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RESEARCH ARTICLE

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Valine in diets for juvenile Nile tilapia (*Oreochromis niloticus*): growth performance, chemical composition, blood parameters and skeletal muscle development

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Abstract

Valine belongs to the group of branched-chain amino acids, has an important structural role and is primarily deposited as body protein. The present study evaluated the effects of valine in diets of juvenile Nile tilapia. A total of 216 juveniles with weight of 21.40 ± 0.42 g and length of 10.07 ± 1.00 cm were distributed into 18 aquarium in a completely randomized design with six treatments and three replicates. Six diets containing 24.30% digestible protein, 3,100 kcal/kg digestible energy, and increasing valine levels (0.54; 0.63; 0.72; 0.81; 0.90; 0.99%) were prepared. The following factors were evaluated: performance, chemical composition, blood hematological and biochemical, and skeletal muscle development. Differences were observed in productive performance between treatments for weight gain, daily weight gain and apparent feed conversion, where inclusion levels from 0.81% to 0.99% provide better performances. According to the equation of quadratic regression, the inclusion of 0.86% of valine provided greater weight gain. Regarding the hematological and biochemical parameters, there were differences among the treatments for hemoglobin, triglycerides, and cholesterol. Not difference was observed for the chemical composition and muscle fiber growth. It is recommended the inclusion of 0.86% of valine in the diet of juveniles of Nile tilapia because it provides greater weight gain.

Additional keywords: animal health; aquaculture; branched-chain amino acids; essential amino acid; fish nutrition; hematology; histology.

Authors' contributions: Conceived and designed the experiments: RBR, DHN, FB and WRB. Coordinating the research project and supervising the work: FB and WRB. Performed the experiments: RBR, MZH and IWAM. Analyzed the data: RBR, DHN and FB. Contributed reagents/materials/analysis tools: RBR, DHN, FB and WRB. All authors wrote and approved the final manuscript.

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Introduction

The Nile tilapia (*Oreochromis niloticus*) is a teleost fish belonging to the family Cichlidae. It is one of the most cultivated species, mainly in intensive fish farming. In this culture system, fish require complete diets, since the natural food available in the aquatic environment does not meet their nutritional requirements.

Fish feed represents the highest production cost in commercial aquaculture. According to Wilson (2002), protein is the main and most costly component in formulated diets for aquatic animals. Thus, it is essential to maximize the efficiency of dietary protein use by the fish and present the amino acids in balanced quantities (Wilson, 1985).

Valine is described as hydrophobic and belongs to the group of branched-chain amino acids (BCAA) together with leucine and isoleucine (NRC, 2011). This group contains approximately 35% of the amino acids essential for muscle protein and 40% of the amino acids required for mammals (Harper *et al*., 1984), constituting 18-20% of the total amino acids present in plant and animal proteins (Li *et al*., 2009). These amino acids

are involved in a series of metabolic reactions as in the neurotransmitter synthesis and energy production (Fernstrom, 2005), which are essential for the synthesis of glutamine and alanine (Wu, 2009), muscle synthesis (Shimomura *et al*., 2006), maintenance of immune parameters (Calder, 2006), and recently Feng *et al*. (2015) found that valine has a series of benefits the integrity maintainer of gills, in the expression of cytokines among other enzymes and antioxidants.

Santiago & Lovell (1988) determined a requirement of 0.78% valine for Nile tilapia; however, they used purified ration, which is not commonly used in a tilapia culture system and evaluated the effects of valine on muscle growth and blood parameters that provide important information for the assessment of fish health status.

Therefore, the present study evaluated the effects of valine in diets for juvenile Nile tilapia, at performance, body composition, blood hematological and biochemical profiles, and muscle fiber development.

Material and methods

Ethical considerations

The present study was approved by the Ethics Committee for Animal Experiments of UNIOESTE - Universidade Estadual do Oeste do Paraná (Brazil) under protocol no. 11/2015 in July, 2015.

Fish and experimental conditions

The fish were acquired in a commercial fish, located in the city of Toledo-PR, Brazil. A total of 216 juvenile Nile tilapia with a mean weight of 21.40 ± 0.42 g and total length of 10.07 ± 1.00 cm were used. The fish were distributed in 18,500-L glass fiber tanks with an experimental unit comprising one tank containing twelve fish. Each tank was coupled to a water recirculation system (4 L of water/min per tank) with a central biological filter, constant aeration, and controlled temperature.

The water quality was controlled by monitoring the temperature (27.5 ± 0.08 ºC), dissolved oxygen (5.85 ± 0.15 mg/L), pH (7.05 ± 0.22), and conductivity (110.05 ± 1.85 µS/cm) using YSI Professional Plus Multiparameter Water Quality Meter (YSI, Pro Plus, Yellow Springs, OH, USA) apparatus. Daily siphoning was performed to remove the leftover feed and fish feces.

Experimental diets and feeding management

Six isoproteic (24.30% digestible protein) and isocaloric (3100.00 kcal digestible energy) rations were prepared (Tables 1 and 2) that contained increasing valine levels (0.54; 0.63; 0.72; 0.81; 0.90; 0.99%) following the recommendation to meet the nutritional requirements of juvenile Nile tilapia (Furuya, 2010). The ingredients were ground in hammer mill (Vieira, MCS 280, Tatuí-SP, Brazil) with a 0.3 mm sieve and the feed was processed by extrusion (Extec, Ex-Micro, Ribeirão Preto, SP, Brazil) with a 2 mm sieve. The fish were fed four times daily (8:00 am, 11:00 am, 2:00 pm, and 5:00 pm) until apparent satiation, over 15 min, for 77 days.

Performance

After the 77-day experimental period, the fish were fasted for 24 h to empty the gastrointestinal tract and then stunned in 80.0 mg/L eugenol (Deriggi *et al*., 2006) to record the individual weight (g) and total length (cm) measurements. Three fish from each tank were euthanized in 300 mg/L eugenol and then placed on ice to remove the visceral fat and liver. These same animals were freezer (-80 ºC) prior to the performance of the following analyses.

The following productive performance data were evaluated: weight gain (g) (final body weight – initial body weight); daily weight gain (g) (weight gain/day of the experiment); apparent feed conversion (feed consumed/weight gain); visceral fat (%) [visceral fat weight (g) * 100/final body weight (g)]; hepatosomatic index (%) [liver weight (g) * 100 /final body weight (g)]; condition factor (%) [(body weight (g)/total length (cm)^3) *100]; specific growth rate (%/day) [(ln (final weight) – ln (initial weight))/time) * 100]; protein efficiency ratio (weight gain/protein consumed); protein retention efficiency (%) ((final carcass protein content * final biomass) – (initial carcass protein content * initial biomass))/protein consumed); survival (%) [(final number of fish/initial number of fish) * 100]; and fish uniformity (%) (quantity of fish with body weight within the mean ± SD/(total number of fish * 100).

Feed and fish chemical composition

Three fish from each experimental unit (9 fish per treatment) were used to determine the body composition. The determination of the fish chemical composition followed the method suggested by the AOAC (2005) for the analysis of the moisture content (predrying at 55 ºC for 72 h, followed by drying at 105 ºC for 8 h) (Method 950.46), crude protein (Kjeldahl method; MA-036 model, Piracicaba, SP, Brazil) (Method 981.10), ether extraction (Soxhlet extractor with ether as the solvent; TE-0.44 model, Piracicaba,

Table 1. Ingredients of the experimental diets.

Ingredients[1] (g/kg)	Dietary valine (%)					
	0.54	**0.63**	**0.72**	**0.81**	**0.90**	**0.99**
Corn, kernel	399.50	398.50	397.50	396.50	395.50	394.50
Wheat, meal	224.60	225.00	225.40	225.70	226.10	226.50
Corn, gluten 60	90.00	90.00	90.00	90.00	90.00	90.00
Meat and bones, flour	25.00	25.00	25.00	25.00	25.00	25.00
Soybean oil	23.20	22.90	22.70	22.40	22.10	21.80
Fish, flour	20.00	20.00	20.00	20.00	20.00	20.00
Poultry viscera, flour	20.00	20.00	20.00	20.00	20.00	20.00
Glutamic acid	61.00	62.40	63.80	65.10	66.50	67.90
L-alanine	47.00	45.60	44.20	42.80	41.40	40.00
Histidine	1.60	1.60	1.60	1.60	1.60	1.60
L-lysine	10.90	10.90	10.90	10.90	10.90	10.90
Valine	0.00	0.90	1.90	2.80	3.70	4.60
L-threonine	8.50	8.50	8.50	8.50	8.50	8.50
Isoleucine	5.50	5.50	5.50	5.50	5.50	5.50
L-arginine	5.30	5.30	5.30	5.30	5.30	5.30
DL-methionine	3.30	3.30	3.30	3.30	3.30	3.30
L-tryptophan	3.30	3.30	3.30	3.30	3.30	3.30
Mineral and vitamin supplement[2]	10.00	10.00	10.00	10.00	10.00	10.00
Dicalcium phosphate	14.50	14.50	14.50	14.50	14.50	14.500
Limestone	21.60	21.60	21.60	21.60	21.60	21.60
Salt	3.00	3.00	3.00	3.00	3.00	3.00
Calcium propionate (antifungal)	2.00	2.00	2.00	2.00	2.00	2.00
BHT[3]	0.20	0.20	0.20	0.20	0.20	0.20

[1]All ingredients were bought at local market. [2]Levels of guarantee per kg of product - Premix (DSM-Roche®): Vit. A, 24,000 IU; Vit. D3, 6,000 IU; Vit. E, 300 mg; Vit. K3, 30 mg; Vit. B1, 40 mg; Vit. B2, 40 mg; Vit. B6, 35 mg; Vit. B12, 80 mg; Folic acid, 12 mg; Ca pantothenate, 100 mg; Vit. C, 600 mg; Biotin, 2 mg; Choline, 1,000 mg; Niacin; Iron, 200 mg; Copper, 35 mg; Manganese, 100 mg; Zinc, 240 mg; Iodine, 1.6 mg; Cobalt, 0.8 mg. [3]BHT = butylated hydroxytoluene (antioxidant).

SP, Brazil), and mineral matter (calcination of the samples at 550 ºC for 6 h; 2000B model, Belo Horizonte, MG, Brazil) (Method 920.153). All analyses were performed in triplicate.

The determination of the centesimal composition of the experimental diets followed the method suggested by AOAC (2005): Moisture (method 950.46); Crude protein (method 981.10); Ash (method 920.153); Ethereal extract (method 920.39); Crude fiber (method 962.09). The composition analyzes of the experimental diets were performed in triplicates.

Hematological and biochemical analyses

For blood collection, three fish were randomly captured from each experimental unit to sample a 2.0 mL aliquot by caudal puncture using a syringe containing 10% EDTA. The total erythrocyte count was performed in whole blood using a Neubauer chamber. The hemoglobin was evaluated using the method described by Collier (1944), and the hematocrit percentage was evaluated following the method described by Goldenfarb *et al*. (1971).

Blood smears were prepared on frosted glass slides, air-dried, and stained using the method of Rosenfeld (1947). The reading was performed under an optical microscope with 100x magnification using immersion oil. Total leukocytes and thrombocytes were counted using the indirect method (Ranzani-Paiva *et al*., 2013). A total of 100 leukocytes were counted under an ordinary light microscope with 100x immersion increase to determine the ratio of different leukocytes: lymphocytes, neutrophils and monocytes. The number of each element is expressed as a percentage.

Biochemical analyses of the total plasma proteins (g/dL), triglycerides (mg/dL), total cholesterol (mg/dL), and plasma glucose (mg/dL) were performed. The analyses were performed using specific kits (Gold

Table 2. Chemical composition of the experimental diets.

Chemical composition	Dietary valine (%)					
	0.54	0.63	0.72	0.81	0.90	0.99
	Calculated values (%)[1]					
Digestible energy (kcal)	3100.00	3100.00	3100.00	3100.00	3100.00	3100.00
Digestible protein	24.30	24.30	24.30	24.30	24.30	24.30
Calcium	1.80	1.80	1.80	1.80	1.80	1.80
Total phosphorus	0.90	0.90	0.90	0.90	0.90	0.90
Available phosphorus	0.66	0.66	0.66	0.66	0.66	0.66
Glutamic acid	5.98	6.11	6.25	6.38	6.52	6.65
Arginine	1.14	1.14	1.14	1.14	1.14	1.14
Histidine	0.47	0.47	0.47	0.47	0.47	0.47
Isoleucine	0.84	0.84	0.84	0.84	0.84	0.84
Leucine	1.49	1.49	1.49	1.48	1.48	1.48
Lysine	1.38	1.38	1.38	1.38	1.38	1.38
Methionine	0.47	0.47	0.47	0.47	0.47	0.47
Threonine	1.07	1.07	1.07	1.07	1.07	1.07
Tryptophan	0.27	0.27	0.27	0.27	0.27	0.27
Valine	0.54	0.63	0.72	0.81	0.90	0.99
	Analysed values (%)					
Moisture	7.65	7.62	7.58	7.61	7.59	7.64
Crude protein	27.88	27.86	27.87	27.85	27.86	27.89
Ash	7.59	7.66	7.57	7.60	7.61	7.63
Ethereal extract	5.45	5.43	5.40	5.37	5.34	5.34
Crude fiber	2.96	2.96	2.96	2.97	2.97	2.97

[1]Based on the available nutrients (NRC, 2011), and calculated based on the nutritional requirements of tilapia (Furuya, 2010; NRC, 2011) by Super Crac Software (TD Software, Viçosa, MG, Brazil).

Analisa Diagnóstica, Belo Horizonte-MG, Brazil) and processed according to the manufacturer's instructions. The results were read in a spectrophotometer.

Muscle fiber development

A white muscle sample was removed from the left side of each fish (12 fish per treatment) above the lateral line using a scalpel. These samples were placed in 10% buffered formalin for 24 h and then preserved in 70% alcohol. The samples were subjected to the paraffin embedding process and then cut from the paraffin blocks using a microtome (MICROM, International GmbH 69190, Walldorf, Germany). Cross-sections (5 μm) were subjected to hematoxylin-eosin (HE) staining. An image analysis system was used for morphometry (Image-Pro Plus® v. 4.5.0.29). The smallest diameter (μm) of 200 muscle fibers was determined per fish; then, the fish were grouped into diameter classes (<20 μm, 20-50 μm, and >50 μm) to evaluate the contribution of hyperplasia and hypertrophy to muscle growth (Almeida *et al*., 2008).

Experimental design and statistical analysis

The experiment was a completely randomized design with six treatments and three replicates. The data in the tables are shown as mean ± SD. The data were submitted to the Kolmogorov-Smirnov normality and the Levene's homogeneity tests and subjected to analysis of variance (ANOVA). When significant differences were observed, Duncan's mean comparison test at a 5% significance level was applied. Quadratic regression analysis was applied on weight gain data to obtain a better inclusion level of valine. The analyses were performed using the Statistical Analysis System 9.4 and GraphPad Prism 7.0.

Results

There were significant differences (p<0.05) for the variables weight gain, daily weight gain and apparent feed conversion. Not significant differences were observed (p>0.05) for the parameters condition factor,

specific growth rate, protein efficiency ratio, survival, protein retention efficiency, visceral fat, hepatosomatic index, and fish uniformity (Table 3).

Weight gain and daily weight gain were higher in the fish fed diets containing 0.81% valine; however, did not significantly differ from the fish that received the 0.90% and 0.99% valine. Fish fed diets containing 0.54% valine demonstrated the worst results; however, they did not differ significantly from the fish fed diets containing 0.63% and 0.72% valine. There was better apparent feed conversion in the fish that received 0.54%, 0.81%, 0.90% and 0.99% valine in the diet; however, the results did not differ from the treatment with 0.63% valine, which in turn did not differ from the treatment with 0.72% valine.

The quadratic regression equation applied to the weight gain data showed a good level of valine 0.86% inclusion ($R^2 = 0.55$) (Fig. 1).

There were no significant differences ($p>0.05$) for the chemical composition variables moisture content, crude protein, ether extract, or mineral matter (Table 4).

The hemoglobin showed a difference ($p<0.05$), with the highest value of 12.3 g/dL observed in the treatment containing 0.54% valine in the diet; however, did not differ from the treatments containing 0.81%, 0.99% and 0.99% valine. The lowest hemoglobin (9.69 g/dL) was observed in the fish fed 0.63% valine (Table 5).

Fish fed 0.81 and 0.99% valine in the diet exhibited higher rates of triglyceride in the blood (171.71 and 170.25 g/dL, respectively), however, they did not differ from the treatments containing 0.63% and 0.90% valine. Fish fed with diets containing 0.54% and 0.72% valine had the lowest triglyceride levels. Tilapia fed diets containing 0.81% and 0.90% valine showed higher levels of cholesterol in the blood, differing from the other treatments. Tilapia fed diets containing 0.81% and 0.90% valine showed higher levels of cholesterol in the blood, differing from the other treatments (Fig. 2).

There were no significant differences ($p>0.05$) for muscle fiber distribution in the diameter classes between below 20 μm, 20-50 μm and above 50 μm among the different treatments (Fig. 3).

Discussion

The results of growth performance of Nile tilapia juveniles were influenced by the use of valine in the diet. This amino acid deserves greater attention because it is involved in various metabolic processes and is considered essential for adequate animal productive performance. The only study in the scientific literature for Nile tilapia was conducted by Santiago & Lovell (1988), who used younger animals, purified ration, breeding conditions and variables evaluated in the study different from our work. The authors found valine requirement 0.78% for Nile tilapia larvae through the supply of purified feed. The nutritional requirement of fish is affected by several factors, including the physiological state of the animals, the environmental conditions, and the animal size and age (Wilson, 2002).

Table 3. Growth performance of juvenile Nile tilapia fed diets with increasing levels of valine (Means values with their standard deviation).

Variables[1]	Dietary valine (%)						*p*-value[2]
	0.54	0.63	0.72	0.81	0.90	0.99	
IW (g)	21.90 ± 0.79	21.59 ± 0.74	21.59 ± 0.15	21.08 ± 1.04	21.43 ± 0.28	20.84 ± 1.09	0.75
WG (g)	76.75 ± 1.62c	94.06 ± 22.84bc	99.42 ± 16.23bc	111.76 ± 11.46a	105.72 ± 2.39ab	106.65 ± 5.39ab	0.01
AFC	1.18 ± 0.02a	1.29 ± 0.13ab	1.38 ± 0.12b	1.17 ± 0.05a	1.14 ± 0.04a	1.17 ± 0.02a	0.02
DWG (g/day)	0.99 ± 0.02c	1.22 ± 0.29bc	1.29 ± 0.21bc	1.45 ± 0.14a	1.37 ± 0.03ab	1.38 ± 0.07ab	0.01
PER (%)	2.31 ± 0.03	2.01 ± 0.20	2.06 ± 0.17	2.10 ± 0.09	2.26 ± 0.08	2.09 ± 0.04	0.06
CF (%)	2.06 ± 0.16	2.15 ± 0.09	2.09 ± 0.09	2.07 ± 0.15	2.11 ± 0.01	2.19 ± 0.06	0.50
Sur (%)	100.00 ± 0.00	88.86 ± 9.64	91.66 ± 14.43	97.23 ± 4.79	94.46 ± 4.79	100.00 ± 0.00	0.36
PRE	36.48 ± 1.62	34.93 ± 1.77	34.66 ± 2.02	36.23 ± 1.09	39.23 ± 0.34	36.27 ± 1.65	0.10
VF (%)	2.04 ± 0.26	2.54 ± 0.29	2.46 ± 0.31	2.59 ± 0.45	2.24 ± 0.77	2.89 ± 0.30	0.30
HSI (%)	1.77 ± 0.59	2.08 ± 0.72	1.73 ± 0.62	2.05 ± 0.29	2.11 ± 0.12	1.92 ± 0.10	0.87
Uni (%)	66.66 ± 14.43	78.33 ± 10.40	65.74 ± 15.29	60.10 ± 8.87	64.64 ± 9.25	69.44 ± 12.72	0.58
SGR (%/day)	1.95 ± 0.03	2.16 ± 0.29	2.23 ± 0.17	2.39 ± 0.16	2.31 ± 0.03	2.35 ± 0.11	0.06

[1]IW = initial weight; WG = weight gain; AFC = apparent feed conversion; DWG = daily weight gain; PER = protein efficiency rate; CF = condition factor; Sur = survival; PRE = protein retention efficiency; VF = visceral fat; HIS = hepatosomatic index; Uni = uniformity; SGR = specific growth rate. [2]Values followed by different letters in the same row significantly differ by the Duncan's test ($p<0.05$).

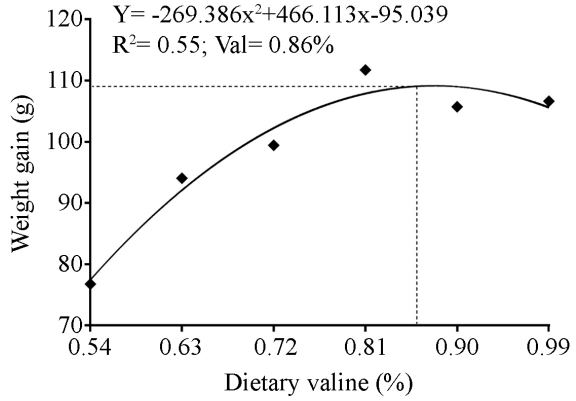

Figure 1. Weight gain of Nile tilapia juveniles fed diets containing increasing levels of valine.

The growth performance results demonstrated that an increased valine supplement level provided better performance, with weight gain, daily weight gain and apparent feed conversion showing better results in the fish fed a diet containing 0.81-0.99% valine. The valine levels in the diet did not significantly affect the other parameters of performance. The regression equation applied to the gain data by weight showed that the level of inclusion of valine that gave the best growth to the animals was 0.86%. It was observed that the requirement of valine obtained by Santiago & Lovell (1988) of 0.78% for tilapia larvae fed with purified ration was lower than that found in our current study of 0.86% for juveniles of tilapia fed with practical diets.

We believe that the development phase may have influenced this observed difference since older and larger fish have a greater contribution of growth due to muscular hypertrophy in relation to younger fish that depend more on the hyperplasia process. This may explain the higher (0.86%) requirement of the animals in our study since valine acts in conjunction with other branched-chain amino acids in muscle deposition and growth. In addition, in a purified diet the nutrients are readily available for absorption by the body of the fish, unlike the practical diets, where the body needs to digest the ingredients to later be able to absorb the nutrients. Thus, the higher requirement observed in the current study is expected because the nutrients were not readily available for absorption as in the purified diet, in which case it is expected that a greater % inclusion of the nutrient will be necessary, due to the loss of nutrients during the digestion process.

Although it is not clear how different levels of dietary valine influenced the weight gain of fish, some studies indicate possible mechanisms of action of this amino acid in fish growth, such as increased secretion of digestive enzymes and also in balancing with other amino acids of branched chain, since the excess or deficiency of one of these amino acids can hinder the use of others. Dong *et al*. (2012) suggest that increased valine in the diet increases the activity of digestive enzymes such as trypsin, chymotrypsin, lipase and amylase, thus providing better digestion and, consequently, better utilization of nutrients by the fish organism. Some studies indicate that the balance between the three branched-chain amino acids is essential because antagonism may occur between them, that is, the excess or deficiency of one of the amino acids may impair the use of another. This is because the three amino acids compete for the same metabolic pathway of absorption and active transport (Hughes *et al*., 1984).

In contrast to the results in the present study, the protein efficiency ratio and protein retention efficiency are thought to be affected by different valine levels in the diet because branched-chain amino acids (leucine, isoleucine, and valine) are essentially anabolic and exhibit translation regulation and the onset of protein synthesis in various tissues as their main function (Shimomura *et al*., 2006).

The body composition of the fish remained unaffected by the different valine levels in the diets, corroborated with Han *et al*. (2014) in a study with *Paralichthys olivaceus*. In contrast, Dong *et al*. (2012) found differences in the body composition of *Cyprinus carpio* var. Jian, Hughes *et al*. (1984) found an effect in *Salvelinus namaycush*, Rahimnejad & Lee (2013) found an effect in *Pagrus major*. In the current study, the diets were most likely prepared for isoproteic and

Table 4. Chemical composition of juvenile Nile tilapia fed diets with increasing levels of valine (Means values with their standard deviation).

Variables[1]	Dietary valine (%)						*p*-value[2]
	0.54	0.63	0.72	0.81	0.90	0.99	
MC (%)	71.50 ± 1.03	70.44 ± 1.07	70.83 ± 2.01	70.16 ± 1.27	69.19 ± 1.20	70.19 ± 1.55	0.50
CP (%)	15.63 ± 0.74	16.88 ± 0.74	16.47 ± 0.48	16.91 ± 0.95	17.72 ± 0.88	16.93 ± 0.75	0.10
EE (%)	9.79 ± 0.60	10.28 ± 0.56	9.92 ± 1.47	10.96 ± 1.48	11.32 ± 1.39	10.44 ± 1.33	0.61
Ash (%)	4.26 ± 0.37	4.29 ± 0.36	4.17 ± 0.03	4.10 ± 0.34	4.07 ± 0.08	4.29 ± 0.53	0.93

[1]MC = moisture content; CP = crude protein = Nitrogen × 6.25, being n = 9 fish per treatment; EE = ether extract.
[2]Not significant (p>0.05).

Table 5. Hematological variables of juvenile Nile tilapia fed diets with increasing levels of valine (means values with their standard deviation).

Variables[1]	Dietary valine (%)						*p*-value[2]
	0.54	0.63	0.72	0.81	0.90	0.99	
TE (10^6/µL)	1.86 ± 0.07	1.9 ± 0.16	1.95 ± 0.29	1.83 ± 0.07	1.89 ± 0.10	1.81 ± 0.12	0.77
He (g/dL)	12.3 ± 2.14a	9.69 ± 1.01c	10.13 ± 1.61bc	10.32 ± 1.86abc	11.69 ± 2.94abc	11.93 ± 2.25ab	0.03
Hem (%)	35.83 ± 4.32	39.61 ± 5.17	35.94 ± 3.13	34.67 ± 3.91	36.28 ± 2.54	35.50 ± 3.46	0.13
TL (µL)	34721 ± 20070	27667 ± 12779	35257 ± 7325	36541 ± 14063	30871 ± 10961	34553 ± 9152	0.70
TT (µL)	12002 ± 15213	10148 ± 6450	11130 ± 11716	9183 ± 11165	13949 ± 10687	11072 ± 11090	0.82
Lym (%)	88.33 ± 1.65	88.11 ± 2.36	88.44 ± 1.66	87.33 ± 2.59	88.55 ± 2.60	88.0 ± 1.93	0.77
Neut (%)	9.11 ± 0.78	8.77 ± 2.04	8.77 ± 1.30	10.77 ± 3.03	9.11 ± 2.66	8.77 ± 1.30	0.58
Mon (%)	2.55 ± 1.42	3.11 ± 1.32	2.77 ± 1.20	1.88 ± 1.61	2.33 ± 1.11	3.22 ± 0.97	0.38

[1]TE = total erythrocyte count; He = hemoglobin; Hem = hematocrit; TL = total leukocytes; TT = total thrombocytes; Lym = lymphocytes; Neut = neutrophils; Mon = monocytes; n = 9 fish per treatment. [2]Values followed by different letters in the same row significantly differ by Duncan's test (p<0.05).

isocaloric maintenance to avoid the occurrence of changes in the fish chemical composition.

The hematological analysis of the fish is very important because it is indicative of the fish's state of health and physiological normality. Little information is available in the scientific literature regarding the effect of valine on the hematological parameters of fish (Rahimnejad & Lee, 2013). In the present study, only the hemoglobin differed significantly among the treatments, with the fish that received feed containing 0.54% valine exhibiting the highest hemoglobin. Abidi & Khan (2007) reported that branched-chain amino acids were important for hemoglobin production, glucose regulation in the blood, and resistance to stress. It is difficult to state the influence of valine on hemoglobin levels since this amino acid apparently does not act directly on iron metabolism. So, differences obtained could be related to intrinsic variations among animals.

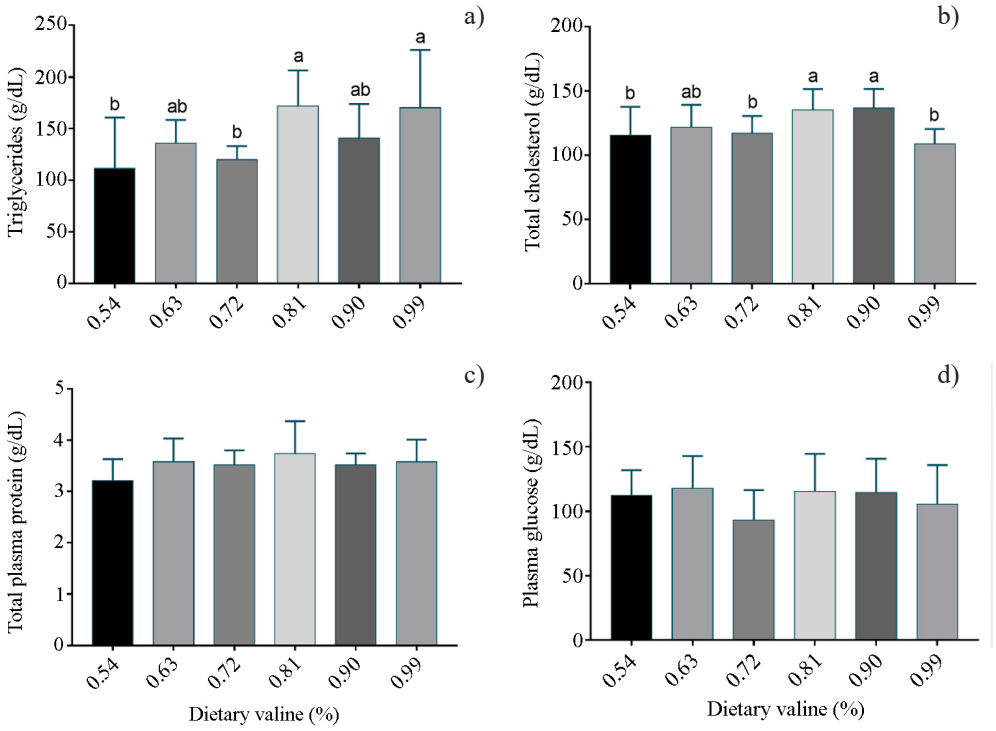

Figure 2. Blood biochemical values found in juveniles of Nile tilapia fed diets containing increasing levels of valine. A: Triglycerides; B: Total cholesterol; C: Total plasma protein; D: Plasma glucose. Different letters indicate significant differences by the Duncan's test (p<0.05); n = 9 fish per treatment.

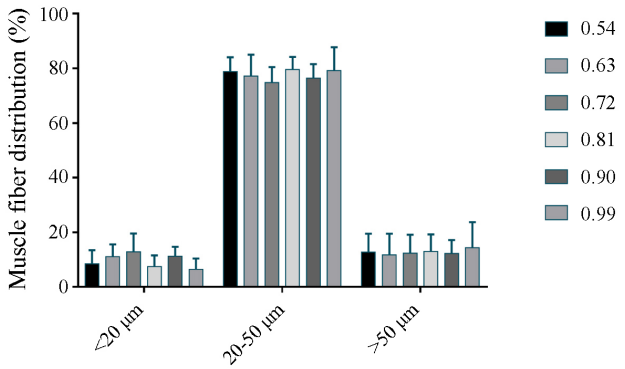

Figure 3. Muscle fiber distribution into three diameter classes (<20 µm, 20-50 µm and >50 µm) in juvenile Nile tilapia fed diets with increasing levels of valine. Not significant by analysis of variance (p>0.05); n = 12 fish per treatment. Muscle fiber diameters (µm) were measured, using the Image-Pro Plus® v. 4.5.0.29 software. Stain: Hematoxylin and eosin (HE).

The white blood cells of the juvenile Nile tilapia remained unaffected by the different valine levels in the diet. The leukocyte values were within the range considered normal for Nile tilapia (Hrubec *et al*., 2000). Similarly, the number of total thrombocytes remained within the range considered adequate for healthy fish (Tavares-Dias & Moraes, 2004).

The triglyceride and cholesterol levels differed in the tilapia fed increasing valine levels in the diet; however, these values were within the range considered normal for the species (Hrubec *et al*., 2000). The total plasma protein and plasma glucose did not differ among the treatments. The fish fed diets containing 0.81% and 0.99% valine exhibited the highest triglyceride levels. No information in the literature that supports a possible relationship between the weights of the animals with triglyceride levels has been described for fish diets with low lipid levels. Plasma proteins include albumins, globulins, and fibrinogens; a reduction in their levels may indicate liver alterations or that the feed is unbalanced. Thus, the levels of these proteins can be used to evaluate the health conditions of the fish. The total plasma protein concentration in this study ranged from 2.96 to 3.30 g/dL; these values were considered normal for healthy tilapia according to Chen *et al*. (2003). In contrast, Rahimnejad & Lee (2013) found that increasing valine (1.22 to 2.04%) in the diet increased the plasma protein level and total cholesterol in the blood of *Pagrus major* and explained this result as the hypercholesterolemic effect conferred by this amino acid. This last claim corroborated the results in the present study because the feed containing 0.81% and 0.90% valine resulted in high blood cholesterol values.

Increasing levels of valine have no effect on skeletal muscle growth in juvenile tilapia. Muscle fibers with diameters smaller than 20 µm suggest the presence of hyperplasia, and muscle fibers with diameters larger than 50 µm indicate the presence of hypertrophy (Valente *et al*., 1999). In the present study, muscle growth due to hypertrophy and hyperplasia was observed in fish from all treatment groups. The fiber diameter did not differ among the treatments, indicating that the different valine levels in the diet provided similar contributions in muscle development.

Muscle growth can be affected by several factors, including nutrition (Koumans & Akster, 1995). Although valine is a branched-chain amino acid with important physiological functions, muscle tissue maintenance and the growth and regulation of protein synthesis (Suryawan *et al*., 2011), the levels included in the diets were sufficient for normal muscle growth. More studies on nutrition related to muscle growth are necessary (especially those linked to amino acids) because they uniquely contribute to the development of myofibrils and are key elements for muscle deposition in fish.

Based on the information obtained in the present study, we concluded that diets containing 0.81% valine (2.90% of dietary protein) to 0.99% (3.55% of dietary protein) provided better performance results for juvenile Nile tilapia. It is recommended the inclusion of 0.86% of valine in the diet of juveniles of Nile tilapia because it provides greater weight gain.

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References

Abidi SF, Khan MA, 2007. Dietary leucine requirement of fingerling Indian major carp, *Labeo rohita* (Hamilton). Aquac Res 38: 478-486. https://doi.org/10.1111/j.1365-2109.2007.01687.x

Almeida FLA, Carvalho RF, Pinhal D, Padovani CR, Martins C, Dal Pai-Silva M, 2008. Differential expression of myogenic regulatory factor MyoD in pacu skeletal muscle (*Piaractus mesopotamicus* Holmberg 1887: Serrasalminae, Characidae, Teleostei) during juvenile and adult growth phases. Micron 39: 1306-1311. https://doi.org/10.1016/j.micron.2008.02.011

AOAC, 2005. Official methods of analysis of association of official analytical chemists. Official Analytical Chemists International, 18th ed. Washington, DC, USA.

Calder PC, 2006. Branched-chain amino acids and immunity. J Nutr 136: 288-293. https://doi.org/10.1093/jn/136.1.288S

Chen CY, Wooster GA, Getchell RG, Bowser PR, Timmons MB, 2003. Blood chemistry of healthy, nephrocalcinosis affected and ozone treated tilapia in a recirculation system, with application of discriminant analysis. Aquaculture 218: 89 102. https://doi.org/10.1016/S0044-8486(02)00499-4

Collier HB, 1944. The standardization of blood haemoglobin determinations. Can Med Assoc J 50: 550-552.

Deriggi GF, Inoue LAKA, Moraes G, 2006. Stress responses to handling in Nile tilapia (*Oreochromis niloticus* Linnaeus): assessment of eugenol as an alternative anesthetic. Acta Sci Biol Sci 28: 269-274.

Dong M, Feng L, Kuang SY, Liu Y, Jiang J, Hu K, Jiang WD, Li SH, Tang L, Zhou XQ, 2012. Growth, body composition, intestinal enzyme activities and microflora of juvenile Jian carp (*Cyprinus carpio* var. Jian) fed graded levels of dietary valine. Aquac Nutr 19: 1-14. https://doi.org/10.1111/j.1365-2095.2011.00926.x

Feng L, Luo JB, Jiang WD, Liu Y, Wu P, Jiang J, Kuang SY, Tang L, Zhang YA, Zhou XQ, 2015. Changes in barrier health status of the gill for grass carp (*Ctenopharyngodon idella*) during valine deficiency: Regulation of tight junction protein transcript, antioxidant status and apoptosis-related gene expression. Fish Shellfish Imunn 45: 239-249. https://doi.org/10.1016/j.fsi.2015.04.023

Fernstrom JD, 2005. 4th amino acid assessment workshop: Branched chain amino acids and brain function. J Nutr 135: 1539-1546. https://doi.org/10.1093/jn/135.6.1539S

Furuya WM, 2010. Tabelas brasileiras para a nutrição de tilápias. GFM, Toledo-PR, Brazil. 101 pp.

Goldenfarb PB, Bowyer FP, Hall E, Brosius E, 1971. Reproducibility in the hematology laboratory: the microhematocrit determinations. Am J Clin Path 56: 35-39. https://doi.org/10.1093/ajcp/56.1.35

Han Y, Han R, Koshio S, Ishikawa M, Yokoyama S, Gao J, 2014. Interactive effects of dietary valine and leucine on two sizes of Japanese flounder *Paralichthys olivaceus*. Aquaculture 432: 130-138. https://doi.org/10.1016/j.aquaculture.2014.05.004

Harper AE, Miller RH, Block KP, 1984. Branched-chain amino acid metabolism. Annu Ver Nutr 4: 409-454. https://doi.org/10.1146/annurev.nu.04.070184.002205

Hrubec TC, Cardinale JL, Smith SA, 2000. Haematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis* hybrid). Vet Clin Pathol 29: 7-12. https://doi.org/10.1111/j.1939-165X.2000.tb00389.x

Hughes SG, Rumsey LJ, Nesheim MC, 1984. Effects of dietary excesses of branched-chain amino acids on the metabolism and tissue composition of lake trout (*Salvelinus namaycush*). Comp Biochem Physio l A Comp Physiol 78: 413-418. https://doi.org/10.1016/0300-9629(84)90569-3

Koumans JTM, Akster HA, 1995. Myogenic cells in development and growth of fish. Comp Biochem Physio 110: 3-20. https://doi.org/10.1016/0300-9629(94)00150-R

Li P, Mai KS, Trushenski J, Wu GY, 2009. New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. Amino Acids 37: 43-53. https://doi.org/10.1007/s00726-008-0171-1

NRC, 2011. Nutrient requirements of fish and shrimp. National Research Council, National Academy Press, Washington, DC. 390 pp.

Rahimnejad S, Lee KJ, 2013. Dietary valine requirement of juvenile red sea bream *Pagrus major*. Aquaculture 416-417: 212-218. https://doi.org/10.1016/j.aquaculture.2013.09.026

Ranzani-Paiva MJT, Benites de Pádua S, Tavares-Dias M, Egami MI, 2013. Métodos para análise hematológica em peixes. Eduem, Maringá-PR, Brazil. 140 pp. https://doi.org/10.7476/9788576286530

Rosenfeld G, 1947. Corante pancrômico para hematologia e citologia clínica. Nova combinação dos componentes do May-Grünwald e do Giemsa num só corante de emprego rápido. Mem Inst Butantan 20: 329-334.

Santiago CB, Lovell RT, 1988. Amino acid requirement for growth of Nile tilapia. J Nutr 118: 1540-1546. https://doi.org/10.1093/jn/118.12.1540

Shimomura Y, Yamamoto Y, Bajotto GS, 2006. Nutraceutical effects of branched-chain amino acids on skeletal muscle. J Nutr 36: 529S-532S. https://doi.org/10.1093/jn/136.2.529S

Suryawan A, Orellana RA, Fiorotto ML, Davis TA, 2011. Leucine acts as a nutrient signal to stimulate protein synthesis in neonatal pigs. J Anim Sci 89: 2004-2016. https://doi.org/10.2527/jas.2010-3400

Tavares-Dias M, Moraes FR, 2004. Hematologia de peixes teleósteos. Editora Eletrônica e Arte Final, Ribeirão Preto-SP, Brazil. 144 pp.

Valente LMP, Rocha E, Gomes EFS, Silva MW, Oliveira MH, Monteiro RAF, Fauconneau B, 1999. Growth and dynamics of white and red muscle fibres in fast- and slow-growing strains of rainbow trout. J Fish Biol 55: 675-691. https://doi.org/10.1111/j.1095-8649.1999.tb00710.x

Wilson RP, 1985. Amino acid and protein requirements of fish. In: Nutrition and feeding fish; Cowey CB, Mackie AM, Bell JG (eds.). pp: 1-16. Acad Press, London.

Wilson RP, 2002. Amino acids and proteins. In: Fish Nutrition; Halver JE, Hardy RW (eds.). pp: 143-179. Acad Press, NY. https://doi.org/10.1016/B978-012319652-1/50004-5

Wu G, 2009. Amino acids: metabolism, functions, and nutrition. Amino Acids 37: 1-17. https://doi.org/10.1007/s00726-009-0269-0