



Short Communication

Protein requirement for *Trichogaster lalius*, blue variety

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ABSTRACT - The objective of the present study was to evaluate the protein requirement of juvenile *Trichogaster lalius*, blue variety. The experimental design was of randomised blocks (B1 = initial weight of 1.04±0.05 g and B2 = 1.36±0.02 g), with two replicates within each block and five treatments (230, 270, 310, 350 and 390 g CP/kg diet). The fish were fed to satiation, three times a day for 90 days. The study evaluated: survival rate, weight gain, final length, feed intake, feed conversion ratio, protein efficiency ratio, specific growth rate and condition factor. There was a linear effect of dietary protein levels for protein efficiency ratio, specific growth rate (positive linear effect) and feed conversion ratio (negative linear effect). For weight gain, final length, feed intake and condition factor a quadratic effect of dietary protein levels was observed, with estimated values of 409.8, 366.2, 317.4 and 365.0 g CP/kg diet, to improve their performance parameters. Analysis of growth based on the length of the fish shows that 366.2 g of CP/kg diet meets the protein requirement of juvenile *Trichogaster lalius*, blue variety.

Key Words: dwarf gourami, growth performance, nutrient requirements, ornamental fish

Introduction

Feeding is one of the main factors for the successful production of ornamental fish. The adequate supply of nutrients is essential for proper development and growth, as well as the reproduction and maintenance of animal health. Therefore, knowledge of nutritional requirements is essential for the formulation of complete and balanced diets that enhance the growth performance of ornamental fish.

For the formulation of fish feed, priority is given to meet the protein requirements, since this nutrient is the main structural constituent of tissues, and is, therefore, responsible for the growth of the animals. The dietary protein is also important for synthesis of enzymes, hormones and neurotransmitters, and thus, it is essential for maintaining homeostasis and animal health (NRC, 1993).

The use of diets deficient in protein and/or essential amino acids causes a reduction in fish growth due to the reduced protein synthesis; whereas, in excess, only part of the protein is used for growth and the surplus is deposited as fat tissue reserve and/or used for energy (Wilson, 2002) by deamination of amino acids. Thus, excess protein in the

diet causes increased excretion of ammonia by the fish, causing eutrophication of cultivation water and effluent (Wu, 1995).

The knowledge of protein requirements allows for the use of balanced rations to improve the growth of fish and reduce water eutrophication. The use of suitable rations for different species can also contribute to reducing the use of live foods, and consequently for the usage of intensive systems of ornamental fish farming.

Among the ornamental species, *Trichogaster lalius*, previously classified as *Colisa lalia*, belonging to the Osphronemidae family, is the smallest and most popular among dwarf gouramis, and is widely marketed worldwide (Welcomme, 1988), both in the wild and as red and blue varieties. Being a species of peaceful behaviour and significant beauty, it is much appreciated by aquarists. The blue variety has received the most attention from producers of ornamental fish due to the high prices achieved for both males and females.

Thus, the objective of the present study was to evaluate the protein requirements of juvenile *Trichogaster lalius*, blue variety.

Material and Methods

The experimental design was of randomised blocks (B1 = initial weight of 1.04±0.05 g and B2 = initial weight of 1.36±0.02 g), with two replicates within each block and five treatments (230, 270, 310, 350 and 390 g of CP/100 g feed).

The experimental diets (Tables 1 and 2) were formulated based on the chemical composition of foods (Rostagno et al., 2005) and the availability of nutrients for

Nile tilapia (Miranda et al., 2000; Pezzato et al., 2002). The ingredients were mixed manually, pelletized and dried in a forced-ventilation oven (50 °C for 24 hours). The diets were ground, sieved and stored in a freezer at -20 °C.

Juvenile *Trichogaster lalius*, blue variety, were kept in 7-L aquariums with a biological filter, aeration and a maintained temperature (27±1 °C) via the use of heaters and thermostats. Each aquarium containing 10 fish was considered an experimental unit. Fish were fed three times a day until satiation.

At the end of 90 days the study assessed the following productive performance parameters: survival rate, weight gain, final length, apparent feed intake, feed conversion ratio, protein efficiency ratio, specific growth rate and condition factor.

The total ammonia in the aquarium water was assessed biweekly through an ammonia photometer meter. The content of non-ionised ammonia was calculated based on the values of temperature and pH of the aquarium water.

The evaluation of the effect of crude protein of the diet on the productive performance parameters and ammonia content in water was performed by analysis of variance and polynomial regression at 5% probability using the software SAEG (Sistema para Análises Estatísticas e Genéticas, 2007). To choose the regression model, the significance of the regression coefficients, the magnitude of the coefficient of determination (calculated on the sum of squares of treatments) and the behaviour of the variables under study were considered.

Results and Discussion

The significant effects of blocks (initial size) for weight gain, feed conversion ratio, final length, specific growth rate and condition factor were observed. Fish of block B1, with

Table 1 - Formulation of experimental diets for juvenile *Trichogaster lalius*, blue variety

Ingredients	Crude protein levels of experimental diets (g/kg)				
	230	270	310	350	390
Soybean meal	00.0	80.0	142.7	180.0	196.0
Corn gluten	00.0	00.0	30.0	80.0	145.0
Fish meal	300.0	300.0	300.0	300.0	300.0
Corn meal	310.0	386.4	318.3	233.2	156.4
Wheat bran	200.0	200.0	180.0	180.0	180.0
Corn starch	145.5	0.00	0.00	0.00	0.00
Cellulose	10.0	0.00	0.00	0.00	0.00
L - lysine	9.2	6.6	4.6	3.0	1.8
DL - methionine	4.0	3.7	3.1	2.5	1.5
Soybean oil	9.0	11.0	9.0	9.0	7.0
Dicalcium phosphate	7.0	7.0	7.0	7.0	7.0
Vitamin C ¹	0.6	0.6	0.6	0.6	0.6
Common salt	2.5	2.5	2.5	2.5	2.5
Vitamin supplement ²	1.0	1.0	1.0	1.0	1.0
Mineral supplement ³	1.0	1.0	1.0	1.0	1.0
BHT ⁴	0.2	0.2	0.2	0.2	0.2
Total	100	100	100	100	100

¹ Ascorbyl monophosphate with 35% of active ingredient.

² Levels of guarantee of the vitamin supplement (Mogiana Alimentos S/A - Guabi): vitamin A - 16,000 IU; vitamin D - 4,500 IU; vitamin E - 250 mg; vitamin K - 30 mg; vitamin B1 - 32 mg; vitamin B2 - 32 mg; vitamin B12 - 32 mcg; vitamin B6 - 32 mg; pantothenic acid - 80 mg; niacin, 170 mg; biotin - 10 mg; folic acid - 10 mg; choline - 2,000 mg.

³ Levels of guarantee of the mineral supplement (Mogiana Alimentos S/A - Guabi): cobalt - 0.5 mg; copper - 20 mg; iron - 150 mg; iodine - 1 mg; manganese - 50 mg; selenium - 1 mg; zinc - 150 mg.

⁴ Butylated hydroxytoluene (antioxidant).

Table 2 - Chemical composition of the experimental diets for juvenile *Trichogaster lalius*, blue variety

Ingredients	Crude protein levels of experimental diets (g/kg)				
	230	270	310	350	390
Crude protein (g/kg)	230.6	272.5	310.3	350.1	390.0
Digestible protein (g/kg) ¹	188.5	226.9	262.3	300.1	338.3
Gross energy (kcal/kg)	4038.69	4096.11	4125.29	4184.82	4246.34
Digestible energy (kcal/kg) ¹	3178.74	3177.67	3171.17	3181.46	3190.28
Crude fiber (g/kg)	32.6	31.2	32.1	33.2	33.3
Ether extract (g/kg)	43.9	49.5	46.5	46.0	44.1
Total calcium (g/kg)	15.6	16.0	16.0	16.1	16.2
Available phosphorus (g/kg) ²	7.2	7.1	7.2	7.3	7.4
Methionine (g/kg)	8.0	8.1	8.0	8.1	8.0
Lysine (g/kg)	17.1	17.2	17.2	17.2	17.1
Digestible protein:digestible energy (mg/kcal)	59.3	71.4	82.7	94.3	122.2

¹ Calculated based on the values of digestible energy and protein for Nile tilapia (Pezzato et al., 2002).

² Calculated for Nile tilapia (Miranda et al., 2000).

lower initial weight, showed higher values for weight gain and specific growth rate and better feed conversion ratio, while fish of block B2, with greater initial weight, showed higher values for final length and condition factor.

For protein efficiency ratio and specific growth rate, positive linear effects of dietary protein levels were observed. For feed conversion ratio, negative linear effect of dietary protein levels was observed. There was a quadratic effect of protein level of the diet on weight gain, final length, feed intake and condition factor, and 409.8, 366.2, 317.4 and 365.0 g of CP/kg of diet were respectively, the estimated values to improve their productive performance parameters. There was no significant difference in the survival rate of the fish (Table 3).

The worst results for the growth and efficiency of nutrient utilisation by fish fed diets with lower protein indicate that they did not ingest the amount of protein needed to meet their nutritional requirements. These results can also be related to the higher carbohydrate content of these diets (Martinez-Palacios et al., 2007; Giri et al., 2011). Diets with high levels of carbohydrates decrease the activity of digestive enzymes and digestibility of carbohydrates and proteins, resulting in lower growth (Ufodike & Matty, 1983).

The improvement in feed conversion ratio for juvenile *Trichogaster lalius*, with increasing levels of crude protein in the diet, was probably due to the lower feed intake to meet the protein requirement. Similar results were observed for hybrid *Clarias batrachus* × *Clarias gariepinus* (Giri et al., 2003) and *Horabagrus brachysoma* (Giri et al., 2011).

A quadratic effect of dietary protein levels was observed on the concentration of total ammonia nitrogen in water, where the highest estimated value was obtained in water of fish fed 374.0 g CP/kg. For the amount of non-ionised ammonia in the water there was a positive linear effect of protein level in the diet (Table 4), which corroborated the results observed by Dosdat et al. (1996) for five species of fish (marine and freshwater). In general, the ammonia content in water is related to the excretion of nitrogen from amino acid catabolism by fish (Melo et al., 2006). Although higher levels of protein in the diet improved the efficiency of protein utilisation by juvenile *Trichogaster lalius*, the catabolism of amino acids may have been greater due to the low supply of lipids by the diets. Diets with lower lipid contents cause greater protein catabolism to meet the requirement for energy and consequent increase in nitrogen excretion (Santinha et al., 1999; Green et al., 2002; Li et al., 2010). Thus, further studies are needed to evaluate the

Table 3 - Mean values of productive performance parameters of juvenile *Trichogaster lalius*, blue variety, according to the levels of crude protein in the diet

Variables	Crude protein levels in the diet (g/kg)					CV (%)	P-value		
	230	270	310	350	390		Linear	Quadratic	Lack of fit
Survival rate (%) ^{ns}	95.0	97.5	100.0	90.0	95.0	5.80	0.3083	0.2511	0.3449
Weight gain (g) ¹	0.70	0.82	0.94	1.15	0.97	9.74	0.0001	0.0081	0.0235
Final length (mm) ²	35.76	36.48	36.87	38.14	37.30	1.11	0.0001	0.0117	0.0089
Apparent feed intake (g) ³	4.06	4.31	4.24	4.48	4.01	4.02	0.7840	0.0030	0.0508
Feed conversion ratio ⁴	6.26	5.31	4.59	3.92	4.19	14.02	0.0002	0.0704	0.2652
Protein efficiency ratio ⁵	0.93	1.16	1.32	1.57	1.56	3.89	0.0001	0.0018	0.0144
Specific growth rate (%/day) ⁶	0.54	0.59	0.65	0.75	0.66	9.86	0.0012	0.0520	0.1258
Condition factor ⁷	4.05	4.20	4.21	4.35	4.18	1.96	0.0064	0.0065	0.0814

CV - coefficient of variation; ns - not significant.

¹ - $0.00002x^2 + 0.01639x - 1.88150$ ($R^2 = 0.823$).

² - $0.00010x^2 + 0.07323x + 24.03740$ ($R^2 = 0.820$).

³ - $0.00005x^2 + 0.03174x - 0.56507$ ($R^2 = 0.636$).

⁴ - $0.01381x + 9.1345$ ($r^2 = 0.856$).

⁵ $0.00416x + 0.0196$ ($r^2 = 0.942$).

⁶ $0.00101x + 0.32413$ ($r^2 = 0.639$).

⁷ - $0.00002x^2 + 0.01460 + 1.84269$ ($R^2 = 0.770$).

Table 4 - Mean values of total ammonia nitrogen and non-ionised ammonia in the water of aquaria of juvenile *Trichogaster lalius*, blue variety, according to the levels of crude protein in the diet

Variables	Crude protein levels in the diet (g/kg)					CV (%)	P-value		
	230	270	310	350	390		Linear	Quadratic	Lack of fit
N-NH ₃ (mg/L) ¹	2.83	5.38	8.72	9.85	9.44	19.87	0.0001	0.0092	0.5107
NH ₃ (mg/L) ²	0.0055	0.0081	0.0161	0.0156	0.0169	33.16	0.0003	0.1761	0.2623

N-NH₃ - total ammonia nitrogen; NH₃ - non-ionised ammonia; CV - coefficient of variation.

¹ N-NH₃ = $-0.00036x^2 + 0.26929x - 40.1961$ ($R^2 = 0.980$).

² NH₃ = $0.00008x - 0.01128$ ($r^2 = 0.824$).

interaction between protein and lipid levels in diets for this species.

The results obtained in this study are similar to those reported by Shim et al. (1989), who observed that the diet with 350 g CP/kg diet resulted in higher growth, and a higher percentage of vitellogenic oocytes for *Trichogaster lalius* (the wild variety).

As body length is a defining characteristic of prices in the marketing of ornamental fish, it is recommended to use diets with 366.2 g of CP/kg diet for feeding *Trichogaster lalius*. Moreover, the possibility of using diets with low protein levels without affecting the performance of the animal contributes to reducing the amount of nitrogen excreted in the water, making fish farming environmentally sustainable, especially in intensive systems, which rely exclusively on balanced diets (Furuya et al., 2005).

Conclusions

Diets with 366.2 g of CP/kg meet the nutritional requirement of protein for juvenile *Trichogaster lalius*, blue variety.

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