LYSINE IN THE DIET OF COMMON CARP (*Cyprinus carpio)* FINGERLINGS

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ABSTRACT

The aim of the present study was to determine the lysine requirement of common carp fingerlings. Two hundred and eighty common carp fingerlings, with mean weight and mean length initial of 0.62 ± 0.05 g and 3.38 ± 0.11 cm, respectively were randomly distributed into 28 squares net cages of 150 litres arranged inside a masonry circular tank with water capacity of 25 m^{-3} (seven treatments and four replications). The fish were fed until apparent satiation, four times per day, with isoproteic and isoenergetic diets supplemented with lysine at 1.58, 1.81, 2.05, 2.29, 2.53, 2.76, and 3.0% of the diet for 57 days. Productive performance, survival rate, and chemical composition of the fish body were evaluated at the end of the study. The data obtained was subjected to regression analysis and, when significantly different (P<0.05), the Tukey multiple comparison test was used. Additionally, linear response plateau (LRP) analysis was performed. Lysine supplementation significantly improved (P<0.05) mean final weight, mean weight gain, and specific growth rate. However, lysine levels above 2.29% compromised the feed efficiency. Lysine requirements of common carp fingerlings were found to be 1.89% of the diet or 4.73% of the crude protein in the diet for obtain better growth.

Key words: amino acids; nutrients; requirements; feed.

LISINA EM DIETAS PARA ALEVINOS DE CARPA COMUM

ABSTRACT

O presente estudo teve por objetivo determinar a exigência de lisina para alevinos de carpa comum (*Cyprinus carpio*). Foram distribuídos inteiramente ao acaso 280 alevinos de carpa comum com peso e comprimento inicial médio de 0,62 ± 0,05 g e 3,38 ± 0,11 cm, respectivamente, em 28 tanques-rede quadrados com volume útil de 150 litros, dispostos no interior de um tanque circular de alvenaria com capacidade para 25 m^3 de água (sete tratamentos e quatro repetições). Os peixes foram alimentados até a saciedade aparente quatro vezes ao dia com dietas isproteicas e isoenergéticas suplementadas com lisina nas concentrações de 1,58; 1,81; 2,05; 2,29; 2,53; 2,76 e 3,0% da dieta por 57 dias. Ao final do estudo foi avaliado o desempenho produtivo, sobrevivência e composição centesimal da carcaça dos peixes. Os dados obtidos foram submetidos a análise de regressão (p<0,05) e ao teste múltiplo de média Tukey. A análise do platô de resposta linear (LRP) foi aplicado adicionalmente. A suplementação de lisina melhorou (p<0,05) o peso final médio, ganho de peso médio e taxa de crescimento específico e a partir de 2,29% de suplementação de lisina na dieta prejudicou a eficiência alimentar dos peixes. A exigência de lisina para alevinos de carpa comum é de 1,89% da dieta ou 4,73% da proteína bruta para obtenção do melhor crescimento.

Palavras-chave: aminoácidos; nutrientes; exigências; ração.

Nota Científica: Recebido em 17/04/2017; **Aprovado em** 15/07/2017

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INTRODUCTION

The common carp (*Cyprinus carpio* L.) is one of the most successful freshwater species in aquaculture. Commercially, this species is farmed in intensive or semi-intensive systems (KHAN *et al*., 2016). The common carp is considered a species with great potential in fish farming due to its tolerance to low levels of dissolved oxygen, handling, and wide temperature range; easy reproduction; good adaptation to farming systems; and good acceptance of a variety of diets (BARBIERI *et al*., 1998; OMAR *et al*., 2012; TANG *et al*., 2013). Although its meat is not the most sought after by the food industry, the demand for its by-products and processed meats is increasing (GRAEFF and TOMAZELLI, 2007).

Fish kept in confined spaces do not get the quantity or quality of food necessary to meet the nutritional requirements for optimal productive and reproductive performance (GRAEFF and MONDARDO, 2006; MARKOVIC *et al*., 2009; FURUYA *et al*., 2010), consequently demand nutritionally balanced diets (NRC, 2011). Fish diets should not only meet the nutritional requirements of the species but also reduce nutrient surplus in order to minimize the negative impacts on the breeding systems and aquatic ecosystems (HENRY-SILVA *et al*., 2006; TRBOVIC *et al*., 2013). However, the profile and bioavailability of essential and non-essential amino acids that make up these diets must be evaluated; especially for lysine, methionine, threonine, and tryptophan, as these amino acids are considered to be the most limiting (FURUYA *et al*., 2010; DIEMER *et al*., 2014). Several protein feeds of vegetal origin have been used as replacement to fishmeal due to their greater availability and, consequently, lower cost (HU *et al*., 2008; RAWLES *et al*., 2013). However, their deficiency in some amino acids requires supplementation with synthetic amino acids (FURUYA *et al*., 2006; RAWLES *et al*., 2013) as the deficiency of a single amino acid in the diet can limit the use of the whole protein by the fish (SAAVEDRA *et al*., 2015; RAGGI *et al*., 2016). Lysine is considered one of the main limiting amino acids in fish diets of vegetable origin (FURUYA *et al*., 2006; COLDEBELLA *et al*., 2011) and lysine supplementation has been associated with to weight gain, better food conversion, greater nitrogen retention, lower lipid content in the carcass and better reproductive performance in fish (COLDEBELLA *et al*., 2011; DIEMER *et al*., 2014).

Fish growth and feed efficiency can be maximized by manipulating the amino acid composition of the diet; however, the formulation of balanced diets requires precise information on the species amino acid requirements (ZHANG *et al*., 2008; SAAVEDRA *et al*., 2015). Advances in nutrition and the search for balanced diets to improve the use of each nutrient and reduce the polluting power of excrement have led to the formulation of diets with balanced levels of digestible protein and amino acids (RAGGI *et al*., 2016). Thus, the aim of this study was to determine the lysine requirement of common carp fingerlings with practical diets.

METHODS

The study took place at the experimental greenhouse of the Study Group in Aquaculture Management (GEMAq) of the Universidade Estadual do Oeste do Paraná (Unioeste, Toledo-SP, Brazil) and was approved by Ethics Committee on the Use of Animals (Unioeste, CEUA, protocol 05812). Two hundred and eighty common carp (*Cyprinus carpio)* fingerlings, with mean (± standard error) initial weight and length of 0.62 ± 0.05 g and 3.38 ± 0.11 cm; respectively, were randomly distributed into 28 square net cages of 150 litres arranged in a circular tank with water capacity of 25 m^3 (7 treatments and 4 repetitions). Continuous aeration was provided by a central air pump. The photoperiod was natural.

The diet ingredients (Table 1) were individually milled using a hammer-mill with 0.5 mm mesh sieve, weighed, homogenized with a "Y" type mixer. The micronutrients, oil and the amino acid test were homogenized to a portion of the diet and subsequently to the diet as a whole, rehydrated according to the standard laboratory procedure and extruded (extrutec®) using a 1.2 mm matrix. The pellets were dried in a forced air circulation oven at 55ºC for 12 hours, cooled, and stored in at -4ºC. A portion of the feed was milled and provided as bran up to the 15th day, and as pellets thereafter (up to 57th day). The fish were feeding four times a day (at 8, 11, 14, and 17 hours) until apparent satiation, for 57 days. The physical and chemical parameters of the water, pH, electric conductivity (mS cm^{-1}), and dissolved oxygen (mg L^{-1}) were measured weekly. Water temperature (°C) was monitored four times a day at the time of feeding. At the end of the experimental period, fasting of 24 hours was introduced to empty the gastrointestinal tract. The fish were anaesthetized in 100 mg L^{-1} benzocaine solution (GOMES *et al*., 2001; BITTENCOURT *et al*., 2013), counted, weighed, and measured (total and standard length (cm)) individually.

Table 1. Percentage and nutritional composition of the experimental diets, supplemented with different levels of lysine, fed to common carp (*Cyprinus carpio*) fingerlings

Ingredients	Lysine supplementation levels %						
	1.58	**1.81**	**2.05**	**2.29**	**2.53**	**2.76**	**3.00**
Corn (%)	34.33	34.54	34.74	34.95	35.16	35.36	35.57
Poultry by-products meal (%)	18.89	18.92	18.95	18.97	19.00	19.03	19.06
Corn gluten (%)	14.96	14.69	14.43	14.17	13.90	13.64	13.38
Fish meal (%)	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Poultry feather meal (%)	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Soy Oil (%)	5.93	5.95	5.97	5.99	6.01	6.02	6.04
Glutamic acid (%)	2.00	1.70	1.40	1.10	0.80	0.50	0.20
Mineral and vitamin supplement (%)[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DL-Methionine (%)	0.45	0.45	0.45	0.46	0.46	0.46	0.47
Sodium chloride (%)	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Antifungal (%)	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Tryptophan (%)	0.03	0.03	0.03	0.03	0.03	0.04	0.04
Butyl hydroxy toluene (%)	0.02	0.02	0.02	0.02	0.02	0.02	0.02
L-Lysine HCl (%)	0.00	0.30	0.61	0.91	1.22	1.52	1.83
Nutrients[2]							
Crude protein (%)	40.00	40.00	40.00	40.00	40.00	40.00	40.00
Digestible energy for tilápia (kcal/kg)[3]	3.310.00	3.310.00	3.310.00	3.310.00	3.310.00	3.310.00	3.310.00
Fat (%)	10.92	10.94	10.96	10.98	11.00	11.02	11.04
Dry matter (%)	94.46	94.64	94.82	95.00	95.18	95.36	95.54
Mineral matter (%)	6.66	6.61	6.45	6.95	6.37	6.61	6.54
Total arginine (%)	2.24	2.24	2.24	2.24	2.24	2.24	2.24
Crude fibre (%)	1.21	1.21	1.21	1.21	1.21	1.21	1.21
Total phosphorus (%)	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total methionine (%)	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Total threonine (%)	1.60	1.60	1.60	1.60	1.60	1.60	1.60
Total tryptophan (%)	0.32	0.32	0.32	0.32	0.32	0.32	0.32

[1] Guarantee levels per kilogram of product: Vit. A, 1.750.000UI; Vit. D3, 375.000UI; Vit. E, 20.000UI; Vit. K3, 500mg; Vit. B1, 2.000mg; Vit. B2, 2.500mg; Vit. B6, 2.500mg; Vit. B12, 5.000mg; Folic acid, 625 mg; Pantothenate, 7.500mg; Vit. C, 37.500mg; Biotin, 50mg; Inositol, 12.500mg; Niacin, 8.750mg; Choline, 100.000mg; Co, 50mg; Cu, 1.250mg; Fe, 15.000mg; I, 100mg; Mn, 3.750mg; Se, 75mg; Zn, 17.500mg.

[2] The nutrients requirement of Common Carp was considered following NRC (2011) and ZHOU *et al.* (2008). The protein requirement of Common Carp was considered based on GRAEFF and PRUNER (2003).

[3] Energy, protein and nutrients values of ingredients was estimated based on datas for tilapia by BOSCOLO *et al.* (2002), PEZZATO *et al.* (2002) and BOSCOLO *et al.* (2008).

The data was used to determine mean final weight (FW) = (wet final weight in g – wet initial weight in g); weight gain (WG) = (wet final weight in g – wet initial weight in g); specific growth rate (SGR) = ((Ln (wet final weight in g) – Ln (wet initial weight in g) / times in day) x 100); survival rate (SR) = ((fish final number/fish initial number)*100); Feed efficiency (FE) = (wet weight gain in g / feed intake in g); Protein efficiency rate (PER) = (wet weight gain in g/ intake protein in g); and condition factor (CF) = ((mean final weight in g/(mean body length in cm at cube elevated))*100). For chemical composition of the fish carcass analysis of dry matter (DM), mineral matter (MM), protein, and lipid content was realized in three fish per experimental unit euthanized by 300 mg L-1 benzocaine solution (RODRIGUES et al., 2016) and maintained at -20°C in the Laboratory for Food Quality (Unioeste), in accordance with the AOAC (2005).

The data obtained was subjected to regression

analysis and, when significant, differences were compared by Tukey multiple comparisons test using the statistical program Statistical Analysis System (SAS, 2004). Significance was considered at 5% (P<0.05). Additionally, linear response plateau (LRP) analysis was performed.

RESULTS AND DISCUSSIONS

The physical and chemical parameters of the water during the experimental period (23.77 ± 1.98 ºC, pH 7.33 ± 0.19, dissolved oxygen 5.38 ± 0.50 mg L^{-1}, and electric conductivity 117.2 ± 8.18 mS cm^{-1}) remained within the recommended range for tropical fish species (BHATNAGAR and DEVI, 2013).

Lysine supplementation in the diet had a positive influence on the productive performance of carp fingerlings (Table 2), with quadratic effects observed on mean final weight and mean weight gain, and linear effects on apparent feed conversion and protein efficiency rate. For final weight and weight gain and specific growth rate, the best results (P<0.05) were observed in the fish fed diets containing 2.05 to 2.29% lysine. The lysine supplementation with 2.05% improved (p<0.05) the protein efficiency rate. For apparent feed conversion was obtained in the fed diets supplemented with 1.58%, 1.81%, 2.05%, and 2.53% lysine. The linear response plateau (LRP) showed that the lysine requirement of common carp fingerlings for better FW and WG is 1.89% (Figure 1) of the diet or 4.73% of the crude protein in the diet.

Table 2. Productive performance of common carp (*Cyprinus carpio*) fingerlings fed diets supplemented with different levels of lysine for 57 days

	Lysine supplementation levels								
Parameters	**1.58**	**1.81**	**2.05**	**2.29**	**2.53**	**2.76**	**3.00**	CV%	p
Mean initial weight (g)	0.62	0.62	0.62	0.62	0.62	0.62	0.62	1.39	ns
Mean final weight[1] (g)	2.62b	2.75ab	2.92a	2.86a	2.81a	2.84a	2.77ab	2.60	0,003
Mean weight gain[2] (g)	2.00b	2.13ab	2.30a	2.25a	2.19ab	2.23a	2.15ab	3.48	0.004
Specific growth rate[3]	2.54b	2.61ab	2.72a	2.70a	2.65ab	2.68ab	2.63ab	2.19	0.02
Total length (cm)	5.50	5.60	5.56	5.64	5.53	5.57	5.59	1.61	ns
Survival rate (%)	100.00	100.00	96.67	100.00	100.00	96.67	96.67	3.58	ns
Protein efficiency rate	3.80abc	3.62bc	4.51a	3.39bc	4.07ab	2.63d	3.12cd	8.07	0.0001
Feed efficiency[5]	0.84a	0.76ab	0.87a	0.66bc	0.84a	0.52d	0.65c	5.65*	0.0001
Condition factor	1.57	1.56	1.70	1.60	1.66	1.59	1.65	4.28ns	0,1667

**Rows followed by different letters indicate significant difference (P<0.05) by Tukey's test;*
[1]Quadratic effect ($y = -0.00003x^2 + 0.0165x + 0.8937$; $R^2 = 0.76$);
[2]Quadratic effect ($y = -0.00003x^2 + 0.0165x + 0.2752$; $R^2 = 0.75$);
[3]Quadratic effect ($y = 0.00002189x^2 + 0.0105x + 1.436$; $R2 = 0.43$);
[4]Quadratic effect ($y = -0.0000972x^2 + 0.038x + 0.2022$; $R^2 = 0.32$);
[5]Linear effect ($y = 0.0016x - 1.0980$; $R^2 = 0.37$);
CV: coefficient of variation

Lysine is present in high proportions in the muscle of fish and its requirement must be met through the diet (ESPE *et al*., 2007; RAWLES *et al*., 2013) because the animals are unable to synthesize it (CHENG *et al*., 2003). Thus, determining the lysine requirements of species of interest is essential for the formulation of diets that best meet their nutritional requirements (WANG *et al*., 2005). Lysine supplementation in diets improved the productive performance of carp fingerlings corroborating with the results observed by ZHOU *et al*. (2008) that lysine supplementation in diets for juvenile carp (*Cyprinus carpio*) improve the final weight, protein deposition and protein production value and AHMED and KHAN (2004) with Indian major carp (*Cirrhinus mrigala*) showed better productive performance and higher protein and crude fat concentration in the carcass with lysine supplementation in diet. Those results suggest that the carp can use better the nutrients available in the diet with an ideal level of lysine supplementation in relation the protein concentration in diets. Furthermore, the presence of lysine in the diet as well

as an amino acid pool, can optimize the amino acid profile of the diet (DENG *et al*., 2006; SAAVEDRA *et al*., 2015; NGUYEN and DAVIS, 2016), improving consumption and productive performance (DENG *et al*., 2011), which lead to greater tissue deposition and consequently greater fish productive performance (WANG *et al*., 2005; HU *et al*., 2008; NGUYEN and DAVIS, 2016). However, the protein analysis in body composition of carp in this study (Table 3) not corroborate with the above statements because the better results in productive performance (2.05% lysine supplementation in diet) produced the smaller protein in body composition of carp. HU *et al*. (2008) at the supplemented lysine and methionine in diets with inclusion of plant ingredients in fishmeal substitution not report differences in body composition of fish. These results reinforce the need of deepen research in order to better understand the metabolic interactions that occur in *C. carpio* with lysine supplementation in the diet. Another situation that limits the discussion about lysine influence in the diet of fish is the small growth of carp fingerlings in the present study.

Specific growth rate represents the daily growth rate of fish and its evaluation revealed that 1.58% lysine supplementation in diet of common carp fingerlings was deficient and compromised fish growth (Table 2). When protein efficiency rate, specific growth rate, mean final weight and mean weight gain were evaluated together, it was observed that 2.05% lysine supplementation in the diet resulted in the greatest productive performance in common carp fingerlings. Diets with deficient or imbalances of amino acid result in lower fish growth (ZHOU *et al*., 2008; SARDAR *et al*., 2009; OSTASZEWSKA *et al*., 2010; HU *et al*., 2015).

Linear response plateau (LRP) analysis for weight gain and mean final weight of carp fingerlings revealed optimum lysine supplementation to be 1.89% of diet (Figure 1) or 4.73% of the crude protein in the diet. WANG *et al*. (2005) reported lysine requirements of juvenile of grass carp (*Ctnopharyngodon idella*) to be 2.07% of the diet or 5.44% of the crude protein of the diet and ZHOU *et al*. (2008) indicated 5.9% of protein for juveniles of Jian carp (*C. carpio*) and the NRC (2011) suggest 2.2% of diet or 5.7% of crude protein for fingerlings of common carp, demonstrating different requirements for species of the same genus and in different phases. According to the NRC (2011), nutritional requirements are influenced by several factors, such as feeding habits, type of food used in the formulation of diets, physiology, and metabolism of fish. Food containing protein of greater quality and nutritional availability can lead to lower demands for amino acid supplementation in the diet (CHENG *et al*., 2003; RAWLES *et al*., 2013). However, the lysine requirement levels obtained in the present study were lower than those reported by MURTHY and VARGHESE (1997) in Indian major carp (*Labeo rohita*) and FAGBENRO *et al*. (1998) in African catfish (*Clarias gariepinus*) being 2.24 and 2.29% of lysine in the diet or 5.6% and 5.73% of crude protein of diet, respectively, as well as WANG *et al*. (2005) with juvenile of grass carp being 2.07% of diet or 5.44% of protein and ZHOU *et al*. (2008) with juvenile of Jian carp being 1.89% of diet or 5.90%. The available and quality nutrients of diets, phase of development and species physiologic characteristics are factors that influence the amino acids requirements in fish (FURUYA *et al*., 2010; NRC, 2011; FURUYA *et al*., 2012; HU *et al*., 2015).

Lysine supplementation had no significant effect ($P>0.05$) on the remaining variables analysed as survival rate and condition factor and total length. These parameters appear not to be influenced by dietary lysine supplementation. ZHOU *et al*. (2008) and WANG *et al*. (2005) have not report the behavior of these parameters in their studies with carp. Supplementation of amino acids in fish diets tends to have a positive influence until the requirement is meet (ESPE *et al*., 2007; NRC, 2011; JIN *et al*., 2015). Amino acid and/or micronutrient supplementation above or below of required level can compromise productive performance and nutrient deposition in the reared fish tissue (WANG *et al*., 2005; TANG *et al*., 2013; RAGGI *et al*., 2016). So, its demonstrates the importance of determining the nutritional requirements of species to formulate nutritionally balanced diets that will provide better use of available nutrients and to reduce the organic matter of the environment in which these animals are being reared (NRC, 2011; LEWANDOWSKI *et al*., 2017).Many factors can influence the nutritional requirement of a species, including lineage, growth, husbandry, physiological state, physical and chemical parameters of the water, evaluation methods, genetic variations, and especially the type of food used in the formulation of the diet (HU *et al*., 2008; RAWLES *et al*., 2013; JIN *et al*., 2015). These factors influence the results; therefore, biological interpretation is necessary in each case (GONÇALVES *et. al*., 2009).

The nutritional composition of diets can influence

the percentual composition of fish body (SIGNOR *et al*., 2007; MESSINA *et al*., 2013). In the present study, lysine supplementation had a significant influence (P<0.05) on crude protein concentration in the fish carcass (Table 3). Diets consisting of different protein sources, supplemented or not with amino acids, are known to influence fish body composition (CHENG *et al*., 2003; YANG *et al*., 2010) and lysine supplementation in the diets influences the body composition of farmed fish (WANG *et al*., 2005; RAWLES *et al*., 2013; JIN *et al*., 2015). The remaining variables carp analysed in the present study had no significant influence (P>0.05) on the composition percentage of fish.

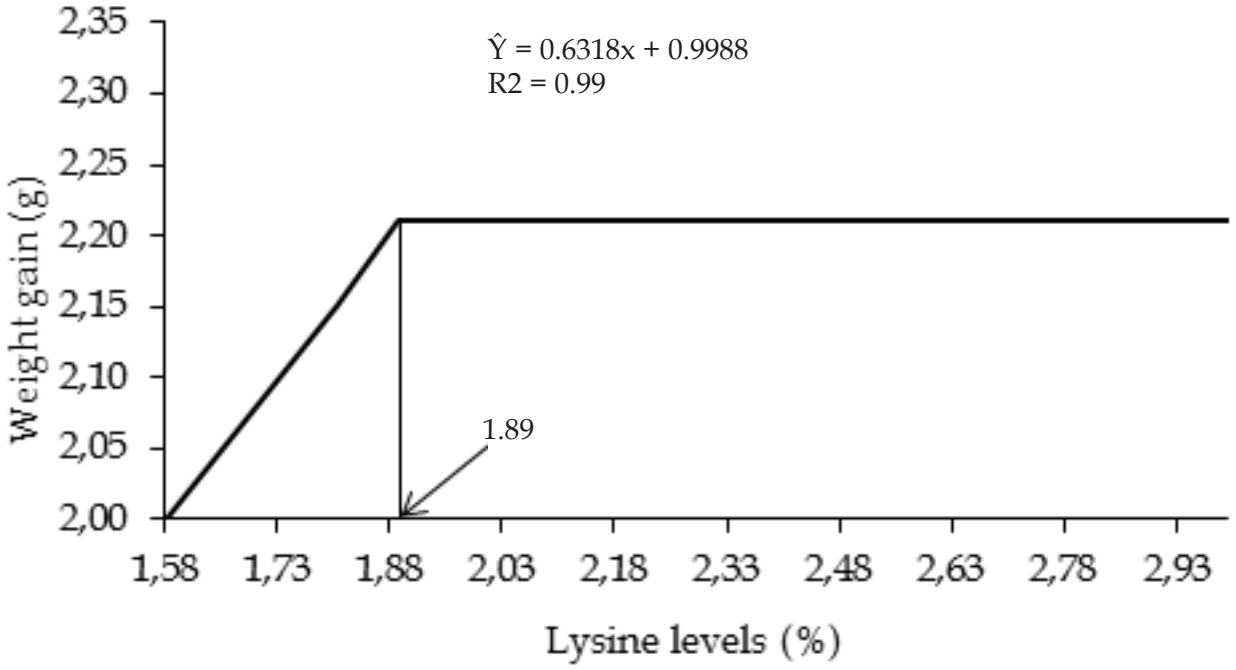

Figure 1. Weight gain of common carp (*Cyprinus carpio*) fingerlings according to the levels of lysine in the diet by *Linear Plateau Response* (LRP)

Table 3. Body composition of common carp (*Cyprinus carpio*) fingerlings fed diets supplemented with different levels of lysine for 57 days

Parameters	Lysine supplementation levels							CV%
	1.58	1.81	2.05	2.29	2.53	2.76	3.00	
Humidity	74.97	75.18	76.16	75.12	75.28	74.42	74.87	1.02^{NS}
Crude protein	13.90^{ab}	13.69^{ab}	12.78^{b}	14.42^{ab}	13.73^{ab}	14.65^{a}	13.27^{ab}	5.84*
Lipids	8.93	9.62	8.93	8.84	9.64	8.63	8.79	7.09^{NS}
Mineral matter	3.49	4.33	3.96	4.01	4.00	4.34	4.53	12.99^{NS}

* Different letters on the same row indicate significant difference (P<0.05) by the Tukey test.

The use of synthetic amino acids speeds gastrointestinal evacuation and their availability for absorption is greater than those from "intact" proteins; leading to greater deposition of body protein when these amino acids are supplemented in fish diets (NRC, 2011). Diets deficient in lysine are known to compromise protein and amino acid deposition in fish tissues (PERES and OLIVA-TELES, 2008; YANG *et al*., 2011) but to *Cyprinus carpio* in the present study the better result for weight gain and protein efficiency rate promoted less concentration crude protein in fish carcass. Essential amino acid supplementation in fish diet leads to progressive increase in the tissue protein synthesis until the requirements of the species are

met (YANG *et al*., 2011; JIN *et al*., 2015). The results obtained are contradictory to those observed in the literature. The fact that the fish have low growth in the experimental period may have compromised the attainment of more consistent results.

CONCLUSIONS

We can conclude that lysine requirements of common carp *Cyprinus carpio* fingerlings is 1.89% of diet or 4.73% of crude protein of the diet.

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