25±0.08	7.85[a]±2.07	1.01[a]±0.23	-	
Lys	2.26±0.07	5.96[ab]±2.08	0.73[b]±0.29	15.60	100
Met+Cys	2.24±0.03	3.72[d]±0.51	0.38[b]±0.11	9.90	64
Thr	2.30±0.03	3.59[d]±0.26	0.34[b]±0.05	14.50	93
Trp	2.28±0.10	4.46[cd]±0.92	0.51[b]±0.14	3.70	24
Arg	2.25±0.04	5.25[bc]±1.68	0.62[b]±0.26	19.50	125
His	2.28±0.03	5.52[bc]±1.99	0.65[b]±0.30	5.40	34
Ile	2.26±0.03	5.77[ab]±1.71	0.69[b]±0.26	8.80	57
Leu	2.26±0.04	4.03[d]±0.58	0.44[b]±0.11	15.00	96
Phe+Tyr	2.22±0.05	6.54[ab]±1.50	0.83[a]±0.18	15.70	101
Val	2.25±0.09	3.62[d]±0.35	0.35[b]±0.07	11.80	76

[§] Values in the same column followed by different letters differ significantly ($P<0.05$) by Student's t test
[Ⱡ] Values within the same column followed by different letters differ significantly from the balanced diet (BD) group by Dunnett's test ($P<0.05$).

Table 5. Comparison between the ideal amino acid profiles expressed as g/ 16 g N

Amino acid	Present study	Santiago and Lovell[1] (1988)	Rollin *et al.* (2003)[2]	Peres and Oliva-Teles (2009)[3]
Lys	5.01	5.12	8.50	5.13
Met+Cys	3.19	3.21	3.70	2.60
Thr	4.67	3.75	4.60	2.98
Trp	1.19	1.00	-	0.75
Arg	6.27	4.20	6.90	5.55
His	1.72	1.72	2.50	1.89
Ile	2.84	3.11	4.60	2.55
Leu	4.83	3.39	7.60	4.75
Phe+Tyr	5.03	5.54	8.50	5.76
Val	3.78	2.80	5.80	3.21

[1]Based on dietary protein content for Nile tilapia (*Oreochromis niloticus*) juveniles.
[2]Based on body protein content for Atlantic salmon (*Salmo salar L.*) fry.
[3]Based on dietary protein content for gilthead sea bream (*Sparus aurata*) juveniles.

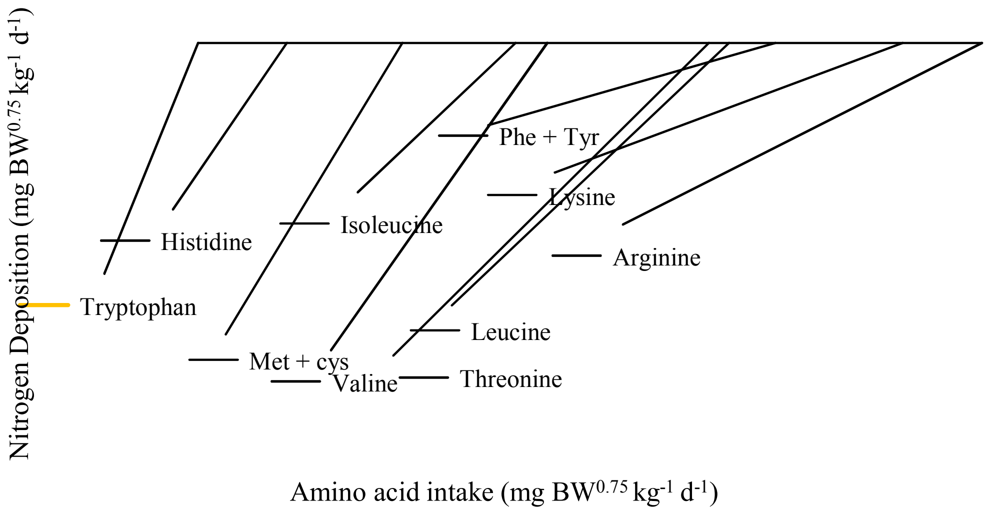

Figure 1. Effect on nitrogen retention of Nile tilapia juveniles when 45% of each essential amino acid is removed from the balanced diet.