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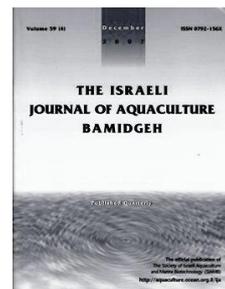
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## Effect of Dietary Protein Level on Growth Performance and Nitrogen Excretion of the Yellow Tail Cichlid, *Pseudotropheus acei*

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### Abstract

A 12-week growth trial was conducted to determine the effects of dietary protein on the growth of the yellow tail cichlid, *Pseudotropheus acei*, and on water quality in closed recirculating systems. Six test diets were formulated to contain protein levels ranging 25-50% by substituting corn oil and  $\alpha$ -starch for fishmeal. Diets were assigned to triplicate groups of 10 fish in a completely randomized design. The feed conversion ratios of fish fed the 35% and 50% crude protein diets were significantly ( $p < 0.05$ ) lower than that of fish fed the 30% protein diet. The protein efficiency ratio declined as the dietary protein level increased. Dietary protein levels significantly influenced total ammonia-nitrogen excretion (TAN); fish fed 50% protein excreted higher concentrations of ammonia-nitrogen than fish fed 25-35% dietary protein ( $p < 0.05$ ). Results suggest that inclusion of more than 35% protein in diets containing 10% crude lipid does not benefit juvenile yellow tail growth performance. Further, as TAN excretion and feed costs increased at protein levels beyond 35%, we recommend a diet containing 35% protein and 10% lipid for promoting good growth of juvenile yellow tail cichlids under the conditions used in the present trial.

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### Introduction

Ornamental fish are of growing importance because of their great commercial value in the pet market. Freshwater aquarists represent 90% of the global market of the ornamental fish industry, worth an estimated US\$1 billion annually (FAO, 2010). Cichlids are popular freshwater ornamental fish (Alderton, 2008) and amongst the nearly 4000 species and varieties traded worldwide. The yellow tail cichlid, *Pseudotropheus acei*, is popular due to its brilliant colors and because it may be kept in small aquaria (Axelrod et al., 2007).

Nutrition plays an important role in maintaining good health and normal behavior, and in enhancing the external appearance and color of ornamental fish (Miller and Mitchell, 2008; Baron et al., 2008). However, nutritional data for ornamental fish are largely based on extrapolation of results derived from related aquacultured fish species reared under intensive farming conditions. Protein is a major dietary nutrient affecting growth performance and health of fish (NRC, 1993). Dietary protein supplies essential and nonessential amino acids to synthesize body protein and energy for maintenance. Because feed proteins are expensive, their inclusion in fish diets significantly impacts overall feed costs. Thus it is important to optimize, for economic and environmental reasons, the amount of dietary protein in fish feeds (Wilson, 2002). Other advantages associated with an optimized dietary protein level include rapid and cost-effective growth, reduced environmental impact, and improved water quality. Generally, increasing dietary protein in fish diets leads to improved fish production, however, excess dietary protein is metabolized as energy and increases excretion of nitrogenous waste materials into the aquatic environment, which may be detrimental to fish growth and the culture environment.

Most ornamental fish are grown in closed aquarium systems where water is at least partially recycled. However, the excretion of ammonia, which is directly correlated to dietary protein and protein intake in fish (Wagner et al., 1995; Brunty et al., 1997), is toxic to fish and limits the carrying capacity of culture systems (Wright, 1995). Total ammonia-nitrogen (TAN) levels can reduce fish vigor and/or growth and, in extreme cases, cause mortality. Protein in fish feeds is the primary source of nitrogen excreted through fish gills as ammonium ions. Consequently, determination of the optimum dietary protein level is important to attain maximum growth with cost-effective and environmentally-friendly artificial diets. The objectives of the present study were to determine the effects of the dietary protein level on the growth of yellow tail cichlid, *P. acei*, and on nitrogen excretion.

### Materials and Methods

*Diets.* Six isoenergetic (20 MJ/kg) diets were formulated containing crude protein levels of 25%, 30%, 35%, 40%, 45%, and 50% (Table 1). Fishmeal and full-fat soybean meal were the protein sources, wheat was the carbohydrate 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 stored frozen at -20°C until use.

*Rearing systems and fish.* Eighteen 80-l circular tanks (diameter 50 cm), within a 4000-l recirculation freshwater system, were annexed to a 650-l sand filter system. The aquaria flow rate was 3 l/min and water quality was monitored daily. Water temperature was maintained at 26.7±0.06°C, dissolved oxygen at 8.1±0.06 mg/l, and pH at 8.14±0.06. The system was housed in a climate-controlled laboratory with controlled photoperiod (12 h light:12 h dark). Yellow tail were obtained from a local commercial aquarium (Zonguldak, Turkey) and transported to the Ornamental Fish Unit of Armutlu Community College, University of Yalova, Turkey. Prior to the start of the feeding trial, fish were transferred to a 100-l aquarium and fed a commercial tropical fish diet (48% protein, 10% lipid, 2% fiber, 10% ash; Tetra) for three weeks for acclimation. Juvenile yellow tails were randomly distributed amongst 18 tanks at a density of 10 fish per tank with three replicate tanks for each dietary treatment. Fish were fed three times daily (08:30; 12:30, 16:30) by hand to visual satiation. Daily feed intake was recorded to

Table 1. Ingredients, chemical composition, and amino acid contents of experimental diets.

	Dietary protein level (%)					
	25	30	35	40	45	50
Fishmeal <sup>1</sup>	31.5	39.2	47	54.8	62.5	70.2
Starch <sup>2</sup>	36.2	29.2	22	14.9	7.9	0.8
Wheat feed <sup>3</sup>	14	14	14	14	14	14
Soybean meal <sup>4</sup>	12	12	12	12	12	12
Fish oil <sup>5</sup>	4.4	3.7	3.1	2.4	1.7	1.1
Vitamin C <sup>6</sup>	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin mix <sup>7</sup>	1.0	1.0	1.0	1.0	1.0	1.0
Mineral <sup>8</sup>	0.5	0.5	0.5	0.5	0.5	0.5
Binder <sup>9</sup>	0.2	0.2	0.2	0.2	0.2	0.2
<i>Chemical composition</i>						
Crude protein (%)	24.99	29.96	34.99	40.02	44.99	49.96
Crude lipid (%)	9.98	9.96	10.04	10.03	10.00	10.08
Crude ash (%)	5.56	6.59	7.63	8.67	9.70	10.73
Crude fiber (%)	1.22	1.15	1.09	1.02	0.96	0.89
Nitrogen free extract (%)	58.26	52.34	46.25	40.25	34.34	28.33
Gross energy (MJ/kg)	19.9	20.0	20.2	20.4	20.5	20.7
<i>Essential amino acid composition</i>						
Methionine	0.73	0.85	0.98	1.10	1.22	1.34
Cystine	0.35	0.39	0.42	0.46	0.50	0.54
Lysine	2.19	2.61	3.03	3.46	3.87	4.29
Tryptophan	0.32	0.36	0.40	0.45	0.49	0.53
Threonine	1.09	1.26	1.43	1.61	1.78	1.95
Isoleucine	1.56	1.81	2.07	2.32	2.57	2.82
Histidine	0.68	0.78	0.88	0.99	1.09	1.20
Valine	1.44	1.66	1.88	2.10	2.32	2.53
Leucine	2.03	2.35	2.67	3.00	3.31	3.63
Arginine	1.66	1.91	2.18	2.44	2.70	2.96

<sup>1</sup> Anchovy fishmeal, Can Kardesler Fish Meal Corp., Samsun, Turkey

<sup>2</sup> Kepez Un, Çanakkale, Turkey

<sup>3</sup> Agromey Feed Mill Co., İzmir, Turkey

<sup>4</sup> Turtaş Corp., İzmir, Turkey

<sup>5</sup> Anchovy fish oil, Can Kardesler Fish Meal Corp., Samsun, Turkey

<sup>6</sup> Kartal Chemical Inc., Kocaeli, Turkey

<sup>7</sup> per g mixture: vitamin A 342 IU, vitamin D<sub>3</sub> 329 IU, vitamin E 0.0274 IU, vitamin K<sub>3</sub> 5.48 mg, vitamin B<sub>1</sub> 2.05 mg, vitamin B<sub>2</sub> 3.42 mg, vitamin B<sub>3</sub> 20.5 mg, vitamin B<sub>5</sub> 5.48 mg, vitamin B<sub>6</sub> 2.05 mg, vitamin B<sub>12</sub> 2.74 mg, vitamin C 24.0 mg; Kartal Chemical Inc., Kocaeli, Turkey

<sup>8</sup> per g mixture: biotin 0.411 mg, folic acid 0.685 mg, Zn 12.3 mg, Mn 4.80 mg, Cu 1.64 mg, I 0.274 mg, Se 0.0274 mg, Ca 125 mg, K 189 mg; Kartal Chemical Inc., Kocaeli, Turkey

<sup>9</sup> Guar gum, Kartal Chemical Inc., Kocaeli, Turkey

(% protein + % lipids + % ash + % fiber). Gross energy was calculated using the conversion factors of 23.7 kJ/g for protein, 39.5 kJ/g for lipid, and 17.2 kJ/g for carbohydrates (Brett and Groves, 1979). For essential amino acid analysis (excluding tryptophan), 50 mg of the diet was hydrolyzed in 10 ml 6 N HCl at 110°C for 24 h. After removal of the HCl by evaporation under vacuum, the amino acids were separated by ion-exchange chromatography on a Shimadzu RF-10AXL sodium column and detected following postcolumn derivatization with ninhydrin, by measuring absorbance at 350-450 nm.

*Evaluation of growth performance and production cost.* The feed conversion ratio (FCR), specific growth rate (%SGR; %/day), protein efficiency ratio (PER), and economic conversion ratio (ECR) were calculated as follows: FCR = feed intake/wt gain, SGR = 100 x ([ln final fish wt] - [ln initial fish wt])/experimental days, PER = (wt gain/dietary

calculate feed conversion ratio (FCR). Growth and FCR were monitored biweekly by collectively weighing the fish from each aquarium.

*Determination of TAN excretion.* After the feeding trial, fish were starved for three days to ensure evacuation of food from the gut. On the morning of the fourth day, tanks were thoroughly cleaned and fish were fed the appropriate diet to apparent satiation. Thirty min postprandial, the water flow was discontinued, uneaten food was removed, and the baseline total ammonia-nitrogen (TAN) level of the water was analyzed by the ammonia salicylate method using a NH<sub>3</sub>-N reagent kit from Hach and measured in a spectrophotometer (DR-2800, Hach, Loveland, CO).

*Sampling and chemical analyses.* Proximate analyses of the feedstuffs and diets 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. Crude fiber was determined by acid alkali hydrolysis and ignition of the dried sample for 3 h. Nitrogen-free extracts (NFE) were calculated as NFE = 100 -

protein intake, voluntary feed intake (%/day) = 100 x (feed intake/[initial fish wt + final fish wt]/2/days), ECR = feed cost x FCR.

**Statistical analysis.** Growth data were subjected to one-way analysis of variance (ANOVA) when differences among treatments were significant, Duncan's multiple range test was used to rank groups using Statgraphics 4.0 (Manugistics Inc., Rockville, MD) statistical software (Zar, 2001). The relationship between nitrogenous excretion rates and dietary protein levels was described by linear regression of the form  $y = a + bx$ , where  $y$  is the excretion rate of ammonia-nitrogen and  $x$  is the dietary protein content %DM (Engin and Carter, 2001). Regression analysis was used to describe the relationships between nitrogen intakes and ammonia-nitrogen excretion rates for individual tanks in each treatment. Differences were considered significant at 5%.

## Results

The yellow tail juveniles readily accepted the experimental diets and were in good health, with no mortality, throughout the experiment. Mean weights at the end of the 12-week nutrition trial ranged 2.68-2.94 g, with no significant differences between groups (Table 2). Although there were no significant differences in feed intake, FCR significantly varied and PER decreased as the dietary protein increased. The cost of diets increased with the dietary protein level. TAN generally increased with the dietary protein level (Fig. 1). TAN of fish fed the 25% (2.41 mg/100 g fish), 30% (2.71 mg/100 g fish), and 35% (2.74 mg/100 g fish) diets were significantly ( $p < 0.05$ ) lower than of fish fed the 50% (4.22 mg/100 g fish) protein diet. The relationship between dietary protein level ( $x$ , %) and mean TAN excretion ( $y$ , mg/100 g fish) was described as  $y = 0.0703x + 0.6276$ ,  $r^2 = 0.9275$ .

Table 2. Growth, feed utilization, ammonia-nitrogen excretion, and economic factors of yellow tail fed diets with different protein levels ( $n = 3$ ).

	Dietary protein level (%)					
	25	30	35	40	45	50
Initial mean wt (g)	0.93	0.93	0.93	0.93	0.93	0.93
Final mean wt (g)	2.76±0.07	2.68±0.05	2.93±0.19	2.86±0.02	2.77±0.09	2.94±0.11
Specific growth rate	1.30±0.04	1.25±0.01	1.37±0.10	1.33±0.02	1.30±0.04	1.37±0.06
Voluntary feed intake	0.40±0.01	0.39±0.02	0.43±0.02	0.41±0.01	0.41±0.01	0.42±0.01
Feed conversion ratio	2.77±0.12 <sup>ab</sup>	2.89±0.11 <sup>b</sup>	2.43±0.32 <sup>a</sup>	2.57±0.04 <sup>ab</sup>	2.64±0.10 <sup>ab</sup>	2.46±0.09 <sup>a</sup>
Protein efficiency ratio	1.45±0.06 <sup>c</sup>	1.17±0.08 <sup>b</sup>	1.18±0.05 <sup>b</sup>	0.97±0.09 <sup>a</sup>	0.84±0.27 <sup>a</sup>	0.81±0.10 <sup>a</sup>
NH <sub>3</sub> -N excretion (mg/100 g fish)	2.41±0.08 <sup>a</sup>	2.71±0.12 <sup>a</sup>	2.74±0.19 <sup>a</sup>	3.69±0.10 <sup>ab</sup>	3.61±0.08 <sup>ab</sup>	4.22±0.14 <sup>b</sup>
Feed cost (€/kg)*	0.71	0.80	0.90	1.00	1.09	1.19
FCR	2.77	2.89	2.43	2.57	2.64	2.46
Economic conversion ratio (€/kg)	1.97 <sup>a</sup>	2.31 <sup>ab</sup>	2.19 <sup>a</sup>	2.57 <sup>bc</sup>	2.88 <sup>c</sup>	2.93 <sup>c</sup>

Values in the same line with different superscripts significantly differ ( $p < 0.05$ ).

\*Costs of feed ingredients as listed by index mundi (<http://www.indexmundi.com/commodities/>) June 2010.

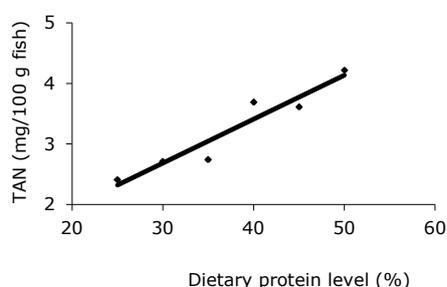


Fig. 1. Relationship between dietary protein level and ammonia-nitrogen excretion in yellow tail juveniles fed diets containing different levels of protein ( $n = 18$ ).

## Discussion

Although no significant differences were found in final weight or SGR, growth performance tended to increase as the protein content increased. The FCR of fish fed the 35% and 50% protein diets were significantly lower than that of fish fed the 30% protein diet. FCR improves as the protein level increases in some fish species (NRC 1993). Compared to the present trial, FCR for African cichlids, *Sciaenochromis ahli*, is relatively low (Güllü et al., 2008). FCR for omnivorous African cichlids, *Pseudotropheus socolofi*, is 2.75-3.35 (Royer et al., 2005).

We estimate that the protein requirement of

juvenile yellow tails fed diets containing 10% lipid is around 35% when using processed full fat soybean meal and fishmeal as the dietary protein sources. This level is within the range of 30-50% for a variety of ornamental fish species (Sales and Janssens, 2003; Güllü et al., 2008; Ergün et al., 2010) and close to the requirement of herbivorous or omnivorous species such as goldfish (29%; *Carassius auratus*; Lochmann and Phillips, 1994) and dwarf gourami (25%; *Colisa lalia*; Shim et al., 1989). In contrast, goldfish larvae require 53% crude dietary protein (Fiogbé and Kestemont, 1995). The difference in goldfish protein requirements between these two studies may be attributed to the age of the fish. Other factors also affect dietary protein requirements, including the dietary protein to energy ratio, the essential amino acid composition, digestibility of the dietary protein, the dietary protein source, environmental conditions, the experimental design, and the amount of non-protein energy sources in the diet (Wilson, 2002).

In the present study, the dietary essential amino acids increased with the dietary protein level. Although the essential amino acid requirements of *P. acei* are unknown, the lack of differences in growth between groups indicates that the dietary essential amino acids were adequate and that the requirements of *P. acei*, under the experimental conditions, must be below the levels provided by the 25% diet. The dietary crude essential amino acid levels determined in this study were near those of discus (*Symphysodon aequifasciata*; Chong et al., 2003) and lower than those of goldfish larvae, possibly because the important growth phase of larvae occurs during a short 20-day period or because a selective system of macromolecular protein absorption is lacking in larvae compared to juveniles (Fiogbé and Kestemont, 1995).

The PER significantly dropped as the dietary protein level increased, as in other ornamental fish species (Royes et al., 2005; Güllü et al., 2008; Ergün et al., 2010). This is likely because, when high protein diets are fed to fish, more dietary protein is used for energy, consistent with the linear increase in ammonia excreted by yellow tail as the level of dietary protein increased. The correlation coefficient of 0.9275 indicates that the linear model can explain 92.75% of the relationship between TAN and dietary protein level. Increased nitrogen content in the feed results in increased potential for ammonia formation because of the increased presence of the nitrogen atoms required to form ammonia. TAN levels in the present trial ranged 2.41-4.22 mg per 100 g feed, similar to values for Nile tilapia, *Oreochromis niloticus* (2.42-5.74 g; Brunty, 1997). Many factors may affect the TAN level including temperature, salinity, fish species, other water parameters, and experimental design.

In the ornamental fish industry, water quality is very important to the health and quality of a fish. The majority of aquaria utilize closed systems that recirculate water through a filter. Ammonia is produced in fish as an end product of protein catabolism and is also generated in the aquatic system by the breakdown of uneaten feed and waste. Ammonia is soluble in water, and minimal amounts are lost through evaporation. Even low levels of ammonia can be toxic to gills and skin, resulting in increased susceptibility to infection. Therefore, appropriate nutrition and good quality water are paramount to maintaining a successful aquarium.

In conclusion, there appears to be no significant benefit to growth of juvenile yellow tail cichlids when more than 35% protein is incorporated into diets containing 10% lipid while higher inclusion levels contribute to the deterioration of water quality.

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