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The effect of protein levels on growth, postprandial excretion and tryptic activity of juvenile mullet *Mugil platanus* (Günther)

Cristina V A de Carvalho[1], Adalto Bianchini[2], Marcelo B Tesser[1] & Luís A Sampaio[1]

[1]Laboratório de Piscicultura Marinha, Instituto de Oceanografia, Universidade Federal do Rio Grande, Rio Grande-RS, Brazil
[2]Laboratório de Zoofisiologia, Instituto de Ciências Biológicas, Universidade Federal do Rio Grande, Rio Grande-RS, Brazil

Correspondence: Luís A Sampaio, Universidade Federal do Rio Grande, Instituto de Oceanografia, Laboratório de Piscicultura Marinha, Rio Grande-RS, CP 474, Brasil, CEP 96201-900, Brazil. E-mail: sampaio@mikrus.com.br

Abstract

The objective of the present work was to determine the optimum dietary protein level for juvenile mullets. Five isocaloric diets were formulated to contain increasing levels (300, 350, 400, 450 and 500 g kg^{-1}) of crude protein (CP) corresponding to 18.7 MJ metabolizable energy kg^{-1}. All diets were tested in triplicate. Each experimental unit was composed of a 50 L tank with 50 juveniles (mean ± SE initial weight and length equal to 1.17 ± 0.02 g and 4.34 ± 0.03 cm respectively). Diets were offered five times a day until apparent satiation for 35 days. No significant difference ($P > 0.05$) was observed in survival rate, feed efficiency and body composition between treatments. However, weight gain, feed consumption and specific growth rate were higher in fish fed the 350 g kg^{-1} CP level than those fed the highest protein content diet (500 g kg^{-1} CP). The amount of postprandial ammonia excreted by mullet was linearly related to protein intake. Intestinal tryptic activity was inversely proportional to the percentage of dietary CP. It is likely that diets containing < 350 g kg^{-1} CP will be needed for on-growing mullet, especially when reared in ponds with abundant natural food.

Keywords: diet, excretion, feeding, fish, growth, nutrition, protein

Introduction

Fish of the Mugilidae family, known as mullets, are found worldwide in tropical and subtropical waters, especially in coastal and estuarine regions (Menezes & Figueiredo 1985). Mullets have been considered to be among the most promising species for coastal aquaculture (Khemis, Zouiten, Besbes & Kamoun 2006). Also, their low position on the food web suggests a high potential for extensive culture, being reared either in monoculture or polyculture with other fish and crustaceans (Benetti & Fagundes Netto 1991).

The mullet *Mugil platanus* (Günther) is of great economical importance for estuarine artisanal fisheries in Southern Brazil (Reis & D'Incao 2000). It is being considered for aquaculture, because Godinho, Kavamoto, Andrade-Talmelli, Serralheiro, Paive and Ferraz (1993) demonstrated the feasibility to obtain induced spawning with human chorionic gonadotropin. Juvenile mullet is euryhaline (Sampaio, Wasielesky & Miranda-Filho 2002) and eurythermic (Okamoto, Sampaio & Maçada 2006), which are important features for coastal and estuarine aquaculture.

The knowledge of the nutritional requirements for mullet is limited. Considering that juveniles are iliophagus and feed on detritus and organic matter (Oliveira & Soares 1996), it is expected a low dietary protein requirement. Ito and Barbosa (1997) compared the performance of juvenile mullet fed on two dietary protein levels [200 and 400 g kg^{-1} crude protein (CP)] and verified a higher growth rate for mullets fed on the higher protein content diet.

Appropriate dietary concentration of high-quality protein generally results in high protein efficiency rate. However, when the protein level is excessively increased relative to the energy content of the diet, its excess can be catabolized and used as an energy

source. This process results in reduced growth rate and increased ammonia excretion, which could be harmful to fish health and the environment (McGoogan & Gatlin III 1999).

Because protein is the most expensive component of fish diets, determination of the optimum protein requirement can lead to the development of a diet that will provide high growth rates at a minimum cost (Lee, Kim & Cho 2002).

Considering the importance of protein in fish feed formulations and the poor understanding of protein requirement for juvenile mullet, the objective of the present study was to estimate the optimum dietary protein level for juvenile mullets when fed isoenergetic semi-purified diets under controlled laboratory conditions.

Materials and methods

Experimental procedures

Juvenile mullets were captured at the Cassino Beach (Rio Grande – RS, Brazil; 32°17′S–52°10′W) and transferred to the Laboratory of Marine Fish Culture of the Federal University of Rio Grande (Southern Brazil), where they were stocked for 2 weeks in a circular tank (1000 L) containing sea water. They were randomly distributed into 15 fibreglass tanks (50 L), at a stocking density of one individual per litre (Sampaio, Ferreira & Tesser 2001). The feeding trial was designed in triplicate for each of five treatments. Mullets were fed a commercial diet (INVE – 570 g kg^{-1} CP, Dendermonde, Belgium) on the first week. Feed was supplied until apparent satiation. On the following week, they were fed with the intermediate protein level experimental diet (400 g kg^{-1} CP) five times a day, until apparent satiation. This procedure was performed to adapt fish to the semi-purified diets.

At the beginning of the experiment and every week, 15 fish from each tank were sampled, anaesthetized with 50 ppm benzocaine, and individually measured and weighed. After these procedures, fish were returned to their respective tanks. Initial total length and weight was 4.34 ± 0.03 cm and 1.17 ± 0.02 g respectively. There was no significant difference of initial biomass among tanks ($P > 0.05$). Feeding was suspended 12 h before weight measurements, and mullet resumed feeding immediately after they were returned to their tanks. Dead fish were removed daily from the tanks. At the end of the experiment, all surviving fish were counted to estimate final survival.

Experimental diets were offered until apparent satiation five times a day at 08:00, 11:00, 14:00, 17:00 and 20:00 hours. The amount of food consumed in each tank was registered daily.

Water quality was monitored daily. Temperature and dissolved oxygen were measured with an oxymeter (YSI, model 55 Hexis, Yellow Springs, OH, USA), pH was measured with a pH metre (Hanna Instruments, model HI 221, Woonsocket, RI, USA), salinity was measured with a hand refractometer (Atago, model 103, Tokyo, Japan), while ammonia and nitrite concentrations were determined by colorimetric methods [American Public Health Association (APHA) 2005]. Average water temperature, salinity, pH, dissolved oxygen and ammonia and nitrite concentrations during the experiment was 24.0 ± 0.2 °C, 29 ± 0‰, 7.93 ± 0.01, 5.67 ± 0.04 mg O_2 L^{-1}, 0.43 ± 0.02 mg TAN-N L^{-1} and 0.08 ± 0.01 mg NO_2-N L^{-1} respectively.

Detritus deposited at the bottom of the tanks were siphoned out daily. Each tank was maintained in a flow-through mode, with a water exchange rate of 0.8 L min^{-1} ($\sim 100\%$ h^{-1}). Tanks were covered with a net in order to prevent fish from jumping out. Photoperiod was fixed at 14-h light/10-h dark. Water was constantly aerated through air stones. Temperature was kept constant by means of a water bath.

Experimental diets

Five semi-purified diets with increasing CP concentrations of 300, 350, 400, 450 and 500 g kg^{-1} (dry weight) were tested. Diets were formulated to be isoenergetic [18.7 MJ estimated metabolizable energy (ME) kg^{-1}] (Table 1). As digestible energy values for the ingredients used have not been determined for juvenile mullets, the dietary ME was estimated based on standard physiological fuel values of 16.7 MJ kg^{-1} for carbohydrate and protein and 37.7 MJ kg^{-1} for lipid (Garling & Wilson Jr 1976). In order to keep the diets isoenergetic, the level of dextrin was reduced accordingly to the protein increase.

Diets were prepared by initially mixing and homogenizing the dry ingredients and subsequently adding oil. After humidification of the mixture with distilled water at 50 °C, the homogenate was forced through a meat grinder with 2-mm-diameter holes. Afterwards, pellets were dried in an oven at 50 °C for 15 h, broken and sieved through 2 mm sieves. Diets were stored in hermetically sealed plastic bags in a freezer (– 20 °C) until use.

Table 1 Formulation and proximate composition of experimental diets

	Dietary protein level (g kg^{-1})				
	300	350	400	450	500
Ingredient (g kg^{-1} diet)					
Fish meal*	230	230	230	230	230
Casein†	100	150	200	250	300
Gelatin†	20	30	40	50	60
Dextrin‡	395	335	275	215	155
Cellulose†	145	145	145	145	145
Fish oil§	62	62	62	62	62
Min. and vit. premix¶	5	5	5	5	5
Corn starch	43	43	43	43	43
Proximate composition					
Dry matter (DM, %)	91.11	90.28	89.88	90.78	91.30
Crude protein (% DM)	29.10	34.95	40.36	45.47	50.98
Crude fat (% DM)	9.16	9.08	8.64	9.35	8.97
Ash (% DM)	2.83	2.97	2.88	2.70	3.03
ME (MJ kg^{-1}) ‖	18.6	18.7	18.7	18.9	18.8
P:E ratio [h] (g MJ^{-1})**	15.7	18.7	21.6	24.0	27.1

*Fish meal contains (as % of dry matter): crude protein, 80; lipid, 11.8; ash, 9.01. Nicoluzzi (Santa Catarina, RS, Brazil).
†Synth (Diadema, SP, Brazil).
‡Rhoster (São Paulo, SP, Brazil).
§Campestre Ind. e Com. de Óleos Vegetais Ltda (São Paulo, SP, Brazil).
¶Socil (São Paulo, SP, Brazil) composition/kg of premix: vitamin A 1 000 000 IU, vitamin D_3 500 000 UI, vitamin E 20 000 IU, vitamin K 500 mg, vitamin C 25 000 mg, vitamin B_1 500 mg, vitamin B_2 1750 mg, vitamin B_6 1125 mg, Ca pantothenate 5000 mg, folic acid 250 mg, biotin 50 mg, niacin 5000 mg, vitamin B_{12} 24 mg, iron 13 700 mg, selenium 75 mg, copper 2000 mg, zinc 20 000 mg, manganese 3760 mg, iodine 100 mg, BHT 250 mg and vehicle 1000 g.
‖Metabolizable energy, calculated from the physiological standard values, where 1 kg of carbohydrate (N-free extract), protein and lipid yields 16.7, 16.7 and 37.6 MJ respectively (Garling & Wilson 1976).
**Crude protein metabolizable energy^{-1}.
DM, dry matter; ME, metabolizable energy.

Diet and whole-body composition analyses

The composition of the experimental diets is shown in Table 1. Diet dry matter (DM) was obtained by keeping samples at 105 °C for 5 h. Ash content was determined after sample incineration at 550 °C. Lipid content was determined by ether extraction with a Soxhlet extractor. Crude protein content was determined using the Kjeldahl method (N × 6.25). All analyses followed the Association of Official Analytical Chemists (AOAC) (1995) standard procedures.

Thirty fish were sampled at the beginning of the experiment. At the end of the experiment (day 35), 10 juveniles were sampled from each tank for whole-body composition analyses. Mullets were individually weighed, sacrificed on ice and frozen (− 20 °C) until analysis. Fish sampled from each tank were pooled, grinded, homogenized and had their body composition analysed. Moisture, ash, fat and CP content were determined according to the same procedure described for the diet analyses (AOAC 1995). Proximal compositions of diets and fish whole body were performed in triplicate.

Postprandial ammonia excretion

At the end of the feeding trial, remaining fish were not fed for a period of 48 h to ensure complete evacuation of any food from the gut. On the morning of the third day, tanks were thoroughly cleaned, water was 100% renewed and water flow was discontinued. Afterwards, fish in all tanks were fed the appropriate diet until apparent satiation for 1 h and debris were siphoned out. The amount of diet ingested by fish in each tank was registered. Total ammonia (TAN) was determined before fish were fed and then 4 h after feeding, following the method described by APHA (2005). Excretion rates were calculated based on the difference between final and initial concentration of TAN, using the following equation: Excretion rate $= [(C_f - C_i)] \times V/(W \times t)$, where C_f is the final TAN concentration (mg L^{-1}); C_i is the initial TAN concentration (mg L^{-1}); W is the wet body weight (kg) of the fish; t is the time (h). Water temperature, salinity, dissolved oxygen concentration and pH during the 24 h were 24 ± 0.5 °C, 28.8 ± 0.0‰, 5.40 ± 0.2 mg O_2 L^{-1} and 7.84 ± 0.0 respectively.

Trypsin assay

The effect of dietary protein level on the intestinal trypsin activity was evaluated at the end of the experiment. Three juveniles were sampled from each tank and immediately frozen in liquid nitrogen (− 196 °C). The intestine was dissected on an ice plate and processed in a Potter micro-homogenizer (Micro Potter Sartorius, Goettingen, Germany) (1 mg sample − 2 μL buffer) using cold Tris-HCl buffer (0.1 M, pH 8.0) containing $CaCl_2$ (0.02 M). The homogenate was centrifuged at 6000 × g for 60 min at 4 °C (Micro 22R, Hettich Zentrifugen, Global Medical Instrumentation, Ramsey, MN, USA). The supernatant was used for enzyme activity assays and protein content analysis. Tryptic activity measurement was performed using a fluorescence technique adapted

from Ueberschär (1988). The reaction was performed with 250 μL of Nα-carbobenzoxy-L-arginine-4-methylcoumarinyl-7-amide (CBZ-L-Arg-MCA) solution (0.2 mM) as substrate and 10 μL of sample supernatant. Increase in fluorescence emission at 450 nm (excitation at 355 nm) was measured every 2 min for 30 min (Victor 2, Perkin-Elmer, Waltham, MA, USA). Only the linear portion of the fluorescence response over time was considered for enzyme activity calculations. Trypsin activity was normalized by the protein concentration in the sample supernatant. Protein was determined using a commercial reagent kit (Proteínas Totais®; Doles, Goiânia, GO, Brazil) based on the Biuret assay. Trypsin activity was expressed as ng trypsin mg protein^{-1}.

Performance parameters

Effects of different dietary protein levels in juvenile mullets were evaluated using the following parameters: survival (S) = [(initial number of fish − number of dead fish)/(initial number of fish) × 100], weight gain (WG) = (final weight − initial weight), specific growth rate (SGR) = [(ln final weight − ln initial weight)/(day) × 100], feed efficiency (FE) = weight gain/feed intake [dry matter (DM)], feed intake (FI) = feed intake (DM)/fish, protein intake (PI) = protein intake (DM)/fish, protein efficiency ratio (PER) = weight gain/protein consumption (DM) and apparent net protein utilization (ANPU) = 100 × [(final weight × final body protein content) − (initial weight × initial body protein content)]/(total dry protein intake).

Experimental design, statistical analysis and estimation of protein requirement

The experimental design was entirely randomized, with five treatments (300, 350, 400, 450 and 500 g kg^{-1} CP) tested in triplicate. Data obtained are expressed as means ± standard error. Before statistical procedures, data were tested for homogeneity of variance and normality using Levene and Kolmogorov–Smirnov tests respectively. Data were subjected to one-way analysis of variance (ANOVA) followed by the Tukey's HSD test when significant differences were detected. A significance level of 95% was adopted in all tests. The relationship between PI and postprandial ammonia excretion rate and between protein content of the diet and trypsin activity was analysed by linear regression.

Table 2 Whole-body composition (wet matter basis, g kg^{-1}) of juvenile mullet *Mugil platanus* after 35 days of feeding on diets containing increasing levels of dietary protein*

Dietary protein	Dry matter	Crude protein	Crude fat	Ash
300	283.6 ± 1	150.4 ± 4^{b}	99.0 ± 1	37.5 ± 0^{b}
350	289.2 ± 1	165.1 ± 3^{a}	100.3 ± 1	39.0 ± 1^{ab}
400	282.4 ± 2	152.7 ± 1^{b}	96.5 ± 1	3.70 ± 0^{b}
450	288.2 ± 0	160.6 ± 1^{ab}	96.1 ± 1	38.7 ± 0^{ab}
500	288.8 ± 1	163.5 ± 2^{a}	99.2 ± 1	40.5 ± 0^{a}

*Data are mean values (± standard error) of three replicates. Initial whole body composition was 260.1 g kg^{-1} dry matter, 592.5 g kg^{-1} protein, 323.9 g kg^{-1} lipid and 158.3 g kg^{-1} ash. Means (n = 3) in each column followed by different letters are significantly different (P < 0.05).

Results

Proximate body composition of juvenile mullet fed diets with different dietary protein content is summarized in Table 2. Fish whole-body protein, fat, ash and moisture contents were not influenced by dietary protein level (P > 0.05). Survival was not influenced by the protein level of the diets (P > 0.05) (Table 3).

The present study was based on a dose–response design. Specific growth rate of juvenile mullet fed diets containing 350 g kg^{-1} CP was significantly higher (P < 0.05) than fish fed 500 g kg^{-1} CP diet. However, it did not differ (P > 0.05) between diets with 300, 350, 400 and 450 g kg^{-1} CP (Table 3). Growth differences between fish fed different CP diets occurred only after 3 weeks of study and the same trend continued until the end of the experiment (Fig. 1).

Feed intake was significantly higher for fish fed diets with 300 and 350 g kg^{-1} CP compared with those fed the highest dietary protein content (500 g kg^{-1} CP). Despite the tendency of lower FI of diets with protein concentration higher than 350 g kg^{-1} CP, protein consumption increased (P < 0.05) as dietary protein content was increased (Table 3).

Feed efficiency did not change (P > 0.05) between fish fed the different experimental diets. However, PER was higher (P < 0.05) for fish fed the lowest dietary protein level (300 g kg^{-1} CP) than for fish fed the highest dietary protein content (500 g kg^{-1} CP). Apparent net protein utilization of fish fed diets containing 300 and 350 g kg^{-1} CP was higher (P < 0.05) than of fish fed 450 and 500 g kg^{-1} protein diets. However, these results did not differ significantly (P > 0.05) from ANPU of fish fed the intermediate dietary protein content (400 g kg^{-1} CP) (Table 3).

Table 3 Survival, growth performance and feed utilization by juvenile mullet *Mugil platanus* fed diets containing graded levels of protein for 35 days*

	Dietary protein level (g kg^{-1})				
Parameters	**300**	**350**	**400**	**450**	**500**
Initial weight (g)			1.17 ± 0.02		
Survival (%)†	97.5 ± 0.6	95.7 ± 3.4	96.3 ± 2.2	98.8 ± 0.6	88.4 ± 2.6^{ns}
SGR (%)‡	3.59 ± 0.1^{ab}	3.84 ± 0.2^{a}	3.60 ± 0.1^{ab}	3.40 ± 0.1^{ab}	3.01 ± 0.1^{b}
FE (%)§	0.36 ± 0.0	0.39 ± 0.0	0.42 ± 0.0	0.41 ± 0.0	0.37 ± 0.0^{ns}
FI (g fish^{-1})¶	8.06 ± 0.2^{a}	8.34 ± 0.47^{a}	6.92 ± 0.4^{ab}	6.94 ± 0.4^{ab}	6.16 ± 0.1^{b}
PI (g fish^{-1})‖	2.34 ± 0.1^{b}	2.92 ± 0.1^{ab}	2.79 ± 0.2^{ab}	3.16 ± 0.2^{a}	3.14 ± 0.1^{a}
PER**	1.23 ± 0.1^{a}	1.12 ± 0.0^{ab}	1.04 ± 0.1^{ab}	0.90 ± 0.0^{bc}	0.74 ± 0.1^{c}
ANPU (%)††	62.00 ± 4.5^{a}	62.89 ± 0.9^{a}	53.92 ± 0.9^{ab}	48.68 ± 1.6^{bc}	40.38 ± 3.3^{c}

*Mean ± standard error of three replicates. Values within the same rows having different superscripts are significantly different ($P < 0.05$).
†Survival = [(initial number of fish – number of dead fish)/(initial number of fish) × 100].
‡SGR = [(ln final weight – ln initial weight)/day] × 100.
§FE = [weight gain/feed intake].
¶FI = feed intake/fish.
‖PI = protein intake/fish.
**PER = [weight gain/protein intake].
††ANPU = [(final weight × final body protein) – (initial weight × initial body protein)]/(total dry protein intake) × 100.
NS, nonsignificant; SGR, specific growth rate; FE, feed efficiency; FI, feed intake; PI, protein intake; PER, protein efficiency ratio; ANPU, apparent net protein utilization.

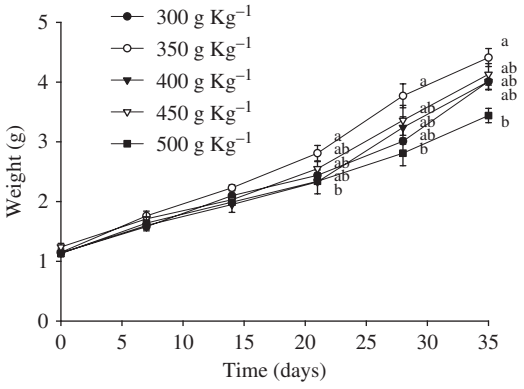

Figure 1 Growth of juvenile *Mugil platanus* fed diets containing increasing levels of protein for 35 days. Values are mean ± standard error of triplicate feeding groups. Points within the same day having different superscripts are significantly different ($P < 0.05$).

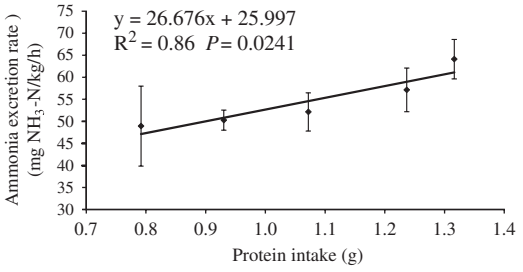

Figure 2 Postprandial ammonia excretion rate as a function of protein intake for juvenile mullet *Mugil platanus*. Values are expressed as mean ± standard error.

The amount of protein ingested by juvenile mullets during the excretion trial was influenced by the dietary protein. Feed intake of fish fed the diet with the highest protein content (500 g kg^{-1} CP) was lower than all other treatments, and they actually ingested the smallest amount of protein. Postprandial ammonia excretion was directly proportional to the amount of protein ingested and it was adequately described by a linear model (Fig. 2).

An inverse relationship was observed between the intestinal trypsin activity of mullets and the dietary protein level (Fig. 3).

Discussion

The dietary protein levels tested did not affect final whole body composition of mullet. Therefore, body composition is not an indicator of protein requirement for juvenile mullets, whereas growth, PER and ANPU reflected the protein requirement. Similar results have been found for juvenile red tailed tinfoil (*Barbodes altus*, Günther) (Elangovan & Shim 1997) and juvenile Mexican silverside (*Menidia*

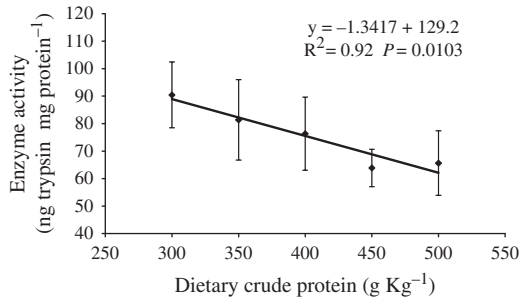

Figure 3 Intestinal tryptic activity in juvenile mullet *Mugil platanus* fed diets containing increasing levels of protein. Values are expressed as mean ± standard error.

estor, Jordan 1879) (Martínez-Palacios, Ríos-Durán, Ambriz-Cervantes, Jauncey & Ross 2007). On the other hand, body protein content increased when the dietary protein level was above the minimum protein requirement for the bydian perch (*Bidyanus bidyanus*, Mitchell) (Yang, Liou & Liu 2002).

Weight gain of juvenile mullet increased with increasing dietary protein level up to 350 g kg^{-1} CP. Thereafter a trend for growth depression as the protein content of the diet was increased was observed, especially at 500 g kg^{-1} CP. Studies with several teleosts have reported similar growth responses in fish, with manifestation of growth depression in response to excessive levels of dietary protein (Papaparaskeva-Papoutsoglou & Alexis 1986; Horn, Mailhiot, Fris & McClanahan 1995; Elangovan & Shim 1997; Yang *et al.* 2002; Jana, Garg, Barman, Arasu & Patra 2006; Martínez-Palacios *et al.* 2007).

Reduction of growth was accompanied by reduced feed ingestion at higher protein content diets. Feed consumption by the omnivorous white seabream *Diplodus sargus* L. was higher when they were offered a diet with low protein content (170 g kg^{-1} CP) compared with a diet with a higher protein level (270 g kg^{-1} CP), possibly trying to compensate the lower dietary protein content (Sá, Pousão-Ferreira & Oliva-Teles 2008). Similar result was observed by Horn *et al.* (1995), where the monkeyface prickleback (*Cebidichthys violaceus*, Girad), an herbivorous marine fish, markedly reduced their food consumption with an increase in dietary protein. The same behaviour was observed for the omnivorous rohu (*Labeo rohita*, Hamilton) (Satpathy, Mukeherjee & Ray 2003) and silver barb (*Puntius gonionotus*, Bleeker) (Mohanta, Mohanty, Jena & Sahu 2008).

It is important to notice that anorexia has been shown to be induced by excess dietary protein. Veldhorst, Smeets, Soenen, Hochstenbach-Waelen, Hursel, Diepvens, Lejeune, Luscombe-Marsh and Westerterp-Plantenga (2008) listed four mechanisms that may contribute to the protein-induced satiety: altered concentration of satiety hormones, increased energy expenditure, higher concentration of metabolites (i.e., amino acids) and gluconeogenesis. Besides that, the proportion of protein to energy was increased as the protein content of the diets increased. The higher protein to energy ratio also resulted in reduced growth for other species, as the excess protein is used as energy source and not for growth (Horn *et al.* 1995; Elangovan & Shim 1997; Martínez-Palacios *et al.* 2007).

Energy content is thought to be one of the major criteria controlling FI in fish (Lee & Putnam 1973; Glencross 2006; Mohanta *et al.* 2008), along with other factors including fish size, temperature or food palatability. However, in the present work fish were fed isoenergetic diets and seemed to control ingestion according to dietary protein level, probably trying to adjust FI to meet their protein requirement (Horn *et al.* 1995; Martínez-Palacios *et al.* 2007).

Feed efficiency of juvenile mullet was not affected by dietary protein level in the present study. However, in other studies the increase in dietary protein above a determined level produced a significant decrease in FE (Elangovan & Shim 1997; Yang *et al.* 2002).

Results from the present study indicated a reduction on PER with increasing dietary protein content. Similar results were reported for the omnivorous red tailed tinfoil (Elangovan & Shim 1997), for the carnivorous Asian redtail catfish (*Mystus nemurus*, Valenciennes) (Ng, Soon & Hashim 2001), omnivorous *Spinibarbus hollandi*, Oshima (Yang, Lin, Liou & Peng 2003), and for the piscivorous olive flounder (*Paralichthys olivaceus*, Temminck & Schlegel) (Kim, Wang, Choi, Park & Bai 2004). These authors suggested that protein in excess to the amount needed for growth is catabolized and used as energy source. The increased ammonia excretion observed for mullet fed excess protein corroborates this idea. In the same manner, ANPU decreased linearly when the dietary protein level of the diet was above 350 g kg^{-1}. Yang *et al.* (2002) also reported a higher ANPU for bydian perch with a lower dietary PI.

Postprandial ammonia excretion can be an indicator of dietary protein adequacy and it is directly related to PI (Merino, Piedrahita & Conklin 2007). Usually, nitrogenous excretion increases linearly with increasing feed consumption (Sun, Chen, Huang & Wang 2006). Similar results were found for monkeyface prickleback (Horn *et al.* 1995), bydian

perch (Yang *et al.* 2002) and the juvenile mullet in the present study.

Trypsin activity decreased with increasing dietary protein levels. A reduction in trypsin activity has also been shown for other species when fish are fed diets with protein content in excess of their requirement (Jana *et al.* 2006; Mohanta *et al.* 2008).

Dietary protein requirement vary between species, with carnivorous fish generally showing higher values than omnivorous and herbivorous species [National Research Council (NRC) 1993]. Comparison of protein requirement among fish species is complicated due to differences in fish size, diet formulation and culture conditions tested (Elangovan & Shim 1997). However, the result obtained in this experiment with mullet is comparable with the protein requirements reported for juveniles of other omnivorous species, such as common carp *Cyprinus carpio* L. 350 g kg $^{-}$ 1 CP (NRC 1993), ayu (*Plecoglossus altivelis*, Temminck & Schlege) 380 g kg $^{-1}$ CP (Lee *et al.* 2002), jundia (*Rhamdia quelen*, Quoy & Gaimard) 373 g kg $^{-1}$ CP (Meyer & Fracalossi 2004) and milkfish (*Chanos chanos*, Forsskål) 400 g kg $^{-1}$ CP (Jana *et al.* 2006). However, Papaparaskeva-Papoutsoglou and Alexis (1986) determined a very low protein requirement (240 g kg $^{-1}$ CP) for juvenile grey mullet *Mugil capito*, Cuvier, while Yoshimatsu, Furuichi and Kitajima (1992) estimated a higher dietary protein requirement (400 g kg $^{-1}$ CP) for juvenile redlip mullet (*Liza haematocheila*, Temminck & Schlegel). Differences observed could be associated with methodological procedures adopted in each study.

Ito and Barbosa (1997) observed a higher growth rate of mullet when fed on a diet containing 400 g kg $^{-1}$ CP compared with a diet with lower protein content (200 g kg $^{-1}$ CP). However, it is also important to notice that optimum dietary requirement is higher for young animals (Yoshimatsu *et al.* 1992), and as such, it is likely that diets containing $<$ 350 g kg $^{-1}$ CP will be needed for on-growing mullet, especially when reared in ponds with abundant natural food.

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