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Crude protein reduction and digestible methionine+cystine and threonine to digestible lysine ratios in diets for Nile tilapia fingerlings[1]

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ABSTRACT - The objective of the present study was to investigate crude protein reduction and the methionine+cystine and threonine to lysine ratios in diets for reversed Nile tilapia fingerlings (*Oreochromis niloticus*), Thai strain. Four hundred and twenty Nile tilapia fingerlings with an average initial weight of 1.83 ± 0.02 g were placed in a completely randomized design, with five treatments, seven replications per treatment and 12 fish per experimental unit, for 37-days. Two crude protein (CP) levels were evaluated (32 and 28%). The diet with 32% CP had methionine+cystine:lysine and threonine:lysine ratios of 63 and 74%, respectively. Three diets with 28% CP had methionine+cystine:lysine and threonine:lysine ratios of 63 and 74%, 60 and 74% and 60 and 69%, respectively. The diet with 28% CP and had methionine+cystine:lysine ratio of 63% and threonine:lysine of 74% and was supplemented with 0.3% glutamic acid. The fish were kept in thirty-five 130 L aquariums supplied with single-pass flow-through water, individual aeration and fed *ad libitum* six times per day. Fish fed the diet with 32% CP had lower protein efficiency ratios and nitrogen efficiency ratios. The diet CP level was 28% and the methionine+cystine and threonine to lysine ratios were 60 and 69%, respectively.

Key Words: digestible lysine, ideal protein, *Oreochromis niloticus*, synthetic amino acids

Redução de proteína bruta e relações de metionina+cistina e treonina digestíveis com lisina digestível em dietas para alevinos de tilápia-do-nilo

RESUMO - Objetivou-se avaliar a redução do nível de proteína bruta e as relações dos aminoácidos metionina+cistina e treonina digestíveis com a lisina digestível em dietas para alevinos revertidos de tilápia-do-nilo (*Oreochromis niloticus*), da linhagem tailandesa. Um experimento com duração de 37 dias foi realizado utilizando-se 420 alevinos com peso inicial de 1,83 ± 0,02 g, distribuídos em delineamento inteiramente ao acaso, composto de 5 dietas, 7 repetições e 12 peixes por unidade experimental. Foram avaliados dois níveis de PB (32 e 28%) em diversas dietas experimentais. Na dieta com 32% de PB, foram mantidas as relações de metionina+cistina e treonina com lisina de 63 e 74%, respectivamente; nas três dietas com 28% de PB, as relações de metionina+cistina e treonina com lisina foram de 63 e 74%, 60 e 74% e 60 e 69%, respectivamente; e, na dieta com 28% de PB, as relações de metionina+cistina e treonina com lisina foram de 63 e 74%, respectivamente, porém com acréscimo de 0,3% de ácido glutâmico. Os peixes foram mantidos em 35 aquários de 130 litros, com abastecimento de água, aeração individual e alimentação à vontade em seis refeições diárias. Os peixes mantidos com a dieta com 32% de PB apresentaram menores eficiências de retenção de proteína e de nitrogênio. O nível de PB de rações para alevinos de tilápia-do-nilo deve ser de 28,0% e as relações de metionina+cistina digestível e treonina digestível com a lisina, de 60 e 69%, respectivamente.

Palavras-chave: aminoácidos sintéticos, lisina digestível, *Oreochromis niloticus*, proteína ideal

Introduction

The rapid expansion of aquaculture and advances in fish farming techniques has increased the demand for balanced diets (Watanabe et al., 2002). However, the achievement of high productivity should be reconciled with the reduction in waste discharge into the aquatic environment, mainly nitrogen and phosphorus, responsible for body of water eutrophication (Furuya et al., 2005; Lanna et al., 2005).

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The great expansion of intensive tilapia farming in earth ponds, recirculation or flow-through water systems, as well as in cages, where the use of diets is the main source of nutrients, tends to raise the nitrogen level excreted to the environment. This problem can be reduced by decreasing the concentration of the protein diet and supplementation of limiting amino acids without affecting the fish development and performance (Boisen, 2003; Yamamoto et al., 2005).

To formulate diets based on the ideal protein concept, which results in lower nitrogen excretion to the environment, and to estimate fish amino acid requirements, it is necessary to reduce the diet protein level (Bomfim et al., 2005a; Furuya et al., 2005).

The determination of the lysine dietary requirements, methionine (methionine + cystine) and threonine can have great impact on tilapia production, since these amino acids are limiting in the majority of feed-stuff used to replace fish meal, such as soybean meal and corn gluten. However, currently they are accessible and their use is economically viable in diets with low protein level. Moreover, amino acids are involved in the intermediary metabolism of various compounds responsible for various functions in the animal's system, such as mucin, which has a high level of threonine, immunoglobulin (cystine and threonine) and glutathione (cysteine) (De Blas et al., 2000, Machado & Fontes, 2005).

As the amino acid requirements, usually expressed as diet protein level percentage or as diet percentage, were obtained from results of a few experiments, more studies are needed to validate the recommendations (Hauler & Carter, 2001; Wilson, 2003).

The objective of the present study was to investigate the crude protein reduction and the digestible methionine+ cystine and threonine to digestible lysine ratios in diets for Nile tilapia fingerlings, based on the ideal protein concept.

Material and Methods

This study was undertaken during 37 days between August and September 2006, at the Laboratório de Nutrição de Peixes of the Departamento de Zootecnia of the Universidade Federal de Viçosa.

Four hundred and twenty Nile tilapia (*Oreochromis niloticus*) fingerlings, Thai strain, with an average initial weight of 1.83 ± 0.02 g, were placed in a complete randomized design, with five diets, seven replications per diet and 12 fish per experimental unit. The experimental diets were isocaloric (3.000 kcal digestible energy) and isolysinic (1.7%), with 32 or 28% CP.

The diet with 32% of CP had methionine+cystine:lysine and threonine:lysine ratios of 63 and 74%, respectively, and had no supplementation of L-lisina-HCL, but the other synthetic amino acids were added as their ratios with digestible lysine were below those estimated in the ideal protein, calculated from the requirement values determined by the NRC (1993).

Four diets contained 28% CP and were formulated with different amino acids ratios: one of the diets with 28% CP had the same dietary amino acids ratios as the diet with 32% CP; another diet was formulated keeping the threonine:lysine ratio, but changing the methionine + cystine:lysine ratio to 60%, according to the recommendations of Furuya et al. (2004) and Bomfim et al. (2007); a third diet was formulated with the same crude protein level and the same methionine + cystine ratio as the previous diet, but the threonine:lysine ratio was changed to 69%, according to the recommendations of Lanna et al. (2007); and, finally, the fourth diet with 28% CP was formulated with methionine + cystine and threonine to lysine ratios of 63 and 74% respectively, but 0.3% glutamic acid was added to verify a possible non-essential amino acid deficiency.

The percentage and chemical compositions of the experimental diets were calculated according to Rostagno et al. (2005) and Furuya et al. (2001) (Table 1).

The digestible amino acid and available phosphorus values were estimated based on ingredient digestibility coefficients according to Rostagno et al. (2000) and Furuya et al. (2001), energy, according to Boscolo et al. (2002) and Pezzato et al. (2002), and the dietary amino acid analyses were performed in the Laboratório da Ajinomoto, in Brazil. The calculated and analyzed amino acids values differed, probably due to the feedstuff composition variations.

Fingerlings were kept in 36 polyethylene aquariums, each one with a volumetric capacity of 150 liters and net volume of 130 liters, with individual aeration systems, single-pass flow-through water supply and draining from the bottom, arranged in a water recirculation system with renewal of at least 25.0% water per day.

The aquarium water supply came from the water treatment system of Universidade Federal de Viçosa - UFV, previously declorified and heated by electrical resistance, with temperature controlled by thermostat.

The water temperature was kept at approximately 28ºC and checked daily at 7:30 a.m. and 5:30 p.m., using an electronic thermometer. The water pH and dissolved oxygen levels were controlled every seven days using a potentiometer and oximeter.

Table 1 - Composition of the experimental diets (as fed)

Item	Crude protein level and amino acid ratios (%)				
	32 (NRC, 1993)	28 (NRC, 1993)	28 (Met+Cys)[1]	28 (Thr)[2]	28 (NRC, 1993)[3]
Ingredient (%)					
Soybean meal	65.342	53.500	53.480	53.560	53.500
Corn	26.167	36.594	36.509	36.588	36.594
Corn starch	1.000	1.922	2.022	1.922	1.673
Soybean oil	3.122	2.843	2.844	2.829	2.793
L-lysine-HCl – 78,4%	0.000	0.351	0.352	0.349	0.351
DL-methionine – 99%	0.197	0.308	0.257	0.308	0.308
L-threonine – 98,5%	0.139	0.305	0.306	0.216	0.305
L-tryptophan – 99%	0.000	0.010	0.063	0.062	0.010
Glutamic acid – 99%	0.000	0.000	0.000	0.000	0.300
Dicalcium phosphate	2.960	3.100	3.100	3.100	3.100
Vitaminic and mineral mix[4]	0.500	0.500	0.500	0.500	0.500
Vitamin C [5]	0.050	0.050	0.050	0.050	0.050
Salt	0.500	0.500	0.500	0.500	0.500
BHT (Antioxidant)	0.020	0.020	0.020	0.020	0.020
Nutritional (calculated)[6]					
Crude protein (%)	32.0	28.0	28.0	28.0	28.2
Digestible energy (kcal/kg)	3,000	3,000	3,000	3,000	3,000
Total calcium (%)	0.9	0.9	0.9	0.9	0.9
Dicalcium phosphate (%)	0.6	0.6	0.6	0.6	0.6
Digestible lysine (%)	1.7	1.7	1.7	1.7	1.7
Digestible tryptophan (%)	0.4	0.3	0.4	0.4	0.3
Digestible isoleucine (%)	1.3	1.1	1.1	1.1	1.1
Met + cys/Lys ratio (%)	63.0	63.0	60.0	60.0	63.0
Thr/lys ratio(%)	74.0	74.0	74.0	69.0	74.0
Nutritional (analyzed)[7]					
Crude protein (%)	33.02	28.81	28.20	29.09	28.41
Digestible lysine (%)	1.7	1.7	1.6	1.7	1.6
Digestible tryptophan (%)	0.5	0.4	0.4	0.4	0.4
Digestible isoleucine (%)	1.3	1.2	1.1	1.1	1.0
Met + cys/Lys ratio (%)	65.0	65.0	62.0	65.0	63.0
Thr/lys ratio(%)	82.0	82.0	81.0	70.0	75.0

[1] Methionine + cystine to lysine ratio of 60% (Furuya et al., 2004; Bomfim et al., 2007).
[2] Threonine to lysine ratio of 69% (Lanna et al., 2007).
[3] Suplemented with 0.3% glutamic acid.
[4] Composition per kilogram of product: vitamin A - 1.200.000 UI; vitamin D_3 - 200.000 UI; vitamin E - 1.200 mg; vitamin K_3 - 2.400 mg; vitamin B_1 – 4,800 mg; vitamin B_2 - 4.800 mg; vitamin B_6 - 4.800 mg; vitamin B_{12} - 4.800 mg; vitamin C - 48 g; folic acid - 1.200 mg; panthotenic acid - 12.000 mg; biotin - 48 mg; cholin - 108 g; niacin - 24.000 mg; Fe - 50.000 mg; Cu - 3.000 mg; Mn - 20.000 mg; Zn - 30.000 mg; I - 100 mg; Co - 10 mg; Se - 100 mg.
[5] Vitamin C: calcic salt, 2- ascorbic acid monophosphate - 42% active principle.
[6] Estimated values based on the digestibility coefficients of the ingredients for the amino acids, phosphorus according to Rostagno et al. (2000) and Furuya (2001), and energy according to Boscolo et al. (2002) and Pezzato et al. (2002).
[7] Ajinomoto Animal Nutrition Laboratory (Brazil).

The photoperiod was kept at 12 hours light using mixing light bulbs, controlled by an automatic timer.

The experimental diets were pelletized and provided daily in six meals at 8 a.m., 10 a.m., 12 p.m., 2 p.m., 4 p.m. and 6 p.m., so that at each meal the food was supplied several times in small quantities to enable maximum intake, without losses, until apparent satiation, to reduce the leaching possibility.

The aquariums were cleaned once a day by siphoning the excreta, always after reading the water temperature in the afternoon.

The following indexes were evaluated: feed intake, lysine consumption, nitrogen consumption, weight gain, specific growth rate, survival rate, feed/gain ratio, protein efficiency for growth, lysine efficiency for growth, protein and body fat daily deposition, body composition (humidity, protein and fat), hepatosomatic index (HSI), carcass yield and nitrogen retention efficiency.

Protein and lysine efficiency for growth were calculated by dividing the weight gain by crude protein or digestible lysine consumption, respectively, according to the expressions described by Jauncey & Ross (1982).

At the beginning of the experimental period, 80 fish were sacrificed, after being anesthetized, for body analysis and, at the end of the trial, eight animals were sacrificed per experimental unit with weights closest to the average to their respective unit.

The viscera and liver weights were obtained after removing the viscera to determine the whole carcass yield (with head and fins). Viscera were weighed in an analytical scale (0.001 g) to determine the percentage of viscera in the body and the IHS.

The chemical analyses of diets and fish samples were performed at the Laboratório de Nutrição Animal of the Departamento de Zootecnia of the Universidade Federal de Viçosa, using the methodology reported by Silva & Queiroz (2002), so that that the body humidity was determined by the lyophilization process.

The body protein and fat depositions were calculated by the difference between the initial and final body protein or fat, respectively, in milligrams, divided by the experimental period (days). The nitrogen retention efficiency, expressed in percentage, was calculated by the difference between the final and initial body nitrogen, divided by the total nitrogen consumed and multiplied by 100.

The statistical analysis was performed using the SAEG program - Sistemas de Análises Estatísticas e Genéticas (UFV, 2000). The data were analyzed by analysis of variance at the level of 5.0% probability and, when there was statistical difference, for the comparison of the obtained means, the Student Neuman Keuls test was used (SNK).

Results and Discussion

The water and aeration system allowed the control of temperature and aeration during the experimental period. Values of 28.0 ± 0.90 °C for water temperature, 7.1 ± 0.20 for pH, 7.4 ± 0.40 ppm for dissolved oxygen and 0.90 ± 0.06 mg/L ammoniac nitrogen were maintained. These values correspond to the range recommended for the production of this species, according to Kubitza (2000).

The reduced diet crude protein level did not influence (P<0.05) weight gain, specific growth rate, the fish survival rate and feed and digestible lysine intake (Table 2).

These results are similar to those reported by Bomfim et al. (2006). There was also no statistical difference (P<0.05) in these parameters among fish fed the diets with different amino acids ratios. Contrary to that observed by Bomfim et al. (2006), there was a numerical increase in weight gain and feed intake of fish fed the diet with the lowest crude protein level (28%) compared to values obtained with the control diet (32%).

There was no difference (P<0.05) in average feed intake among the diets, therefore, the one with the highest CP level resulted in greater nitrogen intake, lower protein efficiency for growth and nitrogen retention efficiency rates (P<0.05). However, fish fed diets with lower protein levels and different amino acids ratios did not differ (P<0.05) for the same parameters.

These results prove that, as described by Halver & Hardy (2002), the preparation of diets for fish using the “ideal protein” concept can reduce nitrogen compound emission to the bodies of water adjacent to fish farms, which with phosphorus, are primarily responsible for algal blooms that lead to eutrophication of ecosystems (Wilson, 2003; Bomfim et al., 2005a). This increased efficiency of the diet protein fraction use may also affect the production cost - since the protein is the most expensive component of the diet - and reduce the total ammoniac nitrogen concentration, which, depending on the water pH, can damage productivity and increase mortality (Losordo, 1997).

All diets provided specific growth rates of approximately 5.5% per day, which were similar to those observed by Hisano et al. (2003), Furuya et al. (2006) and Ribeiro et al. (2006) with tilapia of the same strain and the same weight

Table 2 - Performance of Nile tilapia fingerlings fed experimental diets

Item	CP level and a mino acid ratios (%)					
	32 (NRC, 1993)	28 (NRC, 1993)	28 (Met.+Cys.)[1]	28 (Thr.)[2]	28 (NRC, 1993)[3]	CV (%)
Initial weight (g)	1.83	1.84	1.83	1.82	1.82	1.16
Weight gain (g)	12.17	12.26	12.46	12.40	12.17	8.82
Specific growth rate (%/dia)	5.49	5.50	5.55	5.56	5.51	3.59
Survival rate (%)	100.00	98.81	100.00	100.00	98.81	2.00
Feed intake (g)	14.92	15.06	15.34	15.53	15.29	4.91
Digestible lysine intake (mg/dia)	6.86	6.92	7.05	7.13	7.03	4.91
Nitrogen intake (mg/dia)	20.65a	18.23b	18.57b	18.80b	18.52b	5.00
Feed/gain ratio (g/g)	1.23	1.24	1.22	1.25	1.26	5.98
Protein efficiency for growth (g/g)	2.54b	2.90a	2.90a	2.85a	2.84a	5.80
Lysine efficiency for growth (g/g)	47.74	47.83	47.82	46.95	46.76	5.77

[1] Methionine + cystine to lysine ratio of 60% (Furuya et al., 2004; Bomfim et al., 2007).
[2] Threonine to lysine ratio of 69% (Lanna et al., 2007).
[3] Supplemented with 0.3% glutamic acid.
Means, in the same line, followed by different letters, differ (P<0.05), by SNK test.

category. However, the values were lower than those found by Bomfim et al. (2005 a) and Bomfim et al. (2005b),which were 7.0% per day, the rate of 7.8% reported by Lanna et al. (2005) and 8.7% per day observed by Furuya et al. (2000).

Fish growth rates indicated that the handling used in this experiment and the diets supplemented with free amino acids, even with lower levels of crude protein compared to the requirement of 32% for the species, according to Furuya et al. (2000), were sufficient to enhance the animals´ performance. The “ideal protein” concept has been tested in other species and allows keeping animals performance by reducing the diet protein level through free amino acids supplementation. Yamamoto et al. (2005) obtained similar results in a study with trout.

The protein efficiency for growth (PEG) did not vary among between fish fed diets with 28% CP and different amino acid:lysine ratios, but was higher in absolute value, to that obtained with the 32% CP diet. Similar results were found by Bomfim et al. (2005a), Furuya et al. (2005) and Bomfim et al. (2006), but the results in this study were higher than those observed by Hisano et al. (2003), Boscolo et al. (2006) and Furuya et al. (2006).

This higher PEG may be related to the better amino acid balance of the diet, due to the reduction of amino acid excess in relation to the animal requirement in the diet with higher protein level, that probably would not contribute to the accumulation of lean tissue and were catabolized (Bomfim et al., 2005a; Bomfim et al., 2005b; Bomfim et al., 2006). On the other hand, the absence of significant difference between the means for diets with lower protein level indicated that the ratio of methionine + cystine and threonine to lysine may be lower than those recommended by NRC (1993). It also indicated that in this study, the increase of 0.3% glutamic acid in the diet with 28% CP and ratios of 63 and 74% methionine + cystine and threonine to lysine, respectively, caused no differences ($P<0.05$) in performance and probably there was no deficiency in any non-essential amino acid, contrary to the report by Schumacher et al. (1995) who observed that a source of non-essential amino acids (glutamic acid) had a highly significant effect on rainbow trout growth.

These results suggested that diets formulated based on the ratios between digestible amino acids and lysine may be more appropriate to meet the requirements of tilapia than those made solely based at the crude protein level (Boisen, 2003, Orlando et al., 2005; Bomfim et al., 2005a; Bomfim et al., 2006).

There were no differences ($P<0.05$) between the average performance of animals fed different diets, a result which differed from that reported by Bomfim et al. (2005a), who observed worsening in the feed/gain ratio and lysine use efficiency for growth of fish fed low protein diets supplemented with synthetic amino acids.

This negative result has been attributed to some problems in the use of diets supplemented with an excess of synthetic amino acids, such as the possibility of greater leaching of synthetic amino acids compared to those of vegetable protein in the aquatic environment and the imbalance of the ideal amino acid profile on the sites of protein synthesis due to its higher rate of absorption (Zarate & Lovell, 1997, Zarate et al., 1999, Dabrowski et al., 2003; Lanna et al., 2005).

In this study, the deleterious effects of the synthetic amino acids use may have been minimized by the increased of feed frequency, six times per day in small amounts and several times in each hour to ensure rapid and complete intake of the diets, which may have reduced the leaching and contributed to the stabilization of the amino acid plasma concentration and balance of the amino acid pool on the sites of protein synthesis, reducing its catabolism

Table 3 - Performance and carcass indices of Nile tilapia fingerlings fed experimental diets

Item	CP level and a mino acid ratios (%)					
	32 (NRC, 1993)	28 (NRC, 1993)	28 (Met.+Cys.)[1]	28 (Thr.)[2]	28 (NRC, 1993)[3]	CV (%)
Body humidity (%)	81,7	75,7	74,7	74,8	75,6	74,2
Body fat(%)[4]	1,7	5,0	5,0	5,2	5,3	5,0
Body crude protein (%)[4]	7,5	11,3	11,7	11,6	11,3	12,0
Fat deposition (mg/dia)	-	17,73	17,96	19,11	19,37	18,19
Protein deposition (mg/dia)	-	38,88	40,80	40,77	39,91	41,37
Carcass yield (%)	-	88,9	88,3	88,0	87,9	89,3
Hepatosomatic index	-	1,14b	1,22ab	1,26ab	1,45a	1,17b
Nitrogen retention efficiency (%)	-	30,1b	35,7a	35,5a	34,0a	36,0a

[1] Methionine + cystine to lysine ratio of 60% (Furuya et al., 2004; Bomfim et al., 2007).
[2] Threonine to lysine ratio of 69% (Lanna et al., 2007).
[3] Supplemented with 0.3% glutamic acid.
[4] Natural matter.
Means, in the same line, followed by different letters, differ ($P<0.05$) by SNK test.

and subsequent excretion (Tantikitti & March, 1995; Zarate, 1999; Rodehutscord et al., 2000).

The reduction of the diet protein level did not influence (P<0.05) the carcass yield, fish body protein content, body humidity and body fat (Table 3).

These results differ from those reported by Bomfim et al. (2005a), who observed lower levels of body fat in fish fed diets containing higher protein levels than fish fed diets with lower protein levels. They are also contrary to those observed by Yamamoto et al. (2005), in research on trout. These results may be related to the amino acid excess, that was catabolized (Dabrowski & Guderley, 2002) that may have reduced the deposition of body fat, because of the lower net energy with the higher heat increment produced (Noblet, 2001).

Fish receiving the diet with 32% CP (lower digestible energy: crude protein ratio) and those fed the diet with 28% CP supplemented with glutamic acid had lower HSI (hepatosomatic index), which may be related to excess amino acid catabolism, which may have produced a higher heat increment level and resulted in a smaller fraction of net energy to be deposited as energy reserve (Bureau et al., 2000; Noblet, 2001). The metabolic activity implied the use of nutrients obtained from food intake and energy reserves held in different parts of the body. Therefore, it is expected that the weight of the liver reflects the metabolism (Costa, 1999).

The daily body fat and protein deposition did not differ (P<0.05) among the diets, in spite of the decrease, in absolute values, of these parameters in fish fed the diet with higher crude protein levels (32%) compared to the values obtained with other protein levels. However, fish fed the diet with lower CP levels had higher body protein and fat deposition rates in absolute values, in relation to those fed the higher CP level, in contrast with observations by Bomfim et al. (2005a), and the lack of difference for these variables may be related to high variation coefficients.

The nitrogen retention efficiency of fish fed the diets with lower protein levels were similar (P<0.05) to that obtained in other studies on tilapia (Furuya et al., 2005).

These results corroborate the theory that the use of diets elaborated based on the ideal protein concept is an efficient alimentary strategy to reduce nitrogen excretion to the environment, because it does not significantly affect the animal performance (Ferreira et al., 2003; Bomfim et al., 2006).

Conclusions

The dietary crude protein level for fingerlings of Nile tilapia is 28%, as long as the diets are supplemented with limiting essential amino acids. Ratios of methionine + cystine and threonine to digestible lysine are 60.0 and 69.0%, respectively, in diets for Nile tilapia fingerlings.

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