UNIVERSIDADE FEDERAL DO RIO GRANDE

PROGRAMA DE PÓS-GRADUAÇÃO EM AQÜICULTURA

Cristina Vaz Avelar de Carvalho

Exigência protéica de juvenis de tainha *Mugil platanus*

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Dissertação apresentada como parte dos requisitos para obtenção do grau de Mestre em Aqüicultura no Programa de Pós-Graduação em Aqüicultura da Universidade Federal do Rio Grande.

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Co-orientador: Prof. Dr. Marcelo Borges Tesser

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Sumário

Dedico *in memoriam* esta dissertação à minha avó Ernestina

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RESUMO

A alimentação é um dos principais custos da piscicultura, sendo importante desenvolver estudos que busquem uma maior eficiência alimentar para o aumento do sucesso da atividade e também para a redução do impacto da emissão de nutritientes ao meio ambiente. Levando em conta o potencial de criação da tainha *Mugil platanus* na região Sudeste e Sul do Brasil e a carência de informações sobre suas exigências nutricionais, o presente trabalho foi realizado com o objetivo de determinar a exigência protéica para seus juvenis. As tainhas foram alimentadas com cinco dietas com três repetições cada, sendo cada unidade composta por um tanque de 50L com 50 juvenis com peso inicial 1,17 ± 0,02 g e 4,34 ± 0,03 cm (média ± EP). As cinco dietas isocalóricas foram formuladas para conter níveis crescentes de proteína bruta (PB) de 30% , 35%, 40%, 45% e 49% e 18,7 MJ/Kg de dieta (energia metabolizável). As dietas foram oferecidas até a saciedade 5 vezes ao dia durante 35 dias. As dietas não apresentaram diferenças significativas ($P > 0,05$) para sobrevivência, eficiência alimentar e composição corporal. Os resultados indicaram que o nível de 35% PB foi estatisticamente superior ($P > 0,05$) com relação ao ganho em peso, ingestão de alimento e taxa de crescimento específico do que de tainhas alimentas com o maior nível protéico. A necessidade de proteína para os juvenis de tainha foi estimada em 35,8% PB.

Palavras chaves: proteína, crescimento, excreção, alimentação, nutrição, peixe

ABSTRACT

Feed is one of the main costs for fish culture. Studies looking for higher feed efficiency are important to increase the success of aquaculture and reduce impacts of nutrient emission into the environment. Considering the potential of the mullet *Mugil platanus* for aquaculture, as well as the lack of information on its nutritional demands, the main goal of the present work was to determine the dietary protein requirement of juvenile mullets. Five isocaloric diets were formulated in order to contain increasing levels (30, 35, 40, 45, and 50%) of crude protein (CP) corresponding to 18.7 MJ metabolizable energy/Kg. 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 increase, feed ingestion and specific growth rate was higher in fish fed the 35% CP level than those fed the highest protein content diet (50% 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. The dietary protein requirement of juvenile mullet was estimated as 34.28% CP with a P:E ratio of 18.7 g/MJ.

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

1. Introdução Geral

Os membros da família Mugilidae, conhecidos popularmente por tainhas, são peixes costeiros encontrados em ambientes marinhos e estuarinos (Menezes e Figueiredo, 1985).

O cultivo de tainhas é uma realidade em várias regiões do mundo, sendo o Egito, atualmente, o maior produtor de *Mugil cephalus* (SOFIA, 2006). A criação de tainha vem sendo realizada em mono e ou policultivo em vários países como Itália, Israel, Taiwan, Egito, China, Cuba e Colômbia (Godinho et al., 1988).

No Brasil, os mugilídeos são de grande importância para a pesca artesanal (Reis et al., 1994). Na região do estuário da Lagoa dos Patos, no Rio Grande do Sul, a pesca de *Mugil platanus* é importante economicamente para a comunidade local (Reis e D`Incao, 2000).

Esta espécie ocorre desde o estado do Rio de Janeiro no Brasil, até a Argentina (Menezes e Figueiredo, 1985). Seus juvenis deslocam-se das regiões costeiras para águas estuarinas e lagunares, ricas em alimento, onde passam sua fase de crescimento e na época de desova migram para o mar. No Rio Grande do Sul é frequente a presença de juvenis ao longo do ano no estuário da Lagoa dos Patos (Vieira e Scalabrin, 1991).

O hábito alimentar de *M. platanus* se diferencia de acordo com a fase de seu ciclo de vida, passando de planctófagos a iliófagos (Oliveira e Soares, 1996) e podem atingir cerca de 1 m de comprimento, com peso em torno de 6 kg (Vieira e Scalabrin, 1991).

O protocolo para reprodução em cativeiro e obtenção de juvenis de *M. platanus* ainda não estão bem definidos (Scorvo Filho et al., 1988). Rocha (1981) descreveu os procedimentos básicos que devem ser seguidos para a reprodução e larvicultura de mugilídeos enquanto que as primeiras tentativas de indução para reprodução da tainha *M. platanus* são relatadas por Godinho et al. (1982, 1984).

Além de sua importância econômica para a pesca, a tainha *M. platanus* apresenta características que a qualifica como uma alternativa para a piscicultura nas regiões Sul e Sudeste do Brasil. Está espécie é eurialina e euritérmica (Godinho, 2005), suporta bem condições de confinamento, aceita com facilidade alimentos artificiais e é possível que sua produção seja feita em sistemas de mono e policultivo com outras espécies de peixes e crustáceos (Benetti e Fagundes Netto, 1991; Neto e Spach, 1998/1999; Sampaio et al., 2001).

Estudos realizados sobre a susceptibilidade de juvenis de *M. platanus* à fatores potencialmente limitantes para sua criação foram realizados. Miranda-Filho et al., (1995) avaliaram o efeito da amônia e do nitrito sobre o crescimento da tainha e concluíram que a tainha é um organismo passível de ser aproveitado em sistemas de cultivo desde que os níveis de amônia total sejam inferiores a 4 mg/l, visto que o crescimento é reduzido em concentrações superiores a esta. Neto e Spach (1998/1999) propuseram um protocolo para criação de tainhas em água doce. Sampaio et al. (2001) sugerem uma densidade de estocagem entre três a cinco juvenis por litro em cultivo de laboratório. A toxicidade aguda da amônia e do nitrito para juvenis de tainha é maior em água doce do que em salinidades mais elevadas (Sampaio et al., 2002). Okamoto et al. (2006) observaram que as tainhas crescem melhor em temperaturas de 30°C.

Ito e Barbosa (1997) observaram melhor crescimento de *M. platanus* alimentados com uma dieta contendo 40% proteína bruta (PB) do que quando alimentados com um nível de proteína inferior (20% PB). As dietas foram elaboradas com farinha de peixe, farinha de resíduos de camarão, farelo de soja, fubá de milho, levedura seca de álcool de cana de açúcar, farelo de trigo e farelo de arroz.

Alguns estudos sobre o processo de digestão foram realizados com larvas e juvenis de *M. platanus*. O desenvolvimento estrutural do sistema digestório de larvas da tainha *M. platanus* foi estudado por Galvão et al. (1997a) com o objetivo de aprimorar o seu manejo alimentar. O desenvolvimento do trato digestório das larvas de tainha foi considerado lento e a substituição de organismos vivos por dietas artificiais só é possível ao redor do 40° dia após a sua eclosão, quando o trato digestório torna-se completamente funcional, com a diferenciação do estômago e o aparecimento de glândulas gástricas. Larvas de *M. platanus* com quatro dias de vida já apresentam as enzimas tripsina e carboxipeptidases, embora suas atividades proteolíticas sejam baixas em relação aos juvenis (Galvão et al., 1997b).

Apesar destes estudos, ainda há carência de informação acerca das exigências nutricionais da *M. platanus* para que se possa desenvolver um protocolo alimentar adequado, a fim de possibilitar sua criação intensiva.

As exigências nutricionais de um organismo estão relacionadas com a espécie, fase de desenvolvimento, sexo e estádio de maturação sexual, sistema e regime de produção, temperatura da água, frequência de arraçoamento e qualidade da dieta (Watanabe, 1988; Pezzato et al., 2004), sendo o valor nutricional desta dieta determinada pela sua digestibilidade e absorção pelo animal.

O primeiro passo para determinar as exigências nutricionais de uma espécie é estimar a exigência de proteína (Gao et al., 2005, Martínez-Palacios et al., 2007). Este é o nutriente mais caro da dieta e possui grande importância biológica, desempenhando uma grande variedade de funções: atividade enzimática, transporte (hemoglobina), proteínas nutrientes e de reserva, proteínas contráteis ou do movimento (miosina, actina), proteínas estruturais, de defesa, reguladoras (hormônios) (Pezzato et al., 2004). No trato digestório as proteínas são hidrolizadas enzimaticamente, liberando aminoácidos que são distribuídos através da corrente sanguínea para os órgãos e tecidos, onde são utilizados continuamente no processo de síntese e degradação protéica durante os processos de crescimento, reprodução, ou como fonte de energia (Watanabe, 1988).

As diferenças nas exigências de proteína pelos organismos estão diretamente relacionadas com os hábitos alimentares, tamanho do peixe e funcionalidade do aparelho digestório. Animais carnívoros exigem altas quantidades de proteína na dieta para que sejam obtidos os aminoácidos necessários para a síntese protéica e glicose para atender a demanda energética (Sánchez-Muros et al., 1998), como *Paralichthys olivaceus* (45% PB, Lee et al., 2002), *Epinephelus coioides* (48% PB, Luo et al., 2004). Já outras espécies onívoras como os ciprinídeos, as tiláidas e alguns ictalúrideos, são capazes de manter as taxas de crescimento quando alimentadas com dietas contendo 30% a 40% de proteína bruta (NRC, 1993; Jobling, 1994). Hafedh (1999) verificou que dietas com nível protéico de 30% e 40% foram satisfatórias para tilápias *Oreochromis niloticus* L. com 96 – 264g e 0.51g, respectivamente, demonstrando a alteração na exigência protéica em função do tamanho dos peixes.

O nível de proteína na ração e a quantidade de alimento ofertado são um dos principais fatores que influenciam o crescimento dos peixes, a eficiência alimentar e a qualidade da água (Jana et al., 2006, Mohanta et al., 2008), entretanto o perfil de aminoácidos presentes na proteína é decisivo para sua qualidade e determina seu valor como componente da dieta. Além dos aminoácidos, a garantia de obtenção de uma ótima resposta produtiva e máximo crescimento em peixes depende do atendimento das necessidades protéico-energéticas e demais nutrientes essenciais nas proporções necessárias (NRC, 1993).

Vidotti et al. (2000) testaram diferentes teores protéicos e relações de proteína de origem animal em dietas para juvenis do bagre africano *Clarias gariepinus* e constataram que o bagre é uma espécie exigente com relação a qualidade e quantidade de proteína na dieta, visto que os melhores valores de ganho em peso foram obtidos para dietas com 38% de proteína bruta e com metade da proteína de origem animal. A maior proporção de proteína de origem animal na dieta deve ter melhorado consideravelmente a qualidade da dieta com relação ao perfil de aminoácidos essenciais e também com relação a sua palatabilidade.

Dietas com níveis protéicos que excedam as exigências para crescimento, resultam em um aumento no custo energético para deaminação dos aminoácidos em excesso. Estes são convertidos em compostos energéticos, isto é, as cadeias carbônicas dos aminoácidos são utilizadas para síntese de açúcares e/ou gorduras (Melo et al., 2006). Esta proteína que é usada como fonte de energia, não é utilizada para o crescimento (Horn et al., 1995, Lee et al., 2002, Mohanta et al., 2008). A amônia é o principal produto da excreção de compostos nitrogenados dos peixes, o excesso de proteína na dieta, ou a baixa qualidade da proteína (balanço inadequado de aminoácidos) resultam em um aumento na excreção de amônia (Merino et al., 2007).

O excesso de energia não protéica, como resultado da formulação de dietas com uma baixa relação proteína/energia, não é desejável tanto do ponto de vista dos índices de conversão alimentar, de rentabilidade da dieta, como também porque leva a diminuição do consumo voluntário do alimento e, com isso, há uma menor ingestão de proteína e outros nutrientes essenciais podendo ocorrer excessiva deposição de gordura corporal (Lee et al., 2002).

Embora os lipídios sejam reconhecidos como a principal fonte de energia não protéica para peixes, a disponibilidade e o baixo custo dos carboidratos encorajam a sua inclusão em dietas para atender racionalmente as suas exigências (Hillestad et al, 2001, Martino et al., 2005). Os peixes não têm uma exigência dietética para carboidratos, devido à capacidade de sintetizá-los a partir de substratos como proteínas e lipídios através da gliconeogênese (Pezzato et al., 2004). As espécies onívoras tendem a utilizar mais eficientemente o carboidrato do que as espécies carnívoras. Contudo, espécies carnívoras mostram uma melhora no crescimento quando alimentadas com uma dieta com pouco carboidrato quando comparadas com uma dieta sem adição de carboidrato (Hemre et al., 2002). Portanto, a inclusão de carboidrato nas dietas pode proporcionar uma ação poupadora da proteína como fonte energética direcionando-a para o crescimento (Martino et al., 2005).

Considerando a importância da proteína na formulação de rações para peixes, o objetivo do presente estudo foi determinar o nível de exigência protéica para juvenis de *M. platanus* alimentadas com dietas isocalóricas contendo cinco níveis crescentes de proteína.

2. Objetivos

Objetivo geral:

Determinar a exigência protéica para juvenis de tainha *M. platanus* alimentadas com dietas isocalóricas contendo cinco níveis crescentes de proteína.

Objetivos específicos:

Avaliar o crescimento, sobrevivência, eficiência protéica de juvenis de tainha *M. platanus* alimentados com dietas isoenergéticas contendo cinco níveis crescentes de proteína.

Avaliar a composição corporal de juvenis de tainha *M. platanus* alimentados com dietas isoenergéticas contendo cinco níveis crescentes de proteína.

Avaliar diferenças nos níveis de excreção de amônia de juvenis de tainha *M. platanus* alimentados com dietas isoenergéticas contendo cinco níveis crescentes de proteína.

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4. ARTIGO ANEXO

The dietary protein requirement of juvenile mullet *Mugil platanus* (Günther, 1880)

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The dietary protein requirement of juvenile mullet *Mugil platanus*

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Abstract

Feed is one of the main costs for fish culture. Studies looking for higher feed efficiency are important to increase the success of aquaculture and reduce impacts of nutrient emission into the environment. Considering the potential of the mullet *Mugil platanus* for aquaculture, as well as the lack of information on its nutritional demands, the main goal of the present work was to determine the dietary protein requirement of juvenile mullets. Five isocaloric diets were formulated in order to contain increasing levels (30, 35, 40, 45, and 50%) of crude protein (CP) corresponding to 18.7 MJ metabolizable energy/Kg. 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 increase, feed ingestion and specific growth rate was higher in fish fed the 35% CP level than those fed the highest protein content diet (50% 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. The dietary protein requirement of juvenile mullet was estimated as 34.28% CP with a P:E ratio of 18.7 g/MJ.

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

1. Introduction

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

The mullet *Mugil platanus* is of great economical importance for estuarine artisanal fisheries in Southern Brazil (Reis and D'Incao, 2000). It is being considered for aquaculture, since Godinho et al. (1993) demonstrated the feasibility to obtain induced-spawning with HCG. Juvenile *M. platanus* is euryhaline (Sampaio et al., 2002) and eurythermic (Okamoto et al., 2006), which are important features for coastal and estuarine aquaculture.

The knowledge of the nutritional requirements for *M. platanus* is limited. Considering that juveniles are iliophagus and feed on detritus and organic matter (Oliveira and Soares, 1996), it is expected a low dietary protein requirement. Ito & Barbosa (1997) compared the performance of juvenile *M. platanus* fed on two dietary protein levels (20 and 40% crude protein) and verified a higher growth rate for mullets fed on the higher protein content diet.

Appropriate levels of high quality protein generally result 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 energy source. This process results in reduced growth rate and increased ammonia excretion, which could be harmful to fish health and the environment (McGoogan and Gatlin III, 1999).

Since 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 et al., 2002).

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

2. Materials and Methods

2.1 *Experimental procedures*

Juvenile mullets were captured at 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 two weeks in a circular tank (1,000 L) containing sea water. They were randomly distributed in 15 fiberglass tanks (50L), at a stocking density of 1 individual per liter (Sampaio et al., 2001). Mullets were fed a commercial diet (INVE) on the first week. Food was supplied until apparent satiation. On the following week, they were fed with the intermediate protein level experimental diet (40% 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, anesthetized 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 8:00, 11:00, 14:00, 17:00 and 20:00 h. The amount of food consumed in each tank was registered everyday.

Water quality was monitored everyday. Temperature and dissolved oxygen were measured with an oxymeter (YSI, model 55 Hexis), pH was measured with a pH meter (Hanna Instruments, model HI 221), salinity was measured with a hand-refractometer (Atago, model 103), while ammonia and nitrite concentrations were determined by colorimetric methods (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, 0.43 ± 0.02 mg TAN-N/L and 0.08 ± 0.01 mg NO_2-N/L, respectively.

Detritus deposited at the bottom of the tanks were siphoned out daily. Each tank was maintained in a flow-through system, with a water exchange rate of 0.8 l/min (~100%/h). Tanks were covered with a net in order to prevent fish from jumping off.

Photoperiod was fixed at 14 h-light/10 h-dark. Water was constantly aerated through air stone. Temperature was kept constant by means of a water bath.

2.2 *Experimental diets*

Five semi-purified diets with increasing crude protein (CP) concentrations of 30, 35, 40, 45 and 50% (dry weight) were tested. Diets were formulated to be isoenergetic [18.7 MJ estimated metabolizable energy (ME)/Kg] (Table 1). As digestible energy values for the ingredients used have not been determined for *M. platanus*, the dietary ME was estimated based on standard physiological fuel values of 16.7 MJ/kg for carbohydrate and protein and 37.7 MJ/kg for lipid (Garling and Wilson, 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.

2.3. *Diet and whole-body composition analyses*

The composition of the experimental diets is shown in Table 1. Diet dry matter 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 by the Kjeldahl method (N x

6.25) with the Kjeltec System (Tecator, Sweden). All analyses followed the AOAC (1995) standard procedures.

Thirty fish were sampled at the beginning of the experiment. At the end of the experiment (day 35), ten 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 analyzed. Moisture, ash, fat and crude protein content were determined accordingly to the same procedure described for the diet analyses (AOAC, 1995). Proximal compositions of diets and fish whole body were performed in triplicate.

2.4 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 = $[(C_f - Ci)] \ x \ V/ \ (W \ x \ t)$, where: C_f is the final TAN concentration (mg L^{-1}); Ci 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 and 7.84 ± 0.0 respectively.

2.5. *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 a ice plate and processed in a Potter micro-homogenizer (1mg 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 6,000 x *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.

2.6. *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) x 100], weight gain (WG) = (final weight – initial weight), specific growth rate (SGR) = [(ln final weight – ln initial weight)/(day) x 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 x [(final weight x final body protein content) – (initial weight x initial body protein content)]/(total dry protein intake).

2.7 *Experimental design, statistical analysis and estimation of protein requirement*

The experimental design was entirely randomized, with five treatments (30, 35, 40, 45 and 50% CP) tested in triplicate. Data obtained are expressed as mean ± standard error. Before statistical procedures, data were tested for homogeneity of variance and normality using Levene's test and Kolmogorov-Smirnov test, 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 protein intake and postprandial ammonia excretion rate and between protein content of the diet and trypsin activity was analyzed by linear regression. In addition, the protein requirement was defined as 95% of the maximum weight gain estimated by quadratic regression (Jobling, 1994).

3. 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 ($P > 0.05$), albeit not significant, there was a trend for reduced survival of fish fed the diet with the highest protein content (Table 3).

The present study was based on a dose-response design. Specific growth rate of juvenile mullet fed diets containing 35% CP was significantly higher ($P < 0.05$) than fish fed 50% CP diet. However, it did not differ ($P > 0.05$) between diets with 30, 35, 40, and 45% CP (Table 3). Growth difference between fish fed different CP diets occurred only after 3 weeks of study and the same trend continued until the end of the experiment (Figure 1). Weight gain followed the same trend and its relation with the protein content of the diets was described by a quadratic model, where the dietary protein requirement of juvenile mullet was estimated at 34.2% (Figure 2).

Feed intake was significantly higher for fish fed diets with 30 and 35% CP compared to those fed the highest dietary protein content (50% CP). Despite the tendency of lower feed intake of diets with protein concentration higher than 35% CP, protein consumption increased ($P < 0.05$) as dietary protein content was raised (Table 3).

Feed efficiency did not change ($P > 0.05$) between fish fed the different experimental diets. However, protein efficiency ratio (PER) was higher ($P < 0.05$) for fish fed the lowest dietary protein level (30% CP) than for fish fed the highest dietary protein content (50% CP). Apparent net protein utilization (ANPU) of fish fed diets containing 30 and 35% CP was higher ($P < 0.05$) than of fish fed 45 and 50% protein diets. However, these results did not differ significantly ($P > 0.05$) from ANPU of fish fed the intermediate dietary protein content (40% CP) (Table 3).

It was observed an inverse relationship between the intestinal trypsin activity of mullets and the dietary protein level (Figure 4).

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 (50% 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 (Figure 3).

4. Discussion

Although not significantly different from the other treatments, fish mortality was higher for mullets fed the highest dietary protein concentration (50% CP), but no obvious causes were available to explain it. Higher mortalities were also registered for juvenile *Barbodes altus* (Elangovan and Shim, 1997) and *Paralichthys lethostigma* (Gao et al., 2005) when fed excess protein. According to Kiron et al. (1995), a low-protein diet invariably resulted in greater mortality of *Oncorhynchus mykiss*, but higher levels (around 50% CP) also seemed to depress disease resistance when compared with intermediate protein range (20 - 35% CP).

The dietary protein levels tested did not affect final whole body composition of *M. platanus*. 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 *Barbodes altus* (Elangova and Shim, 1997) and *Menidia estor* (Martínez-Palacios et al., 2007). On the other hand, body protein content increased when the dietary protein level was above the minimum protein requirement for *Bidyanus bidyanus* (Yang et al., 2002).

Weight gain of juvenile *M. platanus* increased with increasing dietary protein level up to 35% CP. Thereafter, it was observed a trend for growth depression as the protein content of the diet was raised, especially at 50% 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 and Alexis, 1986; Horn et al., 1995; Elangovan and Shim, 1997; Yang et al 2002; Jana et al., 2006; Martínez-Palacios et al., 2007).

Reduction of growth was accompanied by reduced food ingestion at higher protein content diets. Feed consumption by *Diplodus sargus* was higher when they were offered a diet with low protein content (17% CP) compared to a diet with a higher protein level (27% CP), possibly trying to compensate the lower dietary protein content (Sá et al., 2008). Similar result was observed by Horn et al. (1995), where *Cebidichthys violaceus*, an herbivorous marine fish, markedly reduced their food consumption with an increase in dietary protein. The same behavior was observed for *Labeo rohita* (Satpathy 2003) and *Puntius gonionotus* (Mohanta et al., 2008).

It is important to notice that anorexia has been shown to be induced by excess dietary protein. Veldhorst et al. (2008) listed four mechanisms that may contribute to the protein-induced satiety: altered concentration of satiety hormones, increased energy expenditure, higher concentration of metabolits (ie: 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 and Shim, 1997; Martínez-Palacios, 2007).

Energy content is thought to be one of the major criteria controlling feed intake in fish (Lee and 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 feed intake to meet their protein requirement (Horn et al., 1995; Martínez-Palacios, 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 and Shim, 1997; Yang et al., 2003).

Results from the present study indicated a reduction on PER with increasing dietary protein content. Similar results were reported for *Barboles altus* (Elangovan and Shim, 1997), *Mystus nemurus* (Ng et al., 2001), *Spinabarbus hollandi* (Yang et al., 2003), and *Paralichthys olivaceus* (Kim et al., 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 35%. Yang et al. (2002) also reported a higher ANPU for *Bidyanus bidyanus* with a lower dietary protein intake.

Postprandial ammonia excretion can be an indicator of dietary protein adequacy and it is directly related to protein intake (Merino et al., 2007). Usually, nitrogenous excretion increases linearly with increasing feed consumption (Sun et al., 2006). Similar results were found for *Cebidichthys violaceus* (Horn et al., 1995), *Bidyanus bidyanus* (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). Thus, the actual protein requirement for juvenile mullet may be lower than it was estimated, approaching 30% CP instead.

Dietary protein requirement vary between species, with carnivorous fish generally showing higher values than omnivorous and herbivorous species (NRC, 1993). Comparison of protein requirement among fish species is complicated due to differences in fish size, diet formulation and culture conditions tested (Elangovan and Shim, 1997). However, the minimum dietary protein required by *M. platanus* for maximum growth is comparable to the protein requirements reported for juveniles of other omnivorous species, such as *Cyprinus carpio* (35% CP; NRC 1993), *Plecoglossus altivelis* (38% CP; Lee et al., 2002), *Rhamdia quelen* (37.3% CP; Meyer and Fracalossi, 2004) and *Chanos chanos* (40% CP; Jana et al., 2006). However, Papaparaskeva-Papoutsoglou and Alexis

(1986) determined a very low protein requirement (24% CP) for juvenile *Mugil capito*, while Yoshimatsu et al. (1992) estimated a higher dietary protein requirement (40% CP) for juvenile *Lisa haematocheila*. Differences observed could be associated with methodological procedures adopted in each study

Ito and Barbosa (1997) observed a higher growth rate of *M. platanus* when fed on a diet containing 40% CP compared to a diet with lower protein content (20% 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 less than 34.2% CP will be needed for on-growing mullet, especially when reared in ponds with abundant natural food.

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Table 1. Formulation and proximate composition of experimental diets (% dry matter)

	Dietary protein level (%)				
	30	35	40	45	50
Ingredient (g/100gdiet)					
Fish meal [1]	23	23	23	23	23
Casein [2]	10	15	20	25	30
Gelatin [2]	2	3	4	5	6
Dextrin [3]	39.5	33.5	27.5	21.5	15.5
Cellulose [2]	14.5	14.5	14.5	14.5	14.5
Fish oil [4]	6.2	6.2	6.2	6.2	6.2
Min. and vit. premix [5]	0.5	0.5	0.5	0.5	0.5
Corn starch	4.3	4.3	4.3	4.3	4.3
Proximate composition (% of 100% dry matter)					
Dry matter	91.11	90.28	89.88	90.78	91.30
Crude protein	29.10	34.95	40.36	45.47	50.98
Crude fat	9.16	9.08	8.64	9.35	8.97
Ash	2.83	2.97	2.88	2.70	3.03
NFE [6]	61.50	56.40	52.21	46.67	41.48
ME (MJ kg $^{-1}$) [7]	18.6	18.7	18.7	18.9	18.8
P:E ratio ^{h} (g MJ $^{-1}$)[8]	15.7	18.7	21.6	24.0	27.1

[1] Fish meal contains (as % of dry matter): crude protein, 80; lipid, 11.8; ash, 9.01. Nicoluzzi (Santa Catarina, RS, Brazil). [2] Synth (Diadema, SP, Brazil). [3] Rhoster (São Paulo, SP, Brazil). [4] Campestre Ind. e Com. de Óleos Vegetais Ltda (São Paulo, SP,

Brazil). [5] Socil (São Paulo, SP, Brazil) composition/kg of premix: vit. A 1.000,000 UI, vit. D3 500.000 UI, vit. E 20.000 UI, vit. K 500mg, vit. C 25.000 mg, vit. B1 500 mg, vit. B2 1750 mg, vit. B6 1125 mg, Ca pantothenate 5000 mg, folic acid 250 mg, biotin 50 mg, niacin 5000mg, vit B12 24 mg, iron 13.700 mg, selenium 75 mg, copper 2000 mg, zinc 20.000 mg, manganese 3.760 mg, iodine 100 mg, B.H.T. 250 mg, vehicle 1000 g. [6] Nitrogen-free extract (100% - %crude protein - %crude fat - %ash). [7] Metabolizable energy, calculated from the physiological standard values, where 1kg of carbohydrate (N–free extract), protein and lipid yields 16.7, 16.7 and 37.6 MJ, respectively (Garling and Wilson, 1976). [8] Crude protein/metabolizable energy.

Table 2. Whole-body composition (% dry matter) of juvenile *Mugil platanus* after 35 days of feeding on diets containing increasing levels of dietary protein[1].

Dietary protein (%)	Dry matter (%)	Crude protein (%)	Crude fat (%)	Ash (%)
30	28.36±0.1	53.02±1.3	34.91±0.2	13.22±0.0
35	28.92±0.1	56.29±0.8	34.69±0.4	13.49±0.3
40	28.24±0.2	54.06±0.5	34.17±0.5	13.11±0.0
45	28.82±0.0	55.73±0.2	33.34±0.4	13.45±0.1
50	28.88±0.1	56.33±0.6	34.17±0.5	13.97±0.0

[1] Data are mean values (± standard error) of three replicates. Figures in the same column were not significantly different ($P > 0.05$). Initial whole body composition was 26.01% dry matter, 59.25% protein, 32.39% lipid and 15.83% ash.

Table 3. Survival, growth performance, and feed utilization by juvenile mullet *Mugil platanus* fed diets containing graded levels of protein for 35 days[1].

Parameters	Dietary protein level (%)				
	30	35	40	45	50
Initial weight (g)			1.17±0.02		
Survival (%)[2]	97.5±0.6	95.7±3.4	96.3±2.2	98.8±0.6	88.4±2.6^{ns}
SGR (%)[3]	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 (%)[4]	0.36±0.0	0.39±0.0	0.42±0.0	0.41±0.0	0.37±0.0^{ns}
FI (g/fish)[5]	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)[6]	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 [7]	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 (%)[8]	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}

[1] Mean ± standard error of three replicates. Values within the same rows having different superscripts are significantly different ($P < 0.05$), ns – non significant.

[2] Survival = [(initial number of fish – number of dead fish)/(initial number of fish) x 100]

[3] Specific growth rate = [(ln final weight – ln initial weight)/day] x 100.

[4] Feed efficiency = [weight gain/ feed intake].

[5] Feed intake = feed intake/ fish.

[6] Protein intake = protein intake/ fish.

[7] Protein efficiency ratio = [(weight gain/protein intake].

[8]Apparent net protein utilization = [(final weight x final body protein) – (initial weight x initial body protein)]/(total dry protein intake) x 100.

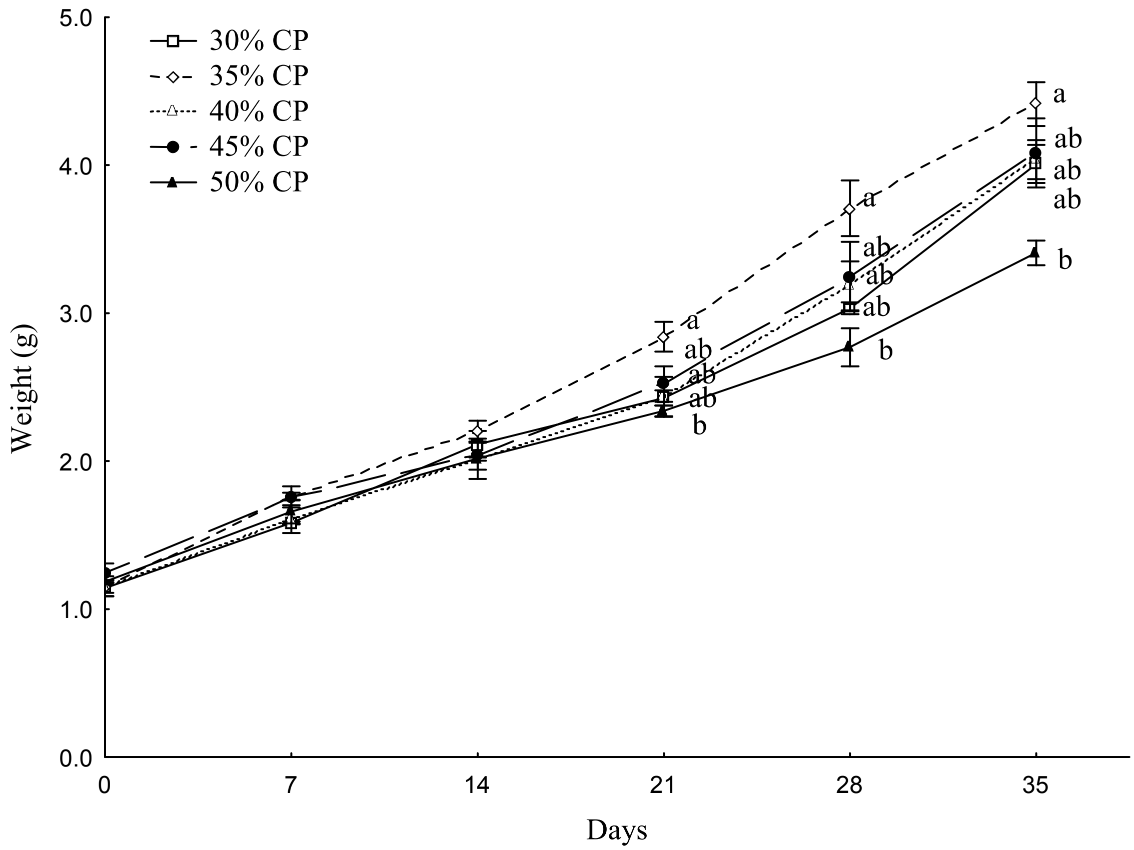

Figure 1. Growth of juvenile *Mugil platanus* fed diets containing increasing levels of protein for 35 days. Values are mean ± standard error of triplicate feeding groups. Points within the same day having different superscripts are significantly different ($P < 0.05$).

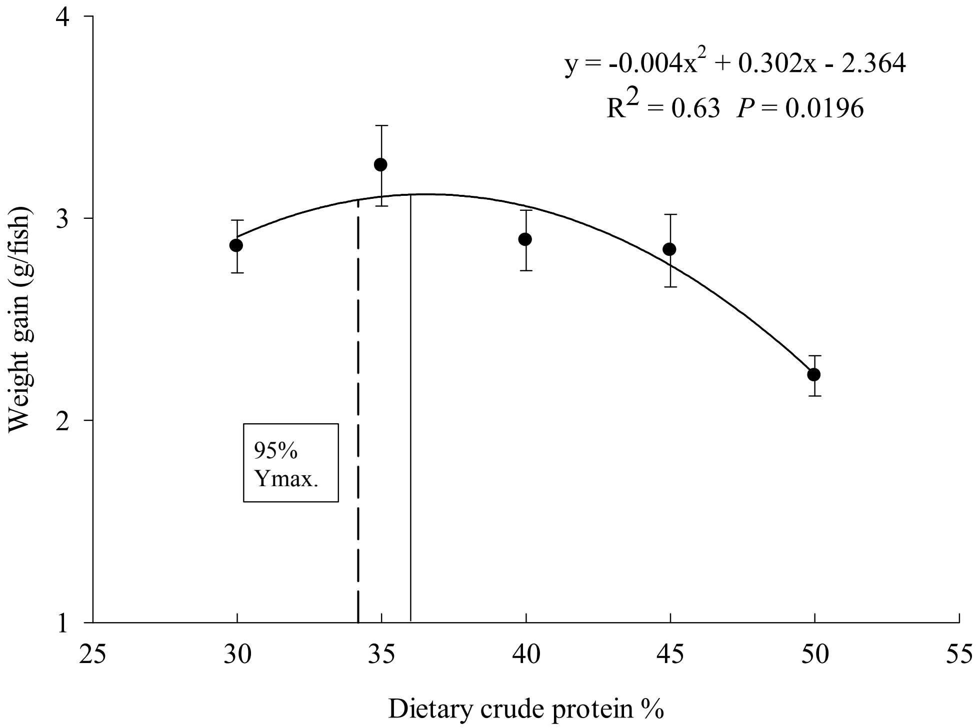

Figure 2. Relation between specific growth rate (%/day) and protein level for juvenile mullet *Mugil platanus* as shown by a quadratic regression, protein requirement is equal to 95% of maximum weight gain. Each point represents mean ± standard error of triplicate treatments.

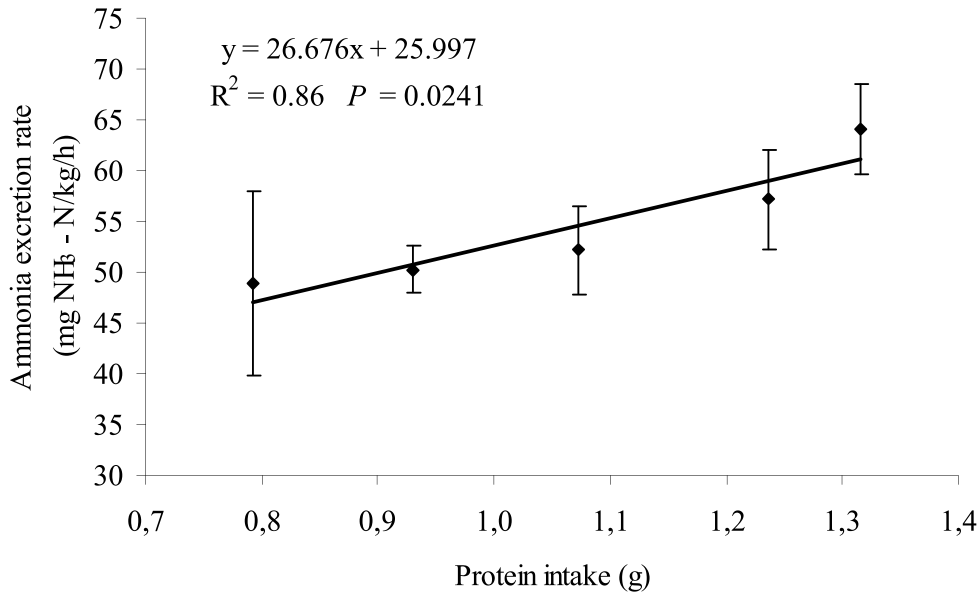

Figure 3. Postprandial ammonia excretion rate (mg NH_3-N/kg/h) as a function of protein intake for juvenile mullet *Mugil platanus*. Values are expressed as mean ± standard error.

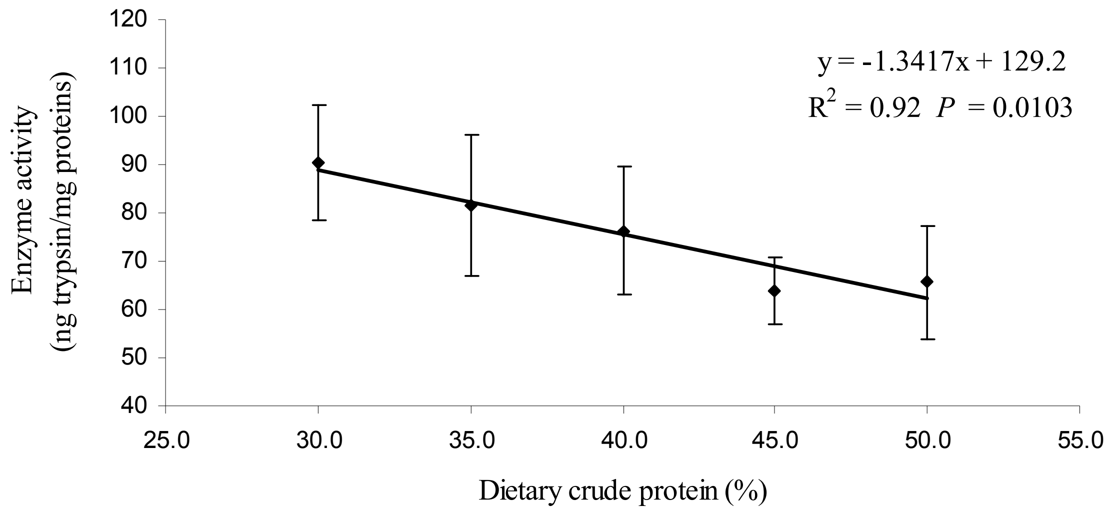

Figure 4. Intestinal tryptic activity in juvenile mullet *Mugil platanus* fed diets containing increasing levels of protein. Values are expressed as mean ± standard error.