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Effect of Dietary Protein Level on Growth Performance and Nitrogen Excretion of the Juvenile Convict Cichlid, *Amatitlania nigrofasciata*

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Abstract: A 8 weeks growth trial was conducted to determine the effects of dietary protein on the growth of the convict cichlid *Amatitlania nigrofasciata* and on water quality in closed recirculating systems. Four test diets were formulated to contain protein levels ranging 30-45%. The fish averaging 0.47±0.07 g were reared in 10 L aquarium. Fish were fed *ad libitum* at 08:30, 12:30 and 16:30 for 8 weeks. A second-order polynomial regression between dietary protein levels and weight gain in each group, demonstrated that the most suitable protein level for maximum growth was determined to be 40.8%. The feed conversion ratios of fish ranged from 1.78-2.78 in the experimental groups. Results showed significant differences in SGR and WG. Dietary protein level significantly influenced ammonia excretion, fish fed 45% protein excreted higher concentrations of ammonia-nitrogen than fish fed 30-40% dietary protein. For optimum growth, feed conversion and ammonia excretion, a diet containing 40.8% protein, 12% lipid and 20.8 kJ gross energy/kg diet is recommended for juvenile convict cichlids, *A. nigrofasciata*.

Key words: Growth, protein requirements, *Amatitlania nigrofasciata*, ornamental fish, ammonia excretion

INTRODUCTION

Ornamental fish farming is an important industry because of their great commercial value in the pet market (Lim and Wong, 1997). Cichlids are popular freshwater ornamental fish (Alderton, 2008). The convict cichlid (*Amatitlania nigrofasciata*) is a member of Cichlidae, it is traded as a commercial ornamental aquarium fish all over the world (Lever,1996).

For efficient and economical fish production it is important to know the nutritional requirements of species. There are some studies on the nutritional requirements of ornamental fish species (Ergun *et al*., 2010; Guroy *et al*., 2012; Gullu *et al*., 2008; Zuanon *et al*., 2006). Nutrition plays an important role in fish health, behavior and improving the external appearance and color of ornamental fish (Miller and Mitchell, 2008; Baron *et al*., 2008). Protein is a major dietary nutrient for good growth performance and health of fish (NRC, 2011). Dietary proteins are expensive and their inclusion in fish diets causes increasing enhancementof the feed cost as well as the excretion of nitrogen compounds with negative effects on the environment (Guroy *et al*., 2012).

Generally, ornamental fish are grown in aquarium systems. The excretion of ammonia is directly correlated to dietary protein and protein intake in fish (Wagner *et al*., 1995; Brunty *et al*., 1997). The excess protein supplied in feeds increases the nitrogen compound excretion in the culture water and affects fish growth and welfare (Catacutan and Coloso, 1995; Tibbetts *et al*., 2000). Determination of the optimum dietary protein level is therefore important to obtain maximum growth with cost effective and environmentally friendly diets.

Currently, there is little information on the dietary protein requirements of the convict cichlid. The present study was conducted to determine the effects of dietary protein levels on growth performance, feed utilization and nitrogen excretion of convict cichlid's *Amatitlania nigrofasciata*.

MATERIALS AND METHODS

Four isoenergetic (20 kJ kg^{-1}) diets were formulated containing crude protein levels of 30, 35, 40 and 45% (Table 1). Fishmeal and defatted soybean meal were the main protein sources, wheat meal was the carbohydrate

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Table 1: Ingredients and chemical composition of experimental diets

	Dietary protein level (%)			
Compositions	30	35	40	45
Ingredients (%)				
Fish meal	27.50	35.50	43.00	51.00
Corn starch	26.50	19.50	12.00	5.50
Soybean meal	20.00	20.00	20.00	20.00
Wheat meal	15.00	15.00	15.00	15.00
Fish oil	9.00	8.00	8.00	6.50
Vitamin-mineral mix[1, 2]	2.00	2.00	2.00	2.00
Total	100.00	100.00	100.00	100.00
Chemical analyses (%)				
Protein	30.00	35.20	40.00	45.20
Fat	12.10	11.90	12.70	12.00
Ash	5.35	6.55	7.67	8.87
NFE[3]	50.50	44.40	37.70	32.00
Energy (kJ g^{-1})[4]	20.44	20.55	20.86	20.84

[1]Vitamin Mix: Vit. A: 18000 IU; Vit. D3: 2500 IU; Vit. E: 250 mg kg^{-1}; Vit. K3: 12 mg kg^{-1}; Vit. B1: 25 mg; Vit. B2: 50 mg; Vit. B3: 270 mg; Vit. B6: 20 mg; Vit. B12: 0.06 mg; Vit. C: 200 mg; Folic acid: 10 mg; Calcium d-pantothenate: 50 mg; Biotin: 1 mg; Inositol: 120 mg; Choline chloride: 2000 mg; [2]Mineral Mix: Fe: 75.3 mg; Cu: 12.2 mg; Mn: 206 mg; Zn: 85 mg; I: 3 mg; Se: 0.350 mg; Co: 1 mg; [3]Nitrogen-Freeextracts (NFE) = Matter-(Crudelipid+Crudeash+Crude protein); [4]Energy calculated according to 23.6 kJ g^{-1} protein, 39.5 kJ g^{-1} lipid and 17.0 kJ g^{-1} NFE

source and fish oil the major lipid source. Diets were prepared by thoroughly mixing the dry ingredients with oil and water in a laboratory mixer and extruding the moist mixture through a 1 mm die. The moist pellets were fan-dried and the pellets were crushed into desirable particle sizes (100-150 μm) then stored frozen at -20°C until use.

Convict cichlid, *Amatitlania nigrofasciata* were produced in Aquarium Unit of Inebolu Vocational School, Kastamonu University, Turkey. Prior to the start of the feeding trial, fish were transferred to a 100 L aquarium and fed a commercial diet (35% protein and 10% fat) for 2 weeks to adjust to the experimental conditions. After the conditioning period, ten fish weighing 0.47±0.07 g were randomly stocked into the twelve glass aquariums of 10 L each filled with dechlorinated tap water. There were three replicates per treatment with controlled photoperiod (12 h light: 12 h dark). Fish were fed to apparent satiation three times a day at 08:30, 12:30 and 16:30 h for 8 weeks.

The aquariums water quality was monitored daily. Water temperature was maintained at 26.6±0.7°C, dissolved oxygen at 7.08±0.37 mg L^{-1} and pH at 8.19±0.3.

Determination of TAN excretion: At the end of the growth trial (8 weeks), each group of fish was kept in a tank (30 L) at 26°C in order to measure ammonia excretion rates. Fish were fed the diets to apparent satiation. After 25 min, the water inflow and air flow was stopped. Water samples were taken every hour for 6 h and total ammonia concentrations (NH_4^+ and NH_3) were analyzed by the Nessler Method with a LovibondMultidirect photometer (Lovibond, Germany) (Ergun *et al*., 2010) according to the following equation:

$$A = \frac{(N2\text{-}N1XV2)/B}{T2\text{-}1}$$

Where:

- A = Ammonia excretion rate (mg total NH_3-N/kg wet fish weight/h)
- N1 = Ammonia concentration at time 1 (mg total NH_3-N/L)
- N2 = Ammonia concentration at time 2 (mg NH_3-N/L)
- V2 = Volume of the medium at time 2 (mL)
- B = Wet weight of the fish (g)
- T2-1 = Time interval between samplings 1 and 2 (h)

Sampling and chemical analyses: Proximate analyses of the feedstuffs, diets and fish were performed using standard methods (AOAC, 2000). Dry matter was measured by drying at 105°C until a constant weight was achieved, crude lipid was determined by ether extraction, crude protein by the Kjeldahl Method after acid digestion using a Behr System and crude ash by incineration at 525°C for 12 h in a muffle furnace. Nitrogen-Free Extracts (NFE) were calculated as:

$$NFE = 100\text{-}(Protein\% + Lipids\% + Ash\% + Fiber\ \%)$$

Gross energy was calculated using the conversion factors of 23.7 kJ g^{-1} for protein, 39.5 kJ g^{-1} for lipid and 17.2 kJ g^{-1} for carbohydrates.

Growth performance of convict cichlid fed with the different protein level diets was evaluated by calculating mean Weight Gain (WG), Specific Growth Rate (SGR), Feed Conversion Rate (FCR):

$$WG\ (\%) = \frac{\text{Final weight (g)-Initial weight (g)}}{\text{Initial weight (g)}} \times 100$$

$$SGR\ (\%\ day^{-1}) = \frac{\text{ln Final weight (g)-ln Initial weight(g)}}{\text{Days of the expr.}} \times 100$$

$$FCR = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$$

RESULTS AND DISCUSSION

The convict cichlid juveniles accepted the experimental diets well no mortality was recorded, throughout the experiment. Weight gain (%) at the end of experiment of the 8 weeks showed significant differences between groups (Table 2).

The best specific growth rate and feed conversion rate were obtained fed with 40% CP diet. The second-order polynomial regression between dietary protein levels and weight gain in each group, demonstrated that the most suitable protein level for maximum growth was 40.8% (Fig. 1).

The protein level in whole body of fish significantly differed among treatments and increased with increasing of protein level. Crude lipid and ash content of fish did not influenced by the dietary protein level (Table 3). The total ammonia excretion by fish in a 6 h period is shown in Fig. 2. Peak values developed at 4 h after feed distribution. The average amount of ammonia excretion in the water was obtained for the 30% CP (3.90 mg kg^{-1} fish/6 h), 35% CP (4.00 mg kg^{-1} fish/6 h), 40% CP (5.15 mg kg^{-1} fish/6 h) and 45% CP (5.67 mg kg^{-1} fish/6 h) groups (Fig. 2).

Table 2: Growth, feed utilization, ammonia-nitrogen excretion of convict cichlid fed diets with different protein levels

	Dietary protein level (%)			
Parameters	30% CP	35% CP	40% CP	45% CP
Initial weight	0.47±0.02	0.50±0.05	0.44±0.05	0.45±0.02
WG (%)	118.43±3.02^a	149.70±3.00^b	184.14±4.34^c	163.64±4.46^d
SGR (1% day^{-1})	1.30±0.02^a	1.52±0.03^b	1.74±0.04^d	1.61±0.03^c
FCR	2.78±0.09^c	2.11±0.18^b	1.78±0.13^{ab}	1.82±0.02^a
NH_3-N excretion (mg kg^{-1} fish)	3.90	4.00	5.15	5.67
Feed cost ($ kg^{-1})*	1.03	1.13	1.25	1.33

n = 10 fish per each group. Mean values sharing the same superscripts in the same row are significantly different (p>0.05); *Cost of feed ingredients as listed by Hammersmith marketing Ltd. (http://hammersmithltd.blogspot.com) May 2013

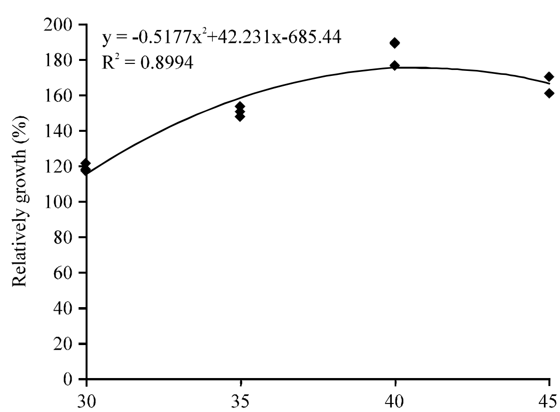

Fig. 1: Relationship between weight gain of convict cichlid and dietary protein level

The present study showed that the dietary protein level clearly influenced growth performance, feed utilization and ammonia excretion of convict cichlid. Polynomial regression analysis of the RGR in the study shows that 40.8% dietary protein was optimum when fishmeal was the main protein sources. Comparison of dietary protein levels of fish species is complicated because of fish size, diet formulation and rearing conditions among studies. Although, the optimum dietary protein level for juvenile convict cichlid determined by this study was lower than other carnivorous ornamental fish such as 45% for swordtails (*Xiphorus helleri*; Kruger *et al*., 2001) and 50% for discus (*Symphysodon* sp.; Chong *et al*., 2000). The protein requirement values for optimal growth for convict cichlid was similar to many other omnivorous ornamental fish such as redhead cichlid (41%; Olvera-Novoa *et al*., 1996), yellowtail (35%; Guroy *et al*., 2012) and electric blue cichlid (38.8%; Gullu *et al*., 2008). Royes *et al*. (2006) did not obtain significant increases in weight gain in *Haplochromis ahli* fed with increasing protein level and they suggested 36% protein level in diets. Royes *et al*. (2006) used average weight of about 2.0 g fish were fed for 8 weeks and gained twofold in final weight. In this study, fish of 0.17 g were fed experimental diets and gained 348.92 and 513.85% increases in final weight. The FCR values in this study obtained (1.81-3.18) showed similarities with omnivorous African cichlids *P. socolofi* (Royes *et al*., 2005). Body composition traits serve as important indicators for the requirements of essential

Table 3: Proximate body composition (%) of convict cichlid fed with different level of protein

Body composition	Dietary protein level (%)			
	30% CP	35% CP	40% CP	45% CP
Moisture	72.00±0.60	71.50±0.40	70.43±0.50	70.62±0.50
Protein	15.72±0.10^a	16.84±0.17^b	18.17±0.14^d	16.50±0.20^c
Lipid	6.41±0.04	6.44±0.02	6.43±0.05	6.43±0.02
Ash	3.89±0.04	3.88±0.05	3.90±0.02	3.88±0.04

Mean values sharing the same superscripts in the same row are significantly different (p>0.05)

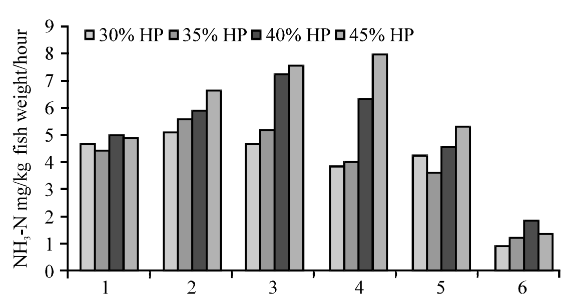

Fig. 2: Ammonia excretion rate of convict cichlid fed with different protein levels diet during the experiment

nutrient. From metabolic point, the data suggest that feeding dietary protein earlier optimum level could not be used for body protein synthesis or tissue building. Body protein content of the fish tented to increase significantly with the increasing of dietary protein up to 400 g kg^{-1}. Similar results were also recorded by Zehra and Khan (2012).

The other important factor which can minimize the nitrogenous waste is output of the fish (Ergun *et al*., 2010). In this study, protein level affected the total ammonia excretion at 3.90, 4.00, 5.15 and 5.67 mg kg^{-1} fish/6 h, respectively (Fig. 1). Similar results reported by Guroy *et al.* (2012) for yellow tail cichlid. It is known that the main internal source of ammonia in fish is through catabolism of proteins (Ip *et al*., 2001). The less protein that is catabolized, the more nitrogen is accumulated in the fish body, indicating that the dietary protein is used for growth than as source of energy (Yigit *et al*., 2003). Many other factors may affect the ammonia excretion level such as temperature, fish species, fish size, experimental design and other water parameters (Guroy *et al*., 2012).

The ornamental fish industry makes use of aquarium systems that are often closed systems. For this reason, water quality is very important to the health of fish. Ammonia is the end product of protein catabolism, uneaten feed and waste products. High levels of ammonia are toxic for the fish in the rearing systems. Therefore, suitable protein level and improved water quality are essential for successful aquarium rearing of fish.

CONCLUSION

Based on growth performance and ammonia excretions results 35.5% protein with 12% crude lipid is recommended for juvenile convict cichlids. Future, studies should investigate the effects of digestibility and amino acid requirements of this important ornamental species.

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