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FEEDING FREQUENCY OF NILE TILAPIA FED RATIONS SUPPLEMENTED WITH AMINO ACIDS[1]

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ABSTRACT – The present study evaluated the feeding frequency of Nile tilapia (*Oreochromis niloticus*) fed low-protein diets (29% crude protein) supplemented with commercial amino acids (L-lysine-HCl, DL-methionine, and L-threonine). Sex-reversed Nile tilapia (240) of Thai origin, with initial body weight of 0.93 ± 0.03 g each, were allocated in a completely randomized design, to five groups with various feeding frequencies (2, 3, 4, 5, and 6 times a day). Six replications were conducted on separate experimental units comprising eight fishes each. The fish were maintained in 30 130-L aquariums, each equipped with individual water supply and controlled temperature and aeration. Fish were fed identical quantities among all treatments over a period of 30 days. Performance parameters, feed conversion efficiency, body composition, daily protein and fat deposition rates, and nitrogen retention efficiency were evaluated. Increased feeding frequency affected neither performance parameters nor body composition. Values for protein and fat deposition rates and nitrogen retention efficiency were significantly lower in fish fed twice daily than in those fed five times daily. Fish subjected to other feeding frequencies yielded intermediate values without any significant differences. The minimum feeding frequency of Nile tilapia fingerlings fed a low-protein ration supplemented with commercial amino acids is three times a day, owing to increased protein deposition rates and nitrogen retention efficiency.

Keywords: Pair feeding. Feed management. *Oreochromis niloticus.* Low crude protein.

FREQUÊNCIA DE ALIMENTAÇÃO DE TILÁPIAS DO NILO ALIMENTADAS COM RAÇÃO SUPLEMENTADA COM AMINOÁCIDOS

RESUMO – Objetivou-se avaliar os efeitos da frequência de alimentação em tilápias do Nilo (*Oreochromis niloticus*), utilizando rações de reduzido teor proteico (29% de proteína bruta) suplementadas com aminoácidos industriais (L-Lisina-HCl, DL–Metionina e L-Treonina). Foram utilizados 240 alevinos sexualmente invertidos, da linhagem tailandeza, com peso inicial de 0,93 ± 0,03 g, em delineamento inteiramente casualizado, composto por cinco frequências alimentares (2, 3, 4, 5 e 6 vezes/dia), seis repetições e oito peixes por unidade experimental. Os peixes foram mantidos em 30 aquários de 130 litros dotados de abastecimento de água, temperatura controlada e aeração individuais e alimentados em quantidades idênticas de ração entre os tratamentos, com valores corrigidos diariamente, durante 30 dias. Avaliaram-se os parâmetros de desempenho e eficiência alimentar, a composição corporal, a deposição de proteína e gordura corporais e a eficiência de retenção de nitrogênio dos peixes. A variação da frequência de alimentação da dieta não influenciou os parâmetros de desempenho e composição corporal. As taxas de deposição de proteína e gordura corporal e a eficiência de retenção de nitrogênio dos peixes alimentados duas vezes por dia apresentaram valores menores em relação aos alimentados cinco vezes por dia, enquanto que os demais tratamentos apresentaram valores intermediários, sem variar entre os alimentados duas e cinco vezes por dia. Concluiu-se que a frequência de alimentação mínima de alevinos de tilápias do Nilo é de três vezes por dia, quando são utilizadas rações de baixo teor proteico suplementadas com aminoácidos, por aumentar a deposição de proteína e eficiência de retenção de nitrogênio.

Palavras-chave: Alimentação pareada. Manejo alimentar. *Oreochromis niloticus*. Redução de proteína bruta.

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INTRODUCTION

Protein is the most abundant macronutrient in formulated fish feed. Regarding metabolism in fish, specific protein requirements do not exist. However, fish require an adequate (quantitatively balanced) supply of essential and non-essential amino acids. Therefore, feed formulations based on values of crude protein or even digestible protein are not guaranteed to meet the nutritional requirements for amino acids in fish (NWANNA et al., 2012; BOMFIM, 2013).

The concept of ideal protein is currently being implemented in the formulation of fish feeds. Ideal protein refers to a mixture of amino acids (which is entirely available for digestion, absorption, and metabolism) the composition of which meets the nutritional needs of the animal (BOTARO et al., 2007; BOMFIM et al., 2008; QUADROS et al., 2009; TAKISHITA et al., 2009; FURUYA et al., 2012).

The formulation of feed based on the ideal protein profile may facilitate the use of lower levels of crude protein than have been previously recommended. In addition, studies have suggested that more precise supplementation with commercially-available limiting amino acids to meet the nutritional requirements of Nile tilapia (FURUYA et al., 2005; BOTARO et al., 2007; BOMFIM et al., 2008; QUADROS et al., 2009; RIGHETTI et al., 2011), tambatinga (ARARIPE et al., 2011), and rainbow trout (YAMAMOTO; SUGITA; FURUITA, 2005) could effectively reduce the use of excessive levels of other amino acids in the feed, thereby reducing levels of nitrogen excreted into the environment.

However, the efficiency of feed formulations based on excessively used commercial amino acids might not be similar to that of formulations based on crude protein. This could be attributed to the possibility of leached amino acids in the aquatic environment, higher rates of gastric emptying, and faster absorption of commercial amino acids, which can lead to temporary imbalance in the profiles amino acids that are needed for protein synthesis (SCHUHMACHER; WAX; GROPP, 1997; ZARATE et al., 1999; RONNESTAD et al., 2000; AOKI; AKIMOTO; WATANABE, 2001; NWANNA et al., 2012).

On the other hand, the moderate use of commercial amino acids at higher feeding frequencies can diminish these negative effects, thereby minimizing the incidence of temporary excesses; maintaining the levels of amino acids and other nutrients in the blood to a more constant, compatible rate with that of protein synthesis; and improving protein utilization in specialized tissues (TANTIKITTI; MARCH, 1995; BARROSO et al., 1999; ROLLIN et al., 2003; BOMFIM, 2013).

It is possible to reduce the protein content in the feed of Nile tilapia by as much as 4% based on the ideal protein concept. However, studies aimed at defining the minimum feeding frequency required to confirm the feasibility of such diets at the growing and finishing stages (for which low feeding frequencies are normally used) have been scarce.

Therefore, the present study was designed to evaluate the effects of feeding frequency on the performance and body composition of Nile tilapia fed a reduced-protein diet, supplemented with amino acids.

MATERIAL AND METHODS

The study was conducted over 30 days in the Laboratory of Fish Nutrition, Department of Animal Science, Federal Universidade of Viçosa (UFV), in the municipality of Viçosa, Minas Gerais, Brazil.

Sex-reversed Nile tilapia fry (240) of Thai origin, with initial body weight of 0.93 ± 0.13 g, were selected in a completely randomized design experiment, consisting of five treatments, with six replications per treatment, and eight fishes allotted to experimental unit.

The treatments comprised five feeding frequencies: two (8 am and 6 pm); three (8 am, 1:30 pm, and 6 pm); four (8 am, 10:30 am, 2:30 pm, and 6 pm); five (8 am, 10:30 am, 1:30 pm, 3:30 pm, and 6 pm); and six (8 am, 10 am, 12 pm, 2 pm, 4 pm, and 6 pm) times daily.

The fish were fed a pelleted feed formulated according to the nutritional recommendations of the NRC (1993), with the exception of the crude protein content, which was maintained at 3% below the recommended minimum protein content. The level of digestible lysine in the diet was 1.5%. The feed was supplemented with the other amino acids, based on the concept of ideal protein. Besides digestible lysine, we aimed to maintain the following balance in the diet derived from the requirements outlined by the NRC (1993): methionine plus digestible cysteine, no less than 63.0%; digestible threonine, 74%; and digestible tryptophan, 20%.

Polyethylene aquariums (34) were used in the study: 30 for the treatment groups and 4 for the controls. Each aquarium had a net volume capacity of 130 L and was equipped with a water supply, aeration, and bottom drainage systems, arranged in a system of constant recirculation and minimum daily water input of 25%.

The chemical composition of the experimental fish feed is presented in Table 1.

Table 1. Chemical and nutritional composition of the experimental fish feed (on a natural matter basis).

Ingredients	g kg^{-1}
Soybean meal	480.6
Corn	277.8
Corn gluten 60	72.6
Soybean oil	24.8
Corn starch	98.3
L-Lysine HCl – 784 g/kg	2.0
DL-Methionine – 990 g/kg	1.1
L-Threonine – 985 g/kg	1.3
Limestone	0.3
Dicalcium phosphate	30.5
Vitamin C [3]	0.5
Vitamin and mineral supplement [4]	5.0
Common salt	5.0
BHT (Antioxidant) [5]	0.2
Nutritional Information	
Crude protein (g kg^{-1}) [1]	290.0
Digestible protein (g kg^{-1}) [2]	263.0
Digestible energy (kcal kg^{-1}) [2]	3000.0
Linoleic acid (g kg^{-1}) [1]	23.3
Total calcium (g kg^{-1}) [1]	9.3
Available phosphorus (g kg^{-1}) [2]	6.0
Digestible lysine (g kg^{-1}) [2]	15.0
Methionine + Digestible cysteine (g kg^{-1})[2]	9.5
Digestible threonine (g/kg) [2]	11.1
Digestible tryptophan (g/kg) [2]	3.2
Digestible isoleucine (g/kg) [2]	11.3

[1]Calculated according to the methods outlined by Rostagno et al. (2005).
[2]Estimates based on the methods outlined by Rostagno et al. (2005) and Furuya et al. (2001) for the digestibility coefficients of crude protein, amino acids, and phosphorus, and Pezzato et al. (2004) for energy values.
[3]Vit. C: ascorbic acid 2-monophosphate calcium salt, 420 g kg^{-1} of active ingredient.
[4]Product composition by kg: Vit. A, 1,200,000 IU; Vit. D_3, 200,000 IU; Vit. E, 1,200 mg; Vit. K_3, 2,400 mg; Vit. B_1, 4,800 mg; Vit. B_2, 4,800 mg; Vit. B_6, 4,800 mg; Vit. B_{12}, 4,800 mg; Vit. C, 48 g; Folic acid, 1,200 mg; calcium pantothenate, 12,000 mg; Vit. C, 48,000 mg; biotin, 48 mg; choline chloride, 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]BHT; Butylated hydroxytoluene

To avoid any confounding of the results due to probable variations in consumption, feed was provided in equal quantities across all treatments, and values were corrected daily. The quantities provided were determined by calculating the average consumption of four additional aquariums (the control group), that is, the difference in weight of the food containers before feeding and at the end of the day. The control group was fed six meals (at 8 am, 10 am, 12 pm, 2 pm, 4 pm, and 6 pm) *ad libitum* and consisted of the same number of fish per aquarium. Feed was provided in small quantities until apparent satiety at each meal, to facilitate maximal ingestion, with minimal waste.

Water used in the aquariums originated from the water treatment system of the Federal University of Viçosa – UFV and was dechlorinated and heated by electrical resistance. Water temperature was controlled by a digital thermostat.

The water temperature was monitored daily, at 8 am and 6 pm, using a mercury bulb thermometer, with a scale from 0–50°C. The pH and water-dissolved oxygen content were controlled and monitored every seven days using a potentiometer and oximeter, respectively.

The photoperiod was maintained at 12 hours of light and 12 hours of darkness. Light conditions were provided by a blended lamp and controlled by an automatic timer. Aquariums were cleaned by syphoning twice daily to remove feces. Cleaning was done following measurements of water temperature.

The following parameters were assessed: weight gain, specific growth rate, survival rate, feed intake, digestible lysine consumption, feed conversion, protein efficiency ratio, lysine utilization efficiency, daily deposition rates of protein and body fat, chemical composition (moisture content, protein, and fat), and nitrogen retention efficiency.

To determine the specific growth rate (SGR), the following equation was applied, using logarithmic transformation.

$$SGR = \frac{[\text{Ln of initial weight (g) - Ln of final weight (g)}]}{\text{Duration of the study (d)}} \times 100$$

Protein efficiency ratio and lysine utilization efficiency were calculated by dividing weight gain of the fish by the consumption of crude protein and digestible lysine, respectively.

After being desensitized on ice, an additional group of 70 fishes was sacrificed, to determine body composition at the start of the study. Similarly, at the end of the experimental period, all fishes in the various experimental units were desensitized and sacrificed to determine body composition.

Protein and body fat deposition were determined by calculating the differences between protein and body fat at the beginning and end of the experimental period, respectively (in mg), and dividing these values by the experimental period (in days).

The nitrogen retention efficiency, expressed as a percentage, was calculated by finding the difference between final and initial body nitrogen (g), and dividing this value by the total amount nitrogen consumed (g), and multiplying by 100.

The bromatological analysis of feed and fish samples were performed at the Laboratory of Animal Nutrition, Department of Animal Sciences (LNA/DZO), Federal University of Viçosa – UFV, according to the procedures described by Detman et al. (2012).

Statistical analyses were performed using the SAEG - Genetic and Statistical Analysis System software, version 5.0, developed at UFV (1993).

Data were interpreted using analysis of variance. The effects of feeding frequency on the variables under investigation were compared using the Duncan's multiple range test, at α = 0.05.

RESULTS AND DISCUSSION

The water supply and aeration systems facilitated the control and maintenance of temperature, pH, and aeration throughout the experimental period. These values were as follows: 29.0 ± 1.21°C for water temperature, 6.6 ± 0.15 for pH, and 6.24 ± 0.46 mg.L^{-1} for dissolved oxygen. These values fall within the ranges recommended for efficient production of this species: 26–30°C for temperature; 6.0–8.5 for pH; and > 4.0 mg.L^{-1} for dissolved oxygen (ZIMMERMANN; FITZSIMMONS, 2004).

Feeding frequency affected neither the performance parameters of the fish nor the feed conversion efficiency (P > 005) (Table 2).

Table 2. Feeding frequency, performance parameters, and feed conversion efficiency of Nile tilapia fry fed reduced-protein feed supplemented with commercially available limiting amino acids.

Parameter	Feeding frequency (times/day)					CV [1] (%)	$P > F$ [2]
	2	3	4	5	6		
Initial weight (g)	0.95	0.94	0.95	0.95	0.95	3.19	0.937
Weight gain (g)	8.60	8.62	8.93	9.21	8.79	4.48	0.499
Specific growth rate (%)	7.70	7.75	7.82	7.89	7.77	1.84	0.669
Survival rate (%)	97.91	95.83	95.83	100.00	100.00	4.88	0.382
Feed consumption (g)	9.87	9.87	9.87	9.87	9.87	–	–
Digestible lysine consumption (mg)	148.05	148.05	148.05	148.05	148.05	–	–
Feed conversion (g/g)	1.15	1.15	1.11	1.08	1.12	4.43	0.503
Protein efficiency ratio (g/g)	29.67	29.72	30.79	31.74	30.31	4.48	0.500
Lysine utilization efficiency (g/g)	58.12	58.21	60.32	62.18	59.37	4.48	0.499

[1] = coefficient of variation; [2] = F test significance in the analysis of variance.

The average specific growth rate of 7.9% in the present study was similar to those observed by Bomfim et al. (2008) and Takishita et al. (2009), who reported values of 7.3% and 7.9%, respectively. However, the value observed in the present study was lower than those observed by Furuya et al. (2000) and Bomfim et al. (2010), who reported 8.7% and 8.9%, respectively. In addition, the specific growth rate of the present study was greater than the values between 3.2% and 5.5% reported by Furuya et al. (2005), Boscolo et al. (2006), Furuya et al. (2006), and Quadros et al. (2009), who studied tilapia of the same lineage and weight category.

These results are consistent with the observations of Furuya et al. (2005), Botaro et al. (2007), Bomfim et al. (2008), Quadros et al. (2009), and Righetti et al. (2011). These researchers suggest that the use of fish feed formulated with a moderate reduction in the recommended crude protein levels (of 32%) (NRC, 1993; FURUYA et al., 2000; NRC, 2011), that based on the ideal protein concept has also been supplemented with commercial amino acids, does not adversely affect the performance of fish, even at low feeding frequencies (twice daily).

A lack of significant differences between treatments was not consistently observed in the studies of Barroso et al. (1999) and Zarate, Lovell and Payne (1999), who confirmed that increasing the feeding frequency from two to four or five times daily, significantly increased the observed values for performance parameters and feed conversion efficiency in trout and channel catfish, respectively. The subjects of those studies were fed diets in which the protein source was supplemented with commercial amino acids. The quantity of feed consumed by the fish in each frequency group might have contributed to differences in the results. Barroso et al. (1999) reported that the quantity of feed (and hence, the energy and nutrient levels) consumed by fish fed at the highest frequency (six times daily) was greater than that of fish fed twice daily (126.7 g and 109.5 g, respectively).

Nwanna et al. (2012) evaluated the effects of feeding frequency (twice daily vs. continuous feeding) in the common carp. In that study, feed was either supplemented with DL-methionine or not and provided in similar amounts (pair feeding). The researchers reported that continuous feeding improved protein digestibility and performance parameters regardless of amino acid supplementation.

In these cases, regardless of feed consumption and amino acid source, increasing the feeding frequency improved protein digestibility, possibly because of increased trypsin activity (ZHAO et al., 2016). In addition, increased feeding frequency facilitated continuous absorption of nutrients, and thereby minimized the negative effects of varying postprandial levels of free amino acids and intact proteins, and optimized the overall performance of fish (BARROSO et al., 1999; NWANNA et al., 2012; ZHAO et al., 2016).

Fish in the present study received similar amounts of feed in all treatments. No significant leftovers were observed, even in the aquarium with the lowest feeding frequency. Therefore, the nutrient and energy levels consumed by the animals were similar and the differences eventually observed among treatments could be attributed to variations in feeding frequency among the various treatment groups.

On the other hand, regardless of feeding frequency or the effects of variation in consumption, the use of feed formulated with excessive levels of amino acids can adversely affect performance in fish. In studies conducted by Barroso et al. (1999) and Zarate, Lovell and Payne (1999), the diets were supplemented with 15% and 8% commercial amino acids, respectively, to maintain levels similar to the respective control diets, in which the source of amino acids was intact proteins. In both cases, regardless of feeding frequency, fish fed with feed supplemented with commercial amino acids showed inferior performance to those fed with amino acids from intact proteins.

Bomfim et al. (2008) in an experiment with Nile tilapias fed six times daily, evaluated the effects of reduced crude protein levels (32%, 31%, 30%, 29%, 28%, and 27% CP) in digestible isolysinic fish feed supplemented with amino acids, based on the concept of ideal protein. They reported that feed conversion and lysine utilization efficiencies were lower in fish fed feed containing 27% CP compared to those fed 32% CP (control group).

Regarding the body composition of fish (moisture content, protein, and body fat), no significant effects were observed ($P > 0.05$) among the various treatments. However, daily deposition of protein and body fat and the nitrogen retention efficiency in fish fed twice daily were significantly lower ($P < 0.05$), when compared to those fed five times daily. The other treatments showed intermediate values, without any significant differences between fish fed twice daily and those fed five times daily (Table 3).

These results suggest that a feeding frequency of twice daily can compromise the feed conversion efficiency of the dietary protein fraction (amino acids) as it relates to the deposition of lean muscle in fish. These effects could be attributed to reductions in nitrogen retention efficiency particularly, as well as the rate of protein deposition in fish fed feed supplemented with limiting amino acids.

These findings were likely due to an imbalance of amino acids in specialized tissues, caused by early catabolism of commercial amino acids, as a consequence of faster rates of gastric emptying and absorption (TANTIKITTI; MARCH, 1995; SCHUHMACHER; WAX; GROPP, 1997; AOKI; AKIMOTO; WATANABE, 2001; BOMFIM et al., 2008; QUADROS et al., 2009).

The lower fat deposition rate in fish fed twice daily might be related to the higher levels of excess amino acids that needed to be catabolized, which provided greater caloric increments and lower fractions of excess net energy for storage as body fat (BOMFIM et al., 2008; NRC, 2011).

The results of the present study are consistent with those reported by Tantikitti and March (1995) and Schuhmacher, Wax and Gropp (1997) in trout; Aoki, Akimoto and Watanabe (2001) in yellowtail; and Zarate, Lovell and Payne (1999) in channel catfish, who confirm the negative implications of using commercial amino acids at low feeding frequencies. In these studies, free amino acids induced early increases in their plasma

Table 3. Feeding frequency, body composition, daily deposition of protein and body fat, and nitrogen retention efficiency of Nile tilapia fry fed reduced-protein feed supplemented with commercially available limiting amino acids.

Parameter	Feeding frequency (times/day)						CV [3] (%)	$P > F$ [4]
	Initial	2	3	4	5	6		
Body moisture (%) [1]	79.29	76.55	76.35	76.03	76.05	76.04	1.00	0.380
Body fat (%) [1]	5.90	7.82	7.87	7.84	8.02	7.78	5.73	0.631
Body protein (%) [1]	10.76	11.57	11.65	11.73	11.58	11.69	3.60	0.949
Body fat deposition (mg/day) [2]	–	22.95^{B}	23.18^{AB}	23.90^{AB}	25.19^{A}	23.32^{AB}	6.54	0.250
Body protein deposition (mg/day) [2]	–	33.41^{B}	33.72^{AB}	35.23^{AB}	35.69^{A}	34.52^{AB}	4.71	0.235
Nitrogen retention efficiency (%) [2]	–	35.02^{B}	35.34^{AB}	36.92^{AB}	36.84^{A}	36.18^{AB}	4.71	0.237

[1] = on a natural matter basis; [2] = means followed by the same letter in the same row are not significantly different (P > 0.05), Duncan's multiple range test; [3] = coefficient of variation; [4] = *F* test significance in the analysis of variance.

concentrations compared to those derived from the digestion of intact protein. As a result, it caused a temporary imbalance in the amino acids required for protein synthesis, thereby also reducing the efficiency of use of other amino acids derived from intact proteins. On the other hand, these researchers indicated that these negative effects could be minimized by increasing the feeding frequency; maintaining more stable amino acid concentrations compatible with the capacity of use by specialized tissues; and verifying the improvements observed in the variables of interest.

Based on the results of the present study, it is possible to culture Nile tilapia using feed formulated with a moderate reduction in protein levels (3%), as it relates to that recommended (32%) for the stage of production evaluated (NRC, 1993; FURUYA et al., 2000; NRC, 2011). The feed can be supplemented with commercially available limiting amino acids, and fed at a minimum frequency of three times daily. This management strategy is also effective for the reduction of nitrogen excretion in the aquatic environment, without any adverse effects on fish performance (BOMFIM et al., 2008).

CONCLUSIONS

The minimum feeding frequency of Nile tilapia fed low-protein rations (29% CP) supplemented with commercial amino acids is three times a day, which does not adversely affect performance or body composition of the fry.

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