DIETARY CRUDE PROTEIN LEVELS FOR JUVENILE BETA

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ABSTRACT

The aim of this study was to evaluate the dietary crude protein requirements of juvenile *Betta splendens*. Isocaloric diets were used with six levels (27, 31, 35, 39, 43 e 47%) of crude protein (CP) in entirely randomised design with four repetitions. Fish with an average weight of 0.11 ± 0.02g were fed three times a day for 30 days. At the end of the experiment were evaluated: survival rate (SR); weight gain (WG); feed intake (FI); protein intake (PI); feed conversion (FC); protein efficiency ratio (PER); and specific growth rate (SGR). For FI, FC and PER, a negative linear effect of dietary CP levels was observed ($p < 0.05$). A quadratic effect of dietary CP levels for SR, WG and SGR was also observed and the estimated values which maximise the respective parameters were 30.95, 33.13 and 29.55% of CP. Thus, we concluded that the requirement for protein for juvenile *Betta splendens* is between 30 and 33% CP.

Keywords: growth; nutritional requirements; ornamental fish; productive performance

NÍVEIS DE PROTEINA BRUTA EM DIETAS PARA JUVENIS DE BETA

RESUMO:

Com o presente estudo objetivou-se avaliar as exigências por proteína bruta para juvenis de *Betta splendens*. Foram utilizadas dietas isocalóricas com seis níveis de proteína bruta (27, 31, 35, 39, 43 e 47%), em delineamento inteiramente casualizado, com quatro repetições. Peixes com peso médio de 0,11 ± 0,02g foram alimentados três vezes ao dia, por 30 dias. Ao final do experimento avaliou-se: taxa de sobrevivência (TS), ganho de peso (GP), consumo de ração (CR), consumo de proteína (CP), conversão alimentar (CA), taxa de eficiência protéica (TEP) e taxa de crescimento específico (TCE). Para os parâmetros de desempenho produtivo CR, CA e TEP observou-se efeito linear negativo dos níveis de proteína bruta na dieta ($p < 0,05$). Observou-se efeito quadrático dos níveis de proteína da dieta para TS, GP e TCE, sendo 30,95%, 33,13% e 29,55% de PB os valores estimados para maximizar os respectivos parâmetros de desempenho produtivo. Dessa forma, conclui-se que a exigência por proteína para juvenis de *Betta splendens* está entre 30 e 33% PB.

Palavras-chave: crescimento; desempenho produtivo; peixes ornamentais; exigências nutricionais

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INTRODUCTION

The ornamental fish trade may be considered a significant sector of economic aquaculture, due to the high values achieved in exports. Approximately 1 billion ornamental fish are exported annually, involving the trade of more than 1000 species (Dykman, 2012). Ornamental fish exports are worth US$ 278 million annually in the world and the wholesale trade value is around US$ 1 billion per year (FAO, 2005). However, despite their economic importance research on ornamental fish has only recently earned a prominent space in literature.

The successful domestication and captive breeding of a particular fish species depends on a thorough knowledge of genetic, environmental and reproductive factors, as well as nutritional requirements (WOODS III, 2001). Among these factors, nutritional requirements should be highlighted for ensuring rapid growth and high efficiency use of nutrients necessary for the health of the fish.

In order to establish the optimal nutritional requirements of an animal species, it is essential to first estimate dietary protein requirements, since this is the main nutrient required for growth, enzyme synthesis, and hormone and neurotransmitter production. Moreover, protein levels should be optimized for economic reasons, because it is an expensive dietary component. Utilization of diets lacking in protein results in reduced growth and weight loss. However, when dietary protein levels are excessive only a part of the protein is actually used for protein synthesis (growth) and the remainder is used for obtaining energy (WILSON, 2002) by deamination of amino acids. Therefore, excess protein in diets causes fish to excrete more ammonia, resulting in eutrophication of water, increased fish stress and mortality rates (WU, 1995).

Among the many different species of ornamental fish the beta *Betta splendens* stands out as a species of lush beauty: a function of its wide variety of colors and fin shapes. In addition, their hardiness and the presence of an accessory air breathing system allows beta to be reared in small aquariums with no need for an aeration system. Such characteristics create a differentiated consumer market for this species (Zuanon et al., 2007). The *B. splendens* is a carnivorous species (THONGPRAJUKAEW et al., 2010; 2011) that feeds on their natural habitat live foods as tubifex worms, Daphnia, small aquatic insects and larvae of mosquitoes and Chironomus (JAMES and SAMPATH, 2003).

JAMES and SAMPATH (2003) evaluated the protein requirements for beta and observed that fish fed with a diet containing 35% crude protein had higher growth rates and feeding efficiency. However, further studies are necessary to evaluate more precisely the protein demands of the species because in the above mentioned study fish were fed only once a day. In fact, feeding rate has been reported to be one of the main sources of variation among results from experiments performed to evaluate nutritional requirements in fish (LUPATSCH *et al.,* 2001). As growth is usually used as a criterion to estimate protein nutritional requirements it is essential that fish be fed until they are satiated, in order to ensure adequate nutrient intake (LUPATSCH *et al.,* 2001; LUPATSCH, 2005). Thus, the aim of the present study was to determine the crude protein requirements for juvenile *Betta splendens*.

MATERIAL AND METHODS

In the present study an entirely randomised design was used with six treatments (isocaloric diets with six levels of crude protein: 27, 31, 35, 39, 43 e 47%CP) and four repetitions. The experimental units were tanks containing eight fish each. Juvenile beta, *Betta splendens* were obtained from multiple spawns from a fish farm in Rosario da Limeira MG region (Brazil), with 30 days age and initial average weight and standard deviation of 0.11 ± 0.02g. Fish were kept in 24 tanks (6L) with biological filtration (internal filter with mechanical filtration and substrate for development of nitrifying bacteria), airing and temperature maintenance systems (26 ± 1 °C). Fish were fed ad libitum three times a day at 07:00, 13:00 and 17:00, for 30 days. The tanks were siphoned weekly to remove the feces.

Experimental feeds (Tables 1 and 2) were designed to be isocaloric (3,180 kcal of digestible energy/Kg of feed) based on the feed chemical composition (ROSTAGNO *et al.*, 2005) and availability of nutrients for Nile tilapia (MIRANDA et al., 2000; PEZZATO *et al.*, 2002). In order to prepare the feeds, ingredients were finely ground, mixed manually, moistened with 50 ± 5 °C water for starch gelatinisation and then pelleted using a meat grinder. Feeds were dried in a forced-air oven at 50°C for 24 hours, ground and sieved in order to obtain grains suitable in size for the mouths of the fish (2 mm diameter).

Table 1. Percentage composition of the experimental diets for juvenile *Betta splendens* fed diets containing different levels of crude protein for a period of 30 days.

	Crude protein levels of experimental diets (%)					
Ingredients	**27**	**31**	**35**	**39**	**43**	**47**
Soybean meal	8.00	14.27	18.00	19.60	21.90	22.00
Corn gluten	0.00	3.00	8.00	14.50	21.00	28.50
Fish meal	30.00	30.00	30.00	30.00	30.00	30.00
Corn meal	38.64	31.83	23.32	15.64	9.13	2.80
Wheat bran	20.00	18.00	18.00	18.00	16.50	15.57
L - Lysine	0.66	0.46	0.30	0.18	0.07	0.00
DL - Methionine	0.37	0.31	0.25	0.15	0.07	0.00
Soybean Oil	1.10	0.90	0.90	0.70	0.20	0.00
Dicalcium Phosphate	0.70	0.70	0.70	0.70	0.60	0.60
Vitamin C[a]	0.06	0.06	0.06	0.06	0.06	0.06
Common salt	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin supplement[b]	0.10	0.10	0.10	0.10	0.10	0.10
Mineral supplement[c]	0.10	0.10	0.10	0.10	0.10	0.10
BHT[d]	0.02	0.02	0.02	0.02	0.02	0.02

[a] Ascorbyl monophosphate with 35% active principle.
[b]Assurance levels of the vitamin supplement calculated for this feed (Mogiana Alimentos S/A – GUABI): Vitamin A, 16,000 UI; Vitamin D, 4,500 UI; Vitamin E, 250 mg; Vitamin K, 30 mg; Vitamin B_1, 32 mg; Vitamin B_2, 32 mg; Vitamin B_{12}, 32 mcg; Vitamin B_6, 32mg; Vitamin C, zero; Panthotenic Acid, 80 mg; Niacin, 170 mg; Biotin, 10 mg; Folic Acid, 10 mg; Colin, 2,000 mg.
[c] Assurance levels of the mineral supplement calculated for this feed (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.
[d] Butylated hydroxy toluene (antioxidant).

The following productive performance parameters were evaluated: survival rate (SR); weight gain (WG); feed intake (FI); protein intake (PI); feed conversion (FC); protein efficiency ratio (PER); and specific growth rate (SGR). Parameters were obtained according to the following formulas:

SR = (final number of fish * 100)/initial number of fish

WG = final weight (g) – initial weight (g);

FI = (initial feed weight (g) - final feed weight (g))/number of fish;

PI = FI (g) x protein content of diet (%)/100;

FC = feed intake (g)/weight gain (g);

PER = PI (g)/weight gain (g);

SGR = ((Ln final weight (mg) – Ln initial weight (mg))/experimental period (days)) x 100

Table 2. Calculated chemical composition of the experimental diets for juvenile *Betta splendens* fed diets containing different levels of crude protein for a period of 30 days.

	Crude protein levels of experimental diets (%)					
	27	**31**	**35**	**39**	**43**	**47**
Crude protein (%)	27.25	31.03	35.01	39.00	43.17	47.04
Digestible protein (%)[a]	22.69	26.23	30.01	33.83	37.82	41.56
Gross energy (kcal/kg)	4096.11	4125.29	4184.82	4246.34	4296.36	4367.70
Digestible energy (kcal/kg)[a]	3177.67	3171.17	3181.46	3190.28	3186.65	3200.84
Crude fibre (%)	3.12	3.21	3.32	3.33	3.27	3.15
Crude Lipids (%)	4.95	4.65	4.60	4.41	3.91	3.75
Total calcium (%)	1.60	1.60	1.61	1.62	1.60	1.60
Available phosphorus (%)[b]	0.71	0.72	0.73	0.74	0.72	0.73
Methionine (%)	0.81	0.80	0.81	0.80	0.81	0.83
Lysine (%)	1.72	1.72	1.72	1.71	1.72	1.73

[a] Values were calculated based on the digestible energy and protein for nile tilapia (PEZZATO *et al*., 2002).
[b] Values calculated for nile tilapia (MIRANDA *et al.*, 2000).

The evaluation of the effect of dietary crude protein levels on productive performance parameters was conducted using analysis of variance and regression to the 5% probability level. In order to choose the most suitable regression model we considered the significance of the regression coefficients using the t test, the magnitude of determination coefficients, calculated as a function of the sum of treatment squares and the behaviour of the studied variables.

RESULTS

Results showed negative linear effect ($p < 0.05$) of dietary crude protein levels on feed intake, feed conversion, and protein efficiency ratio. A quadratic effect of dietary protein levels for survival rate, weight gain, and specific growth rate was observed (Table 3). The values that maximize the survival rate (30.95% CP), weight gain (33.13% CP) and specific growth rate (29.55% CP) were estimated from the mathematical expressions obtained. No significant effect of crude protein levels for protein intake was observed (Table 3).

Table 3: Average values and variation coefficients of productive performance parameters for juvenile *Betta splendens* fed diets containing different levels of crude protein for a period of 30 days.

Productive performance parameters	**Crude protein levels of the experimental diets (%)**						
	27	**31**	**35**	**39**	**43**	**47**	**CV (%)**
Survival rate (%) [a]	100.00	96.88	93.75	100.00	90.63	81.25	11.55
Weight gain (g) [b]	0.55	0.58	0.61	0.50	0.47	0.40	19.45
Feed intake (g) [c]	1.24	1.13	1.12	0.95	0.89	0.69	12.85
Protein intake (g) [ns]	0.33	0.35	0.39	0.37	0.38	0.33	14.17
Feed conversion [d]	2.24	1.97	1.82	2.01	1.93	1.75	10.98
Protein efficiency ratio [e]	1.66	1.64	1.57	1.32	1.21	1.22	9.85
Specific growth rate (%/day) [f]	6.17	6.15	6.41	5.67	5.49	5.12	9.09

[a] $SR = -0.0628x^2 + 3.8867x + 38.8156$; with $R^2 = 78.36\%$
[b] $WG = -0.0008x^2 + 0.053x - 0.2611$; with $R^2 = 89.58\%$
[c] $FI = -0.0258x + 1.9575$; with $R^2 = 95.27\%$
[d] $FC = -0.0172x + 2.59$; with $R^2 = 57.01\%$
[e] $PER = -0.0267x + 2.4259$; with $R^2 = 90.03\%$
[f] $SGR = -0.004x^2 + 0.2364x + 2.6957$; with $R^2 = 87.71$
ns – not significant ($p>0.05$)

DISCUSSION

The feed intake by juvenile beta *Betta splendens* decreased with increasing levels of dietary crude protein. These results demonstrate that fish are capable of self-regulating their feed intake to meet protein demands for growth. Therefore, the quantity in grams of protein consumed by fish did not vary as a function of protein levels in the experimental diets (Table 3). Similar results were observed for *Melanogrammus aeglefinus* (KIM and LALL, 2001) and *Pseudobagrus fulvidraco* (KIM and LEE, 2005).

The feed conversion ratio for juvenile beta was improved with increasing levels of dietary crude protein. These results are probably due to the fact that intake of diets with higher protein content allows the fish to consume smaller quantities of feed to meet protein demands for growth. Similar results were observed in the *hybrid Clarias batrachus* × *Clarias gariepinus* (GIRI *et al.*, 2003).

In general, carnivorous fish require high protein levels in the diet (ITUASSÚ *et al.*, 2005; VERAS *et al.*, 2010). However, for some carnivorous species of ornamental fish the protein requirements were relative low, like for the *Pterophyllum scalare* (ZUANON *et al.*, 2006; RIBEIRO *et al.*, 2007; ZUANON *et al.*, 2009). For *Betta splendens*, crude protein levels higher than 29.55% promoted reduction in the specific growth rate while levels higher than 33.13% promoted a reduction in weight gain, indicating that excess protein in the diet reduces fish growth. The reduction in fish growth may have occurred due to the higher energy expenditure for the catabolism of excess protein and amino acids of feed (JAUNCEY 1982; MOHANTA *et al.*, 2008; CORNELIUS *et al.*, 2014), increasing ammonia levels in water. Chronic exposure to ammonia can worsen the growth performance of the fish, as observed in *Dicentrarchus labrax* (LEMARIE *et al.*, 2004), in *Pelteobagrus vachelli* (LI *et al.*, 2014) and *Cyprinus carpio* (DEVARAJ *et al.*, 2014). To LI *et al.* (2014), the lowest growth in fish exposed to ammonia is due to worsen in feed conversion, as observed for *D. labrax* (DOSDAT *et al.*, 2003) and *Scophthalmus maximus* (RASMUSSEN and KORSGAARD 1996).

The reduction in protein efficiency ratio, as a function of increased dietary protein levels observed in beta in the present study, has also been reported to occur in other species: for example juvenile *Bidyanus bidyanus* (YANG *et al.*, 2002) and *Pseudobagrus fulvidraco* (KIM and LEE, 2005). This effect probably occurs because part of the dietary protein is used as energy when fish are fed with diets containing excessive protein (YANG *et al.*, 2002; MELO *et al.*, 2006), thus decreasing the efficiency with which dietary protein is used for growth. Moreover, diets with lower protein levels have higher carbohydrate contents due to the higher percentage of corn meal (Table 1) and lower protein / energy ratio. The greater availability of energy from non-protein ingredients may have contributed to the increased nitrogen retention, resulting in lower elimination of ammonia by fish (ENGIN and CARTER, 2001).

The higher the efficiency with which dietary protein is used, the lower the catabolism of amino acids and the quantity of nitrogen excreted in water (RUOHONEN *et al.*, 1999; MELO *et al.*, 2006), thus advancing the maintenance of quality fish-farming water and minimising potential pollutants from fish-farming effluents. Decreased nitrogen excretion in water is particularly important in ornamental fish farming because these fish are raised in small tanks and ponds (ZUANON *et al.*, 2011) where the chances of ammonia levels reaching values harmful to fish is higher. Therefore, the use of feed containing 27% CP in beta feeding may contribute to a reduction of eutrophication in fish-farming water and effluents.

The reduction of fish survival rates with increasing dietary crude protein levels (from 30.95% CP) is probably related to increased catabolism of amino acids and excretion of ammonia in water. Even with the use of biological filters ammonia may have reached critical levels for the species, thus increasing the mortality among fish fed with diets containing excessive protein. The increased excretion of nitrogenous products in fish fed with diets containing high protein levels was also reported for the Australian eel *Anguilla australis australis* (ENGIN and CARTER, 2001), for the silver perch *Bidyanus bidyanus* (YANG *et al.*, 2002) and for catfish *Rhamdia quelen* (MELO *et al.*, 2006).

JAMES and SAMPATH (2003) evaluated protein nutritional requirements for beta and observed that fish fed with a diet containing 35% CP displayed higher growth rates and feed efficiency. Differences between the optimal dietary protein levels obtained in the present study (30 - 33% CP) and those reported by JAMES and SAMPATH (2003) (35% CP) might be related to the feeding rates used, three times a day in the present study versus once a day in JAMES and SAMPATH's study (2003), since nutritional requirements are affected by the feed intake (ARZEL *et al.*, 1998; MCGOOGAN and GATLIN III 1998). CHO and JO (2002) observed improved weight gain, specific growth rates and protein efficiency rates for nile tilapia *Oreochromis niloticus* fed twice a day compared to fish fed once a day with the same feed quantity. In addition, LUPATSCH *et al.*, (2001) observed that protein demands for *Sparus aurata* were influenced by the feed intake rate and attributed such difference to the capacity of that species to intake large feed quantities due to the increased stomach capacities of some fish, thus enabling more efficient use of diets with lower energy and protein levels.

CONCLUSION

We concluded that the requirement for protein for juvenile *Betta splendens* is between 30 and 33% crude protein.

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