AQUACULTURE CENTER,
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UNIVERSIDADE ESTADUAL PAULISTA

Optimum biological and economic balanced digestible protein requirements of juvenile, pre-adult and adult pacu (*Piaractus mesopotamicus* Holmberg, 1887)

Exigências de proteína digestível balanceada para juvenis, pré-adultos e adultos de pacu (*Piaractus mesopotamicus* Holmberg, 1887)–Portuguese

Kifayat Ullah Khan

PhD supervisor: *Prof. Dr. João Batista Kochenborger Fernandes*

Jaboticabal, São Paulo–Brazil

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Thesis presented to the Aquaculture Post-Graduate Program of the Aquaculture Center at São Paulo State University (UNESP), as part of the requirements for obtaining a doctoral degree in aquaculture.

Jaboticabal, São Paulo–Brazil

2019

CAUNESP

Khan, Kifayat Ullah
K45o Optimum biological and economic balanced digestible protein requirements of juvenile, pre-adult and adult pacu (*Piaractus mesopotamicus* Holmberg, 1887) / Kifayat Ullah Khan. -- Jaboticabal, 2019
vii, 174 p. ; il. ; 29 cm

Tese (doutorado) - Universidade Estadual Paulista, Centro de Aquicultura, 2019
Orientador: Prof. Dr. João Batista Kochenborger Fernandes
Banca examinadora: Dr. Joao Batista Kochenborger Fernandes, Dra. Margarida Maria Barros, Dra. Marta Verardino de Stefani, Dr. Eduardo Makoto Onaka, Dra. Rosangela Kiyoko Jomori Bonichelli
Bibliografia

1. Amino acids. 2. Balanced protein. 3. Modeling. 4. Cost-benefit-analyses. 5. Fish nutrition. I. Título. II. Jaboticabal-Centro de Aquicultura.

CDU 639.3.043

Ficha Catalográfica elaborada pela STATI - Biblioteca da UNESP
Campus de Jaboticabal/SP - Karina Gimenes Fernandes - CRB 8/7418

unesp

UNIVERSIDADE ESTADUAL PAULISTA

Unidade Complementar - Jaboticabal

CERTIFICADO DE APROVAÇÃO

Título: Optimum biological and economic balanced digestible protein requirements of juvenile, pre-adult and adult pacu (Piaractus mesopotamicus Holmberg, 1887)

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Aprovado como parte das exigências para obtenção do Título de Doutor em AQUICULTURA, pela Comissão Examinadora:

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Jaboticabal, 28 de fevereiro de 2019.

Centro de Aquicultura - Unidade Complementar - Jaboticabal -
Via de Acesso Prof. Paulo Donato Castellani, s/nº, 14870000, Jaboticabal - São Paulo
http://www.caunesp.unesp.br/#!/pos-graduacao/

IN THE NAME OF ALLAH, THE MOST BENEFICENT, THE MOST MERCIFUL

"Knowledge enables its possessor to distinguish what is forbidden from what is not; it lights the way to Heaven; it is our friend in the desert, our society in solitude, our companion when bereft of friends; it guides us to happiness; it sustains us in misery"

– Prophet Muhammad (P.B.U.H)

"Knowledge exists potentially in the human soul like the seed in the soil; by learning the potential becomes actual"

– Imam Al-Ghazali (R.A)

SUMMARY

DEDICATION

This thesis is dedicated

To my

Beloved **Grandparents** (maternal & paternal),

Beloved Parents **Amma Jaan** and **Abbu Jaan**,

Respected **Teachers**,

&

Beloved **family**

For their love, support, encouragement, sacrifices, well wishes and prayers

Kifayat Ullah Khan

ACKNOWLEDGEMENTS

First of all, I thank Almighty Allah for making my dream come true and blessing me to successfully complete my doctoral research work. I also express my sincere and whole hearted thanks to our beloved Holy Prophet Muhammad (PBUH) whose teachings guide us towards the light of knowledge.

I would like to express my special appreciation and thanks to my PhD supervisor Prof. Dr. João Batista Kochenborger Fernandes for his kind help, continuous support and encouragement during my PhD studies. His guidance helped me in all the time of my PhD research and writing of this thesis. It was fantastic to have the opportunity to work under his kind supervision at the Aquaculture Center.

I offer my special thanks to The World Academy of Sciences – TWAS and Brazilian National Council for Scientific and technological Research – CNPq for offering me a full time PhD fellowship, with a special mention to the São Paulo Research Foundation – FAPESP for financially supporting my research work. I am also grateful to Nei André Arruda Barbosa, Senior Regional Marketing Manager at Evonik São Paulo, Brazil and the Evonik industries AG, Essen, North Rhine-Westphalia, Germany for prviding us the amino acids and aminograms.

I am very grateful to Prof. Dr. Sergio Ricardo Batlouni (Director of the Aquaculture Center) and Prof. Dr. Julieta Rodini Engrácia de Moraes (Coordinator of the postgraduate program at the Aquaculture Center) for prviding me the necessary help during my PhD studies. I extend my special thanks to Prof. Dr. Nilva Kazue Sakomura for her kind help, useful advises and financial support of my PhD research. I am thankful to Prof. Dr. Robert M. Gous for his kind help in the designing of my PhD research project. I would like to express my thanks to Prof. Dr. Dalton Carneiro and Prof. Dr. Marta Verardino De Stéfani for providing me the necessary Lab. facilities during my research work with a special mention to Prof. Dr. Jesaías Ismael da Costa for his kind assistance in the economic analyses. I am also thankful to Prof. Dr. Fabiana Pilarski for her kind help in the pathological analyses of fish prior to each feeding trial of this study.

I would like to thank Prof. Dr. Margarida M Barros, Prof. Dr. Marta Verardino De Stéfani, Prof. Dr. Eduardo Makoto Onaka, and Prof. Dr. Rosangela Jomori, for their kind help in the evaluation of my PhD thesis. I am also thankful to Prof. Dr. Cyntia Peralta de Almeida Prado, for providing me the opportunity of teaching internship at the Department of Animal Morphology and Physiology, UNESP, Jaboticabal.

I am grateful to all the professors/ teaching staff of the Aquaculture Center, UNESP for their support, help and guidance during my PhD studies. I would like to offer my sincere thanks to my Portuguese language teacher Prof. Adriana Gonçalves for her kind help and continuous encouragement during my Portuguese classes.

A very special gratitude goes to all my spiritual, school, college and university teachers especially my previous research supervisor Prof. Dr. Amina Zuberi for their kind help, support and useful advises. I would have been lost without their inspiration, support and guidance. Many Thanks!

I would like to express my special thanks to my friends and labfellows Dr. Thiago Nascimento, Dr. Cleber Mansano, Andre Zuffo, Andressa Tellechea, Daniel Monge, Rafael Romaneli, Thais Silva, Monique Virães, Amanda Zucatto, and Fabio Henrique, with a special mention to Natalia Bassi, Hariany Ferreira and Allana Feitoza, for their continuous help and support during my research work. I am thankful to all my research colleagues at the poultry sector of UNESP especially Dr. Matheus Reis and Jefferson Azevedo, for their kind help during my research work.

I appreciate the help of Silvia, Renata and Andreia during the laboratory analyses. Special thanks to the Tuiuiu fish farm, located at the Guaira City, SP for providing fish to the present study.

I express my special gratitude to all the technical and supporting staff of the Aquaculture Center, UNESP particularly Valdecir Fernandes, Marcio Reche, Luis and Donizete, for their help and support during my experimental work. I am also thankful to the coordination staff of the Aquaculture Center including David Oliveira, Veralice (Ex. Member), Mayara, Robson, Elisandra and Monica for their sincere help during my PhD studies.

I wish to thank my entire extended beloved family especially my beloved Sister, Brothers and first degree relatives incluing my Sisters-in-law, Nephews, Aunts, Uncles, and First-cousins for their kind love, encouragement, support, prayers and useful advises.

Last but not least, I'd like to thank my beloved parents my sweet Amma Jaan and Abbu Jaan. They raised me, supported me, taught me and loved me. Their kind love is a source of major moral support in my life and their wholehearted prayers are the basic source of my success. Many thanks!

I am thankful to all my friends and colleagues for their well fishes and emotional support. Thanks all the people who helped me to achieve my academic goals and made this thesis possible.

Kifayat Ullah Khan

FINANCIAL SUPPORT

TWAS and CNPq have provided a full time PhD fellowship to the author of this thesis [CNPq PG FT/Kifayat Ullah Khan 190123/2014-1]

FAPESP supported the present study under the thematic project (2013/25761-4)

Evonik Industries AG (Germany) provided us the amino acids and aminograms.

Generalized abstract

This study aimed to determine the whole-body amino acid (AA) composition based dietary essential amino acid (EAA) requirements and optimum biological and economic balanced digestible protein (BDP) requirements of juvenile, pre-adult and adult pacu (*Piaractus mesopotamicus,* Holmberg 1887). No significant differences were observed in the present estimated dietary EAA requirements for the three growth phases of pacu. For juvenile (average body weight (BW), 54.3 ± 8.2 g), pre-adult (average BW, 822.5 ± 33.9 g), and adult pacu (average BW, 1,562.8 ± 41.8 g), the average EAA requirements were estimated to be histidine (0.42%), arginine (1.36%), threonine (0.82%), valine (0.90%), methionine (0.45%), isoleucine (0.82%), leucine (1.29%), phenylalanine (0.74%), lysine (1.64%) and tryptophan (0.14%). These EAA ratios were then used in the formulation of basal diets for three consecutive feeding trials being designed to determine the optimum biological and economic balanced digestible protein (BDP) requirements of juvenile, pre-adult and adult pacu. The basal diets consisted of high protein (HP) and low protein (LP) diets were formulated according to the diet-dilution approach. In the present study, dietary protein was defined as the balanced digestible protein because basal diets were provided with an essential amino acid balance using lysine as the reference amino acid. Lysine was provided in basal diets at a constant and optimum ratio of digestible protein. The ratios among lysine and minimum requirements of all other essential amino acids were kept constant. The two basal diets were mixed in proper proportions to achieve six isoenergetic experimental diets containing 163, 201, 238, 272, 315, and 348 g BDP kg^{-1} (dry matter-DM basis), respectively. The present study was performed in an experimental setup which was connected to a closed re-circulation aquaculture system (RAS). The RAS consisted of a mechanical and biological filter being provided with a heating system. The obtained results showed that growth performance and feeding parameters were significantly ($P < 0.05$) improved with diets containing optimum digestible protein levels and considerably worsened with the diet provided in protein beyond the optimum level. For juvenile pacu (initial average BW, 10.82 ± 0.14), the quadratic model calculated optimum biological balanced digestible protein (BBDP) requirement of 326 g kg^{-1} (dry matter−DM basis) and optimum digestible protein to energy (DP: DE) ratio was calculated to be 22.13 g MJ^{-1}. For whole-body, eviscerated and sliced juvenile pacu, the economic model estimated optimum economic balanced digestible protein (EBDP) requirement of 311, 316 and 319 g kg^{-1} (DM basis) and the optimum DP: DE ratio was estimated to be 21.11, 21.47, and 21.67 g MJ^{-1}, respectively. For pre-adult pacu (initial average BW, 490.60 ± 14.39), the quadratic model estimated optimum BBDP requirement of 292 g kg^{-1} (DM basis) and the optimum DP: DE ratio was estimated to be 19.82 g MJ^{-1}. For whole-body, eviscerated and sliced pre-adult pacu, the economic model estimated optimum EBDP requirement of 248, 253, and 260 g kg^{-1} (DM basis) and the optimum DP: DE ratio was calculated to be 17.00, 17.32, and 17.80 g MJ^{-1}, respectively. For adult pacu (initial average BW, 1158.50 ±

21.59), the quadratic model estimated optimum BBDP requirement of 245 g kg^{-1} (DM basis) and the optimum DP: DE ratio was estimated to be 16.78 g MJ^{-1}. For whole-body, eviscerated and sliced adult pacu, the economic model estimated optimum EBDP requirement of 215, 217, and 222 g kg^{-1} (DM basis) and the optimum DP: DE ratio was estimated to be 14.65, 14.79, and 15.13 g MJ^{-1}, respectively. According to the the present data, formulating diets for maximum profit rather than maximum fish performance may lead to higher profitability and better growth, particularly when the selling prices of protein-rich ingredients are high in the market. Moreover, it was revealed that the optimum economic balanced digestible protein requirements are subjected to the fluctuations in the selling prices of protein rich ingredients and the marketing of pacu such as whole body or in cut-up portions and fluctuations in the selling price of the feed ingredients. Thus, the present study suggests that the optimum economic protein should be adjusted according to the fluctuations in the selling prices of feed ingredients and whole fish and cut-up parts.

Keywords: Amino acids; Balanced protein; Modeling; Cost-benefit analyses; Fish nutrition

Resumo geral (Portuguese)

O estudo teve como objetivo determinar as exigências de aminoácidos essenciais (AAE's) baseado na composição aminoacídica do corpo inteiro. Como também, determinar os níveis ótimos biológicos e econômicos de proteína digestível balanceada (PDB) para juvenis, pré-adultos e adultos de pacu (*Piaractus mesopotamicus*, Holmberg 1887). Não foram observadas diferenças significativas nas exigências de AAEs estimadas para as três fases de crescimento do pacu. Para o estudo, foram utilizados juvenis com peso médio de 54,3 ± 8,2 g, pré-adultos de 822,5 ± 33,9 g e adultos de 1.562,8 ± 41,8g, cujas exigências médias de AAEs foram estimadas para a histidina (0,42%), arginina (1,36%), treonina (0,82%), valina (0,90%), metionina (0,45%), isoleucina (0,82%), leucina (1,29%), fenilalanina (0,74%), lisina (1,64%) e triptofano (0,14%). Para determinar os níveis ótimos biológicos e econômicos de proteína digestível balanceada (PDB) para pacu em diferentes fases de crescimento, duas dietas isoenergéticas, uma com alto teor de proteína e outra com baixo teor de proteína foram formuladas de acordo com o método de diluição. No presente estudo, a proteína foi definida como proteína digestível balanceada, porque as dietas basais foram fornecidas com um perfil balanceado de aminoácidos essenciais usando lisina como aminoácido de referência. A lisina foi fornecida nas dietas basais em proporções constantes ao nível ótimo de proteína digestível e, as relações entre os níveis de lisina e as exigências mínimas de todos os outros aminoácidos essenciais permaneceram constantes. Estas duas dietas foram misturadas em proporções adequadas para alcançar seis dietas

experimentais isoenergéticas contendo 163, 201, 238, 272, 315 e 348 g de PDB kg^{-1} (com base na matéria seca-MS), respectivamente. Os peixes foram mantidos em sistema de recirculação de água, equipado com filtragem mecânica e biológica, trocador de calor e composto por 18 unidades experimentais de alvenaria com volume de água de 2,000-L. De acordo com os resultados obtidos no presente estudo, os peixes alimentados com diferentes níveis de proteína digestível apresentaram melhor ($P < 0,05$) desempenho zootécnico até atingirem o platô. Para juvenis de pacu (PC médio inicial de 10,82 ± 0,14), o modelo quadrático calculou o nível ótimo biológico de proteína digestível balanceada (PDB) de 326 g kg^{-1} de MS, com relação proteína digestível e energia (PD: ED) de 22,13 g MJ^{-1}. Ao avaliar o modelo econômico para juvenis de pacu utilizando três formas de comercialização (corpo inteiro, eviscerado e em postas), o modelo econômico estimou o nível ótimo econômico de proteína digestível balanceada (PDB) de 311, 316 e 319 g kg^{-1} de MS, com relação PD: ED de 21,11; 21,47 e 23,67 g de MJ^{-1}, respectivamente. Na fase de pré-adulto (PC médio inicial, 490,60 ± 14,39), o modelo quadrático estimou o nível ótimo biológico de proteína digestível balanceada (PDB) de 292 g kg^{-1} de MS, com relação PD: ED de 19,82 g MJ^{-1}. Na fase de pré-adulto, os pacus vendidos como corpo inteiro, eviscerado e em postas, o modelo estimou o nível ótimo econômico de proteína digestível balanceada (PDB) de 248, 253 e 260 g kg^{-1} de MS, com relação PD: ED de 17,00; 17,32 e 17,80 g MJ^{-1}, respectivamente. Para adultos de pacu (PC médio inicial, 1158,50 ± 21,59), o modelo quadrático estimou o nível ótimo biológico de proteína digestível balanceada (PDB) de 245 g kg^{-1} de MS, com relação PD: ED de 16,78 g MJ^{-1}. Na fase de adulto, os pacus vendidos como corpo inteiro, eviscerado e em postas, o modelo estimou o nível ótimo econômico de proteína digestível balanceada (PDB) de 215, 217 e 222 g kg^{-1} de MS, com relação PD: ED de 14,65; 14,79 e 15,13 g MJ^{-1}, respectivamente. De acordo com a descrição de modelo econômico atua, a formulação de dietas para máximo lucro, em vez de máximo desempenho do peixe, pode resultar em alta lucratividade e melhor crescimento. O modelo econômico foi capaz de predizer que o nível ótimo econômico da proteína digestível pode ser submetida a flutuações de preço de venda dos ingredientes, que compõem as rações de pacu comercializados como pacu eviscerado, em postas ou em corpo inteiro.

Palavras-chave: Aminoácidos; Proteína balanceada; Modelagem; Análises de custo-benefício; Nutrição de peixe

CHAPTER 1

GENERAL CONSIDERATIONS

1. Introduction

Feed is a critical factor in intensive aquaculture systems and protein and amino acids are essential and important components of fish feeds due to their significant role in the structure formation and metabolism in all living organisms (Lee et al., 2001, 2003; National Research Council, 2011; Ma et al., 2019). Fish require adequate amount of dietary protein for growth and maintenance purpose but excess supply of this important nutrient may result in its conversion into energy in the liver and thus, increases the nitrogen excretion/ total ammonia production and causes a decline in growth (Wood, 1993; McGoogan and Gatlin III, 1999). Beside an essential nutrient, protein is considered the most expensive nutrient in fish nutrition and may lead to increased feeding costs if provided in excess in fish feeds (National Research Council, 2011).

Appropriate formulations and feeding strategies could help to prevent the use of protein as an energy source and determine the accurate protein requirements. The formulation of diets with optimum energy and providing an amino acid balance is necessay for the proper metabolism of the available dietary protein only for muscle growth (Gao et al., 2011; NRC, 2011). The diet-dilution approach which was originally developed by Fisher and Morris (1970) allowing the formulation of isoenergetic diets with an amio acid balance. The amino acid balance could be achived through the implementation of protein bound-amino acid concept rather than crystalline amino acids (Gous et al., 2018). This method makes possible the establishment of balanced diets which improve the body composition of the animal and may avoid the supplementation of higher protein content in the diet (Gous and Morris, 1985). On the other hand, diets formulated without providing an amino acid balance and optimum energy may negatively affect the body composition of fish and usually produce a fatty fish which is less acceptable in the consumer market. Also, such diets may lead to the the determination of inaccurate protein requirements because in the absence of sufficient energy and amino acid balance fish utilize the available dietary protein being composed of important amino acids as an energy source rather than muscle growth.

Pacu (*Piaractus mesopotamicus*), is an important freshwater fish species endemic to Latin American States (Fernandes et al., 2000, 2001, Machado-Allison, 1982; Paula et al., 1988;

Machado et al., 1991). This is an omnivorous freshwater species which is resitant to low temperatures, has a better growth rate and thus is considered an important cultivable species (Urbinati and Gonc¸alves, 2005). The available protein requirements have been determined for mostly for juvenile pacu (*Piaractus mesopotamicus*) with the concept to achieve maximum performance rather than obtaining maximum profits. Due to the increasing feed costs, the determination of economic protein levels is getting popularity in aquaculture industry. The establishment of balanced diets provided with economic balanced protein levels may result in a better growth response and higher profitability index (Eits et al., 2005b). Beside the determination of optimum economic protein levels, another important strategy should be the development of size-specific diets i.e., estimation of optimum protein requirements during different growth phases of fish (National Research Council (NRC), 2011; Ye et al., 2017). Such practices could play a significant role in the optimization of pacu nutrition and may help to boost the aquaculture production and achieve maximum profits of the final products at low feeding costs.

Keeping in view all the above concerns and to test the hypothesis "if exists any considerable difference between the optimum biological and economic balanced digestible protein requirements on the basis of performance data and cost-benefit analyses", the present study was designed with the following objectives.

2. Objectives

2.1. General objective

To determine optimum biological and economic balanced digestible protein requirements of juvenile, pre-adult and adult pacu

2.2. Specific objectives

2.2.1: To determine the whole body chemical composition and AA pattern of pacu in different growth phases

2.2.2: To estimate the quantitative dietary essential amino acid requirements using whole body composition AA pattern

2.2.3: To know if exist any difference in the essential amino acid requirements estimated according to the whole body AA composition among different growth phases

2.2.4: To determine the effect of different balanced digestible protein levels on the growth performance, feeding parameters and body chemical composition

2.2.5: To assess the optimum biological and economic balanced digestible protein requirements through appropriate biological and economic models

2.2.6: To determine the difference if exists between the optimum biological and economic requirements on the basis of performance and cost-benefit analyses data.

2.2.7: To establish nutritionally balanced size specific diets for pacu

2.2.8: To know if the optimum economic balanced digestible protein requirements depend on how the fish is marketed such as whole body or in the form of cut-up portions

1. Introdução (Portuguese)

A proteína e os aminoácidos são nutrientes essênciais devido a importância na formação da estrutura e metabolismo em todos os organismos vivos, incluindo peixes (NRC, 2011, Lee et al., 2001, 2003). Os peixes necessitam de uma quantidade adequada de proteína na dieta para o crescimento e manutenção, mas o excesso deste nutriente pode resultar na sua conversão em energia no fígado e, assim, aumentar a produção de nitrogênio/ amônia total e um declínio no crescimento (Wood, 1993; McGoogan e Gatlin III, 1999). Além de um nutriente essencial, a proteína é considerada o nutriente mais oneroso na nutrição de peixes e pode levar a um aumento nos custos de alimentação, se fornecido em excesso em rações para peixes (NRC, 2011).

Formulações apropriadas e estratégias de alimentação poderiam ajudar a prevenir o uso da proteína como fonte de energia (Gao et al., 2011; NRC, 2011). No entanto, uma dieta

formulada pela técnica da suplementação, sem o balanço adequado de aminoácidos e o nível nível ótimode energia pode afetar negativamente a composição corporal e resultar em um peixe com maior teor de gordura, que é menos aceitável no mercado consumidor e, também pode resultar em uma exigência de proteína inadequada. O segundo método baseia-se no princípio da diluição, no qual é formulado dietas com perfil balanceado de aminoácidos (Fisher and Morris, 1970; Gous et al., 2018). Esta última é uma boa alternativa que permite o estabelecimento de dietas balanceadas que melhoram a composição corporal do animal e que colaboram para a diminuição do teor proteico nas dietas (Gous e Morris, 1985).

O pacu (*Piaractus mesopotamicus*) é uma importante espécie de peixe de água doce endêmica da América Latina (Fernandes et al., 2000, 2001; Machado-Allison, 1982; Paula et al., 1988; Machado et al., 1991). Trata-se de uma espécie onívora, resistente a baixas temperaturas, apresenta boa taxa de crescimento e, portanto, é considerada uma importante espécie para cultivo (Urbinati e Gonçalves, 2005). As exigências de proteína disponíveis foram determinadas para juvenis de pacu com o conceito de atingir o máximo desempenho. No entanto, devido ao aumento dos custos de alimentação, a determinação dos níveis de proteína pela avaliação econômica está crescendo no setor de aquicultura. O estabelecimento de dietas balanceadas visando os níveis econômicos de proteína balanceada, podem resultar em melhores respostas para o crescimento dos peixes e consequentemente melhorar o índice de lucratividade da produção (Eits et al., 2005b). Além da determinação dos níveis ótimos de proteína econômica, outra estratégia importante deve ser o desenvolvimento de dietas de acordo com o tamanho do corpo de peixe (NRC, 2011; Ye et al., 2017). Tais estratégias poderiam resultar na otimização da nutrição da espécie, além de poder propiciar maior lucratividade no produto final, assim, impulsionando a sua produção a baixos custos de alimentação.

Com a hipótese de saber se existe alguma diferença entre os níveis de proteína digestível balanceada biológica e econômica com base em dados de desempenho e análises de custo-benefício, o presente estudo foi desenhado com os objetivos seguintes;

2. Objetivos

2.1. Objetivo geral

Exigências de proteína digestível balanceada para juvenis, pré-adultos e adultos de pacu (*Piaractus mesopotamicus* Holmberg, 1887)

2.2. Objetivos específicos

2.2.1. Determinar a composição de corpo inteiro do pacu em diferentes fases de crescimento

2.2.2. Estimar as exigências de aminoácidos

2.2.3 Avaliar as possíveis diferenças nas exigências de aminoácidos essênciais estimados de acordo com a composição do corpo inteiro entre diferentes fases de crescimento

2.2.4 Determinar o efeito de diferentes níveis de proteína digestível balanceada no desempenho zootécnico, nos parâmetros de alimentação e na composição química do corpo

2.2.5 Avaliar os níveis ótimos de proteína digestível balanceada biológica e econômica por modelos biológicos e econômicos apropriados

2.2.6 Determinar as diferenças entre os níveis ótimos de proteína digestível balanceada biológica e econômica com base em dados de desempenho e análises de custo-benefício

2.2.7 Estabelecer dietas de acordo com o tamanho do pacu

2.2.8 Avaliar se as exigências de proteína digestível balanceada econômica dependem de como o pacu é comercializado

3. Review of literature

3.1. Fish farming and socio-economic growth

Fisheries and aquaculture industry produces per year more than 90 million metric tons of fish as a source of nutritious food for the growing population globally (Food and Agriculture Organization (FAO), 2018). Fish provide biologically valuable nutrients particularly protein which help to reduce malnutrition in the under developed regions of the world (Albert et al., 2015; Bennet et al., 2018). In developing countries majority of people get approximately 30 percent of the total animal protein from the fisheries and aquaculture sector (FAO, 2018). In the current scenarios, acquiring only food security is not sufficient; food safety requires the same concern as well, because food safety is important for the successful establishment of food security. Consequently, the nutrition and health status of poor populations being suffered from serious malnutrition could be improved. Fish performance and meat quality should be properly focused and enhanced in order to better cope with increasing malnutrition problems. Such practices may help to provide an inexpensive, protein-rich and healthy food particularly to the people in the low-income societies. According to Yang et al. (2002), Eroldogan et al. (2008) and NRC (2011) improving feed utilization efficiency through the establishment of well-balanced diets may increase the production and improve the meat quality of fish at low feeding costs (Bennet et al., 2018; Khan et al., 2019b). The establishment of balanced diets help to avoid the extra supplementation of protein, economize the extra feeding costs and reduce the high nitrogen excretion in water (El-Saidy and Gaber, 2005; Kaushik and Seiliez, 2010; Deng et al., 2011; Marammazi and Kahkesh, 2011; Chen et al., 2012; El-Abed et al., 2013; Khan et al., 2019b). Recent research should be focused on securing the food security and food safety in order to boost and strengthen the socio-economic growth of the developing countries (Sargent and Tacon, 1999; Bennet et al., 2018; FAO, 2018).

3.2. Fish nutrition, feed quality and effective feeding practices

For sustainable and profitable aquaculture, nutrition and physiology oriented research in fish needs to be improved according to new practicable approaches keeping in view the growth response and production costs particularly feeding costs. Nutritional requirements can be

optimized by improving the quality of feeds and establishment of balanced diets provided with optimum energy and essential amino acid balance (Khan et al., 2019b). Fish farmers usually prefer diets to achieve maximum profit from the production systems at low feeding costs (Aliu et al., 2017). Diets with high protein content, lack of amino acid balance and insufficient energy along with irregular feeding frequencies and mismanaged feeding practices may lead to increase the feeding costs and cause high nitrogen excretion in water. Consequently, the profit of the final product declines and both the aquatic environment and fish health are negatively affected (Zeitoun et al., 1976; Abdel-Tawab et al., 2010; NRC, 2011). Feeding costs usually account for about 50 to 65 percent of the total production costs in intensive aquaculture systems and if such costs are properly managed then maximum profit coupled with good environmental effects could be obtained even from small investments (El-Saidy and Gaber, 2005; El-Sayed, 2006; Glencross et al., 2007, 2008; Ali et al., 2008; Nguyen et al., 2009; Abdel-Tawab et al., 2010).

Successful aquaculture production systems and profitability of the final product are closely linked to proper nutrition, feed quality and effective feeding programmes (Besson et al., 2014). The amount of feed which a fish actually needs should be provided at propter time and intensity. Prior to feeding, exploring the eating behaviour of fish is extremely important to supply proper amount of feed and at accurate time in order to attain maximum performance (El-Saidy and Gaber, 2005). This technique is helpful in avoiding the extra supplementation of feed and its application in fish farming reduces increasing feed costs and nitrogen losses into water (Hardy, 2002). Feeding fish with balanced diets at proper time and intensity are essential factors in the healthier fish production at viable feeding costs (Cho and Bureau, 2001; Houlihan, 2001; Halver and Hardy, 2002; Hamre et al., 2003; Eroldog˘an et al., 2008; NRC, 2011).

3.3. Protein research in fish

Protein is amongst the most studied nutrients and its requirements have been extensively studied for different fish species may be due to its high price or significant role in growth performance and physiological fucntions of the animal body (Halver and Hardy, 2002; Yang et al., 2003; Meyer and Fracalossi, 2004; Kim and Lee, 2009; NRC, 2011). Being a rich source of all essential and non-essential amino acids, the supplementation of dietary protein improves the performance, feeding aspects and body composition of fish. Its proper supplementation enhances the feed utilization efficiency of fish (El-Sayed and Teshima, 1992; Shiau and Lan, 1996; Yang

et al., 2002; Satpathy et al., 2003; Luo et al., 2004; NRC, 2011). On the other hand its inadequate or surplus amounts in the diet may result in reduced growth performance, poor feed utilization efficiency and increased feeding costs coupled with high nitrogenous wastes (Engin and Carter, 2001; Abimorad and Carneiro, 2004; Monentcham et al., 2010; Abdel-Tawwab et al., 2010; Bicudo et al., 2010).

Rearing environment, genetic pattern of the species, growth phase, feeding rates, available source of food and feeding habits are the main factors that influence the protein requirements (Craig, 2017; Ye et al., 2017). Most of the previous studies have been focused on the determination of protein requirements only for juvenile fish. From the market point of view, large size fish should be properly focused as well, in order to reduce the extra nitrogen supplementation which may cause extra feeding costs and exerts negative effects on the profit, fish health and water quality of the rearing system. The estimation of dietary protein requirements of fish in different growth phases may help feed industries to establish balanced diets for different growth phases of fish so that the fish farmers provide accurate amount of protein to a fish and get maximum profits (De Silva and Anderson, 1995; Nelson and Cox, 2005; Deng et al., 2011).

Proper dietary energy and protein to energy (DP: DE) ratios should be used in the formulation of diets which are important to obtain better performance and determine the accurate protein requirements (Bicudo et al., 2010; Gous et al., 2018; Khan et al., 2019b). When high protein content is added to feed with an insufficient energy level, fish utilize a part of the available dietary protein as an energy source to fulfil energy requirements rather than only for body growth. Such diets usually lead to the determination of inaccurate protein requirements (Meer et al., 1995; Ai et al., 2004; Sweilum et al., 2005; Gous et al., 2018). Using the recommended optimum amounts of dietary energy may help in proper metabolism of balanced dietary protein and make possible to determine the actual protein requirement of fish (Catacutan and Coloso, 1995; Wang et al., 2013). To avoid the use of protein as an energy source maintaining optimum dietary protein to energy ratios is a key factor in effective protein research (Hillestad and Johnsen, 1994; Cho and Bureau, 2001; El-Abed et al., 2013; Lemos et al., 2014).

3.4. Dietary protein quality and the concept of balanced protein

Low quality of dietary protein usually have low digestibility due to the lack of amino acid balance which negatively influence the determination of optimum dietary protein (Hamre et al., 2003; Sadasivam et al., 2010; Gous et al., 2018). The use of feed ingredients of low quality in terms of amino acid profile and without maintaining an essential amino acid balance can result in the supplementation of high amount of dietary protein (Wethli et al., 1975; Gümüş and ikiz, 2009). Such diets usually lead to the estimation of improper protein requirements (Thoman et al., 1999). This problem can be resolved by using protein ingredients of good quality and providing an essential amino acid balance in diets (Jauncey and Ross, 1982; Halver and Hardy, 2002; El-Saidy and Gaber, 2005). In the concept of balanced dietary protein, lysine is used as the reference amino acid and according to an ideal protein profile, the ratio among lysine and the minimum requirements of all other essential amino acids are kept constant (Mack et al., 1999). In addition, lysine is kept at a constant and optimum proportion of dietary protein (Eits et al., 2005a).

3.5. Essential amino acid balance

The formulation of diets provided with amino acid balance improves the quality and digestibility of the diet (Gous and Morris, 1985; Houlihan et al., 2001; Furuya et al., 2015). The AA pattern should be preferably that being determined in dose-response trials but if such data are unavailable for a species then whole-body AA composition based estimated AA pattern can be used in the formulation of diets (Wilson, 2002; Khan et al., 2019a). In previous studies, a close correlation has been determined among the amino acid requirements estimated in the dose response trials and whole-body AA pattern based estimated AA requirements (Wilson and Poe, 1985; Wilson, 2002; Abimorad et al., 2010). The dose response trials are expensive and time consuming and have been conducted for few amino acids in some of the fish species, however, whole-body amino acid patterns have been determined in several fish species because such estimations are less expensive and can be obtained in a short time. The dose-response based essential AA requirements are available for some most limiting essential amino acids such as lysine, methionine and threonine but requirements for all essential amino acids are still unavailable in majority of fish species including pacu (*Piaractus mesopotamicus*) (Portz and Cyrino, 2004; Gurure et al., 2007; Bicudo and Cyrino, 2009; Abimorad et al., 2010; Furuya et al., 2015; Khan et al., 2019a). Until dose response data are available, whole-body AA pattern

based estimated requirements have been recommended to be used in the formulation of practical and experimental diets (Wilson and Poe, 1985; Tacon, 1989; Kaushik, 1998; Wilson, 2002; Monentcham et al., 2010; NRC, 2011; Khan et al., 2019a). Such estimated requirements could be different than the actual requirements but they may provide a base-line for future dose-response trials to determine the accurate amino acid and protein requirements (Khan et al., 2019a, b).

The amino acid balance can be achieved through the supplementation of crystalline amino acids or protein-bound amino acids i.e., using protein-rich ingredients. The addition of high quantities of synthetic amino acids in fish diets has several issues such as; some of the synthetic amino acids are very costly and may increase the feeding costs; the leaching losses may reduce the amino acid digestibility and efficiency; and the use of any coating substance such as gelatin or agar may lead to improper texture and palatability of the feed (Lall and Anderson, 2005; Ambardekar and Reigh, 2007). Fish will not accept such diets favorably, feed intake will be restricted. The growth rate and profit will be declined due to the high quantity of synthetic amino acids and increased feeding costs. Another serious concern among aqua nutritionists regarding the synthetic amino acids are that they absorb more rapidly than protein-bound essential amino acids and thus limiting the utilization efficiency of fish due to the higher catabolism rates (Cowey, 1995; Ambardekar and Reigh, 2007). On the other hand, maintaining the amino acid balance through the protein rich ingredients i.e., concept of protein-bound amino acids overcomes all the above problems. This approach seems more practical in the formulation of aquaculture diets and may lead to increased productions at viable feeding costs. If necessary, i.e., in the case of using any plant ingredient, some most limiting essential amino acids such as lysine, methionine and threonine could be added to the diet at a low quantity (Gous and Morris, 1985; Schuhmacher et al., 1997; Ambardekar et al., 2009; Lemme, 2010).

3.6. Modeling optimum biological and economic balanced dietary protein requirements

To attain better performance and maximum profit, the formulation of bio-economically balanced diets is getting popularity in fish farming (Kim and Lee, 2005, 2009; Zhang et al., 2010). Balanced diets generally have essential amino acid balance and optimum energy and protein to energy ratios (Catacutan and Coloso, 1995; El-Saidy and Gaber, 2005; Okorie et al., 2007; Eroldogan et al., 2008; NRC, 2011; Khan et al., 2019b). In poultry and pigs proper biological and economic models are available to determine the optimum biological and economic

requirements by taking into account biological and economic responses (Gous and Morris, 1985; Eits et al., 2005a, b; Gous et al., 2018). In fish although biological models have been widely used to estimate the optimum biological requirements on the basis of performance data (Khan et al., 2019b) but still no appropriate economic models are available to model optimum economic protein requirements by taking into account both biological and economic responses (outputs). The optimization of biological models and development of economic models in fish along with the implementation of new feed approaches are highly important to establish nutritionally balanced diets for different fish species. Introducing such diets in fisheries and aquaculture sector can help the farmers to produce healthier fish and achieve maximum profit at low feeding costs.

For the formulation of experimental diets in dose response studies, so far two methods have been practiced in animal and fish nutrition, one is the grade supplementation method being practiced commonly in animal nutrition including fish (Fernandes et al., 2000, 2001; Abimorad et al., 2007; Bicudo et al., 2010; Kpogue et al., 2013; Klein et al., 2014; Sankian et al., 2017; Wang et al., 2017) and the other is the diet-dilution method which has been well studied in animals like pigs and poultry (Fisher and Morris, 1970; Gous and Morris, 1985; Gous et al., 2018) but in fish nutrition it has been reported in few studies such as by Abboudi et al. (2007) and Liebert and Benkendorff (2007).

The graded supplementation method has been widely used for the formulation of protein diets to determine the protein requirements in different fish species (Fernandes et al., 2000, 2001; Yang et al., 2002; Sweilum et al., 2005; Ozo´rio et al., 2006; Abimorad et al., 2007; Mohanta et al., 2007; Bicudo et al., 2010; Signor et al., 2010; Ye et al., 2017). It is based on the principle of the graded supplementation of a test nutrient to a basal diet. The main disadvantage of this method is that a systematic change occurs in essential amino acid balance among different diets which may negatively affect the growth response in animals (Fisher and Morris, 1970; Gous and Morris, 1985; Gous et al., 2018).

A good alternative to the graded supplementation method is the diet dilution method which was basically established for the evaluation of growth responses to methionine intake in laying hens by Fisher and Morris (1970). The same method with some modifications was then used by Pilbrow and Morris (1974) to determine laying hen response to dietary lysine intakes. Freeman (1979) used it for the determination of tryptophan requirement in chicks. Similarly,

Gous and Morris (1985) used the same technique for evaluating the response of broiler chickens to increasing levels of lysine. Later on, it was also tested in fish species like in Atlantic salmon (*Salmo salar* L.) to determine threonine requirement for maintenance by Abboudi et al. (2007) and in *Oreochromis niloticus* to determine lysine requirements by Liebert and Benkendorff (2007). This concept is based on the hypothesis that AA utilization is independent of the dietary protein level and is based on the principle of diluting a high protein diet with a protein free or low protein diet in order to achieve different intermediary protein levels (Fisher and Morris, 1970). According to this method, the high protein diet usually contains all the essential amino acids in excess than their optimum requirement and the low protein diet provides an amount of EAAs lower than their requirement but the energy content should be kept the same in both basal diets. Both the basal diets will be provided with the same amount of energy, vitamins, minerals and other necessary supplements. This method has an advantage over the graded supplementation method because it avoids the problem of the change of essential amino acid balance among different dietary treatments. By this method consecutive increasing of levels of dietary protein could be achieved by maintaining the same EAA balance in all the test diets (Gous and Morris, 1985). This method focuses on the supplementation of protein bound amino acids rather than synthetic amino acids, and thus avoids the problems of high feeding costs and restricted feed intake and low feed utilization efficiency which may occur if large amounts of synthetic AAs are added to the diets in order to achieve the desired balance (Gous and Morris, 1985; Hauler and Carter, 2001). It allows the development of well-balanced diets at a low-cost (Fisher and Morris, 1970; Gous and Morris, 1985; Halver and Hardy, 2002; Abboudi et al., 2007; Liebert and Benkendorff, 2007).

Diets formulated according to the dilution approach considerably improve the feed intake and growth performance in fish and reduce nitrogen leaching (Gous and Morris, 1985; Abboudi et al., 2007). The energy level should be maintained optimum so that fish use the available balanced dietary protein only for muscle growth and maintenance purpose rather than as an energy source (Gous et al., 2018; Khan et al., 2019b). Based on the above arguments, in the case of the studies determining the biological and economic protein requirements, the diet dilution method is an important option for the formulation of balanced protein diets for both experimental and practical purposes.

3.7. Dietary protein requirements

Most of the nutritional requirements that have been reported in literature for different fish species including pacu are determined for juvenile growth phase and for adult fish such information is almost unavailable (NRC, 2011). Feed industries are usually using the nutritional requirements of the juvenile fish for the formulation of diets for fish of all size. Sometimes it leads to the supplementation of higher nitrogen in feed, especially in the case of adult fish which may increase the feeding costs and profit and also deteriorate the water quality. The establishments of optimum protein requirements in different growth phases have beneficial effects on fish health, growth, profit of the final product and aquatic environment. Thus for sustainable aquaculture, it is extremely important to determine protein requirements of fish in different growth phases by taking into consideration both biological and economic outputs (Jauncey and Ross, 1982; El-Saidy and Gaber, 2005; Ali et al., 2008; Nguyen et al., 2009; Abdel-Tawab et al., 2010; Dumas et al., 2010; NRC, 2011; Ye et al., 2017).

Pacu (*Piaractus mesopotamicus*) is an important freshwater fish which belongs to the characidae family and is a favorable cultivable species in several countries of Latin America, Asia, Europe, North and Central America and Oceania (Britski, 1973; Borghetti and Canzi, 1993; Lovshin, 1995; Pullela, 1997; Fernandes et al., 2000, 2001; Urbinati and Gonçalves, 2005; Cagauan, 2007; Abimorad et al., 2010; Bicudo et al., 2010; Cáleta et al., 2011; Froese and Pauly, 2013; Uchoi et al., 2015; Nico and Loftus, 2018). It is as an omnivore/ herbivore fish and favorably eats leaves, flowers, fruits, and seeds of plants (Merola, 1988), zooplankton, insects, snails and decaying plants (Froese and Pauly, 2013). Like in several other fish species, protein requirements have been determined mostly for juvenile pacu and little is known about the nutritional requirements of large size pacu. The previously protein requirements estimated in dose response trials through graded supplentation approach for juvenile pacu are too much diversified and ranging from 23 to 29 g kg^{-1} (Fernandes et al., 2000, 2001, Abimorad et al., 2007; Bicudo et al., 2010, Signor et al., 2010; Klein et al., 2014). These requirements have been mostly evaluated through the biological responses such as weight gain, specific growth rate and feed conversion ratio and simple statistical comparisons among different treatments and the broken-line regression (Brenner, 1988; Fernandes et al., 2000, 2001; Abimorad et al., 2007; Bicudo et al., 2010; Signor et al., 2010; Klein et al., 2014). Similar to other fish, for pacu no appropriate

economic models are available to model the optimum economic dietary protein requirements by taking into account both the biological and economic responses.

Fisheries and aquaculture industry is rapidly growing and due to the problems of increased feeding costs and high nitrogen excretion in water, the optimization of pacu nutrition should be properly focused in order to increase its healthier production and profit of the final product through nutritionally balanced and economically viable diets. The protein requirements for pacu and other fish species should be estimated through biological responses cost-benefit analyses. Such efforts are essential and important towards the development of sustainable and profitable aquaculture.

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CHAPTER 2

Whole body amino acid pattern of juvenile, pre-adult and adult pacu, *Piaractus mesopotamicus,* with an estimation of its dietary essential amino acid requirements

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Article first published: 17 February, 2019**: Journal of the World Aquaculture Society,** 1–11p. **DOI:** https://doi.org/10.1111/jwas.12600

ABSTRACT

In the present study, juvenile (live body weight, 54.3 ± 8.2 g), pre-adult (live body weight, 822.5 ± 33.9 g), and adult (live body weight, 1,562.8 ± 41.8 g) pacu, *Piaractus mesopotamicus*, were used to estimate their dietary essential amino acid (EAA) requirements using the whole-body amino acid (AA) pattern. The results showed that whole-body moisture, crude protein, total lipid, and ash contents expressed on a wet weight basis (%) were significantly different among the studied growth phases. No significant differences were observed in the dietary EAA requirements estimated for the three growth phases of pacu. These dietary EAA requirements were found to be different than those previously estimated for the same fish through its muscle AA pattern. Based on whole-body EAA to total EAA ratios {A/E ratios; [(each EAA/total EAA) × 1,000]}, EAA requirements were estimated to be histidine (0.42%), arginine (1.36%), threonine (0.82%), valine (0.90%), methionine (0.45%), isoleucine (0.83%), leucine (1.29%), phenylalanine (0.74%), lysine (1.64%), and tryptophan (0.14%) for pacu. These estimated requirements may serve as a reference line in the formulation of practical and experimental diets until dose–response-based optimum EAA requirements are available for pacu.

***Keywords*:** A/E ratios, amino acid research, balanced diets, fish nutrition, ideal protein concept, pacu, whole-body amino acid pattern

1. Introduction

Protein is an important source of both essential (EAAs) and nonessential (non-EAAs) amino acids and plays a significant role in the optimum growth and physiological functions of animals including fish (Meyer and Fracalossi, 2005; National Research Council, 2011; Khan, 2012). It is considered the most expensive and essential nutrient in animal feeds that directly

affects animal performance (Martinez-Palacios et al., 2007; Zuanon et al., 2009). A suitable level of dietary protein is essential for growth and enzymatic activities in all animals including fish (Arai, 1981; Ketola, 1982; Ogata et al., 1983; Rumsey and Ketola, 1975; Zhang et al., 2008). However, if supplied at an insufficient level, a decline in animal performance will occur (Wilson, 2002). On the other hand, if it is supplied in excess, the animal will only use a portion for maintenance and growth while converting the rest into energy (Shiau, 2002).

To control feed costs and minimize nitrogen pollution, it is necessary to formulate diets by supplementing a wellbalanced amino acid (AA) profile (Poston and Rumsey, 1983; National Research Council, 2011). A diet with the mixture of optimal EAA ratios is usually considered well-balanced (Gous and Morris, 1985; Wilson, 2002), and its supplementation improves animal growth and feed utilization efficiency (Davies and Morris, 1997; Hardy, 2002; Yamamoto et al., 2005; Zhang et al., 2008; Li et al., 2009; Diogenes et al., 2016). Moreover, to obtain a better performance at viable feeding costs, the oversupplementation of EAAs should be reduced in order to help reduce feed costs. This strategy also helps to reduce aquatic pollution, which is mainly caused by the oversupplementation of nitrogen (Gaylord and Barrows, 2009; Ng and Hung, 1995).

The accurate EAA requirements of an animal can be determined only through dose–response trials (Ahmed, 2012; Akiyama et al., 1997). However, until dose–response data are available, whole-body AA composition might be an important factor to estimate the quantitative dietary EAA requirements of animals including fish (Arai, 1981; Brown, 1995; Gurure et al., 2007; Monentcham et al., 2010; Small and Soares, 1998). Thus, in the absence of dose–response-based AA data, a whole body-based estimated AA profile may serve as a good alternative (Miles and Chapman, 2007; Monentcham et al., 2010). This method is usually considered less time consuming and less costly than AA dose–response trials (Akiyama et al., 1997), and the estimated AA profile provides a reference line for future dose–response studies regarding protein and AA research.

In the absence of dose–response data, if we are to formulate efficient practical and experimental feeds, we need to estimate the EAA requirements using the concept of "ideal protein," which has been widely studied in several animals including fish (Boisen et al., 2000; Dari et al., 2005; Fuller et al., 1989; Mansano et al., 2017; Wang and Fuller, 1989). Diets

formulated according to such a concept contain optimum EAA ratios and may help to improve growth performance and AA retention (Wilson, 1991; Wilson and Poe, 1985). Thus, the use of this concept is important in the formulation of economically and biologically viable and environment-friendly diets in the aquaculture industry (Gaylord and Rawles, 2005).

In previous studies, whole-body EAA data of animals have been used to estimate their quantitative dietary EAA requirements using the concept of A/E ratios [(each EAA/total EAA) × 1,000] (Arai, 1981), which have already been applied to several aquatic species (Bicudo and Cyrino, 2009; Hossain et al. 2011; Mansano et al., 2017; Meyer and Fracalossi, 2005). According to the available literature, whole body- and dose–response-based established EAA requirements are closely related. This phenomenon has been reported in several species, including hybrid striped bass, *Morone chrysops* × *Morone saxatilis* (Brown, 1995); common carp, *Cyprinus carpio* (Cowey and Tacon, 1983); channel catfish, *Ictalurus punctatus* (Wilson and Poe, 1985); milkfish, *Chanos chanos* (Borlongan and Coloso, 1993); and white sturgeon, *Acipenser transmontanus* (Ng and Hung, 1995).

Pacu, *Piaractus mesopotamicus*, is an important fish species that belongs to the characidae family and is a favorable cultivable species in different regions of the world, including Asia, Europe, North and Central America, and Oceania (Abimorad et al., 2010; Cáleta et al., 2011; Fernandes et al., 2000; Froese and Pauly, 2013). To support the aquaculture industry, it is necessary to improve the optimum protein and AA requirement data and increase the production of this important fish by formulating diets with a balanced EAA profile. As dose–response-based AA data (except lysine) is unavailable for the species under study, whole body-based estimated EAA requirements could provide accurate information to formulate future practical and experimental diets. Hence, the present study was designed to determine the whole-body AA pattern of pacu, *P. mesopotamicus*, in different growth phases and estimate the quantitative dietary EAA requirements.

2. Materials and methods

2.1 Animals

All procedures followed in the trial were according to the ethical guidelines described by the Brazilian College of Animal Experimentation and were approved by the "Ethics Committee on Animal Use" of the Faculty of Agricultural and Veterinary Sciences, São Paulo State University (UNESP) via protocol no. 009999/14.

Fish in three different growth phases [20 juvenile (54.3 ± 8.2 g), 15 pre-adult (822.5 ± 33.9 g), and 10 adult (1,562.8 ± 41.8 g) pacu] were sampled from the fish ponds of the Aquaculture Center at UNESP, Jaboticabal. The origin of the fish was the Paraná River located at the Ilha Solteira city, SP, Brazil. Fish were immediately euthanized, and their body weights were recorded. The whole-body fish samples were classified in three sizes and separately packed in polyethylene bags and frozen immediately in a freezer for subsequent analysis. The whole-body samples were ground in a meat grinder and then refrozen and lyophilized at −80 °C in a Thermo VLP200 freeze dryer to obtain predry matter and were ground again in a ball mill to obtain a fine powder prior to conducting AA analysis.

2.2 Analytical procedures

Well-prepared whole-body fish samples in triplicate were sent to the Laboratory of Protein Sources, University of Campinas (UNICAMP), SP, Brazil, for the determination of the AA pattern. Total AAs were analyzed by acid hydrolysis and ion exchange chromatography high performance liquid chromatograph at the laboratory according to the methods of White et al. (1986) and Hagen et al. (1989). The analysis of crude protein, total lipid, ash, and moisture content was performed at the Bromatology Laboratory, Department of Animal Science, São Paulo State University (UNESP), Jaboticabal, SP, Brazil. Moisture, crude protein, total lipid and ash content in the whole body samples were analyzed according to the standard methods of AOAC (2000).

2.3 Estimation of theoretical dietary EAA requirements

The concentration of each specific EAA was expressed relative to the total EAA content of the sample according to the formula of Arai (1981): A/E ratio = [(individual EAA/total EAAs) × 1,000]. The lysine requirement, determined through a previous dose–response trial by

Abimorad et al. (2010) for the species under study, was used to estimate the present dietary EAA requirements using whole-body AA composition and A/E ratios estimated in the present study. The estimation of quantitative dietary EAA requirements was performed according to the formula of Kaushik (1998): EAA requirement = (requirement for lysine × specific A/E) (A/E for lysine)$^{-1}$.

3. Results

Whole body analyses for moisture, crude protein, total lipid and ash content are presented in Table 1 with significant differences observed across different life stages.

Whole body amino acid composition (expressed as a percentage of dietary protein) and the estimated A/E ratios for the three growth phases of pacu are presented in Table 2.

No significant differences were observed in the estimated quantitative dietary EAA requirements and A/E ratios among the three growth phases of pacu (Table 3).

Based on the whole body EAA to total EAA ratios (A/E ratios, ((each EAA/total EAA) × 1000), the average EAA requirements of pacu were estimated to be histidine (0.42%), arginine (1.36%), threonine (0.82%), valine (0.90%), methionine (0.45%), isoleucine (0.83%), leucine (1.29%), phenylalanine (0.74%), lysine (1.64%) and tryptophan (0.14%).

The estimated EAA requirements of pacu estimated in the present study were found different to those previously estimated on the basis of its muscle tissue AA composition (Table 4).

Table 1

Whole body chemical proximate composition of pacu on wet weight basis (%).

Growth phase	Moisture	Crude protein	Total lipid	Ash
Juvenile	75.92 ± 1.40^{a}	13.68 ± 0.38^{c}	5.86 ± 1.23^{c}	3.56 ± 1.73^{b}
Pre-adult	68.63 ± 0.54^{b}	14.88 ± 0.78^{b}	9.72 ± 0.22^{b}	4.96 ± 0.24^{a}
Adult	59.96 ± 0.35^{c}	15.62 ± 1.09^{a}	15.66 ± 1.22^{a}	5.79 ± 0.24^{a}

Values are mean ± SD. Averages with different superscript letters within the same column are significantly different ($P < 0.05$) by Duncan's test.

Table 2

Whole body amino acids (AAs) composition and A/E ratios of pacu.

EAAs and Non-EAAs composition (g/100 g crude protein)				A/E Ratios [Arai (1981)]		
	Juvenile	Pre-adult	Adult	Juvenile	Pre-adult	Adult
Histidine	1.86	2.11	2.20	18.62	21.94	23.07
Arginine	6.05	6.91	6.73	60.61	71.82	70.41
Threonine	3.62	4.12	4.23	36.23	42.79	44.32
Valine	3.94	4.42	4.69	39.47	45.96	49.05
Methionine	2.00	2.25	2.30	20.06	23.35	24.07
Isoleucine	3.59	4.06	4.35	35.95	42.21	45.53
Leucine	5.62	6.38	6.74	56.37	66.23	70.61
Phenylalanine	3.31	3.65	3.74	33.14	37.95	39.18
Lysine	7.22	8.24	8.38	72.33	85.58	87.73
Tryptophan	0.61	0.63	0.84	6.11	6.51	8.76
Cysteine	1.03	1.21	1.23	10.35	12.59	12.89
Tyrosine	2.27	2.43	2.63	22.72	25.27	27.50
Aspartic acid	8.17	7.42	7.23	81.92	77.07	75.64
Glutamic acid	15.01	12.62	12.46	150.44	131.12	130.44
Serine	4.59	3.75	3.91	46.03	38.95	40.89
Glycine	12.50	10.66	9.14	125.23	110.68	95.69
Taurine	1.63	1.15	0.82	16.30	11.93	8.56
Alanine	8.92	7.64	7.84	89.41	79.41	82.09
Proline	7.86	6.61	6.07	78.73	68.65	63.56

Note. AA: amino acid; EAA: essential amino acid.

Table 3

Whole body AA composition based EAA and Non-EAA requirements of pacu.

	Requirements (%)		
	Juvenile	Pre-adult	Adult
Histidine	0.42	0.42	0.43
Arginine	1.37	1.38	1.32
Threonine	0.82	0.82	0.83
Valine	0.89	0.88	0.92
Methionine	0.45	0.45	0.45
Isoleucine	0.82	0.81	0.85
Leucine	1.28	1.27	1.32
Phenylalanine	0.75	0.73	0.73
Lysine	1.64	1.64	1.64
Tryptophan	0.14	0.12	0.16
Cysteine	0.23	0.24	0.24
Tyrosine	0.52	0.48	0.51
Aspartic acid	1.86	1.48	1.41
Glutamic acid	3.41	2.51	2.44
Serine	1.04	0.75	0.76
Glycine	2.84	2.12	1.79
Taurine	0.37	0.23	0.16
Alanine	2.03	1.52	1.53
Proline	1.79	1.32	1.19

Note. AA: amino acid; EAA: essential amino acid.

Table 4

Present estimated EAA requirements of pacu and some previous related literature.

EAAs	Present study‡	Abimorad et al. (2010)	Nascimento (2015)
Histidine	0.42	0.47	0.37
Arginine	1.36	1.24	0.90
Threonine	0.82	0.90	0.53
Valine	0.90	0.86	0.72
Methionine	0.45	0.41	0.48
Isoleucine	0.82	0.78	0.64
Leucine	1.29	1.56	1.19
Phenylalanine	0.74	0.80	0.59
Lysine	1.64	1.77	1.76
Tryptophan	0.14	Not reported	0.17
Cysteine	0.24	0.13	0.24
Tyrosine	0.50	0.63	0.51

‡ An average EAA pattern obtained for pacu in the present study.

4. Discussion

It is well known that the chemical composition, including the moisture, crude protein, total lipid, and ash content, of fish does not remain constant and changes during the growth phases (Dumas et al., 2010; Ye et al., 2017). Sufficient literature is available about the body chemical composition of fish, which shows that it may be influenced by several internal and external factors such as fish size, strain, diet nature, rearing conditions, and season (Brown, 1957; Caulton and Bursell, 1977; Groves, 1970; Love, 1980; Phillips et al., 1960; Salam et al. 2001; Salam and Davies, 1994; Ye et al., 2017). In the preset study, body chemical composition was significantly affected by wet body weight among the three growth phases of pacu. According to previous studies, lipid and moisture contents are inversely related (Dumas et al., 2010; Ye et al., 2017). In the present study, body lipid content significantly increased with increasing fish size, resulting in reduced moisture content of the fish. Body protein and ash content also increased in relation to increases in fish size. Similar results have been reported by Salam and Davies (1994) and Salam et al. (2001), concluding that the body chemical composition of pike, *Esox lucius* Linnaeus, 1758 and *Oreochromis niloticus*, respectively changes in relation to its body size.

According to the National Research Council (2011), AAs play an important role in the synthesis of protein and other compounds. Inadequate amounts can cause reduced growth and severe health problems in fish (Walton et al., 1985; Akiyama et al., 1985a, 1985b; Akiyama and Arai, 1993). Therefore, dose–response-based optimum AA requirements should be determined. However, before conducting dose–response trials, the whole-body AA pattern of an animal species needs to be determined in order to provide some reference line for the formulation of experimental diets. Whole-body AA composition, when expressed as a percentage of dietary protein, typically does not change in relation to fish body size (Kaushik, 1998; Monentcham et al., 2010). In our study, AA composition expressed as a percentage of dietary protein showed no significant difference among different growth phases of pacu. Namulawa et al. (2012) also observed no significant differences in the AA composition among different growth phases of Nile perch, *Lates niloticus* Linnaeus 1758. In African bonytongue, *Heterotis niloticus*, no significant differences were observed in the AA composition among different developmental

stages (larva up to adult size) (Monentcham et al., 2010). Results reported by Kaushik et al. (1991) on Siberian sturgeon, *Acipenser baeri*; Kaushik (1998) on European sea bass, *Dicentrarchus labrax*, gilthead sea bream, *Sparus aurata*, and turbot, *Psetta maxima*; and Meyer and Fracalossi (2005) on *Rhamdia quelen* conclude that AA composition, when expressed as a percentage of dietary protein, is consistent among different growth phases of a fish. Similarly, Wilson and Poe (1985) also observed in channel catfish, *I. punctatus* (weighing between 30 and 863 g) that AA composition, when expressed as a percentage of dietary protein, does not change in relation to body size. These previous findings support the current data.

According to the concept of "ideal protein," quantitative EAA requirements of an animal can be estimated using whole-body AA composition when only one EAA is known through the ideal ratio of the determined EAA relative to the EAA totals in the fish body (Arai, 1981; Monentcham et al., 2010). If no such information is available regarding any EAA, then a known EAA requirement for another closely related species can be used to estimate the quantitative EAA requirements (Meyer and Fracalossi, 2005; Monentcham et al., 2010). In the present study, quantitative dietary EAA requirements were estimated using the lysine requirement of the juvenile pacu determined by Abimorad et al. (2010). No significant differences were observed in EAA requirements among different growth phases of pacu. Similar results have been reported by Namulawa et al. (2012) on Nile perch, *L. niloticus* Linnaeus 1758; Monentcham et al. (2010) on African bonytongue, *H. niloticus*; and Wilson and Poe (1985) on channel catfish, *I. punctatus*, indicating that quantitative EAA requirements do not change in relation to body size. This consistency may be possibly because of the use of lysine requirement known for only one growth phase of a species to calculate the quantitative dietary EAA requirements for its different growth phases. The lysine requirement usually changes in relation to fish body size; thus, if lysine requirements determined through dose–response trials for different growth phases of a fish species are used for the calculation of quantitative EAA requirements, significant differences may occur in the EAA requirements (Tibaldi and Lanari, 1991). Differences in the AA requirements among different growth phases of a fish but within the same species and among different species may arise because of differences in rearing environment, available source of food, or the relative proportions of body structural proteins among different growth phases of the same species or different species. In addition, the metabolic or physiological needs for specific

AAs may also differ among different growth phases or among different species (Wilson and Poe, 1985), which may lead to differences in EAA requirements.

As no significant differences were found in the quantitative EAA requirements among the three growth phases of pacu, an average EAA profile was obtained for pacu and compared to that estimated by Abimorad et al. (2010) using muscle tissue AA composition for the same fish (Abimorad et al., 2008). The total EAAs, including lysine, histidine, threonine, phenylalanine, arginine, valine, methionine, leucine, cysteine, and tyrosine requirements estimated in the present study, were found to be different from those estimated by Abimorad et al. (2010). Similar differences were also reported in common carp, *C. carpio*, by Buchtová et al. (2007) and yellow tail lambari, *Astyanax altiparanae*, by Abimorad and Castellani (2011). These results reflect the fact that the AA content of specific tissue varies depending on the function of that tissue. Hence, the use of select tissue versus whole-body composition will result in different AA patterns (Wilson and Poe, 1985).

Tryptophan requirement was not reported in the study of Abimorad et al. (2010). The present estimated requirement of tryptophan appears to be low compared to that estimated for other fish species. The low requirement of tryptophan has been estimated by Nascimento (2015) for tambaqui, *Colossoma macropomum* and Meyer and Fracalossi (2005) for jundiá, *R. quelen*. Regarding the estimation of such low levels of tryptophan, one contributing factor could be the analysis technique. Thus, in the case of tryptophan, it is important to perform analyses using different methodologies in order to know if some errors exist in commonly used methods. According to the National Research Council (2011), tyrosine and cysteine requirements can replace about 50% (50:50 ratio) of the supplementation of phenylalanine and methionine, respectively. Thus, because of their important role in the substitution of phenylalanine and methionine, these AAs were also estimated in the present study.

Pacu, *P. mesopotamicus* and tambaqui, *C. macropomum* are sister species and belong to the same family (Characidae) and subfamily (Serraassalminae) and have some common characteristics. Thus, the present data were compared to the results of Nascimento (2015), who estimated digestible EAA requirements for juvenile tambaqui, C. macropomum, using muscle tissue AA composition. We have estimated the whole-body AA composition-based total EAA requirements, while Nascimento (2015) determined the muscle AA composition-based digestible

EAA requirements. The present total EAA requirements for histidine, arginine, threonine, valine, isoleucine, phenylalanine, lysine methionine, leucine, tryptophan, cysteine, and tyrosine were found to be different than those estimated by Nascimento (2015) for tambaqui juveniles. These results show that the present whole-body AA composition-based estimated EAA requirements of pacu were not only different from those estimated previously on the basis of its muscle tissue AA composition but were also found to be different than those estimated for its sister species tambaqui juveniles.

A positive correlation has been observed between whole-body AA composition and established EAA requirements in channel catfish, *I. punctatus* (Wilson and Poe, 1985). Wilson (2002) reported the same positive correlation between EAA requirements determined by dose–response trials and those estimated by whole-body AA composition in channel catfish. In the species under study, any such correlation, if it exists, can be confirmed through future dose–response studies.

The present study indicates that the whole-body AA composition of pacu when expressed as a percentage of dietary protein is not affected by its body size, at least within the size ranging from juvenile (54.3 ± 8.2 g, average live body weight) to preadult (822.5 ± 33.9 g, average live body weight) and adult (1,562.8 ± 41.8 g, average live body weight). The whole-body chemical proximate composition was significantly influenced by body size. No significant differences were observed in the EAA requirements between different growth phases. The present whole-body AA composition-based estimated quantitative EAA pattern of pacu was found to be different from that previously estimated through its muscle tissue AA composition. Thus, until dose–response trials are conducted to determine accurate EAA requirements of *P. mesopotamicus*, the EAA requirements estimated in this study could be used in the formulation of experimental and practical diets.

Acknowledgments

We thank The World Academy of Sciences (TWAS), Brazilian National Council for Scientific and Technological Development (CNPq), and São Paulo State Research Foundation (FAPESP) for supporting the present research work. We also acknowledge the help of our technical staff members, particularly Valdecir Fernandes de Lima and Marcio Roberto Reche.

Conflicts of interest

The authors declare no potential conflict of interests.

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CHAPTER 3

Optimum biological and economic balanced digestible protein requirements of Juvenile pacu (*Piaractus mesopotamicus*)

ABSTRACT

A 45-day feeding trial was conducted to determine the response of pacu (*Piaractus mesopotamicus*) fed different balanced digestible protein diets with the evaluation of optimum biological and economic requirements. Two basal diets, one with high protein content (HP) and other with low protein content (LP) were formulated according to the diet-dilution approach. Both the diets were provided with a protein-bound essential amino acid balance and equal amounts of optimum digestible energy, minerals, vitamins and other supplements. These diets were properly mixed to obtain six experimental diets containing 163, 201, 238, 272, 315, and 348 g BDP kg^{-1} (dry matter-DM basis), respectively. Four hundred and thirty-two healthy and uniform size fish with an average initial live body weight of 10.82 ± 0.14 g were distributed among 18 fiber of 1000-L capacity each, being connected to a closed re-circulation aquaculture system (RAS). The experimental design was completely randomized and consisted of 6 treatments and 3 replicates each. A total of 24 fish were stocked per tank/ replicate and fed three times a day at 09:00, 12:00 and 17:00 h until apparent satiation. The obtained data revealed that dietary protein supplementation significantly ($P < 0.05$) improved the biological and economic outputs. The quadratic regression model calculated the optimum biological BDP requirement of 326 g kg^{-1} (dry matter) and the optimum digestible protein to energy (DP: DE) ratio was calculated to be 22.13 g MJ^{-1} (dry matter) to gain maximum performance of juvenile pacu. To gain maximum profit from pacu production, the economic model calculated the optimum economic BDP requirement of 311 g kg^{-1} (dry matter) and the optimum DP: DE ratio was calculated to be 21.11 g MJ^{-1} (dry matter) for pacu that will be sold as whole body fish. For pacu that will be sold as cut-up portions i.e., eviscerated and sliced fish, the economic model estimated the optimum economic BDP requirement of 316 and 319 g kg^{-1} and the optimum DP: DE ratio was calculated to be 21.47, and 21.67 g MJ^{-1}, respectively.

Keyword: Balanced diets; Modeling; Aquaculture nutrition; Cost-benefit analyses; Profitable aquaculture

1. Introduction

Protein is an essential nutrient and has significant impacts on growth, feed intake, body composition and physiological functions of animals including fish (Watanabe et al., 2001; Eits et al., 2005a; National Research Council−NRC, 2011). Beside biological importance, protein has a considerable impact on the feeding costs as well (Eits et al., 2005b; Bicudo et al., 2010; NRC, 2011). About 65 percent of the dietary protein could lose to the aquatic environment due to the use of nutritionally unbalanced diets and improper feeding practices (Michael, 1987; NRC, 2011; Craig, 2017). The over supplementation of this important nutrient causes high nitrogen excretion which may lead to deprived growth, higher feeding costs, low profits and poor water quality. On the other hand, diets provided with adequate amounts of balanced protein, optimum energy and other necessary nutrients are defined as nutritionally balanced which along with proper feeding practices and regular supervision of the wastes are essential factors in the healthy and lucrative production of animals without any negative environmental effects (Meer et al., 1995; Hebb et al., 2003; Eits et al., 2005a, b; Wang et al., 2006; Jiang et al., 2015). The proteins provided with an amino acid balance by the use of lysine as a reference amino acid are called balanced protein (Eits et al., 2005a). In this concept the ratios of other essential amino acids are estimated in relation to lysine requirement. The ratios among lysine requirement and minimum requirement of other essential amino acids remain constant according to the ideal protein profile (Mack et al., 1999).

The calculation of optimum economic protein is a matter of great attention in order to estimate such protein levels that are viable both biologically and economically. Such economic protein may help to achieve higher profits and better performance (Eits et al., 2005a). Feed industries and nutritionists are affirming that it would be promising to have appropriate economic models and take into account both the performance and cost-benefit analyses data of animals for the calculation of optimum economic protein (Zeitoun et al., 1976; Eits et al., 2005a, b). In majority of fish species most of the previous dose response trials have been focused on the estimation of protein requirements for maximum performance and have used the graded supplementation method. This method has a major drawback as the amino acid balance does not remain the same in all the experimental diets which can affect the growth response and feed

intake of the animal and may lead to the determination of inappropriate protein requirements (Gous and Morris, 1985; Sa et al., 2014). This disadvantage could be overcome through the diet-dilution method which was basically developed by Fisher and Morris (1970). The diet dilution approach has been widely applied in animals like poultry and pigs (Fisher and Morris, 1970; Gous and Morris, 1985; Halver and Hardy, 2002; Gous et al., 2018) but in fish still little is known about this method. Abboudi et al. (2007) and Liebert and Benkendorff (2007) have successfully used this method in Atlantic salmon (*Salmo salar* L.) fry and Nile tilapia (*Oreochromis niloticus*) juveniles, respectively. This method allows the formulation of diets with increased protein levels and maintaining a balanced ratio among all the essential amino acids in all diets. These ratios are calculated in relation to the optimum requirement of lysine which is to be kept constant and in proportion to dietary protein. Dietary energy, minerals and vitamins are maintained at the same level in the basal diets. The amino acid balance could be obtained by supplementing crystalline amino acids or protein-rich ingredients. The use of high amounts of crystalline amino acids may lead to increased feed costs and restricted feed intake and thus, to avoid the problems associated to the use of crystalline amino acids, protein-rich ingredients should be used to achieve the desire amino acid balance and if necessary the most limiting amino acid can be supplemented in crystalline form but at low amounts (Gous and Morris, 1985; Schuhmacher et al., 1997; Ambardekar and Reigh, 2007; Ambardekar et al., 2009; Lemme, 2010).

Successful aquaculture practices are closely linked to the selection of species that have appropriate characteristics for large scale farming and production and can be easily managed. Pacu (*P. mesopotamicus*) is an important freshwater omnivorous/ herbivorous fish species endemic to the Latin American States and has been successfully introduced as an exotic species in several other regions of the world including Asia, USA, and Europe (Paula et al., 1988; Machado and Sgarbieri, 1991; Fernandes et al., 2000, 2001). The available protein requirements for most of fish species have been usually determined on the basis of maximum performance data (NRC, 2011). Still no appropriate economic models are available to estimate the optimum economic protein requirements through the performance and cost-benefit analyses data. The development of appropriate economic models is very important for the optimization of productive and profitable production of fish. Such models can help to establish nutritionally balanced and economically viable diets in order to achive the profitable production of fish at low

feeding costs and without any negative effects on growth response. Thus, the present study aimed to determine the optimum biological and economic balanced digestible protein requirements of juvenile pacu by taking into account the performance and cost-benefit analyses data together.

2. Materials and methods

2.1. Animals, acclimation and ethical approval

Pacu juveniles reproduced at the Aquaculture Center, Sao Paulo State University (UNESP), Jaboticabal, SP were transferred to the indoor facility connected to a closed re-circulation aquaculture system (RAS). They were acclimated for one week and provided a commercial diet once a day. Before starting the trial, some samples were analyzed to know if they are having any disease. The pathological analyses have shown that the fish were healthy and the fish were then used in the feeding trial.

The experimental design was according to the ethical guidelines of the Brazilian College of Animal Experimentation (COBEA), and all the procedures followed in this trial were approved by the Ethics Committee on Animal Use of the Faculty of Agricultural and Veterinary Sciences, São Paulo State University (UNESP) via protocol No. 009999/14.

2.2. Experimental design and management

The experiment lasted 45 days and the experimental design was completely randomized which consisted of six treatments and three replicates each. The experiment was conducted in 18 1000-L fiber tanks connected to the closed re-circulation system. A total of 432 healthy pacu juveniles with an average live body weight of 10.82 ± 0.14 g were randomly distributed among the 18 tanks such as 24 fish were stocked per tank/ replicate. There was no significant difference in the initial average body weights of fish among the treatments. Each diet provided to its respective treatment three times a day at 09:00; 12:00 and 17:00 until apparent satiation. The feeding behaviour of fish was properly observed and feed was offered to fish with a great

caution. The total quantity of feed offered to fish up to the apparent satiation was regularly noted and the data were used in the calculation of biological and economic variables.

2.3. Experimental setup, experimental units and closed re-circulation aquaculture system (RAS)

The experimental setup consisted of the experimental tanks which have been located inside a laboratory being used for the trial and the closed re-circulation aquaculture system which was located outside the laboratory. The RAS consisted of three main tanks, first tank was receiving a large volume of water from the experimental units but with a low water flow to help in sedimentation of the waste material, second tank provided with a bio mechanical filter which further connected to a heating system to provide a constant temperature to the experimental units and the third tank was receiving the filtered i.e., organic matter-free water from the bio mechanical filter. The filtered water was then continuously throwing back to the experimental units through a water pump.

The tube well water was used in the trial and an electric air blower provided dissolved aeriation in all the experimental units. In all the experimental tanks, equal level of aeration was supplied through aeration stones which were connected with small plastic oxygen supplying pipes. The oxygen supply was regularly monitored and controlled through the regulators being fixed to the small plastic oxygen supplying pipes. The water was continuously re-circulated between the experimental tanks and the filtration set-up throughout the experiment.

2.4. Formulation of basal and experimental diets

The SuperCrac 6.1 Premium software was used for the formulation of the basal (Table 1) and experimental (Table 2) diets. Before the formulation of diets required ingredients were purchased from a local feed industry and analyzed according to the standard methods of AOAC (2016) to determine the contents of dry matter, crude protein, total lipid, ash, total amino acid, minerals and crude energy. The digestible values of the ingredients being used in the present formulations were calculated according to the apparent digestibility coefficients reported by Abimorad et al. (2008), Fabregat et al. (2008) and Gonçalves and Cyrino (2014) for the species under study.

Table 1

Formulation of basal (low-protein and high-protein) diets (g kg^{-1}).

Ingredients	Price (R$ kg^{-1})	Basal diets	
		Low protein-128.3	High protein-366
Fish meal	5.20	47.10	176.40
Corn gluten	5.50	11.30	37.24
Soybean meal	1.84	124.30	441.00
Broken rice	1.15	162.66	0.00
Corn	0.80	151.50	258.72
Corn starch	1.35	255.00	0.00
Rice husk	0.40	75.00	0.00
Soybean oil	2.90	100.00	41.39
L-lysine (99%)	17.30	3.70	10.88
DL-methionine	23.00	0.44	1.86
L-threonine (98.5%)	50.00	0.50	1.47
Dicalcium phosphate	1.65	36.00	17.15
Limestone	0.50	24.00	5.39
Antifungal	23.80	3.00	3.00
Antioxidant BHT	18.30	0.50	0.50
Mineral and vitamin premix[1]	36.00	5.00	5.00

[1]Vitamin A 860.000 UI kg^{-1}, Vitamin D3 240.000 UI kg^{-1}, Vitamin E 10.500 UI kg^{-1}, Vitamin K3 1.400 mg kg^{-1}, Vitamin B1 2.100 mg kg^{-1}, Vitamin B2 2.150 mg kg^{-1}, Vitamin B6 2.100 mg kg^{-1}, Vitamin B12 2.200 mg kg^{-1}, Vitamin C 25,000 mg kg^{-1}, Niacin 10,000 mg kg^{-1}, calcium pantothenate 5.600 mg kg^{-1}, folic acid 580 mg kg^{-1}, biotin 17 mg kg^{-1}, choline chloride 60,000 mg kg^{-1}, inositol 3.750 mg kg^{-1}, copper 1.800 mg kg^{-1}, manganese 5.000 mg kg^{-1}, zinc 8.000 mg kg^{-1}, iodine 90 mg kg^{-1}, selenium 36 mg kg^{-1}, and cobalt 55 mg kg^{-1}.

The basic nutritional requirements of dietary protein, energy, minerals and vitamins that have been recommended for the species under study were maintained during the formulation of basal diets in the present study (Carneiro, 1983; Fernandes et al., 2000, 2001; Abimorad and Carneiro, 2004; Abimorad et al., 2007; Abimorad et al., 2010; Bicudo et al., 2010). The essential amino acid profile reported by Khan et al. (2019a) for the species under study was maintained in

the formulation of basal diets through the protein-rich ingredients. The most limiting essential amino acids including lysine, methionine and threonine were added in crystalline form in small quantities due to the use of plant based ingredients in the present formulations.

Table 2

Experimental diets (g kg^{-1}) and the analyzed chemical composition of the basal and experimental diets.

	LP	HP	163	201	238	272	315	348
Dilution of the two basal diets to achieve six consecutive experimental diets.								
LP			806.68	647.36	488.03	328.71	169.39	10.06
HP			193.32	352.64	511.97	671.29	830.61	989.94
Total			1,000	1,000	1,000	1,000	1,000	1,000
Total cost (R$ kg^{-1})	1.94	2.86	2.12	2.26	2.41	2.56	2.7	2.85
Analyzed proximate chemical composition (dry matter basis g kg^{-1}) of the basal and experimental diets.								
Dry matter	935.29	928.92	954.23	950.56	951.16	955.31	946.18	946.67
Crude protein	147.00	409.80	185.00	227.10	268.00	305.60	353.20	389.70
Digestible protein	128.30	366.00	163.00	201.00	238.00	272.00	315.00	348.00
Total fat	126.80	90.10	117.80	112.40	106.00	99.60	94.50	88.60
Digestible fat	106.40	73.10	98.30	93.40	87.60	81.90	77.20	71.90
Ash	91.50	72.90	85.80	82.80	80.10	77.00	74.60	71.70
Crude energy (MJ kg^{-1})	17.56	19.25	17.38	17.71	17.95	18.13	18.57	18.82
Digestible energy (MJ kg^{-1})	15.06	15.04	14.63	14.68	14.67	14.60	14.73	14.72
DP: DE ratio (g MJ^{-1})			11.14	13.69	16.22	18.63	21.38	23.64

LP = Low protein diet; HP = High protein diet.

Table 3

Amino acid composition (dry matter basis) of the basal diets (g kg^{-1}).

Essential amino acids (EAAs)	LP-total EAA	LP-dig. EAA	HP-total EAA	HP-dig. EAA
Methionine	2.8	2.5	8.5	7.8
Lysine	9.9	9.1	30.2	28
Threonine	5.6	4.8	16.6	14.7
Arginine	8.8	8.4	25.5	24.3
Isoleucine	5.6	4.8	16	14.2
Leucine	9.7	8.8	29.9	27.8
Valine	6.2	5.2	17.4	15.3
Histidine	3	2.8	8.8	8.2
Phenylalanine	6.3	5.8	18	16.8
Tryptophan	1.5	1.3	3.9	3.5
Non-essential amino acids	LP-total NEAAs		HP-total NEAA	
Cysteine	2.3		5.3	
Glycine	8.6		23.4	
Serine	7		18.3	
Proline	9.4		24.1	
Alanine	8.5		21.6	
Aspartic acid	14.3		38	
Glutamate	25		63.5	
Methionine + Cysteine	5.6		13.8	

LP = Low protein diet; HP = High protein diet; dig. = Digestible; NEAAs = Non-essential amino acids.

2.5. Use of diet-dilution approach in the formulation of basal and experimental diets

The diet-dilution concept originally developed by Fisher and Morris (1970) was used in the formulation of the present basal diets, one with high protein content and the other with low protein content (Table 1). In the present study, dietary protein was defined as the balanced digestible protein because basal diets were provided with an essential amino acid balance using lysine as the reference amino acid. Lysine was provided in basal diets at a constant and optimum

ratio of digestible protein (Abimorad et al. 2010; Khan et al., 2019a). The ratios among lysine and minimum requirements of all other essential amino acids were kept constant according to the concept of Eits et al. (2005a).

In the high-protein diet, all the essential amino acids (EAAs) were provided 1.6 percent in excess than the recommended requirements while in the low-protein diet EAAs were provided in 0.5 percent less than the recommended requirements. All of the EAAs requirements were provided in relation to 100 percent lysine requirement. The basal diets were kept isoenergetic providing the digestible energy 15 MJ kg^{-1}, an average value of the previously determined optimum energy levels for the species under study by Carneiro (1983), Abimorad and Carneiro (2004), Abimorad et al. (2007, 2010) and Bicudo et al. (2010).

After the formulation of low-protein and high protein diets, six consecutive experimental diets were obtained by the proper dilution of the high protein diet with the low protein diet. The six experimental diets contained balanced digestible protein at the rate of 163, 201, 238, 272, 315, and 348 g kg^{-1}, respectively (Table 2). All the diets had the same protein-bound essential amino acid balance, only the amount of digestible protein was increased in the diets according to each dietary level.

2.6. Extrusion of diets

All the required ingredients were weighed and properly mixed in a dual mixer with a capacity of 100 kg and then ground in a single hammer mill (Model: MCS 280, Moinhos Viera, Tatuí, Brasil). The specific amount of water and oil was added to each diet and passed through a strainer to properly mix all the ingredients. Each diet was again well mixed in a Y-shaped mixer in order to homogenize. The homogenized feed was extruded into pellets of the diameter of 4 mm under identical processing conditions using a single screw extruder (Ex Micro Model, Exteec® Máquinas, Ribeirão Preto, Brasil) having the extrusion capacity of 15 kg h^{-1}. The extrusion temperature was kept beyond 90 °C. The diets were dried in a Forced Air Circulation Oven at 55 °C for 24 hours. After drying process, the diets were putted in separate plastic bags being specified for each diet and placed in a freezer at -20 °C until use.

2.7. Water quality parameters

To get optimum production from a re-circulation system, it is necessary to maintain the water quality of experimental tanks in optimum conditions. Water temperature, dissolved oxygen and pH were measured daily and water samples were collected once a week from the inlet and outlet of the experimental units to analyze the total ammonia. The experimental tanks were cleaned once a week in order to remove the wastes from the bottom of the experimental tanks.

The water temperature and pH was measured through oxygen meter (peagâmetro YSI pH100A, Brasil), dissolved oxygen through DO_2 meter (YSI $DO_2$00A, Brasil). The total ammonia in the water samples was measured through the phenol-hypochlorite method reported by Solorzano (1969). The average water temperature, dissolved oxygen and pH during the present experiment evaluated to be 27.11 ± 0.24 °C, 6.0 ± 0.12 mg L^{-1} and 7.45 ± 0.41, respectively. The total ammonia remained less than 0.1 mg L^{-1} during the experiment. The water quality parameters were found within the suitable range reported by Sipaúba-tavares and Santeiro (2013) for the species under study.

2.8. Samples collection

At the start and end of the feeding experiment, fish were subjected to 24 h fasting and then anesthetized with benzocaine solution at the rate of 60 mg L^{-1} and carefully weighed one by one. For the analysis of the initial and final whole body composition of fish and those being used in the separation of different body parts were deeply anesthetized with an overdose of benzocaine (200 mg L^{-1}) as reported by Abimorad et al. (2010) for juvenile pacu.

Initially, a representative group of total 30 fish was sampled for the analysis of the initial whole body chemical composition. At the end of the experiment, 6 fish per replicate from each treatment were sampled for the analysis of final whole body composition. For the economic calculations, a total of 12 fish per replicate were sampled and processed. The fish were processed by a local trained operator according to the method followed by Klein et al. (2014). The fish processing technique was performed by a single one operator in order to reduce the chances of variability which may occur if different operators perform the processing to separate the fish parts.

2.9. Analytical procedures

The moisture, dry matter, crude protein, crude energy, total lipid and ash content in the basal and experimental diets and whole body samples of fish were analyzed according to the standard methods of AOAC (2016) (Table 2). The amino acid content in the basal diets was evaluated at the Evonik industries AG, Essen, North Rhine-Westphalia, Germany by the High Performance Liquid (HPLC) technique (Table 3).

The initial and final whole-body fish samples were ground in a meat grinder and then refrozen and lyophilized at −80C in a Thermo VLP200 freeze dryer to get the pre-dry matter samples. The freeze-dried samples were then ground in a ball-grinder obtaining fine homogenized whole body samples. The feed samples (pellets) were also crushed in the ball-grinder in order to get fine homogenized feed samples. The fine homogenized whole body and feed samples were used in the subsequent proximate chemical analyses.

The moisture content was analyzed after drying the samples at a temperature of 105 °C for 24 h to a constant weight. Whole body crude protein was determined by the Kjeldahl method (N × 6.25) after acid digestion. The acid hydrolysis method was used to analyze the total lipid content in the feed samples. In the whole body samples, the total lipid content was extracted with petroleum ether in a fat extractor (Ankon technology®). Ash content was determined by burning the dried samples in a muffle furnace at a temperature of 550 °C for a period of 12 hr. Gross enrgy in the feed samples was analyzed by bomb calorimetry.

2.10. Performance responses

At the end of the feeding experiment, the following performance responses were evaluated.

Weight gain (g) = (final wet weight - initial wet weight)

Feed intake (g) = (total feed consumed/ fish)

Specific growth rate (%) = ((ln final body weight - ln initial body weight)/ days) × 100

Feed conversion ratio = (dry feed intake/ wet weight gain)

Feed efficiency ratio = (wet weight gain/ dry feed intake)

Protein efficiency ratio (g) = (wet weight gain/ protein intake)

2.11. Optimum biological balanced digestible protein requirements

Quadratic regression was used to determine the optimum biological balanced protein requirement of pacu juveniles. The inputs provided to the model consisted of six experimental diets (g kg^{-1}) and weight gain (g). These inputs were submitted to the SAS, 2014 software that generated the model parameters including "a", "b" and "c".

2.12. Optimum economic balanced digestible protein requirements

The model of Eits et al. (2005b) was adapted to determine the optimum economic protein requirements of the pacu juveniles that will be sold as whole body or in the form of cut-up portions i.e., eviscerated and sliced juvenile pacu.

2.13. Economic model description

At the end of the experiment the following three main commercial products were obtained including the whole body, eviscerated, and sliced fish.

Whole body fish = {Skin + Scales + fins + head + visceral content + main body trunk}

Sliced fish = {Whole body fish – scales – fins – head – visceral content}

Eviscerated fish = {Whole body fish – visceral content}

The gross margin GM (R$ g^{-1} $fish^{-1}$) was calculated according to the model developed by Eits et al. (2005*b*) in poultry. The inputs provided to this model consisted of the whole body, eviscerated and sliced juvenile pacu final weights (a) (g $fish^{-1}$), feed intake (b) (g $fish^{-1}$), feed price including the feed processing costs (c) (R$ g^{-1} diet) and the selling price of whole fish and cut-up portions (d) (R$ g^{-1} $fish^{-1}$). The price of the cut-up portions also included the processing costs.

Feed price (R$ g^{-1} diet) equation = {a × BDP + b/1000},

Where,

BDP is the balanced digestible protein while the model parameters "a" and "b" are depending on the feed price.

The selling price of the whole fish and cut-up portions and the processing costs (R$ g^{-1} $fish^{-1}$) were obtained from a local fish farm.

Providing inputs to the model, the outputs such as the feeding cost (R$ g^{-1} $fish^{-1}$), revenue (R$ g^{-1} $fish^{-1}$) and gross margin (R$ g^{-1} $fish^{-1}$) were obtained. The outputs were calculated according to the following formulas;

Feeding cost (R$ g^{-1} $fish^{-1}$) = feed price R$ g^{-1} × feed intake (g)

Revenue (R$ g^{-1} $fish^{-1}$) = final body or cut-up portion weight (g) × whole fish price (R$ $fish^{-1}$)

Gross margin (R$ g^{-1} $fish^{-1}$) = revenue (R$ g^{-1} $fish^{-1}$) - feeding costs (R$ g^{-1} $fish^{-1}$)

The above calculations can be simplified as;

Gross margin (GM) = Revenue – Feeding cost

2.14. Algebraic description of the model

The gross margin can be algebraically expressed as follows;

$GM = (a \times (d)) - (c° \times b)$

Where,

a = Final weight of the whole fish or cut-up portion (g), d = coefficient obtained for the selling price (R$ g^{-1} $fish^{-1}$) of the whole fish and cut-up portion, the processing costs included in the selling price of the cut-up portions, c° = feed price R$ g^{-1} + feed processing cost, and b = feed intake (g)

Thus, the functions in the above equation can be elaborated as follow;

$d = c_0 + c_1x + c_2x^2$, estimates of the quadratic regression obtained for final weight of whole body and cut-up portion + processing costs using exponential data.

$c° = y_0 + y_1x$, estimates of the linear equation obtained for feed price + feed processing cost

$b = f_0 + f_1x + f_2x^2$, estimates of the quadratic regression obtained for feed intake using exponential data

Or, if we expand the equation as;

$GM = (ac_0 + ac_1x + ac_2x^2) – (y_0f_0 + y_0f_1x + y_0f_2x^2 + y_1f_0x \times y_1f_1x^2 + y_1f_2x^3)$

Where, a, c_0, c_1, c_2, y_0, y_1, f_0, f_1, and f_2 are constants while X is unknown

GM is maximized when $\frac{\partial GM}{\partial X} = 0$ or we also can express as;

$\frac{\partial GM}{\partial X} = (ac_1x + 2\ ac_2) – (y_0f_1 + 2\ y_0f_2x + y_1f_0x + 2\ y_1f_1x + 3\ y_1f_2x^2)$

$= (ac_1 - y_0f_1x - y_1f_0) + (2\ ac_2 - 2\ y_0f_2 - 2\ y_1f_1)\ x – (3\ y_1f_2)\ x^2$

Let $k_a = -3\ y_1f_2$

$K_b = 2\ ac_2 - 2\ y_0f_2 - 2\ y_1f_1$

$K_c = ac_1 - y_0f_1 - y_1f_0$

Then the GM is maximized when X takes the value of

$$x^\circ = \frac{-k_b \pm \sqrt{{k_b}^2 - \mathrm{k}ac}}{2k_a}$$

According to the model descriptions, gross margin (GM, R$ g^{-1} $fish^{-1}$) is the profitability of the final product obtained at various feeding costs. The level of BDP g kg^{-1} where maximum gross margin (profit) obtained with better performance and at viable feeding cost was considered to be the optimum economic balanced digestible protein level.

2.15. Statistical analysis

Results were shown as mean ± SD of the three replicates. The performance and whole body composition data were subjected to One-way analysis of variance-ANOVA using the SPSS for Windows, version 16.0. Chicago, SPSS Inc., (2007) software. When a significant ($P < 0.05$)

difference was observed, Tukey's Least Significant Test (LSD) was applied to compare the means.

The average body weight gain, final weight and feed intake data against the dietary protein levels were fitted to the quadratic regression model (second order polynomial) using the SAS Institute Inc. 2014 software. $y = \alpha + \beta x + cx^2$, where x is the protein level, y is response variable, α is the curve intercept, β and c are the slopes, and x = - β/ 2c yield the minimum protein level for maximum response.

3. Results

3.1. Biological responses

During the feeding trial, fishes well accepted the experimental diets and no mortality were observed. The growth performance and feeding parameters of pacu juveniles fed different balanced digestible protein levels are shown in table 4. Table 4 shows that body weight gain (BWG), feed intake (FI), feed efficiency ratio (FER) and specific growth rate (SGR) of juvenile pacu were significantly (P < 0.05) increased with balanced digestible protein (BDP) supplementation up to the diet with 315 g BDP kg^{-1} and thereafter the performance parameters were decreased.

Feed conversion rate (FCR) was significantly (P < 0.05) decreased with increasing dietary protein up to the level 315 g kg^{-1} and beyond it increased. Protein efficiency rate (PER) was gradually decreased (P < 0.05) with the increasing digestible protein (Table 4).

Table 4

Responses of pacu juveniles fed different balanced digestible protein (g kg^{-1}) levels.

[1]BDP	[2]BWG	[3]FI	[4]FCR	[5]FER	[6]SGR (%)	[7]PER
163	18.6 ± 0.4^{e}	22.21 ± 0.7^{d}	1.19 ± 0.01^{f}	0.84 ± 0.01^{e}	2.24 ± 0.01^{d}	5.14 ± 0.06^{a}
201	21.5 ± 1.0^{d}	24.13 ± 0.6^{c}	1.12 ± 0.02^{e}	0.89 ± 0.02^{d}	2.42 ± 0.08^{c}	4.43 ± 0.09^{b}
238	25.2 ± 1.0^{c}	25.95 ± 0.9^{b}	1.02 ± 0.03^{d}	0.98 ± 0.03^{c}	2.68 ± 0.06^{b}	4.13 ± 0.13^{c}
272	27.5 ± 0.9^{b}	26.54 ± 0.5^{ab}	0.97 ± 0.02^{ca}	1.04 ± 0.02^{b}	2.81 ± 0.04^{a}	3.81 ± 0.06^{d}
315	29.4 ± 0.9^{a}	27.23 ± 0.5^{a}	0.93 ± 0.01^{b}	1.08 ± 0.02^{a}	2.90 ± 0.05^{a}	3.42 ± 0.05^{e}
348	27.6 ± 0.7^{b}	25.87 ± 0.4^{b}	0.94 ± 0.01^{ab}	1.07 ± 0.02^{ab}	2.82 ± 0.08^{a}	3.06 ± 0.04^{f}
P-value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

[1]Balanced digestible protein; [2]Body weight gain; [3]Feed intake; [4]Feed conversion ratio; [5]Feed efficiency ratio; [6]Specific growth rate; [7]Protein efficiency ratio.

Data are represented as mean ± SD (n=3), means in the same column with different superscript letters are significantly different ($P < 0.05$).

The weights of the initial and final whole body of pacu and those of the final cut-up portions are shown in Table 5. The final whole body weight was significantly ($P < 0.05$) increased in response to dietary protein. The dietary protein also significantly enhanced the weights of the final cut-up portions.

Table 5

Initial and final average weight (g) of whole body, eviscerated and sliced juvenile pacu fed different BDP (g kg^{-1}).

	Initial WB fish	Final WB fish	Eviscerated fish	Sliced fish
Initial BW			9.32 ± 1.76	5.86 ± 1.17
163	10.70 ± 0.13	29.30 ± 0.55^{e}	28.06 ± 0.85^{d}	18.76 ± 0.98^{c}
201	10.94 ± 0.16	32.45 ± 0.86^{d}	28.68 ± 1.34^{cd}	19.17 ± 0.95^{c}
238	10.77 ± 0.08	35.93 ± 0.94^{c}	30.77 ± 1.40^{bc}	20.56 ± 1.29^{bc}
272	10.83 ± 0.07	38.32 ± 0.99^{b}	32.63 ± 0.33^{b}	21.95 ± 0.24^{b}
315	10.91 ± 0.08	40.26 ± 0.88^{a}	35.46 ± 1.11^{a}	24.77 ± 0.63^{a}
348	10.82 ± 0.23	38.40 ± 0.52^{a}	36.16 ± 1.66^{a}	24.81 ± 1.48^{a}
P-Value	0.001	0.001	0.001	0.001

Data are represented as mean ± SD (n=3), means in the same column with different superscript letters are significantly different ($P < 0.05$). WB = Whole body.

3.2. Biological protein requirement

The optimum biological protein requirement was determined using the quadratic regression model as shown in Figure 1. According to the quadratic regression applied to the BWG data and different BDP levels represented an estimated optimum biological BDP requirement of 326 g kg^{-1} for juvenile pacu (Fig. 1). The optimum digestible protein to energy (DP: DE) ratio was calculated to be 22.13 g MJ^{-1} for the juvenile pacu.

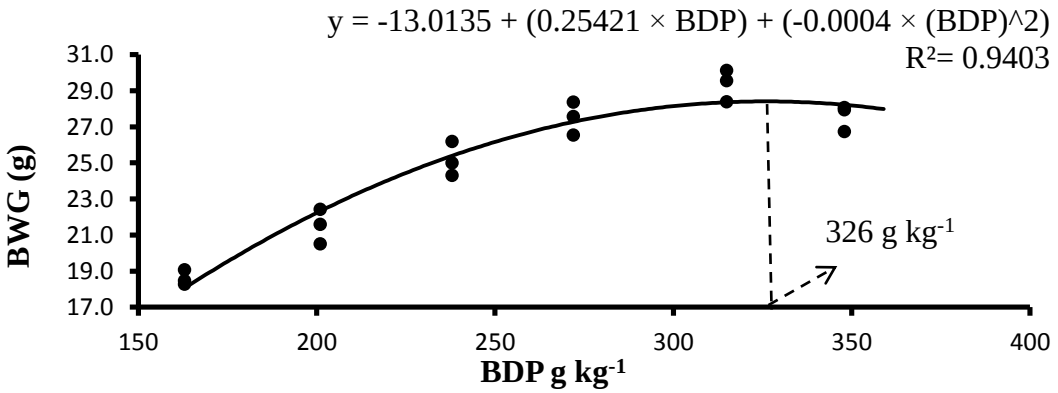

Fig.1. The quadratic regression of body weight gain (BWG) against different balanced digestible protein (BDP g kg^{-1}) levels in pacu juveniles.

3.3. Whole body composition

Whole body proximate composition data of pacu juveniles are shown in Table 6. The whole body moisture and crude protein content in pacu juveniles fed different balanced digestible protein (BDP) levels was significantly ($P < 0.05$) increased while total lipid and ash content significantly ($P < 0.05$) decreased with increasing digestible protein levels.

Table 6

Body composition (wet weight basis g kg^{-1}) of juvenile pacu fed different BDP levels.

BDP (g kg^{-1})	Moisture	Crude protein	Total lipid	Ash
Initial	715.9	142.7	120	18.7
163	671.93 ± 2.41^{d}	125.50 ± 0.20^{e}	158.27 ± 4.72^{a}	32.33 ± 0.49^{a}
201	678.17 ± 4.40^{d}	127.57 ± 0.12^{d}	152.30 ± 2.08^{b}	29.63 ± 1.17^{ab}
238	690.80 ± 4.83^{c}	130.80 ± 2.52^{c}	143.87 ± 1.58^{c}	28.07 ± 2.02^{bc}
272	699.73 ± 5.25^{b}	136.23 ± 0.15^{b}	131.07 ± 0.29^{d}	26.47 ± 2.91^{bcd}
315	711.93 ± 4.78^{a}	137.53 ± 0.42^{ab}	119.33 ± 4.41^{e}	24.17 ± 0.87^{d}
348	709.43 ± 5.23^{a}	138.30 ± 0.52^{a}	118.63 ± 2.05^{e}	25.73 ± 2.56^{cd}
P-value	0.0000	0.0000	0.0000	0.0024

BDP = Balanced digestible protein.

Data are represented as mean ± SD (n=3), means (of three replicates) in the same column with different superscript letters are significantly different ($P < 0.05$) by Tukey Least significant test.

3.4. Economic inputs and outputs (responses)

The optimum feed intake (Fig. 2) and final body weight (Fig.3) was determined by the quadratic regression model. The optimum dietary protein requirements were estimated using the feed intake (FI) and final body weight (FBW) to be 301 g kg^{-1} (Fig. 2) and 325 g kg^{-1} (Fig. 3), respectively according to the quadratic regression. A linear equation was obtained for feed price (Fig.4).

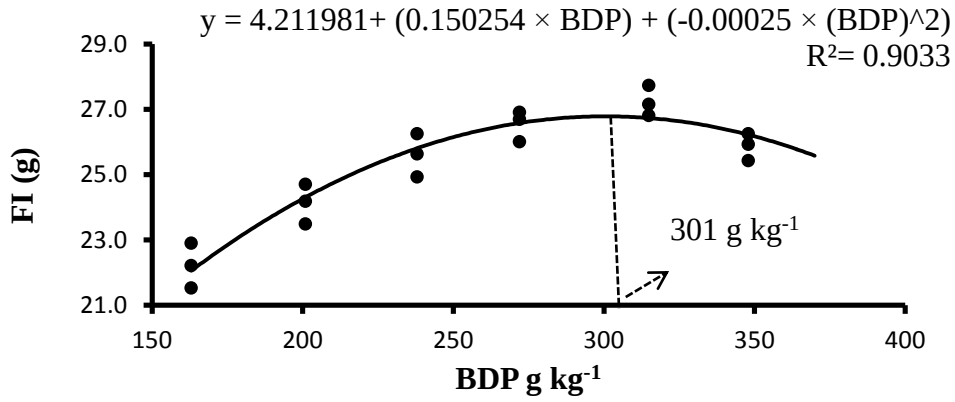

Fig. 2. Quadratic regression model applied to the observed feed intake (FI) data against different balanced digestible protein (BDP g kg^{-1}) levels in pacu juveniles.

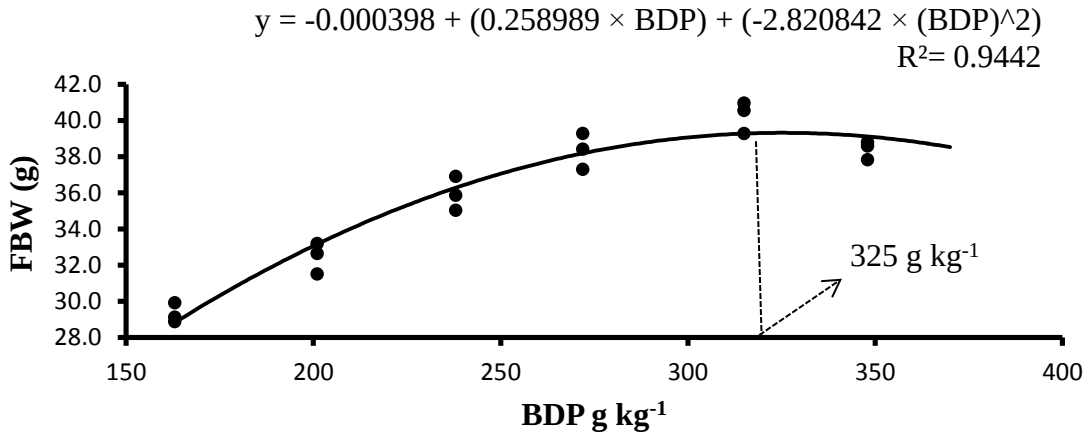

Fig. 3. Quadratic regression model applied to the final body weight (FBW) against different balanced digestible protein (BDP g kg^{-1}) levels in pacu juveniles.

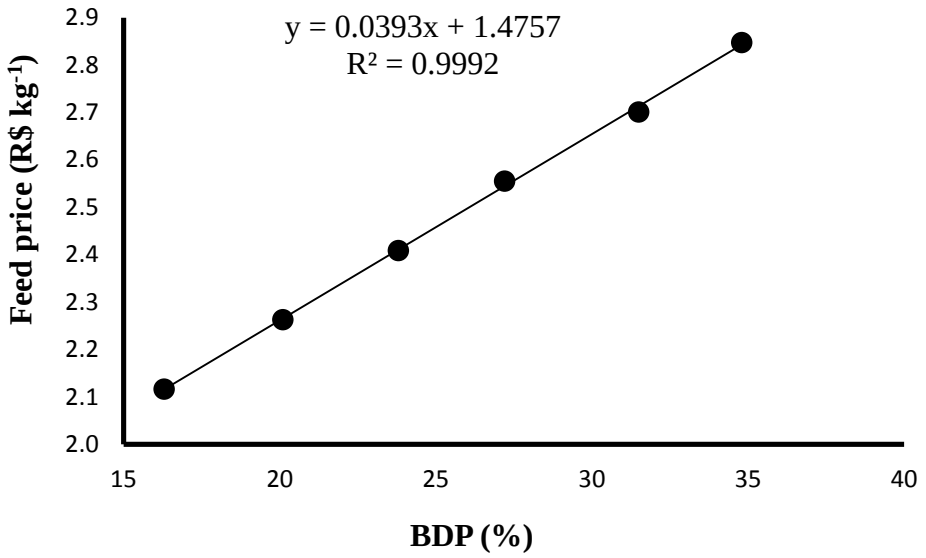

Fig.4. Price (R$ kg^{-1}) of the experimental diets supplemented with different balanced digestible protein (%) levels.

The feed intake (FI), final body weight (FBW), selling price of whole body fish (WFP) and cut-up portions and feed price (FP) equations obtained in the present study were used as inputs in the present economic model. The selling prices of the whole body fish, cut-up portions (including the fish processing labour) and feed ingredient costs being obtained in R$ Kg^{-1} from a local fish farm and feed industry, respectively were converted into R$ g^{-1}.

The natural logarithm (ln) of the weights of eviscerated and sliced juvenile pacu was calculated and the data were used along with different levels of digestible protein to get the equations for eviscerated and sliced juvenile pacu. The following linear equations were obtained for both cut-up portions.

Eviscerate juvenile pacu = [$y = (1.0599x - 0.3049)$ $R^2 = 0.9979$]

Sliced juvenile pacu = [$y = (1.2267x - 1.2876)$ $R^2 = 0.9901$]

These equations were used as inputs in the economic model in order to calculate the optimum economic protein requirement for eviscerated and sliced juvenile pacu.

3.5. Economic protein requirement

The present economic model yielded the outputs including the feeding costs (R$ g^{-1} $fish^{-1}$), revenue (R$ g^{-1} $fish^{-1}$) and most importantly the gross margin (R$ g^{-1} $fish^{-1}$). The level of dietary protein where maximum gross margin occurred was considered to be the optimum economic dietary balanced digestible protein requirement of whole body, eviscerated and sliced juvenile pacu (Fig. 5, 6, 7).

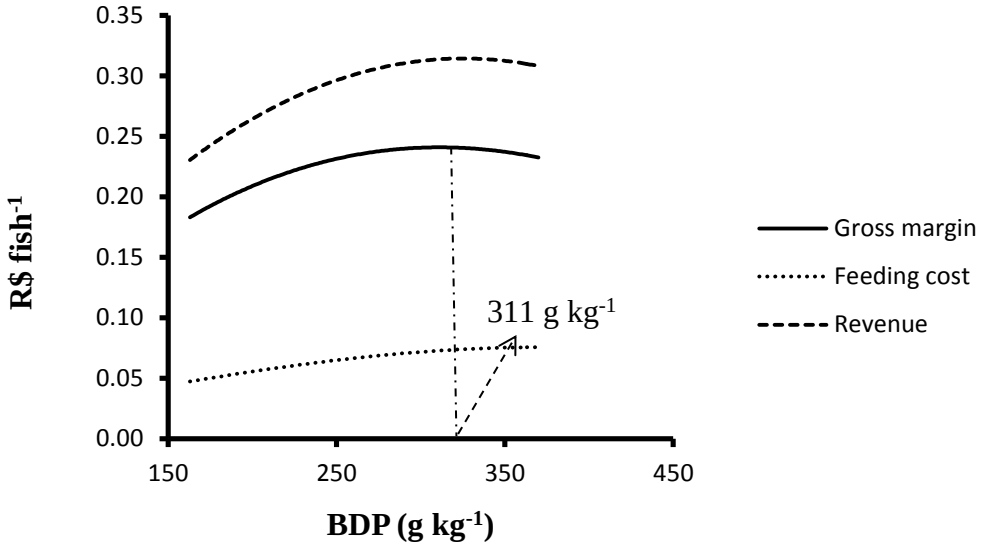

Fig. 5. Effect of different balanced digestible protein (BDP g kg^{-1}) levels on feeding cost (FC), revenue, gross margin (GM) in whole body juvenile pacu.

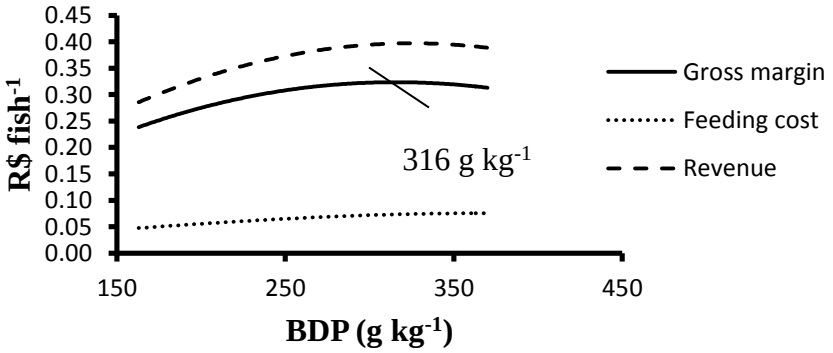

Fig. 6. Effect of different balanced digestible protein (BDP g kg^{-1}) levels on feeding costs, revenue and gross margin in juvenile eviscerated pacu.

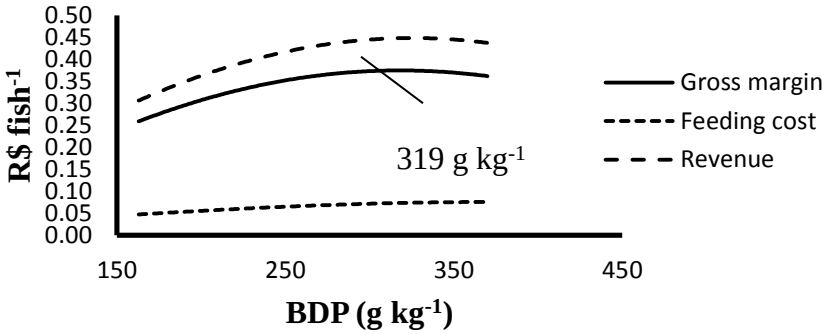

Fig. 7. The effect of different balanced digestible protein (BDP g kg^{-1}) levels on feeding costs, revenue and gross margin in juvenile sliced pacu.

According to the applied economic equation, the level of BDP (311 g kg^{-1}) where maximum gross margin (R$ g^{-1} $fish^{-1}$) achieved was considered to be the optimum economic level of pacu that will be sold as a whole body and the optimum DP: DE ratio was calculated to be 21.11 g MJ^{-1}. The optimum biological protein requirement determined in the present study was 326 g kg^{-1} being 25 g higher than the optimum economic level (Fig. 5). The optimum economic level of 316 g BDP kg^{-1} and the optimum DP: DE of 21.47 g MJ^{-1} was determined to obtain maximum profit from pacu that will be sold as an eviscerated fish and for pacu that will be sold as a sliced fish, the optimum economic level was estimated to be 319 g BDP kg^{-1} and the optimum DP: DE of 21.67 g MJ^{-1} for achieving maximum gross margin. These results showed that feeding pacu that will be sold as eviscerated or sliced fish, the gross margin decreases as compared when it will be sold as a whole fish.

3.6. Scenarios

Beside the normal scenarios, alternate scenarios were hypothesized for juvenile pacu by altering the selling price of whole body, eviscerated and sliced juvenile pacu and fish meal and soybean meal. The selling prices were first increased by 10 percent and then decreased by the same 10 percent than the current selling prices, respectively.

Table 7

Balanced digestible protein resulting in maximum gross margin under different scenarios.

Scenarios		BDP	Feed intake	Feeding cost	Revenue	Gross margin
Biological level	Normal FP	326	26.626	0.074	0.314	0.240
Economic level	Normal FP	311	26.761	0.073	0.314	0.241
Whole fish	(-10)	309	26.770	0.073	0.345	0.210
Whole fish	(+10)	313	26.749	0.073	0.282	0.272
Fish meal	(-10)	313	26.749	0.071	0.314	0.243
Fish meal	(+10)	310	26.766	0.075	0.314	0.239
Soybean meal	(-10)	311	26.761	0.073	0.314	0.241
Soybean meal	(+10)	308	26.774	0.077	0.314	0.237

BDP (g kg^{-1}) = Balanced digestible protein; Feed intake (g); Feeding cost (R$ g^{-1} $fish^{-1}$); Revenue (R$ g^{-1} $fish^{-1}$); Gross margin (R$ g^{-1} $fish^{-1}$); Normal FP = Normal feed price (Table 1).

Table 8

Balanced digestible protein resulting in maximum gross margin under different scenarios.

Scenarios		BDP	Feed intake	Feeding cost	Revenue	Gross margin
Normal feed price	Current feed price	316	26.728	0.073	0.402	0.329
Evis whole fish price	(-10)	314	26.743	0.073	0.362	0.289
Evis whole fish price	(+10)	317	26.720	0.073	0.443	0.369
Fish meal	(-10)	317	26.720	0.071	0.402	0.331
Fish meal	(+10)	315	26.736	0.075	0.402	0.327
Soybean meal	(-10)	315	26.736	0.073	0.402	0.329
Soybean meal	(+10)	314	26.743	0.077	0.402	0.325

BDP (g kg^{-1}) = Balanced digestible protein; Feed intake (g); Evis = Eviscerated; Current feed price (Table 1); Feeding costs, revenue and gross margin = (R$ g^{-1} $fish^{-1}$).

Table 9

Balanced digestible protein resulting in maximum gross margin under different scenarios.

Scenarios		BDP	Feed intake	Feeding cost	Revenue	Gross margin
Normal feed price	Current feed price	319	26.703	0.074	0.474	0.400
Sliced whole fish price	(-10)	318	26.712	0.073	0.426	0.353
Sliced whole fish price	(+10)	319	26.703	0.074	0.521	0.447
Fish meal	(-10)	319	26.703	0.071	0.474	0.402
Fish meal	(+10)	318	26.712	0.076	0.473	0.398
Soybean meal	(-10)	318	26.712	0.074	0.473	0.400
Soybean meal	(+10)	317	26.720	0.078	0.473	0.396

BDP (g kg^{-1}) = Balanced digestible protein; Feed intake (g); Current feed price (Table 1); Feeding costs, revenue and gross margin = (R$ g^{-1} $fish^{-1}$).

The results shown in table 7, 8, and 9, respectively indicate that the optimum economic digestible protein requirement of juvenile pacu was subjected to the changes in the selling price of whole body, eviscerated and sliced pacu and protein rich feed ingredients. The changes in the selling price considerably affected the optimum economic protein levels.

4. Discussion

An increase in the dietary protein above the optimum requirement causes a decline in the growth rate, feeding aspects and physiological status of fish. Dietary protein requirement can be calculated in the form of concentration of protein in the diet or the quantity of protein consumed per unit weight of fish (Tacon and Cowey, 1985; De Sliva et al., 1989; NRC, 2011). According to De Silva et al. (1989), from aquaculture point of view the protein requirements of fish should be expressed in relative terms due to the better applicability of this approach. The biological and

economic models applied in the present study were chosen due to the characteristics of the present data.

The protein requirements are not only influenced by the experimental design and type of biological response but also by the type of statistical analysis and size of the animal (Lupatsch et al. 2001; Wang et al. 2005; Gous et al., 2018). Weight gain is the most commonly used parameter in the determination of optimum protein requirement studies in fish (NRC, 2011; Ye et al., 2017). The reason may be that weight gain will be low at the insufficient dietary supplementation of any essential nutrient/ protein. Different dietary levels of protein in relation to body weight gain provide fundamental information about the requirements of an animal. Another reason probably is the market or producer preference which is orienting the attention of researchers toward the importance of the body weight gain in nutrient requirement studies (Zeitoun et al., 1976).

Based on biological and economic assumptions and approaches several statistical methods have been/ can be used for the estimation of nutrient requirements. The selection of appropriate models immensely helps to calculate the accurate biological and economic nutrient requirements (Gurure et al., 1995; Shearer, 2000). In fish nutrition, broken-line model has been used very commonly but sometimes it under estimate the nutrient requirements and thus the quadratic regression is a better choice (Zeitoun et al., 1976; Cortes-Jacinto et al., 2003). The quadratic regression model provides noble information about the intake of a nutrient and the gain in weight and describes well the behaviour of growth (Zeitoun et al., 1976; NRC, 2011; Ye et al., 2017). In the species under study, broken line model has been used in a previous study conducted by Bicudo et al. (2010) estimating the optimum crude protein requirement of pacu fingerlings to be 268 g kg^{-1} through the input of specific growth rate. Moreover, they estimated an optimum crude protein to digestible energy ratio of 22.2 g MJ^{-1} for pacu fingerlings. Klein et al. (2014) estimated the optimum CP of 258 g kg^{-1} for juvenile pacu which is near to the recommendation of Bicudo et al. (2010). However, the pacu size in the study of Klein et al. (2014) is large than in the Bicudo et al. (2010) and the culture conditions were also different. In *Barbodes altus* (Elangovan and Shim, 1997), *Scophthalmus maximu* L. (Kim et al., 2003) and *Diplodus Vulgaris* (OzoRio et al., 2009) less growth occurred at high protein levels and according to Qian (2001) it might be because that the available energy content was lower.

In the present study, the optimum biological balanced digestible protein requirement was estimated to be 326 g kg^{-1} and the optimum DP: DE ratio of 22.13 g MJ^{-1} was calculated for juvenile pacu. Fernandes et al. (2000) concluded optimum 260 g CP kg^{-1} and optimum crude energy of 17 g MJ^{-1} for pacu fingerlings. In another study Fernandes et al. (2001) recommended optimum 220 g CP kg^{-1} and optimum crude energy 17 g MJ^{-1} for pacu juveniles of relatively large size. Abimorad et al. (2007) found the better digestible protein level at the rate of 230 g kg^{-1} for juvenile pacu which also seems very low especially in the intensive systems. Signor et al. (2010) suggested crude protein of 250 g kg^{-1} and 13 g MJ^{-1} of digestible energy for better performance of pacu juveniles in cage culture conditions. Thus to avoid the underestimation of protein for juvenile pacu, quadratic regression model was used in the present study to estimate the optimum biological digestible protein requirement of juvenile pacu through the input of body weight gain data rather than specific growth rate. Meyer and Fracalossi (2005) reported the protein requirement of 326-373 g CP kg^{-1} for the Neotropical omnivorous catfish jundia´ (*Rhamdia quelen* Quoy and Gaimard, 1824) which matches the present BDP requirement 326 g kg^{-1} for juvenile pacu. These data show that a difference exist in the present and previously estimated protein requirements for juvenile pacu may be due to the methodology used for the formulation of diets or the biological models being used or may be due to the statistical analyses.

According to Gous et al. (2018), the use of protein diets provided in an essential amino acid balance and optimum protein levels may improve the feed intake of the animal while the diets containing protein beyond the optimum level may lead to a reduced feed intake. In the present study, feed intake (FI) was significantly improved in response to the diets with increasing digestible protein up to the diet with optimum level and at the diet containing protein content beyond the optimum level, the feed intake tended to decrease. The same behavior of fish in response to increasing dietary protein levels has been reported in several other fish species by Coutinho et al. (2016) in Zebra sea bream (*Diplodus cervinus*, Lowe 1838) juveniles; Huang et al. (2016) in juvenile chu's croaker, *Nibea coibor*, Xu et al. (2016) in young grass carp (*Ctenopharyngodon idella*), Ma et al. (2019) in sub-adult triploid rainbow trout. Similarly, the specific growth rate (SGR%) and feed efficiency ratio (FER) were also significantly improved with diets contained the optimum digestible protein but in response to the diet with protein content beyond the requirement declined the above both parameters. Feed conversion ratio (FCR) was significantly reduced with the digestible protein supplementation showing that the

fish efficiently utilized the feed for body growth. According to Ma et al. (2019), fish usually less accepts unbalanced diets and thus feeds without an amino acid balance or high protein content have negative impacts on the growth performance and feeding aspects of fish. Whereas the diets with an amino acid balance and optimum protein levels are usually more efficient and the use of such diets may improves the growth performance and feeding aspects of fish.

Protein efficiency ratio (PER) was declined with the increasing digestible protein levels and similar results have been reported by Abimorad et al. (2007; Bicudo et al. (2010) in the species under study. Similar results have been reported by Hossain et al. (2010), Zhang et al. (2010), Kim et al. (2004, 2005) and Kim et al (2016) in other fish species. The relationship between PER and increasing dietary protein is subjected to various factors such the according to Dabrowski (1979) it may be affected by the species difference and De Silva and Anderson (1995) have reported that PER is not an effective parameter because it does not consider the amount of protein being used for maintenance and considers the lipid deposition as protein retention and additionally, sometimes the protein content of the diet may lost in water due to the poor pellet texture.

The supplementation of balanced digestible digestible protein levels positively affected the whole body chemical composition of pacu juveniles. Whole body moisture and protein content was significantly increased while whole body lipid and ash content significantly decreased with the increasing BDP levels. These results are in concordance to those reported by Abimorad and Carneiro (2007), Bicudo et al. (2010) for the species under study and by Meer et al. (1995), Duan et al. (2001) and Watanabe et al. (2001) for other fish sepcies. The appropriate DP: DE ratios are important to be maintained for achieving better growth and quality of the final product (Bicudo et al., 2010; Nguyen et al., 2017). The optimum DP: DE ratio obtained in the present study for maximum performance was 22.13 g MJ^{-1} (dry matter) which was found near to that obtained by Bicudo et al. (2010) (22.20 g MJ^{-1}) for the same fish. However, DP: DE ratio is subjected to the objective of a research study and it may change due to the composition of diet and species and size differences in fish.

Dietary protein is the major influencing nutrient in feeding costs and consequently has strong effects on total production costs (Ye et al., 2017). Like in several other fish species, the

dietary protein requirements of pacu (*P. mesopotamicus*) have been previously determined by considering only biological responses (outputs) (Fernandes et al., 2000, 2001; Abimorad and Carneiro, 2007; Signor et al. 2010; Bicudo et al., 2010; Klein et al., 2014). In the present study we have determined both biological and economic protein requirements for juvenile pacu using appropriate biological and economic models and comparatively studied the difference between the biological and economic estimations.

Considering the estimated results, the present economic model revealed that it is an appropriate and viable model to calculate the optimum economic dietary level of protein for fish. Nutritionists usually formulate diets with higher protein conent in order to achieve maximum performance. According to the present economic model, the optimum economic balanced digestible protein was estimated to be 315 g kg^{-1} (while biological level was 326 g BDP kg^{-1}) and the optimum DP: DE was calculated to be 21.11 g MJ^{-1} for juvenile pacu that will be sold as whole body fish. For pacu that will be sold as eviscerated and slicedfish, the economic level was 315 and 318 g kg^{-1} with the optimum DP: DE of 21.47, and 21.67 g MJ^{-1}, respectively. In the present situation, feeding pacu with optimum biological protein would decrease the gross margin.

To check what happens if the selling price of whole fish, cut-up parts and fish meal and soybean meal is increased or decreased, different scenarios were hypothesized (Table 7, 8, 9). It was observed that a 10 percent increase in the selling price of whole body, eviscerated and sliced fish permit the addition of some more protein in order to further increase the growth and gross margin (R$ g^{-1} $fish^{-1}$). When the selling prices were decreased, relatively lower levels of protein requirement were estimated. This may be because when the selling prices of whole fish and sliced fish increases in the market, then fish farmers can earn more income if they supply more fish to the market. Thus, feeding fish with relatively high protein content may lead to a rapid growth. On the other hand, in the case of the low selling prices, feeding fish with relatively lower protein content was beneficent from the cost-benefit point of view.

Similarly, the selling price of fish meal and soybean meal was also increased and decreased by 10 percent. The results showed that 10 percent or more increase in the selling price of protein rich ingredients may restrict the high supplementation of dietary protein. However, a

10 percent or more reduction in the selling price of these ingredients permit to increase the protein content in fish feed in order to grow the fish fastly and get more profit. The present scenarios may help to better explain the practicality of the present economic model and provide important information for the feed industries to establish nutritionally balanced and economically viable diets to get maximum profit from the production systems of juvenile pacu at low feeding costs.

By taking into account both biological and economic responses the present economic model efficiently calculated the optimum economic digestible protein requirements for pacu juveniles. The present results indicate that the formulation of diets according to the economic protein levels may considerably increases the gross margin without any negative effects on the performance of fish especially when the selling price of protein rich ingredients increase by 10 percent or more. The optimum biological BDP requirement of 326 g kg^{-1} (dry matter) and the optimum digestible protein to energy (DP: DE) ratio was calculated to be 22.13 g MJ^{-1} (dry matter) to gain maximum performance of juvenile pacu. To gain maximum profit from pacu production, the economic model calculated the optimum economic BDP requirement of 311 g kg^{-1} (dry matter) and the optimum DP: DE ratio was calculated to be 21.11 g MJ^{-1} (dry matter) for pacu that will be sold as whole body fish. For pacu that will be sold as cut-up portions such as eviscerated and sliced fish, the economic model estimated the optimum economic BDP requirement of 316 and 319 g kg^{-1} and the optimum DP: DE ratio was calculated to be 21.47, and 21.67 g MJ^{-1}, respectively.

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CHAPTER 4

Optimum biological and economic balanced digestible protein requirements of pre-adult pacu (*Piaractus mesopotamicus*)

ABSTRACT

The establishment of nutritionally balanced and economically viable diets is considered extremely important for sustainable aquaculture. Feeding costs have significant effects on the profitability index of aquaculture production systems. The evaluation of protein requirements on the basis of performance and cost-benefit analyses data could be helpful to achieve maximum profits and better performance of fish at viable feeding costs. A 49-day feeding trial was conducted to determine the optimum biological and economic balanced digestible protein (BDP) levels for pre-adult pacu (*Piaractus mesopotamicus*). A completely randomized design was used which consisted of six treatments and three replicates each. Fish with initial average live body weight of 490.60 ± 14.39g were randomly distributed among 18 experimental units/ concrete tanks of 2,000L, each. A total of 10 fish were stocked per tank/ replicate and fed with six different levels of balanced digestible protein at the rate of 163, 201, 238, 272, 315 and 348 g kg^{-1}, respectively until apprarent satiation three times a day at 09:00, 12:00 and 17:00 h. In the present study, dietary protein was considered as the balanced digestible protein (DBP) because amino acid balance was maintained among the diets. Lysine was used as a reference amino acid and the ratio among lysine and all other essential amino acids was kept constant. Additionally, lysine requirement was kept at a constant and optimum proportion of digestible protein. At the end of the trial, fish of each treatment were weighed and total feed offered to fish per replicate was calculated. These data were used in performance and economic analyses. A group of 8 pre-adult pacu was sampled at the start of the experiment and 6 fish per replicate were sampled at the end of the experiment to determine the initial and final whole body chemical composition, respectively. The obtained results showed that weight gain (WG), feed efficiency ratio (FER) and specific growth rate (SGR) were significantly increased ($P < 0.05$) and feed conversion ratio (FCR) significantly decreased ($P < 0.05$) with increasing BDP levels up to 315 g kg^{-1} and the last diet which was provided with 348 g BDP kg^{-1} showed significantly reduced ($P < 0.05$) effects on the performance variables. Based on the quadratic regression and economic model, the optimum biological balanced digestible protein requirement of pre-adult pacu was estimated to be 292 g kg^{-1} (dry matter) and the optimum digestible protein to energy (DP: DE) ratio was calculated to be 19.82 g MJ^{-1}. The economic model estimated the optimum economic level of 248 g BDP kg^{-1}

(dry matter) and the optimum DP: DE ratio of 17 g MJ^{-1} was calculated for pre-adult pacu that will be sold as whole body. For pre-adult pacu that will be sold as eviscerated fish and sliced fish, the economic requirement was estimated to be 253 and 259 g BDP kg^{-1} and the optimum DP: DE ratio was calculated to be 17.32, and 17.80 g MJ^{-1}, respectively.

Keywords: Balanced diets; Better performance; Maximum gross margin; Low feeding costs; Fish nutrition; Pre-adult growth phase

1. Introduction

In intensive fish farming, fish nutrition is usually reflected as the most critical factor because it is directly linked to the performance and total production costs (Gatlin, 2002). To avoid reduced growth and high feeding costs, nutritional requirements should be accurately determined for fish in different growth phases. Protein is the most essential and important nutrient and is considered an integral part of fish feeds because of its significant role in maintenance and growth (Hatlen et al., 2005; National Research Council-NRC, 2011). It is composed of essential and non essential amino acids and is needed for the synthesis of body protein (Kim et al., 2003). The supplementation of dietary protein at optimum levels significantly improves the performance of fish but its extra amounts are either utilized by fish as an energy source and result in high fat deposition or lead to high nitrogen excretion in water. Consequently, exerts negative effects on fish growth and health and also increases the feeding costs and reduces the profitability (Cho and Bureau, 2001; Gatlin, 2002). The optimization of dietary protein requirements by taking into account performance and cost-benefit analyses data through appropriate models may result in the establishment of nutritionally balanced diets at low feed costs (Eits et al., 2005b; Sá et al., 2008).

There are several factors which affect protein requirements such as fish species, nature and design of the diet, rearing conditions and size (NRC, 2011). The development of size specific balanced feed formulations, proper feed management and effective feeding strategies are

important efforts toward sustainable aquaculture. Juvenile fish require relatively high dietary protein content in their diet than the large ones (Ye et al., 2017). Thus, protein requirements should be determined in different growth phases. Also, the application of proper mathematical models may help to calculate the optimum protein requirement from economic economic point of view. Pre-adult is an important growth phase and any improper use of dietary protein in this growth phase not only increases the feeding costs but also increases the nitrogen losses and decline the profitability (Jiang et al., 2016; Ye et al., 2017; Ma et al., 2019).

The increasing feed costs and high nitrogen losses have oriented the attention of feed industries to develop nutritionally balanced and economically viable diets. Such diets may play an important role in boosting the production rates and profits at viable feeding costs (Melo et al., 2006; Duong et al., 2011; FAO, 2013; Khan et al., 2019b). Nutritionists and feed industries are trying to develop size specific nutritionally balanced and economically viable diets for fish through efficient feeding approaches (McGoogan and Gatlin, 2000; Webb and Gatlin III, 2003; Wu and Gatlin III, 2014). In fish nutrition, the graded supplementation method has been widely used to formulate experimental and practical diets (Elangovan and Shim 1997; Fernandes et al. 2000, 2001; Alvarez-Gonzaez et al., 2001; Lee et al., 2001; Luo et al., 2004; Alam et al., 2008; Arnason et al. 2009; Bicudo et al., 2010; Kim and Lee, 2009; Zhang et al., 2010; Hung and Quy, 2013; Ye et al., 2017). However, this method has a serious problem as the essential amino acid ratios do not remain the same in the consecutive experimental diets which may influence the growth response and the optimum protein requirement. On the other hand the dilution method developed by Fisher and Morris (1970) allows maintaining essential amino acid balance among experimental diets such that only the protein level increases but the ratio among essential amino acids in relation to lysine remain constant (Gous and Morris, 1985; Abboudi et al., 2007; Liebert and Benkendorff, 2007). The balance among essential amino acids can be easily achieved through the concept of protein-bound amino acids rather than the addition of crystalline amino acids which may increase the feed costs and lead to restricted feed intake (Gous and Morris, 1985). The development of nutritionally balanced and economically viable diets may considerably improves the feed intake and growth performance of animal and maximum profit (Eits et al., 2005b; Vandergeest, 2007; FAO, 2013; Robb and Crampton, 2013; Shipton and Hecht, 2013; White, 2013; Sa et al., 2014; Ye et al., 2017; Gous et al., 2018; Khan et al., 2019b).

Pacu (*Piaractus mesopotamicus*) is an important freshwater fish species being considered favourable for farming in different regions of the world (Saint-Paul, 1986; Machado and Sgarbieri, 1991; Fernandes et al., 2000; Bicudo et al., 2010). The available literature has recommended the protein requirements for juvenile pacu of sizes below 400 g (Fernandes et al., 2000, 2001; Abimorad and Carneiro, 2004; Abimorad et al., 2007; Bicudo et al., 2010; Signor et al., 2010; Klein et al., 2014). According to literature, for pre-adult growth phase of pacu still no dietary protein requirements data are available. Thus, the present study aimed to model the optimum biological and economic balanced digestible protein levels for pre-adult pacu.

2. Materials and methods

2.1. Ethical guidelines, fish, experimental design, closed water re-circulation aquaculture system (RAS)

The 49 days feeding trial was conducted according to the ethical guidelines of the Brazilian College of Animal Experimentation and the all the procedures used were approved by the Ethics Committee on Animal Use of the Faculty of Agricultural and Veterinary Sciences, São Paulo State University (UNESP), Jaboticabal, Brazil via protocol No. 009999/ 14.

Pre-adult pacu were obtained from the Tuiuiu Fish Farm located at the Guaira City, SP. Fish were adapted to the laboratory conditions for one week. At the start of the experiment, a total of 180 fish such as 10 individual were stocked per tank/ replicate with initial average body weight of 490.61 ± 6.58g. Fish were distributed among 18 concrete tanks of 2,000 L^{-1} water capacity each and were fed three times a day at 09:00, 12:00 and 17 h until apparent satiation.

All the experimental tanks were connected to a closed re-circulation aquaculture system provided with a biological and mechanical filter and heating system. The recirculation system consisted of three reservoirs and the filter was receiving the water of the experimental units/ tanks for filtration. After filtering process, waste free water with a constant temperature was again throwing back to the experimental units through a water pump. An electric air blower was producing the aeriation and equal concentration of oxygen supplied in all the experimental units

through small plastic pipes being connected with oxygen pump and were provided with regulators and air stones.

2.2. Preparation and extrusion of experimental diets

The formulation of basal diets is shown in Table 1. The required ingredients were analysed according to the standard methods of AOAC (2016) and the dry matter, crude protein, total lipid and total amino acids were determined. Using the analysed ingredients two basal diets one with high-protein content and the other with low-protein content, were formulated according to the dilution method of Fisher and Morris (1970). The whole-body based balanced essential amino acid pattern reported by Khan et al. (2019a) was maintained in basal diets. The amino acid profile in basal diets was achieved through the protein rich ingredients and only the most limiting essential amino acids including lysine, methionine and threonine were added synthetically at very small amounts due to the use of some plant based ingredients. The dietary protein was considered to be balanced digestible protein (DBP) because amino acid balance was maintained among the diets. According to the approach of Mack et al. (1999), lysine was used as a reference amino acid and the ratio among lysine and all other essential amino acids was kept constant. Furthermore, lysine requirement was kept at a constant and optimum proportion of digestible protein (Eits et al., 2005a).

The required ingredients were finely ground and mixed properly in a hammer mill (Model MCS 280, Brasil). Six consecutive experimental diets were formulated by diluting the high-protein diet with the low-protein diet which contained balanced digestible protein levels at the rate of 163, 201, 238, 272, 315 and 348 g kg^{-1}, respectively (Table 2). An average value i.e., 15 MJ kg^{-1} of the previously determined digestible energy for the species under study by Carneiro (1983), Abimorad and Carneiro (2004) Abimorad et al. (2007, 2010) and Bicudo et al. (2010), was added to the basal diets. The digestible values of the ingredients used in the present formulations were calculated according to the apparent digestibility coefficients reported by Abimorad et al. (2008), Fabregat et al. (2008) and Gonçalves and Cyrino (2014) for the species under study.

After the mixing of proper amounts of high-protein and low-protein diets, six diets were prepared. The specific amount of oil and water was added to each diet, properly mixed in a Y-

shaped mixer and then extruded through an extruder (Ex Micro Model, Exteec, Brazil) into pellets of 4 mm with identical size. The diets were dried in a Force Air Circulation-Oven at 55°C for 24 hours and then kept in a refrigerator at - 20°C until use.

Table 1

Formulation of the basal (low-protein and high-protein) diets (g kg^{-1}).

Ingredients	Price (R$ kg^{-1})	Basal diets	
		Low protein-128.3	High protein-366
Fish meal	5.20	47.10	176.40
Corn gluten	5.50	11.30	37.24
Soybean meal	1.84	124.30	441.00
Broken rice	1.15	162.66	0.00
Corn	0.80	151.50	258.72
Corn starch	1.35	255.00	0.00
Rice husk	0.40	75.00	0.00
Soybean oil	2.90	100.00	41.39
L-lysine (99%)	17.30	3.70	10.88
DL-methionine	23.00	0.44	1.86
L-threonine (98.5%)	50.00	0.50	1.47
Dicalcium phosphate	1.65	36.00	17.15
Limestone	0.50	24.00	5.39
Antifungal	23.80	3.00	3.00
Antioxidant BHT	18.30	0.50	0.50
Mineral and Vitamin premix[1]	36.00	5.00	5.00

[1]Vitamin A 860.000 UI kg^{-1}, Vitamin D3 240.000 UI kg^{-1}, Vitamin E 10.500 UI kg^{-1}, Vitamin K3 1.400 mg kg^{-1}, Vitamin B1 2.100 mg kg^{-1}, Vitamin B2 2.150 mg kg^{-1}, Vitamin B6 2.100 mg kg^{-1}, Vitamin B12 2.200 mg kg^{-1}, Vitamin C 25,000 mg kg^{-1}, Niacin 10,000 mg kg^{-1}, calcium pantothenate 5.600 mg kg^{-1}, folic acid 580 mg kg^{-1}, biotin 17 mg kg^{-1}, choline chloride 60,000 mg kg^{-1}, inositol 3.750 mg kg^{-1}, copper 1.800 mg kg^{-1}, manganese 5.000 mg kg^{-1}, zinc 8.000 mg kg^{-1}, iodine 90 mg kg^{-1}, selenium 36 mg kg^{-1}, and cobalt 55 mg kg^{-1}.

Table 2

Experimental diets and the analyzed chemical composition of the basal and experimental diets.

	LP	HP	163	201	238	272	315	348
Dilution of the two basal diets to achieve six consecutive experimental diets (g kg^{-1}).								
LP			806.68	647.36	488.03	328.71	169.39	10.06
HP			193.32	352.64	511.97	671.29	830.61	989.94
Total			1,000	1,000	1,000	1,000	1,000	1,000
Total cost (R$ kg^{-1})	1.94	2.86	2.12	2.26	2.41	2.56	2.7	2.85
Analyzed proximate chemical composition (dry matter g kg^{-1}) of the basal and experimental diets.								
Dry matter	935.29	928.92	954.23	950.56	951.16	955.31	946.18	946.67
Crude protein	147.00	409.80	185.00	227.10	268.00	305.60	353.20	389.70
Digestible protein	128.30	366.00	163.00	201.00	238.00	272.00	315.00	348.00
Total fat	126.80	90.10	117.80	112.40	106.00	99.60	94.50	88.60
Digestible fat	106.40	73.10	98.30	93.40	87.60	81.90	77.20	71.90
Ash	91.50	72.90	85.80	82.80	80.10	77.00	74.60	71.70
Crude energy (MJ kg^{-1})	17.56	19.25	17.38	17.71	17.95	18.13	18.57	18.82
Digestible energy (MJ kg^{-1})	15.06	15.04	14.63	14.68	14.67	14.60	14.73	14.72
DP: DE ratio (g MJ^{-1})			11.14	13.69	16.22	18.63	21.38	23.64

LP = Low protein; HP = High protein.

Table 3

Amino acid composition (dry matter basis) of the basal diets (g kg^{-1}).

Essential amino acids (EAAs)	LP-total EAAs	LP-dig. EAAs	HP-total EAAs	HP-dig. EAAs
Methionine	2.8	2.5	8.5	7.8
Lysine	9.9	9.1	30.2	28
Threonine	5.6	4.8	16.6	14.7
Arginine	8.8	8.4	25.5	24.3
Isoleucine	5.6	4.8	16	14.2
Leucine	9.7	8.8	29.9	27.8
Valine	6.2	5.2	17.4	15.3
Histidine	3	2.8	8.8	8.2
Phenylalanine	6.3	5.8	18	16.8
Tryptophan	1.5	1.3	3.9	3.5
Non-essential amino acids	LP-total NEAAs		HP-total NEAAs	
Cysteine	2.3		5.3	
Glycine	8.6		23.4	
Serine	7		18.3	
Proline	9.4		24.1	
Alanine	8.5		21.6	
Aspartic acid	14.3		38	
Glutamate	25		63.5	
Methionine + Cysteine	5.6		13.8	

LP = Low protein; HP = High protein. Dig. = Digestible; NEAA.

2.3. Water quality parameters

The water temperature, dissolved oxygen and pH of experimental units were noted daily and ammonia was checked once a week during the entire experiment according to standard methods. Average water temperature, dissolved oxygen, pH and ammonia was observed to be 28.2 ± 0.35 °C; 7.3 ± 0.2 mg L^{-1}, 7.25 ± 0.35, 0.11 ± 0.15 mg L^{-1}, respectively. These values were within the optimum range that was observed in our previous study. The same optimum range was also reported by Sipaúba-Tavares and Santeiro (2013) for the species under study.

2.4. Sample collection

At the begining of the feeding trial, fish were subjected to a 24 h fasting period. A group of 10 fish which were randomly sampled was subjected to the analysis of initial whole body chemical composition. After sampling the fish for initial whole body determination, uniform size and healthy pre-adult pacu were immediately anesthetized with a low dose of benzocaine i.e., 50 mg L^{-1}) as reported by Bicudo et al. (2010) and weighed one by one and per tank/ replicate total 10 fish were stocked. At the end of the trial, all fish were again anesthetized with a low benzocaine solution of 50 mg L^{-1}, and then weighed. The total amount of feed consumed per replicate during the experiment was noted. The initial and final weights of the whole body fish and those of the cut-up portions, body weight gain and feeding data were noted and used in the calculation of biological and economic responses and optimum levels.

At the end of the trial, three fish per replicate were used to determine the final whole body chemical composition of pre-adult. Further four fish per replicate were subjected to the processing procedure to separate different parts including the scales, fins, head, and viscera, main body trunk. Procedure reported by Klein et al. (2014) was followed during the fish processing. The processing procedure was done by a single well trained person to reduce the variability in final weights. The following three market products were obtained and weighed each one separately.

Whole body fish = (Skin + Scales + fins + head + visceral content + main body trunk)

Sliced fish = (Whole fish – scales – fins – head – visceral content) and

Eviscerated fish = (Whole body fish – visceral content)

The fish sampled for the initial and final whole body analysis and those used in the processing procedure were deeply anesthetized with an overdose of benzocaine solution (at the rate of 500 mg L^{-1}) according to the recommendations as reported by Bicudo et al. (2010) for the species under study.

2.5. Feed analysis

Proximate chemical composition of basal and experimental diets (table 2) was determined using standard methods of AOAC (2016). Gross energy was measured only in experimental diets by calorimetry bomb. The samples of the basal diets (high protein and low protein) were sent to the Evonik industries AG, Essen, North Rhine-Westphalia, Germany to determine the AA composition (Table 3).

2.6. Whole body composition analysis

The analysis of the initial and final whole body proximate chemical composition of pre-adult was done according to the standard method of AOAC (2016). The whole body pre-adult pacu were ground in a meat grinder, frozen, and then lyophilized in a freeze dryer to obtain the pre-dry matter. The freeze dried samples were again ground in a ball-grinder to prepare a homogenized mixture for further analytical procedures. Moisture was determined by oven drying the samples at 105 °C for a period of about 24 h to obtain a constant weight. Kjeldahl method was used to determine the crude protein content. Total fat content was measured by ether extraction procedure and ash determined by burning the samples in a muffle furnace at 550 °C for 6 h.

2.7. Biological responses

At the end of the feeding trial, the body weight gain (BWG), feed conversion rate (FCR), feed efficiency ratio (FER), feed intake (FI), protein efficiency ratio (PER) and specific growth rate (SGR) were calculated. The weight gain data were used to model the optimum biological balanced digestible protein.

2.8. Modeling optimum biological balanced digestible protein requirement

According to previous literature, selecting a biological model to estimate optimum biological protein requirement depend on the objective of the study. Several authors claim that quadratic model better explain the behaviour of growth response in relation to different dietary levels of a test nutrient (Zeitoun et al., 1976; Gurure et al., 1995; Ye et al., 2017). On the basis of the characteristics of our data, quadratic regression was selected to model the optimum biological protein requirement of pre-adult pacu. The weight gain data were used to determine the optimum biological protein level. The average weight gain data obtained in response to different dietary

protein levels were fitted to the quadratic regression; $y = \alpha + \beta x + cx^2$, where x is the protein level, y is response variable, α is the curve intercept, β and c are the slopes, and x = - β/ 2c yield the minimum biological protein level for maximum response.

2.9. Economic responses (inputs and outputs)

The economic model developed by Eits et al. (2005b) was adapted to model the optimum economic digestible protein requirements of pre-adult pacu.

The inputs consisted the whole body final weight (a) (g $fish^{-1}$), feed intake (b) (g $fish^{-1}$), feed price including the feed processing costs (c) (R$ $g^{-1\ diet}$) and the selling price (R$ g^{-1} $fish^{-1}$) of the whole body fish or that of the cut-up portions of fish (including the processing costs) (d).

Feed price (R$ g^{-1} diet) = a × BDP + b/1000, where BDP is the dietary balanced digestible protein while the model parameters "a" and "b" are depending on the prices of the diet. The selling price (R$ g^{-1} fish) of the whole body fish or cut-up portions including the processing price for the cut-up portions were obtained from a local fish farmer. The current prices (R$ g^{-1} ingredient) of the feed ingredients were obtained from the Agromix, Jaboticabal, SP, Brazil. The prices of the ingredients were used to calculate the feed cost (R$ g^{-1} diet) per diet.

2.10. Modeling optimum economic balanced digestible protein requirement

The optimum economic protein level was calculated according to the following model of Eits et al. (2005b).

Gross margin (GM) = Revenue – Feeding cost

2.11. Algebraic description of the model

The gross margin can be algebraically expressed as follows;

GM = (a × (d)) – (c° × b)

Where,

a = Final weight of the whole fish or cut-up portion (g), d = coefficient obtained for the selling price of the whole fish and cut-up portion + processing costs (R$ g^{-1} $fish^{-1}$), c° = feed price (R$ g^{-1} diet) + feed processing cost, and b = feed intake (g)

Thus, the functions in the above equation can be elaborated as follow;

$d = c_0 + c_1x + c_2x^2$, estimates of the quadratic regression obtained for final weight of whole body and cut-up portion (including the processing costs) using exponential data.

$c° = y_0 + y_1x$, estimates of the linear equation obtained for feed price + feed processing cost

$b = f_0 + f_1x + f_2x^2$, estimates of the quadratic regression obtained for feed intake using exponential data

Or if we expand the equation as;

$GM = (ac_0 + ac_1x + ac_2x^2) – (y_0f_0 + y_0f_1x + y_0f_2x^2 + y_1f_0x \times y_1f_1x^2 + y_1f_2x^3)$

Where, a, c_0, c_1, c_2, y_0, y_1, f_0, f_1, and f_2 are constants while X is unknown

GM is maximized when $\frac{\partial GM}{\partial X} = 0$ or we also can express as;

$\frac{\partial GM}{\partial X} = (ac_1x + 2\ ac_2) – (y_0f_1 + 2\ y_0f_2x + y_1f_0x + 2\ y_1f_1x + 3\ y_1f_2x^2)$

$= (ac_1 - y_0f_1x - y_1f_0) + (2\ ac_2 - 2\ y_0f_2 - 2\ y_1f_1)\ x – (3\ y_1f_2)\ x^2$

Let $k_a = -3\ y_1f_2$

$K_b = 2\ ac_2 - 2\ y_0f_2 - 2\ y_1f_1$

$K_c = ac_1 - y_0f_1 - y_1f_0$

Then the GM is maximized when X takes the value of

$$x° = \frac{-k_b \pm \sqrt{{k_b}^2 - kac}}{2k_a}$$

According to the present economic model, the dietary protein level where maximum GM (profit) was achieved considered to be the optimum economic protein requirement.

2.12. Statistical analysis

Results were observed as average ± SD of the three replicates per treatment. The obtained growth performance, feeding and whole body composition data were submitted to the SPSS for Windows, version 16.0. Chicago, SPSS Inc., (2007) software. One-way Analysis of Variance-ANOVA was used to evaluate the significant differences. When a significant ($P < 0.05$) difference was perceived, LSD (Tukey's Least Significant Test) was applied to compare the averages.

Growth response in terms of average body weight gain, final weight, and feed intake data against dietary protein levels were fitted to the quadratic regression model using the SAS Institute Inc. 2014 software. $y = \alpha + \beta x + cx^2$

3. Results

3.1. Survival, growth performance and feeding aspects

No mortality was observed during the present experiment and fish well accepted the feed. Growth performance and feeding parameters such as body weight gain (BWG), specific growth rate (SGR %), feed efficiency ratio (FER), and feed intake (FI) were significantly ($P < 0.05$) increased while feed conversion rate (FCR) and protein efficiency ratio (PER) was significantly ($P < 0.05$) decreased by the diets supplemented with balanced digestible protein up to the level 315 g kg^{-1}. The last diet provided with higher protein content (348 g kg^{-1}) resulted in significantly reduced ($P < 0.05$) growth performance and feeding aspects (Table 4).

Table 4

Response of pre-adult pacu to different balanced digestible protein (g kg^{-1}) levels.

BDP	[1]BWG	[2]FI	[3]FCR	[4]FER	[5]SGR (%)	[6]PER
163	170.5 ± 5^{d}	353.17 ± 3^{d}	2.07 ± 0.1^{d}	0.48 ± 0.01^{d}	0.61 ± 0.02^{c}	2.96 ± 0.1^{a}
201	189.3 ± 5^{c}	359.38 ± 1^{c}	1.90 ± 0.1^{c}	0.53 ± 0.01^{c}	0.65 ± 0.02^{d}	2.62 ± 0.1^{b}
238	204.0 ± 5^{b}	364.62 ± 2^{b}	1.79 ± 0.4^{ab}	0.56 ± 0.1^{ab}	0.70 ± 0.02^{b}	2.35 ± 0.3^{c}
272	214.4 ± 6^{a}	370.47 ± 2^{a}	1.73 ± 0.1^{ab}	0.58 ± 0.02^{a}	0.74 ± 0.1^{ab}	2.13 ± 0.1^{d}
315	216.2 ± 5^{a}	371.38 ± 2^{a}	1.72 ± 0.3^{a}	0.58 ± 0.01^{a}	0.76 ± 0.1^{a}	1.85 ± 0.2^{e}
348	202.9 ± 4^{b}	366.10 ± 2^{b}	1.81 ± 0.1^{b}	0.55 ± 0.01^{b}	0.71 ± 0.1^{ab}	1.59 ± 0.03^{f}
P-value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

[1]Balanced digestible protein; [2]Body weight gain; 3Feed intake; [4]Feed conversion ratio; [5]Feed efficiency ratio; [6]Specific growth rate; [7]Protein efficiency ratio.

Values are means of the three replicates per treatment (n=3) and expressed as mean ± SD. Means in the same column with different superscripts are significantly different at $P < 0.05$ by Tukey Least Significant Test.

[1]Weight gain (g) = (final wet weight - initial wet weight)

[2]Feed intake (g) = (total feed consumed/ fish)

[3]Feed conversion ratio = (dry feed intake/ wet weight gain)

[4]Feed efficiency ratio = (wet weight gain/ dry feed intake)

[5]Specific growth rate (%) = ((ln final body weight - ln initial body weight)/ days) × 100

[6]Protein efficiency ratio (g) = (wet weight gain/ protein intake)

The weights of the initial and final whole body pre-adult pacu and those of the final cut-up portions are shown in Table 5. The final whole body weights and cut-up portions were enhanced in response to dietary protein.

Table 5

Initial and final average weight of whole body, eviscerated and sliced pre-adult pacu (g).

	Initial whole fish	Final whole fish	Eviscerated fish	Sliced fish
Initial weight			473.56 ± 77.72	341.72 ± 57.1
163	485.92 ± 6.07	656.37 ± 5.02[b]	623.29 ± 22.70	452.33 ± 25.36
201	502.37 ± 6.28	691.62 ± 9.88[a]	582.75 ± 23.98	422.25 ± 13.87
238	500.97 ± 9.29	704.98 ± 6.98[a]	616.75 ± 3.77	450.21 ± 4.49
272	494.43 ± 6.95	708.78 ± 5.87[a]	631.71 ± 14.46	463.83 ± 4.65
315	480.85 ± 20.90	697.02 ± 16.86[a]	618.42 ± 31.70	445.63 ± 33.29
348	487.35 ± 18.38	690.22 ± 21.76[a]	590.29 ± 10.21	427.29 ± 6.42
P-Value	> 0.05	0.0037	> 0.066	> 0.1184

Data are represented as mean ± SD (n=3), means in the same column with different superscript letters are significantly different (P < 0.05).

3.2. Optimum biological balanced digestible protein requirement

The optimum biological balanced digestible protein requirement of pre-adult pacu was estimated to be 292 g kg^{-1} (dry matter) by the quadratic equation (Fig. 01). The optimum DP: DE for the pre-adult pacu was calculated to be 19.82 g MJ^{-1} for pre-adult pacu

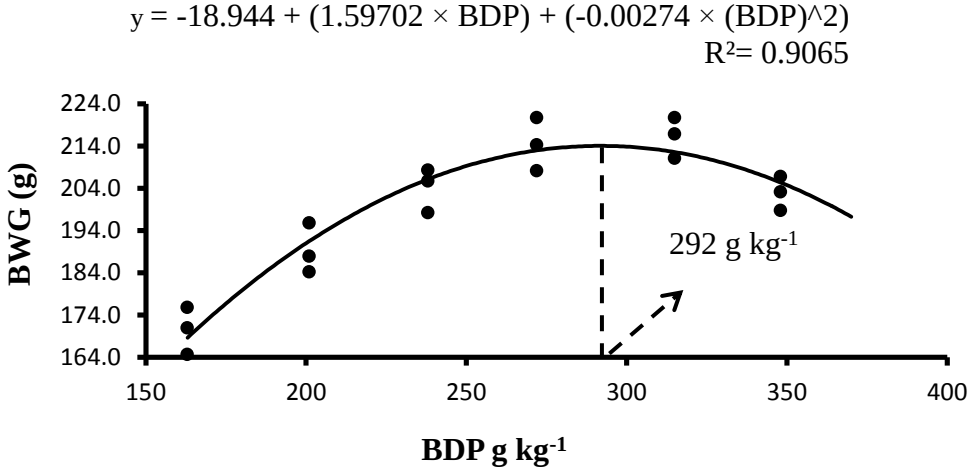

Fig. 1. Body weight gain (BWG) of pre-adult and different balanced digestible protein (BDP g kg^{-1}) levels assessed by the second order quadratic regression model.

3.3. Whole body composition

The whole body chemical composition of pre-adult pacu fed different balanced digestible protein (g kg^{-1}) levels is shown in Table 6. Whole body moisture and crude protein content was significantly ($P < 0.01$) increased while total lipid and ash content significantly ($P < 0.01$) decreased in response to different balanced digestible protein levels.

Table 6

Body composition (wet weight basis g kg^{-1}) of pre-adult pacu fed different BDP levels.

BDP (g kg^{-1})	Moisture	Crude protein	Total lipid	Ash
Initial	669.6	154.6	148	25.7
163	601.90 ± 2.97^{d}	138.20 ± 0.66^{c}	203.67 ± 0.23^{a}	44.97 ± 2.06^{a}
201	600.23 ± 11.56^{cd}	139.37 ± 0.81^{c}	193.23 ± 5.25^{b}	42.77 ± 2.98^{ab}
238	619.03 ± 8.10^{bc}	143.03 ± 0.38^{b}	183.97 ± 0.95^{c}	40.97 ± 1.18^{bc}
272	627.67 ± 1.52^{ab}	144.30 ± 1.42^{b}	177.13 ± 1.27^{d}	35.83 ± 1.04^{d}
315	625.93 ± 2.57^{ab}	147.37 ± 1.00^{a}	173.00 ± 1.04^{d}	35.50 ± 1.25^{d}
348	637.63 ± 13.84^{a}	148.47 ± 0.55^{a}	165.43 ± 6.87^{e}	37.97 ± 0.42^{cd}
P-value	0.0021	0.0000	0.0000	0.0001

BDP = Balanced digestible protein.

Data are represented as mean ± SD (n=3), means (of three replicates) in the same column with different superscript letters are significantly different ($P < 0.05$) by Tukey Least significant test.

3.4. Optimum economic balanced digestible protein requirement

The equations obtained for the final body weight (FBW), feed intake (FI) and feed price (FP) in Figure 2, 3 and 4, respectively were used as inputs in the economic model.

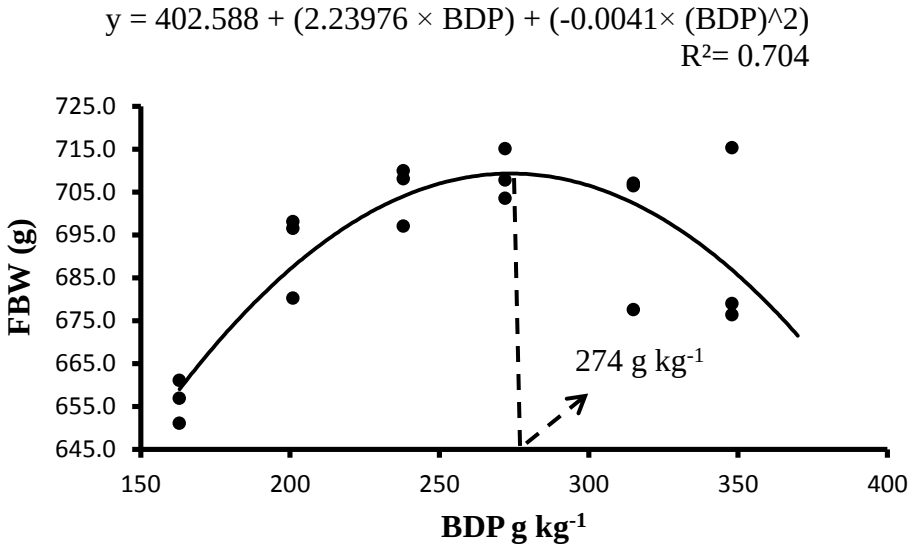

Fig. 2. Final body weight (FBW) of pre-adult pacu in response to different balanced digestible protein levels (BDP g kg^{-1}) estimated through the quadratic regression equation.

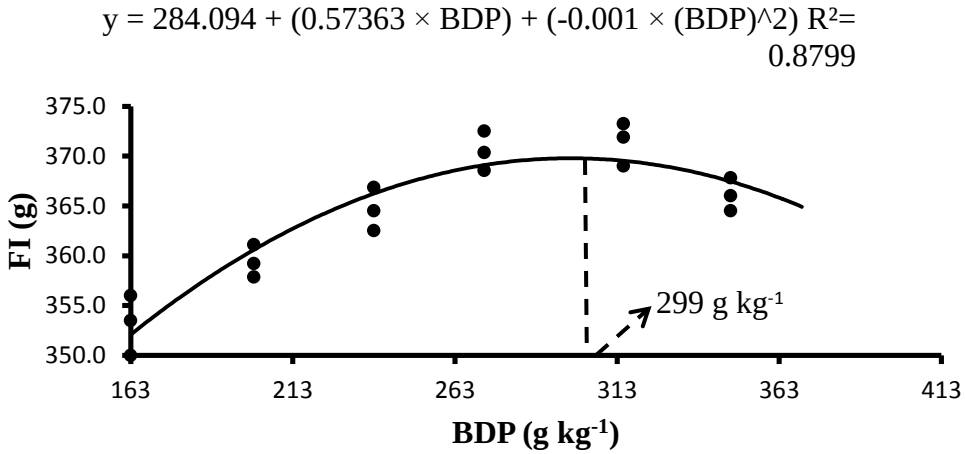

Fig. 3. Observed feed intake response evaluated by the regression equation in pre-adult pacu.

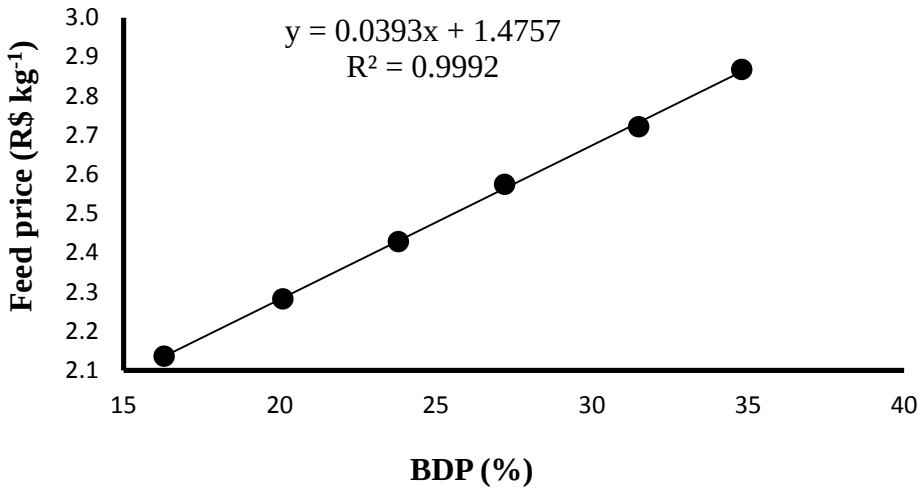

Fig.4. Effect of different balanced digestible protein (BDP) levels on the price of experimental diets.

The weights of eviscerated and sliced fish were calculated in natural logarithm (ln) and these data along with different balanced digestible protein levels were used to calculate the inputs for eviscerated and sliced pre-adult pacu.

Eviscerate pre-adult pacu: $y = [(0.9829x + 0.0054)\ R^2 = 0.9854]$

Sliced pre-adult pacu: $y = [(1.1311x – 1.2806)\ R^2 = 0.9321]$

The above linear equations were provided as inputs to the economic model in order to calculate the optimum economic protein for eviscerated and sliced pre-adult pacu.

The optimum economic balanced digestible protein requirements estimated on the basis of maximum gross margin obtained for whole body, eviscerated and sliced pre-adult pacu are shown in Figure 5, 6 and 7, respectively.

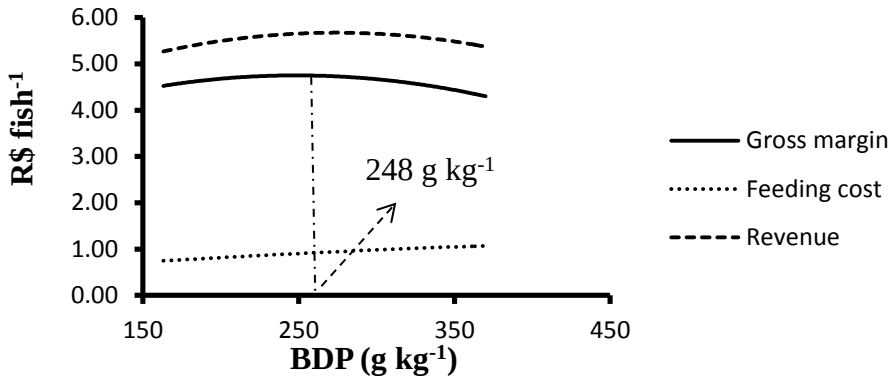

Fig. 5. Optimum economic balanced digestible protein requirement (BDP) of whole body pre-adult pacu based on maximum gross margin (R$ g^{-1} $fish^{-1}$).

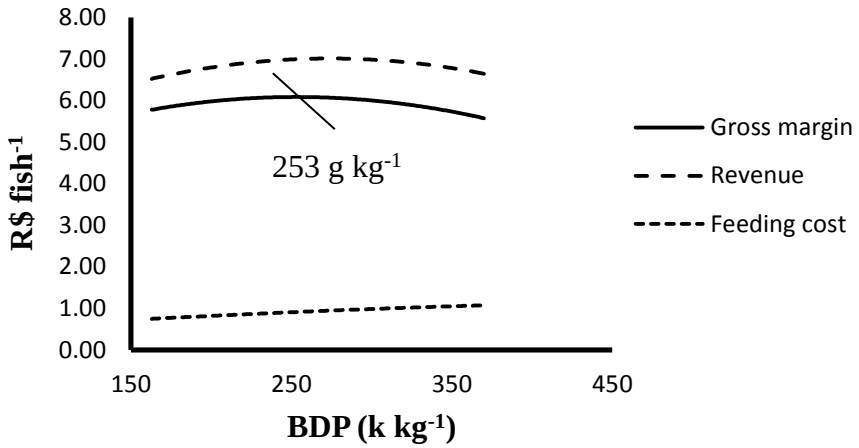

Fig. 6. Effect of different balanced digestible protein (BDP g kg^{-1}) levels on feeding cost (FC), revenue and gross margin (GM) in eviscerated fish.

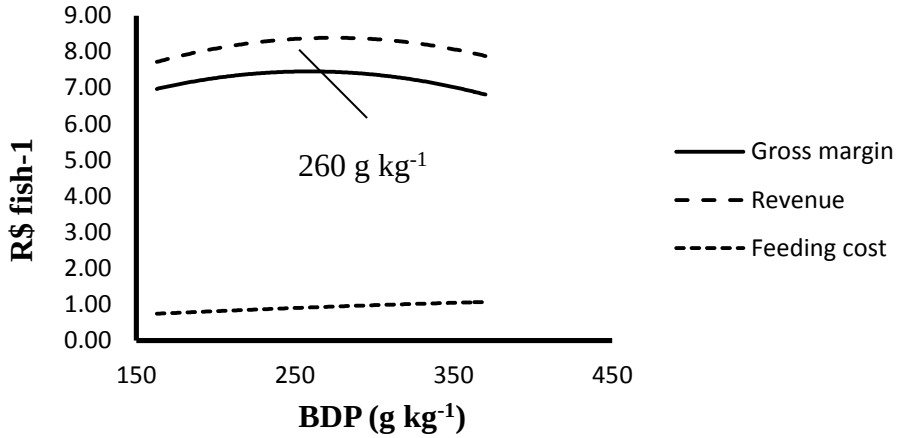

Fig. 7. Effect of different balanced digestible protein (DBDP g kg^{-1}) levels on feeding cost (FC), revenue and gross margin (GM) in sliced fish.

According to the economic model, the level of 248 g BDP kg^{-1} where maximum gross margin (R$ g^{-1} $fish^{-1}$) achieved was considered to be the optimum economic level for pre-adult pacu that will be sold as a whole fish. The optimum DP: DE at the optimum economic BDP was estimated to be 17.00 g MJ^{-1} for the whole body pre-adult pacu. The optimum biological level was estimated to be 292 g BDP kg^{-1} which was higher than the optimum economic level (Fig. 5). The optimum economic level of 253 and 260 g BDP kg^{-1} was determined for pre-adult pacu that will be sold as eviscerated and sliced fish with the optimum DP: DE ratio of 17.32, and 17.80 g MJ^{-1}, respectively. These results showed that feeding pre-adult pacu that will be sold as an eviscerated or sliced fish, the gross margin decreases than in the case if it will be sold as a whole body because the economic requirement slightly increases to obtain pre-adult pacu that will be sold in the form of cut-up portions.

3.5. Hypothesized Scenarios

Alternate scenarios were hypothesized by varying the current selling prices of the whole body (Table 7), eviscerated (Table 8), and sliced pre-adult pacu (Table 9) and those of the fish meal and soybean meal (Table 7, 8 and 9).

Table 7

Optimum economic levels and gross margin in whole body pre-adult pacu.

Scenarios		BDP (g kg^{-1})	FI (g)	FC (R$ g^{-1} $fish^{-1}$)	Rev. (R$ $g^{-1}$$fish^{-1}$)	GM (R$ $g^{-1}$$fish^{-1}$)
Bio-L	Normal	292	369.740	0.971	5.664	4.693
Eco-L	Normal	248	367.311	0.901	5.652	4.752
WFP	(-10)	245	367.010	0.896	5.082	4.187
WFP	(+10)	251	367.594	0.906	6.223	5.317
FM	(-10)	250	367.502	0.881	5.656	4.775
FM	(+10)	246	367.112	0.920	5.649	4.729
SM	(-10)	248	367.311	0.903	5.652	4.749
SM	(+10)	245	367.010	0.939	5.647	4.709

BDP = Balanced digestible protein; FI = Feed intake; FC = Feeding cost; Rev = Revenue; GM = Gross margin; Bio-L = Biological level; Eco-L = Economic level; WFP = Whole fish price; FM = Fish meal; SM = Soybean meal; Normal = Current feed price (Table 1).

Table 8

Optimum protein levels and gross margin in eviscerated pre-adult pacu.

Scenarios		BDP (g kg^{-1})	FI (g)	FC (R$ g^{-1} $fish^{-1}$)	Rev. (R$ g^{-1} $fish^{-1}$)	GM (R$ g^{-1} $fish^{-1}$)
Eco-L	Normal	253	367.774	0.909	7.090	6.181
EviWFP	(-10)	251	367.594	0.906	6.378	5.472
EviWFP	(+10)	255	367.946	0.912	7.802	6.890
FM	(-10)	255	367.946	0.889	7.093	6.204
FM	(+10)	252	367.685	0.931	7.088	6.157
SM	(-10)	253	367.774	0.912	7.090	6.178
SM	(+10)	250	367.502	0.948	6.989	6.041

BDP = Balanced digestible protein; FI = Feed intake; FC = Feeding cost; Rev = Revenue; GM = Gross margin; Bio-L = Biological level; Eco-L = Economic level; EviFP = Eviscerated whole fish price; FM = Fish meal; SM = Soybean meal; Normal = Current feed price (Table 1).

Table 9

Optimum protein levels and gross margin in sliced pre-adult pacu.

Scenarios		BDP (g kg^{-1})	FI (g)	FC (R$ g^{-1} $fish^{-1}$)	Rev. (R$ g^{-1} $fish^{-1}$)	GM (R$ g^{-1} $fish^{-1}$)
EcoL	Normal	260	368.342	0.921	8.843	7.923
SWFP	(-10)	251	367.594	0.906	6.378	5.472
SWFP	(+10)	261	368.416	0.922	9.729	8.807
FM	(-10)	261	368.416	0.898	8.845	7.947
FM	(+10)	259	368.267	0.943	8.842	7.898
SM	(-10)	260	368.342	0.923	8.843	7.920
SM	(+10)	257	368.110	0.961	8.373	7.412

BDP = Balanced digestible protein; FI = Feed intake; FC = Feeding cost; GM = Gross margin; EcoL = Economic level; SFP = Sliced whole fish price; FM = Fish meal; SM = Soybean meal; Normal = Current feed price (Table 1).

The data in the Table 6, 7, and 8, respectively indicate that optimum economic protein requirements change according to the fluctuations in the selling price of whole body, eviscerated and sliced pre-adult pacu and fish meal and soybean meal.

4. Discussion

It has been well-known that in addition to species and size difference, experimental designs, mathematical models and statistical analyses also lead to difference in the protein requirements even for the same fish species (De Silva and Anderson, 1995; Shearer, 2000). To avoid such limitations the biological and economic models being practiced in our previous study conducted with juvenile pacu were also exercised in the present study. According to the quadratic equation, the optimum biological balanced digestible protein requirement was estimated to be 292 g kg^{-1} with an optimum digestible protein to energy (DP: DE) ratio of 19.82 g MJ^{-1} for pre-adult pacu. The present digestible protein requirement for pre-adult pacu was lower than that reported in our previous experiment with juvenile pacu (326 g BDP kg^{-1}). These

results indicate that dietary protein requirement of pacu was declined with the increase in body size which also has been observed in other fish species (Lee et al., 2002a, 2002b; Abboudi et al., 2006; Ye et al., 2017).

Optimum feed intake could be a compensatory mechanism in animals to obtain adequate amount of the limiting nutrient (Meyer and Fracalossi, 2005; Arnason et al., 2009; Gous et al., 2018). In the present study, body weight gain (BWG), specific growth rate (SGR %), feed intake (FI) and feed conversion ration (FCR) feed efficiency ratio (FER) and protein efficiency ratio (PER) were significantly improved with increasing balanced digestible protein upto the optimum levels and beyond that the growth performance and feeding aspects were negatively affected. These results are in accordance to our previous finding with juvenile pacu and those of Bicudo et al (2010) and Klein et al. (2014). According to Sa et al. (2014) and Ma et al. (2019) fish usually do not accept nutritionally unbalanced diets and concluding a negative impact of such diets on the fish growth and feeding aspects. Feed conversion ratio (FCR) is a good indicator of feed efficiency in fish farming which reveals that how the efficiency of a feed or a feeding strategy can be (Alvarez-Gonzaez et al., 2001; Arnason et al., 2010; Jiang et al., 2016). In the present study, significantly lowere FCR was obtained in fish fed with diets containing 315, 272 and 238 g kg^{-1}, respectively. This indicated that pre-adult pacu grew faster with the supplementation of dietary protein up to the optimum levels. The same behaviour of FCR in response to dietary protein has been reported in pre-adult giber carp (*Carassius auratus gibelio* var. CAS III) by Ye et al. (2017) and in hybrid grouper (*Epinephelus lanceolatus* ♂ × *Epinephelus fuscoguttatus* ♀) juveniles by Jiang et al. (2015). In the present study, protein efficiency ratio (PER) significantly reduced with increasing the digestible protein level which has been noticed by Bicudo et al. (2010) in the same fish species. Similarly, Bai et al. (1999) and Kim et al. (2004, 2005) have observed the same response of PER in other fish species. In the present study, fish fed the diets containing higher protein content (348 and 315 g kg^{-1}, respectively) showed significantly decreased PER as compared to the diets with lower protein content. Similar results have been obtained in several other species by Salhi et al. (2004), Catacutan et al. (2001), Bicudo et al., (2010), Hossain et al. (2010), Jiang et al. (2015) and Ye et al. (2017). On the other hand Kikuchi et al. (1992) and Lee et al. (2000) reported that PER increased with the increasing dietary protein. Dabrowski (1979) stated that such differences may occur due to the species difference. According to De Silva and Anderson (1995) PER could not be considered a valid response

because it does not reveal the quantity of dietary protein being used for the maintenance purpose. The behaviour of PER observed in the present study was similar to the data of our previous experiment with juvenile pacu. Thus, in accordance to the above mentioned previous studies and our own finding with juvenile pacu, the present study revealed that growth performance and feeding aspects of pre-adult pacu were significantly improved with increasing balanced digestible protein upto the optimum level and then plateauted.

The whole body chemical composition of pre-adult pacu was improved by balanced digestible protein (BDP) supplementation. The present whole body chemical composition of pre-adult was observed different than the whole body chemical composition of juvenile pacu being evaluated in our previous experiment. These results show that body chemical composition in pacu is not only affected by dietary protein but also by body size which has already confirmed in our previous study (Khan et al., 2019a). Meer et al. (1995) and Ye et al. (2017) have documented the same behaviour of whole body composition in *Colossoma macropomum* (Cuvier) and gibel carp, respectively. In the present study, the whole body moisture and crude protein content was significantly increased by the balanced digestible protein levels but body lipid and ash content was significantly decreased ($P < 0.05$) in response to increasing digestible protein levels. An inverse relationship was observed between the whole body moisture and lipid content in the body of pre-adult pacu which also observed in our previous study (Khan et al., 2019a). The present results show that whole body composition was significantly improved with the supplementation of optimum balanced digestible protein and are in accordance to the findings of several previous researches with the species under study and other species such as those of Meer et al. (1995), Kim et al. (2003), Abimorad and Carneiro (2004), Abimorad et al. (2007), Sa et al. (2008), Bicudo et al. (2010), Signor et al (2010), and Ma et al. (2019). The response of the present whole body composition of pre-adult to different balanced digestible protein levels was found similar to that being observed in our previous study with juvenile pacu.

The economic model of Eits et al. (2005b) being exercised in our previous experiment with juvenile pacu was applied to the present data as well. The economic model calculated the optimum economic balanced digestible protein requirement to be 248, 253, and 259 g kg^{-1} for achieving maximum profit from pre-adult pacu that will be reared to sell as whole body, eviscerated and sliced fish, respectively. The optimum economic requirements were lower than

the optimum biological level of 292 g BDP kg^{-1}. The gross margin (R$ g^{-1} $fish^{-1}$) obtained at the current economic protein levels was higher than that obtained at the biological protein level (Table 7). The present behaviour in terms of optimum economic balanced digestible protein requirements for pre-adult pacu was found closely related to the behaviour of the economic protein levels that has been observed in our previous study with juvenile pacu. Nutritionists usually prefer to formulate diets with high protein content in order to get maximum performance. In the present study it was observed that the supplementation of optimum balanced economic protein could help to achieve maximum profit and better performance. The present results show that in the case of high selling prices of ingredients using the optimum biological protein considerably decline the profit of the aquaculture production which is not considered suitable for sustainable aquaculture. According to this study the use of optimum economic protein not only increases the profitability of the product but also result in less nitrogen excretion in water.

The current economic requirement was subjected to the changes in the selling price of the feed ingredients and whole body, eviscerated and sliced pre-adult pacu (Table 7, 8, 9). Increasing the selling price of whole body, eviscerated and sliced pre-adult pacu by 10% permitted the increase in dietary protein content in order to further increase the gross margin but in the case of a reduction by 10%, it was not necessary to increase the dietary protein content. Additionally, a 10% increase in the selling prices of fish meal and soybean meal resulted in the supplementation of relatively low dietary protein but reducing these prices by 10% permitted to increase slightly the dietary protein level. These results show that an increase of 10% in the selling price of whole fish, cut-up portions and protein rich ingredients considerably affect the gross margin and optimum economic protein. Moreover, any further increase in these prices such as above 10% would affect further the gross margin and optimum economic protein.

The present work provides both biological and economic dietary protein requirements for pre-adult pacu. The quadratic model estimated the optimum biological BDP requirement of 292 g kg^{-1} (DM basis) and an optimum DP: DE ratio of 19.82 g MJ^{-1} was estimated for the better performance of pre-adult pacu. For whole-body, eviscerated and sliced pre-adult pacu, the economic model estimated the optimum economic BDP requirement of 248, 253, and 260 g kg^{-1} (DM basis), with an optimum DP: DE ratio of 17.00, 17.32, and 17.80 g MJ^{-1}, respectively. The present study recommends the the supplementation of economic protein level calculated

according to the present economic model considerably increases the gross margin without any negative affects on the growth and health of fish. Additionaly the present study suggests that the optimum economic protein should be adjusted according to the fluctuations in the selling prices of the whole fish, cut-up parts and protein rich ingredients in order to achieve maximum gross margin and optimize the efficiency of the aquaculture production systems.

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CHAPTER 5

Optimum biological and economic balanced digestible protein requirements of adult pacu (*Piaractus mesopotamicus*)

ABSTRACT

A 44-day feeding trial was designed with the aim to determine the optimum biological and economic balanced digestible protein (BDP) requirements of adult pacu. The experiment was conducted in an indoor experimental set up which connected to a closed re-circulation aquaculture system. A completely randomized design was used which consisted of six treatments and three replicates each. A total of 180 uniform size and healthy fish with an average initial body weight of 1158.50 ± 21.59 g were distributed among 18 concrete tanks of 2,000 L water capacity each. According to the dilution concept two basal diets one with low protein and other with high protein content, were formulated. Both the diets were provided with an essential amino acid balance and the amount of dietary energy, minerals and vitamins were kept at the same level in both the diets. The high protein diet was mixed in proper proportions with the low protein diet in order to achieve six different experimental diets containing balanced digestible protein at the rate of 163, 201, 238, 272, 315 and 348 g kg^{-1}, respectively. The fish were fed three times a day at 09:00, 12:00 and 17:00 h until apparent satiation. The dietary protein used in the present study was defined as balanced digestible protein. According to the concept of balanced protein, the ratios among lysine content and the minimum contents of all other essential amino acids were calculated in relation to lysine. Lysine was used as the reference amino acid and the ratios were kept constant according to an ideal protein profile. The content of lysine was kept at a constant and optimum proportion of digestible protein. The obtained data were submitted to appropriate biological and economic equations to calculate the optimum biological and economic balanced digestible protein (BDP) levels. The optimum BDP for maximum performance was determined to be 245 g kg^{-1} (dry matter) by the quadratic model and the optimum balanced digestible protein to energy (DP: DE) ratio calculated was 16.78 g MJ^{-1}. The economic model estimated the optimum economic BDP requirement of 215, 217 and 222 g kg^{-1} for maximum gross margin (GM) of adult pacu that will be sold as whole body, eviscerated and sliced fish, and the optimum DP: DE ratios were calculated to be 14.65, 14.79, and 15.13 g MJ^{-1}, respectively. The present results revealed that the economic protein requirements were directly related to the fluctuations in the selling price of the feed ingredients and whole body or cut-up portions of adult pacu.

Keywords: Protein research; Balanced diets; High profit; Low cost; Large fish; Fish nutrition; Sustainable aquaculture

1. Introduction

In fish farming, feeding costs have a great impact on the total production costs and profit of the final product (Nguyen, 2013). The use of low-quality feeds and improper feeding practices usually lead to inaccurate dietary requirements, increased feeding costs and low profit coupled with nitrogen excretion in water (Lall and Tibbets, 2009; Nguyen et al., 2009; National Research Council−NRC, 2011; El-Sayed, 2013; Shipton and Hasan, 2013; Uchoi et al., 2015; Khan et al., 2019b). For increasing the feed efficiency in fish, improving the quality of dietary protein and producing appropriate feeding programmes is very beneficial (Houlihan et al., 2001; Broberger, 2005; Lall and Tibbets, 2009). The concept of balanced protein has been successfully applied in animal nutrition (Mack et al., 1999; Eits et al., 2005a, b). The application of this approach in fish nutrition could help nutritionists and feed industries to increase the feed utilization efficiency in fish through diets provided with balanced protein. According to this concept an essential amino acid balance is provided in the experimental diets. Lysine is used as a reference amino acid and the requirements of all other essential amino acids are calculated in relation to lysine. The lysine requirement is kept at a constant and optimum proportion of the dietary protein (Eits et al., 2005a). These efforts help feed industries to establish nutritionally balanced diets in order to reduce the extra supplementation of nitrogen in fish diet which not only increases the feeding costs but also deteriorate the water quality. The modeling of optimum dietary protein requirements by considering the outputs of cost-benefit analyses is another practical approach to avoid the extra supplementation of nitrogen and establish balanced protein diets (Zeitoun et al., 1976; Kaushik, 2000; FAO, 2013; Liu et al., 2013; White, 2013). Feeding fish with such diets may lead to increased profitability and better performance at low feeding costs (De Silva and Hasan, 2007; Duong et al., 2011; Sealey et al., 2013).

Protein requirements have been determined in the juvenile growth phase of different fish species including pacu (Fernandes et al., 2000, 2001; Luo et al., 2004; Alam et al., 2008;

Abimorad et al., 2007; Kim and Lee, 2009; Bicudo et al., 2010; Zhang et al., 2010; Klein et al., 2014) but the requirement of adult fish has been almost neglected. The approach that protein requirements usually decline with the increase in fish size (Pillay, 1990; NRC, 2011; Ye et al; 2017) is clearly indicating that fish size matters a lot in designing a nutritionally balanced diet. To get maximum profit at viable feeding costs, protein requirements should be estimated in different growth phases of a fish taking into consideration both the biological responses and cost-benefit outputs (Craig, 2017, Ye et al., 2017). In the current perplexing issues of increasing feeding costs and high nitrogen losses, the optimization of dietary protein requirements of fish, particularly of large fish through appropriate biological and economic models using the performance and cost-benefit data will probably increase several folds the production rate and economize this important nutrient (Shiau and Lan, 1996; Cho and Bureau, 1998, 2001; Yang et al., 2002, 2003; Kaushik, 2013).

Pacu is an important freshwater fish species which has found in the freshwater bodies in several Latin American countries (Machado and Sgarbieri, 1991; Borghetti and Canzi, 1993) and also has been introduced as an exotic species in some other countries (Pullela, 1997; Cagauan, 2007; Uchoi et al., 2015). Due to its omnivore/ herbivore feeding habits, it has been considered a suitable species for cultivation (Merola, 1988; Froese and Pauly, 2000; Uchoi et al., 2015). The nutritional requirement and feeding cost are closely associated to each other and have a great impact on the efficiency of the production systems and profitability index (Shipton and Hecht, 2013; Guy et al., 2018). The evaluation of protein requirements by taking into consideration both biological and economic responses such as, the protein levels, feed price, feeding cost, and final profit (weight gain or final weight) is extremely important for the profitable and sustainable aquaculture production. Such exercises are necessary to be done in order to develop size specific balanced formulations for different fish species (FAO, 2013; Shipton and Hecht, 2013; Guy et al., 2018). To our knowledge, still no dietary protein requirement is available for adult pacu in the existing literature. Feed industries are producing diets with the recommendations of protein for juvenile pacu which are negatively affecting the fish growth and profit of aquaculture production systems. Thus, the present study was designed with the aim to determine the optimum biological and economic balanced digestible protein (BDP) requirements of adult pacu by taking into consideration both biological and cost-benefit analyses data.

2. Materials and methods

2.1. Ethical guidelines, fish rearing system and experimental conditions

The ethical guidelines of the Brazilian College of Animal Experimentation were adapted in the designing of the present trial and the the present materials and methods were approved by the Ethics Committee on Animal Use of the Faculty of Agricultural and Veterinary Sciences, São Paulo State University (UNESP), Jaboticabal, Brazil via protocol No. 009999/14.

The adult pacu were obtained from the Tuiuiu fish farm located at the Guaira City, SP, Brazil, and transferred to the Aquaculture Center, UNESP, Jaboticabal, Brazil. All the fish were subjected to the acclimation period of 7 days in order to adapt them to the laboratory conditions. During the acclimation period, a commercial feed was offered to fish once a day. Before starting the trial, some fish were clinically analyzed to check if they were healthy. After clinical examination, a total of 180 uniform size and healthy fish with an average initial live body weight of 1158.50 ± 21.59 g were distributed among 18-concrete tanks of 2,000-L each. A total of 10 fish were stocked per tank/ replicate and during the 44-day feeding trial, fish were fed three times a day at 09:00, 12:00 and 17:00 h until apparent satiation.

A complete randomized design consisted of six treatments and three replicates each, was used for the present feeding trial. All the experimental tanks were connected to a closed-recirculation aquaculture system (RAS) which was provided with the biological and mechanical filter and a heat exchange system. The biological and mechanical filter was receiving the freshwater from the experimental tanks in order to filter and the heat exchange system helped in maintaining a constant water temperature. The experimental tanks were supplied about the equal concentration of aeriation from an electric air pump through small plastic oxygen supplying pipes which were provided with air stones. The aeriation was regularly checked and regulated by the regulators being fixed to the small oxygen supplying plastic pipes.

2.2. Feed preparation

Before the formulation of diets, the required feed ingredients were analyzed according to the methods of AOAC (2016) in order to confirm the dry matter, crude protein, total fat, ash and

total amino acid content. Then two basal diets, one with high-protein diet and the other with low-protein diet, were formulated according to the diet dilution concept developed by Fisher and Morris (1970) (Table 1). The basic nutritional requirements that have been recommended in previous literature for the species under study were maintained in the basal diets (Fernandes et al., 2000, 2001; Abimorad and Carneiro, 2004; Abimorad et al., 2007; Abimorad et al., 2010; Bicudo et al., 2010; Khan et al., 2019a).

The basal diets were kept isoenergetic and provided with the same level of digestible energy i.e., 15 MJ kg^{-1}. This digestible energy was an average of the the previous recommendations for the species under study (Carneiro, 1983; Abimorad and Carneiro, 2004; Abimorad et al., 2007, 2010; Bicudo et al., 2010). The high protein diet was diluted with the low protein diet in proper proportions and six experimental diets with different BDP levels such as 163, 201, 238, 272, 315, and 348 g BDP kg^{-1}, respectively were prepared (Table 2). The digestible values of the ingredients being used in the present formulations were calculated on the basis of the apparent digestibility coefficients determined by Abimorad et al. (2008), Fabregat et al. (2008), and Gonçalves and Cyrino (2014) for the species under study.

The dietary protein used in the present study was defined as balanced digestible protein. According to the concept of balanced protein, the ratios among lysine content and the minimum contents of all other essential amino acids were calculated in relation to lysine. Lysine was used as the reference amino acid and the ratios were kept constant according to an ideal protein profile (Mack et al., 1999). The content of lysine was kept at a constant and optimum proportion of digestible protein (Eits et al., 2005a). The essential amino acid balance was achieved by protein rich ingredients and because some plant ingredients were used, therefore a very small amount of the most limiting amino acids such as lysine, methionine and threonine were added synthetically as well.

Table 1

Formulation of the basal (low-protein and high-protein) diets (g kg^{-1}).

Ingredients	Price (R$ kg^{-1})	Basal diets	
		Low protein-128.3	High protein-366
Fish meal	5.20	47.10	176.40
Corn gluten	5.50	11.30	37.24
Soybean meal	1.84	124.30	441.00
Broken rice	1.15	162.66	0.00
Corn	0.80	151.50	258.72
Corn starch	1.35	255.00	0.00
Rice husk	0.40	75.00	0.00
Soybean oil	2.90	100.00	41.39
L-lysine (99%)	17.30	3.70	10.88
DL-methionine	23.00	0.44	1.86
L-threonine (98.5%)	50.00	0.50	1.47
Dicalcium phosphate	1.65	36.00	17.15
Limestone	0.50	24.00	5.39
Antifungal	23.80	3.00	3.00
Antioxidant BHT	18.30	0.50	0.50
Mineral and vitamin premix[1]	36.00	5.00	5.00

[1]Vitamin A 860.000 UI kg^{-1}, Vitamin D3 240.000 UI kg^{-1}, Vitamin E 10.500 UI kg^{-1}, Vitamin K3 1.400 mg kg^{-1}, Vitamin B1 2.100 mg kg^{-1}, Vitamin B2 2.150 mg kg^{-1}, Vitamin B6 2.100 mg kg^{-1}, Vitamin B12 2.200 mg kg^{-1}, Vitamin C 25,000 mg kg^{-1}, Niacin 10,000 mg kg^{-1}, calcium pantothenate 5.600 mg kg^{-1}, folic acid 580 mg kg^{-1}, biotin 17 mg kg^{-1}, choline chloride 60,000 mg kg^{-1}, inositol 3.750 mg kg^{-1}, copper 1.800 mg kg^{-1}, manganese 5.000 mg kg^{-1}, zinc 8.000 mg kg^{-1}, iodine 90 mg kg^{-1}, selenium 36 mg kg^{-1}, and cobalt 55 mg kg^{-1}.

2.3. Diet extrusion

The ingredients were separately weighed for the two basal diets, properly mixed in a dual mixer of 100 kg capacity and ground in a hammer mill (Model: MCS 280, Moinhos Viera, Tatuí, Brasil). Then proper amount of water and oil was added to each diet and passed through a

strainer. Each diet was again properly mixed in a Y-shaped mixer and extruded into pellets of about 4 mm size by a single screw extruder (Ex Micro Model, Exteec, Ribeirão Preto, Brasil). The extruded pellets were dried in a Forced Air Circulation-oven at 55 °C for 24 h. The dried pellets of each diet were saved in separate plastic bags and kept in a cold chamber at -12 °C until use.

Table 2

Experimental diets and the analyzed chemical composition of the basal and experimental diets.

	LP	HP	163	201	238	272	315	348
Dilution of the two basal diets to achieve six consecutive experimental diets (g kg^{-1})								
LP diet			806.68	647.36	488.03	328.71	169.39	10.06
HP diet			193.32	352.64	511.97	671.29	830.61	989.94
Total cost (R$ kg^{-1})	1.94	2.86	2.12	2.26	2.41	2.56	2.7	2.85
Analyzed proximate chemical composition (dry matter basis g kg^{-1}) of the basal and experimental diets.								
Dry matter	935.29	928.92	954.23	950.56	951.16	955.31	946.18	946.67
Crude protein	147.00	409.80	185.00	227.10	268.00	305.60	353.20	389.70
Digestible protein	128.30	366.00	163.00	201.00	238.00	272.00	315.00	348.00
Total fat	126.80	90.10	117.80	112.40	106.00	99.60	94.50	88.60
Digestible fat	106.40	73.10	98.30	93.40	87.60	81.90	77.20	71.90
Ash	91.50	72.90	85.80	82.80	80.10	77.00	74.60	71.70
Crude energy (MJ kg^{-1})	17.56	19.25	17.38	17.71	17.95	18.13	18.57	18.82
Digestible energy (MJ kg^{-1})	15.06	15.04	14.63	14.68	14.67	14.60	14.73	14.72
DP: DE ratio (g MJ^{-1})	-	-	11.14	13.69	16.22	18.63	21.38	23.64

LP = Low protein diet; HP = High protein diet.

2.4. Diet analysis

The basal and experimental diets were analyzed according to the standard methods of AOAC (2016) to determine the dry matter, crude protein, total lipid and ash content and gross energy (Table 2). The amino acid composition was only analyzed in the basal diets in order to

confirm the provided protein-bound amino acid balance (Table 3). Amino acid analyses were done at the Evonik industries AG, Essen, North Rhine-Westphalia, Germany.

Table 3

Amino acid composition (dry matter basis) of the basal diets (g kg^{-1}).

Essential amino acids (EAAs)	LP-total EAAs	LP-dig. EAAs	HP-total EAAs	HP-dig. EAAs
Methionine	2.8	2.5	8.5	7.8
Lysine	9.9	9.1	30.2	28
Threonine	5.6	4.8	16.6	14.7
Arginine	8.8	8.4	25.5	24.3
Isoleucine	5.6	4.8	16	14.2
Leucine	9.7	8.8	29.9	27.8
Valine	6.2	5.2	17.4	15.3
Histidine	3	2.8	8.8	8.2
Phenylalanine	6.3	5.8	18	16.8
Tryptophan	1.5	1.3	3.9	3.5
Non-essential amino acids	LP-total NEAAs		HP-total NEAAs	
Cysteine	2.3		5.3	
Glycine	8.6		23.4	
Serine	7		18.3	
Proline	9.4		24.1	
Alanine	8.5		21.6	
Aspartic acid	14.3		38	
Glutamate	25		63.5	
Methionine + Cysteine	5.6		13.8	

LP = Low protein diet; HP = High protein diet; dig. = Digestible; NEAAs = Non essential ami no acids

2.5. Analysis of water quality indices

Water quality parameters were measured regularly during the entire feeding trial and observed at appropriate levels for the species under study. Average water temperature, pH and

dissolved oxygen was noted daily and observed to be 28.6 ± 0.5 °C, 5.74 ± 0.10 mg L^{-1} and 7.3 ± 0.9 mg L^{-1}, respectively. The total ammonia was measured once a week in the water samples being collected from the inlet and outlet of each experimental tank and observed average value of 0.14 ± 0.4 mg L^{-1}. Once a week all the experimental tanks were regularly siphoned in order to remove the feces and maintain the water quality in optimum condition.

2.6. Sample collection

Fish were subjected to a 24 h fasting period and then properly anesthetized with a benzocaine solution (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) of 60 mg L^{-1} and weighed individually at the begining of the experiment. A group of 10 fish of the initial fish population were anesthetized with an overdose of benzocaine, 500 mg L^{-1} as reported by Bicudo et al. (2010) for pacu and sampled for the analysis of initial whole body chemical composition. At the end of the experiment, fish were again passed through a 24 h fasting period and properly anesthetized with a slight dose of benzocaine solution 60 mg L^{-1} and weighed individually. Then a total of 3 and 4 fish anesthetized with an overdose of benzocaine (500 mg L^{-1}) were sampled for the analysis of the final whole body chemical composition and obtaining cut-up portions, respectively.

The fish sampled for the analysis of the whole body composition were immediately subjected to the subsequent analysis process. While the four fish being sampled for obtaining the cut-up portions were processed by a well-trained operator to separate different parts of fish such as scales, fins, visceral content and head. The method of Klein et al. (2014) was adapted to process the fish and the processing was done by a single operator to minimize the variability in final weights among fish.

2.7. Whole body chemical composition analysis

The standard methods of AOAC (2016) were adapted to determine the whole body chemical composition of the initial and final whole body fish including moisture, crude protein, total lipid and ash content.

2.8. Evaluation of biological and economic responses

At the end of the trial, the growth and feeding parameters such as body weight gain (BWG), specific growth rate (SGR %), feed intake (FI), protein intake (PI), feed conversion ratio (FCR), feed efficiency ratio (FER), protein efficiency ratio (PER), feed price, feeding cost and revenue were calculated according to the following formulas;

Body weight gain g (BWG) = final body weight−Initial body weight

Feed intake g (FI) = total amount of feed consumed per fish

Feed conversion ratio g (FCR) = total dry feed intake ÷ wet weight gain

Feed efficiency ratio (FER) = wet weight gain ÷ total dry feed intake

Specific growth rate (SGR %) = [(ln final body weight - ln initial body weight) ÷ days] × 100

Protein efficiency ratio g (PER) = wet weight gain ÷ protein intake

At the end of the trial, weights of the following three main products were obtained which then used as inputs in the economic model.

Whole body fish = [Skin + Scales + fins + head + visceral content + main body trunk]

Sliced fish = [Whole fish – scales – fins – head – visceral content] and

Eviscerated fish = [Whole body fish – visceral content]

Feed price (R$ g^{-1} diet) = b * BDP + a ÷ 1000, where DBDP is the dietary balanced digestible protein levels and “a” and “b” are the parameters of the linear equation obtained for the feed price.

Feeding costs (R$ g^{-1} $fish^{-1}$) = Feed price * feed intake (g)

Revenue (R$ g^{-1} $fish^{-1}$) = Final whole body or cut-up portion (including the processing price) weight (g) * whole body and cut-up portion price + processing costs (R$ g^{-1} $fish^{-1}$)

Gross margin (R$ g^{-1} $fish^{-1}$) = Revenue (R$ g^{-1} $fish^{-1}$) - feeding costs (R$ g^{-1} $fish^{-1}$)

2.9. Modeling optimum biological balanced digestible protein

Quadratic regression equation $y = a + bx + cx^2$ was used to determine the optimum biological protein levels of adult pacu. In the equation, x is the protein level, y is the response variable, a is the curve intercept, b and c are representing the slopes, and $\{x = -b \div 2c\}$ gives the maximum protein requirement.

2.10. Modeling optimum economic balanced digestible protein

Economic analyses were done using the following mathematical model developed by Etis et al. (2005b);

Gross margin (GM) = Revenue – Feeding cost

The inputs provided in the model consisted of dietary balanced digestible protein levels (g kg^{-1}); feed intake (g); feed price including the feed processing cost (R$ g^{-1} diet); selling price (R$ g^{-1} $fish^{-1}$) of the whole body, eviscerated and sliced fish; and final weight of the whole body, eviscerated and sliced fish (including the processing cost per gram of eviscerated and sliced adult pacu). The model produced the outputs consisted of feeding costs (R$ g^{-1} $fish^{-1}$), revenue (R$ g^{-1} $fish^{-1}$) and gross margin (R$ g^{-1} $fish^{-1}$).

2.11. Algebraic description of the economic model

The gross margin (GM) can be algebraically expressed as follows;

GM = (a × (d)) – (c° × b) where,

d = Final weight of the whole fish or cut-up portion (g), a = coefficient obtained for the selling price of the whole fish and cut-up portion + processing costs (R$ g^{-1} $fish^{-1}$), c° = feed price + feed processing cost (R$ g^{-1} diet), and b = feed intake (g)

Thus, the functions in the above equation can be elaborated as follow;

$d = c_0 + c_1x + c_2x^2$, estimates of the quadratic regression obtained for final weight of whole body and cut-up portion (including the processing costs) using exponential data.

$c° = y_0 + y_1x$, estimates of the linear equation obtained for feed price + feed processing cost

$b = f_0 + f_1x + f_2x^2$, estimates of the quadratic regression obtained for feed intake using exponential data

Or if we expand the equation as;

$GM = (ac_0 + ac_1x + ac_2x^2) – (y_0f_0 + y_0f_1x + y_0f_2x^2 + y_1f_0x \times y_1f_1x^2 + y_1f_2x^3)$

Where, a, c_0, c_1, c_2, y_0, y_1, f_0, f_1, and f_2 are constants while X is unknown

GM is maximized when $\frac{\partial GM}{\partial X} = 0$ or we also can express as;

$\frac{\partial GM}{\partial X} = (ac_1x + 2\ ac_2) – (y_0f_1 + 2\ y_0f_2x + y_1f_0x + 2\ y_1f_1x + 3\ y_1f_2x^2)$

$= (ac_1 - y_0f_1x - y_1f_0) + (2\ ac_2 - 2\ y_0f_2 - 2\ y_1f_1)\ x – (3\ y_1f_2)\ x^2$

Let $k_a = -3\ y_1f_2$

$K_b = 2\ ac_2 - 2\ y_0f_2 - 2\ y_1f_1$

$K_c = ac_1 - y_0f_1 - y_1f_0$

Then the GM is maximized when X takes the value of

$$x^\circ = \frac{-k_b \pm \sqrt{{k_b}^2 - \mathrm{k}ac}}{2k_a}$$

The dietary protein level where maximum GM (profit) was achieved considered being the optimum economic protein requirement.

2.12. Statistical analysis

The growth performance, feeding parameters and whole body composition data were submitted to the SPSS for Windows, version 16.0. Chicago, SPSS Inc., (2007) software. One-way Analysis of Variance-ANOVA was used to evaluate the significant differences. When a significant ($P < 0.05$) difference was perceived, LSD (Tukey's Least Significant Test) was applied to compare the averages.

Growth response in terms of average body weight gain, final weight, and feed intake data against dietary protein levels were fitted to the quadratic regression model $y = \alpha + \beta x + cx^2$ using the SAS Institute Inc. 2014 software.

3. Results

3.1. Growth and feeding aspects

No mortality was observed and the fish well accepted the experimental diets. The body weight gain (BWG), specific growth rate (SGR %), feed intake (FI), and feed efficiency ratio (FER) were significantly increased ($P < 0.05$) with diets up to the level of 272 g BDP kg^{-1} and thereafter significantly decreased. Accordingly, the feed conversion ratio (FCR) significantly decreased up to the level of 272 g BDP kg^{-1} diet and beyond significantly increased. The protein efficiency ratio (PER) remained statistically same up to the level of 238 g BDP kg^{-1} and beyond significantly decreased. The performance data are shown in table 4.

Table 4

Growth and feeding responses of adult pacu to different balanced digestible protein (g kg^{-1}) levels.

BDP	BWG (g)	FI (g)	FCR	FER	SGR (%)	PER (g)
163	122.3 ± 9.15^{c}	302.40 ± 1.45^{c}	2.48 ± 0.19^{cd}	0.40 ± 0.03^{c}	0.22 ± 0.02^{c}	2.48 ± 0.19^{a}
201	146.7 ± 8.13^{b}	306.30 ± 1.65^{b}	2.09 ± 0.11^{bc}	0.48 ± 0.02^{b}	0.27 ± 0.02^{b}	2.38 ± 0.12^{ab}
238	164.2 ± 5.64^{a}	311.72 ± 1.88^{a}	1.90 ± 0.06^{a}	0.53 ± 0.02^{a}	0.30 ± 0.01^{ba}	2.21 ± 0.07^{b}
272	166.9 ± 8.17^{a}	311.98 ± 1.87^{a}	1.87 ± 0.08^{a}	0.53 ± 0.02^{a}	0.31 ± 0.02^{a}	1.97 ± 0.08^{c}
315	129.3 ± 10.16^{c}	296.63 ± 1.65^{d}	2.30 ± 0.18^{c}	$0.44 \pm 0.03b^{c}$	0.24 ± 0.02^{c}	1.38 ± 0.10^{d}
348	95.2 ± 9.38^{d}	294.17 ± 1.40^{d}	3.11 ± 0.30^{e}	0.32 ± 0.03^{d}	0.18 ± 0.02^{d}	0.93 ± 0.09^{e}
P-value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

BDP = Balanced digestible protein; BWG = Body weight gain; FCR = Feed conversion ratio; FER; Feed efficiency ratio; SGR = Specific growth rate; PER = Protein efficiency ratio

Values (mean of three replicates per treatment ± SD) with different letters in the same column indicate statistical differences at the significance level of $P < 0.05$.

The weight of the initial and final whole body and that of the cut-up portions of adult pacu is shown in Table 5. The weights of the final whole body and cut-up portions were increased in response to dietary protein.

Table 5

Initial and final average weight of whole body, eviscerated and sliced adult pacu (g).

	Initial whole fish	Final whole fish	Eviscerated fish	Sliced fish
Initial weight			1046 ± 117.18	805.13 ± 97.15
163	1178.2 ± 28.0	1300.4 ± 21.1^{a}	1087 ± 63.5^{b}	836 ± 49.2^{b}
201	1165.3 ± 18.6	1312.0 ± 16.3^{a}	1147 ± 6.9^{ab}	833 ± 9.8^{ab}
238	1161.1 ± 20.0	1325.3 ± 18.2^{a}	1159 ± 69.0^{ab}	891 ± 54.0^{ab}
272	1139.8 ± 21.1	1306.6 ± 21.0^{a}	1210 ± 33.5^{a}	941 ± 41.5^{a}
315	1169.1 ± 28.0	1298.4 ± 19.5^{a}	1143 ± 16.6^{ab}	885 ± 26.3^{ab}
348	1150.8 ± 7.7	1246.0 ± 16.9^{b}	1080 ± 54.7^{b}	824 ± 42.4^{b}
P- Value	> 0.05	0.0045	0.0451	0.0403

Data are represented as mean ± SD (n=3), means in the same column with different superscript letters are significantly different (P < 0.05).

3.2. Proximate chemical composition

The proximate chemical composition of adult pacu fed different balanced digestible protein (g kg^{-1}) levels is presented in Table 6. Crude protein was significantly ($P < 0.05$) increased while total lipid content significantly ($P < 0.05$) reduced in response to the increased dietary protein content. Moisture and ash content was slightly influenced by the dietary protein.

Table 6

Body composition (wet weight basis g kg^{-1}) of adult pacu fed different BDP levels.

BDP (g kg^{-1})	Moisture	Crude protein	Total lipid	Ash
Initial	580	165	201.9	46.8
163	560.03 ± 15.47^{b}	147.83 ± 1.29^{c}	225.90 ± 3.62^{a}	55.97 ± 1.03^{a}
201	574.53 ± 20.66^{ab}	151.00 ± 2.17^{b}	211.97 ± 4.85^{b}	50.87 ± 4.67^{ab}
238	585.13 ± 3.84^{a}	153.13 ± 0.32^{b}	203.30 ± 1.95^{c}	46.43 ± 4.71^{b}
272	584.50 ± 6.07^{a}	156.23 ± 0.72^{a}	198.03 ± 5.20^{bc}	46.77 ± 2.80^{b}
315	573.03 ± 8.85^{ab}	157.17 ± 1.36^{a}	190.07 ± 0.90^{bc}	51.03 ± 1.70^{ab}
348	579.33 ± 6.61^{ab}	158.17 ± 0.35^{a}	182.77 ± 1.89^{c}	50.90 ± 1.81^{ab}
P-value	0.1764	0.0000	0.0000	0.0294

BDP = Balanced digestible protein.

Data are represented as mean ± SD (n=3), means (of three replicates) in the same column with different superscript letters are significantly different ($P < 0.05$) by Tukey Least significant test.

3.3. Optimum biological digestible protein requirement

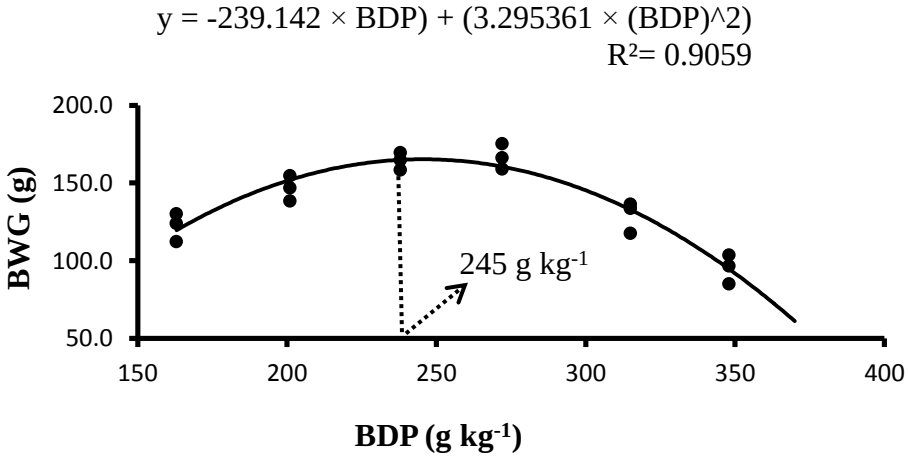

Fig. 1. Optimum biological balanced digestible protein (BDP g kg^{-1}) requirement of adult pacu using the body weight gain and dietary protein levels through the quadratic equation.

The quadratic model estimated the optimum biological balanced digestible protein requirement of 245 g kg^{-1} (dry matter) and the optimum DP: DE ratio was calculated to be 16.78 g MJ^{-1}. The estimated optimum biological digestible protein requirement of adult pacu is shown in Figure 1.

3.4. Economic analyses (inputs and outputs evaluations)

The equations obtained for different inputs such as feed price (FP), feed intake (FI) and final body weight (FBW) are shown in Figure 2, 3, 4, respectively. The current selling price of whole body, eviscerated and sliced adult pacu was obtained from a local fish farmer. The current selling prices of the feed ingredients were obtained from Agromix, Jaboticabal, SP, Brazil.

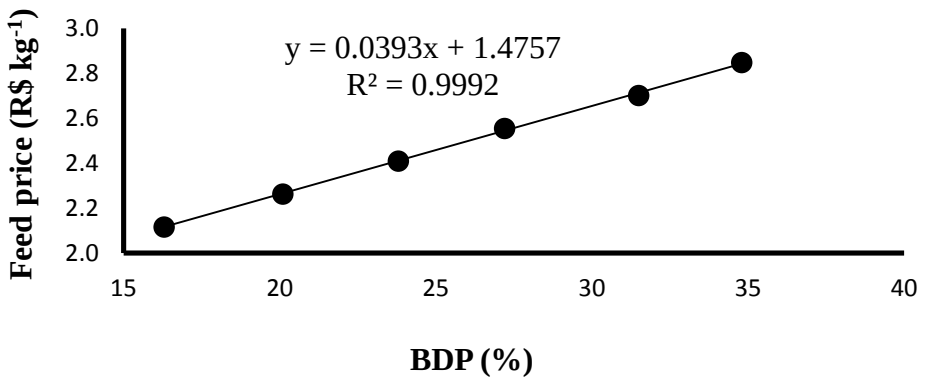

Fig.2. Price of experimental diets (feed price) supplemented with different BDP (g kg^{-1}) levels.

The weights of the eviscerated and sliced fish along with the balanced digestible protein levels were calculated in natural logarithm (ln) and the following linear equations were obtained as inputs.

Eviscerated adult pacu: [y = (0.9155x + 0.4848) R^2 = 0.9865]

Sliced adult pacu: [y = (1.0066x – 0.4277) R^2 = 95.65]

The above linear equations were provided as inputs to the economic model in order to calculate the optimum economic protein for eviscerated and sliced adult pacu.

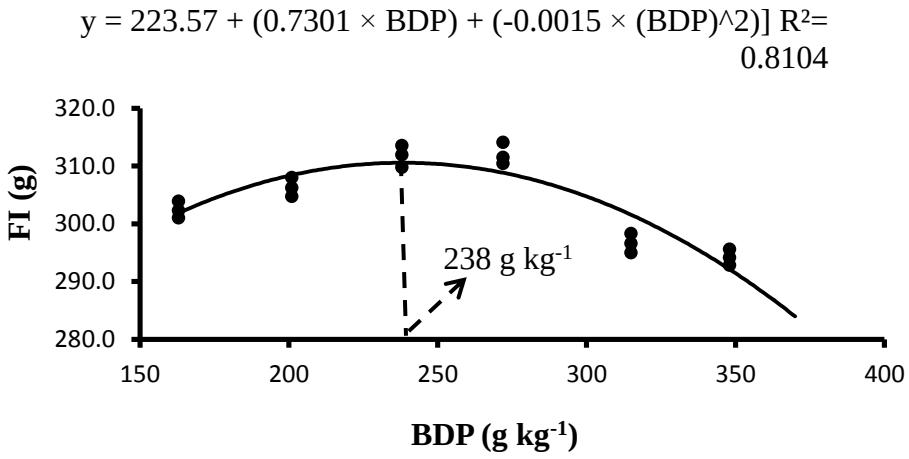

Fig. 3. Observed feed intake (FI) in response to different balanced digestible protein (BDP g kg^{-1}) levels through the quadratic model.

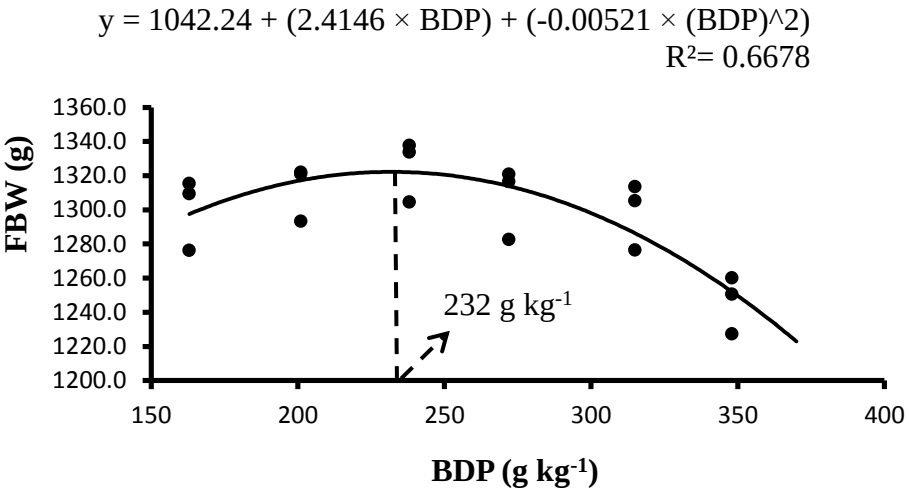

Fig. 4. Maximum final body weight in response to different balanced digestible protein (BDP) levels calculated according to the quadratic model.

3.5. Optimum economic balanced digestible protein requirement

The level of dietary protein where maximum gross margin occurred was considered as the optimum economic dietary balanced protein requirement of the adult pacu that will be sold as whole body (Fig. 5), eviscerated (Fig. 6) and sliced fish (Fig. 7), respectively.

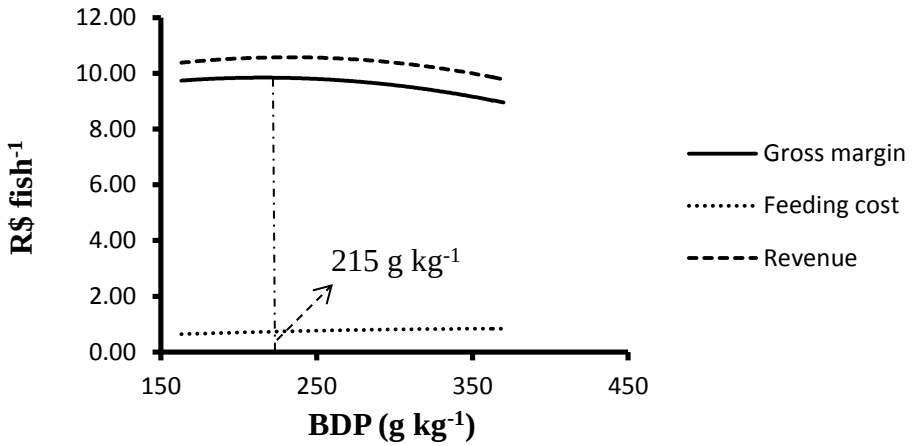

Fig. 5. Effect of balanced digestible protein levels (BDP g kg^{-1}) on the maximum gross margin (GM R$ $fish^{-1}$) and optimum economic dietary protein requirement of whole body adult pacu.

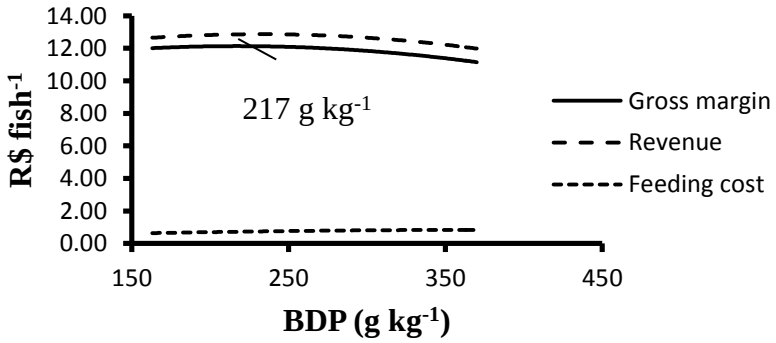

Fig. 6. Effect of different balanced digestible protein (BDP g kg^{-1}) levels on feeding cost (FC), revenue and gross margin (GM) in eviscerated adult pacu.

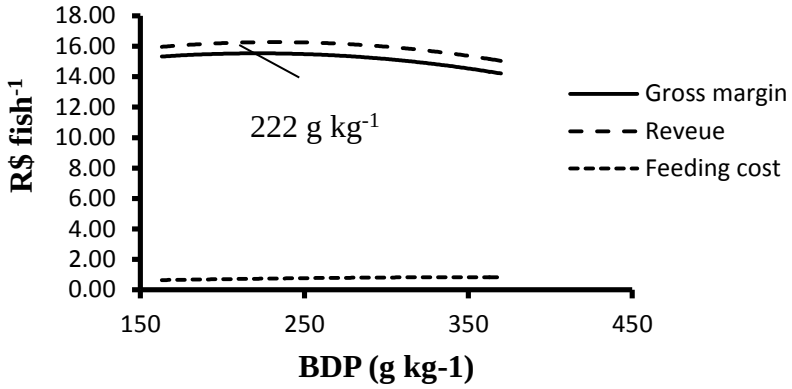

Fig. 7. Effect of different balanced digestible protein (BDP g kg^{-1}) levels on feeding cost (FC), revenue and gross margin (GM) in sliced pacu.

According to the applied economic model, the level of BDP (215 g kg^{-1}) where maximum gross margin (R$ g^{-1} $fish^{-1}$) achieved was considered to be the optimum economic level for pacu that will be sold as a whole fish. The optimum economic level of 217 g BDP kg^{-1} was determined for adult pacu that will be sold as an eviscerated fish and for adult pacu that will be sold as a sliced fish, the optimum economic level was estimated to be 222 g BDP kg^{-1}. The optimum biological level was estimated to be 245 g BDP kg^{-1} (Fig. 1) which was considerably higher than the economic levels. The optimum economic balanced digestible protein to energy (DP: DE) ratios were calculated to be 14.65, 14.79, and 15.13 g MJ^{-1} for the whole body, eviscerated and sliced pacu, respectively.

3.6. Hypothesized scenarios

Some exercises were done to check the effect of fluctuation in the selling price of the fish meal and soybean meal and whole body, eviscerated and sliced adult pacu on the feeding costs and gross margin (profitability index) and optimum economic level (Table 7, 8 and 9, respectively).

Table 7

Balanced digestible protein resulting in maximum gross margin under different scenarios.

Scenarios		[1]BDP	Feed intake	Feeding cost	Revenue	Gross margin
Biological level	ND	245	310.5	0.758	10.571	9.813
Economic level	ND	215	309.7	0.719	10.566	9.846
Whole fish price	(-10)	213	309.6	0.717	9.507	8.790
Whole fish price	(+10)	217	309.9	0.722	11.625	10.903
Fish meal	(-10)	217	309.9	0.706	10.568	9.863
Fish meal	(+10)	214	309.7	0.735	10.564	9.830
Soybean meal	(-10)	215	309.7	0.721	10.566	9.845
Soybean meal	(+10)	213	309.6	0.747	10.563	9.815

ND = Normal feed price (Table 1); [1]Balanced digestible protein (g kg^{-1}); Feed intake (g); Feeding cost (R$ g^{-1} $fish^{1}$); Feeding cost, revenue and gross margin (R$ g^{-1} $fish^{-1}$).

Table 8

Balanced digestible protein resulting in maximum gross margin under different scenarios.

Scenarios		BDP	FI	FC	Rev	GM
EL	ND	217	309.866	0.722	13.032	12.310
EvisWFP	(-10)	216	309.799	0.721	11.728	11.007
EvisWFP	(+10)	219	309.991	0.725	1169.064	14.339
FM	(-10)	218	309.930	0.707	12.858	12.152
FM	(+10)	216	309.799	0.737	13.031	12.294
SM	(-10)	217	309.866	0.724	13.032	12.308
SM	(+10)	215	309.730	0.751	13.029	12.279

BDP (g kg^{-1}) = Balanced digestible protein; FI = Feed intake (g); FC = Feeding cost (R$ g^{-1} $fish^{-1}$); Rev = Revenue (R$ g^{-1} $fish^{-1}$); GM = Gross margin(R$ g^{-1} $fish^{-1}$); EL = Economic level; ND = Normal diet price Table 1 (R$ g^{-1} diet); EvisWFP = Eviscerated whole fish (body adult pacu) selling price; FM = Fish meal; SM = Soybean meal.

Table 9

Balanced digestible protein resulting in maximum gross margin under different scenarios.

Scenarios		[1]BDP	Feed intake	Feeding cost	Revenue	Gross margin
Economic level	ND	222	310.154	0.729	17.169	16.440
Sliced whole fish price	(-10)	221	310.103	0.728	15.451	14.723
Sliced whole fish price	(+10)	223	310.203	0.730	18.887	18.157
Fish meal	(-10)	222	310.154	0.712	16.265	15.553
Fish meal	(+10)	223	310.203	0.748	22.219	21.472
Soybean meal	(-10)	222	310.154	0.731	17.169	16.438
Soybean meal	(+10)	221	310.103	0.760	17.167	16.408

[1]Balanced digestible protein (g kg^{-1}); Feed intake (g); Feeding cost (R$ g^{-1} $fish^{-1}$); Revenue (R$ g^{-1} $fish^{-1}$); Gross margin (R$ g^{-1} $fish^{-1}$); ND = Normal feed price Table 1 (R$ g^{-1} diet).

The data in the Table 7, 8 and 9, respectively indicate that optimum economic protein levels was closely associated with the change in the selling price of fish meal and soybean meal and whole body, eviscerated and sliced adult pacu.

4. Discussion

Shearer (2000) re-evaluated the data of several published research papers via different models and concluded that majority of the published papers had used the broken-line model which truly underestimated the nutrient requirements. The author concluded that requirements achieved in the re-evaluation were higher than those reported in the published papers. According to the author the principle reason might be the use of inappropriate statistical model. In the species under study broken line model has been previously used by Bicudo et al. (2010) and their recommendation seems low particularly for the juvenile pacu in captive conditions. In the case of cost-benefit analyses where the objective should be to get maximum profit at relatively low protein level, using broken line could underestimate the protein requirements which may negatively affect the healthy growth of fish (Zeitoun et al., 1976). Unlike the quadratic regression model, broken line is low efficient in providing a good empirical fit to the growth response of an animal which does not show a sudden change i.e., that grow slowly (Zeitoun et al., 1976). We have exercised both the broken line and quadratic models and both best fitted to our data but quadratic model was selected for the estimation of protein requirement due to the characteristics of our data and the objectives of the present study.

According to previous literature the protein requirement of omnivorous species including the species under study may be ranging from 20 to 40 percent (Caseras et al., 2000; Fernandes et al., 2000, 2001; Carmo de Sá and Fracalossi, 2002; Lundstedt et al., 2004; Melo et al., 2006; Abimorad et al., 2007; Bicudo et al., 2010; Signor et al., 2010). However, the previous requirements are mostly for juvenile fish and have been estimated on the basis of maximum fish performance data rather than cost-benefit analyses. Also, no appropriate models are available to consider together the biological and economic responses and determine optimum economic protein levels to achieve maximum profit. The feed industries are usually using the same

requirements in the development of diets for all growth phases of a species. Such diets not only result in increased feeding costs but also cause reduced growth, lower profit and higher nitrogen excretion (Lee et al. 2003; Abboudi et al., 2006; Arnason et al., 2009; NRC, 2011; Ye et al., 2017; Khan et al., 2019b). The present study is the first attempt regarding the protein requirement of adult pacu. According to literature, protein requirements usually decline with increase in the fish age (NRC, 2011; Ye et al., 2017). In the present study, by quadratic regression model, the optimum biological balanced digestible protein requirement of 245 g kg^{-1} was estimated for adult pacu which was found lower than those being observed in our previous two studies with juvenile (326 g kg^{-1}) and pre-adult pacu (292 g kg^{-1}). These findings showed that protein requirement of pacu decline as it increases in size and thus provide important information about pacu nutrition. The present optimum biological balanced digestible protein requirement was found low as compared to the optimum protein requirement being estimated in our previous studies with juvenile and pre-adult pacu. Besides evaluating the optimum biological digestible protein requirement for adult pacu, optimum economic balanced digestible protein requirement was also calculated to know if any difference exists between these two levels.

In accordance to the results of our previous feeding trials with juvenile and pre-adult pacu, in the present study balanced digestible protein supplementation considerably improved the growth performance of adult pacu. The body weight gain (BWG), feed intake (FI), feed efficiency ratio (FER), specific growth rate (SGR %) were significantly ($P < 0.05$) increased and feed conversion ratio (FCR) was significantly decreased in response to the diets containing 272 and 238 g BDP kg^{-1}, respectively. The present results show that pacu achieved maximum growth via enhanced feed intake in response to the diets with 272 and 238 g BDP kg^{-1}. Similar results have been obtained by Meer et al. (1995) in *Clossoma macropomum* (Cuvier), Bicudo et al., 2010; Signor et al. (2010) and Klein et al. (2014) in pacu. According to Sa et al. (2014) and Ma et al., 2019) balanced diets considerably improve the growth performance and feeding aspects of fish. In the present study, protein efficiency ratio (PER) in fish fed different protein diets up to the optimum level was found statistically same at the low dietary protein levels and significantly reduced at the higher dietary protein levels. The same results for PER have been reported by Ma et al. (2019) in sub-adult triploid rainbow trout, Kim et al. (2005) in Olive flounder *Paralichthys olivaceus,* and Bicudo et al. (2010) in pacu (*Piaractus mesopotamicus*). This shows that beyond optimum requirements, the excess protein may be metabolized as an energy source or increase

the nitrogen excretion in water and thus leading to the described declines in the protein efficiency ratio in fish (Wilson, 2002; Kim et al., 2003). Bai et al. (1999) and Kim et al. (2002, 2005) reported similar results in other fish species. Bicudo et al (2010) stated that usually protein retention efficiency remain high at low protein intake and decline at levels beyond the optimum requirement. According to Dabrowski (1979), PER varies from species to species and depend on several factors including diet composition and fish size and according to De Silva and Anderson (1995), Bicudo et al. (2010) and Kim et al., (2016), PER has the limitation of considering lipid deposition as protein retention and also it does not provide information about the dietary protein being used for maintenance of fish.

In accordance to our previous experiments conducted with juvenile and pre-adult pacu, the optimum economic balanced protein requirement estimated for adult pacu was found lower than the optimum biological protein level. The optimum economic balanced digestible level was estimated to be 215, 217, and 222 g kg^{-1} with the optimum DP: DE ratios of 14.65, 14.79, and 15.13 g MJ^{-1} for adult pacu that will be sold as whole body, eviscerated and sliced fish, respectively. The present results also showed that DP: DE ratio may change according to the biological and economic dietary protein requirements. The future diets of adult pacu supplemented with the present economic dietary protein levels may increase the profitability index at low feeding costs. The present economic levels may be a good option in the case of rearing adult pacu for commercial purposes, however, in studies using adult pacu as a biological model or when the selling price of the protein rich ingredients are lower than the current prices, then the present biological level could be used to get better performance and maximum profit.

Dietary protein supplentation significantly affected the body chemical composition of adult pacu. The body crude protein content was significantly increased while the lipid content significantly reduced in response to the dietary protein levels. The whole body moisture content was statistically same in all the treatments but slightly increased with increasing the dietary protein content. The whole body ash content was significantly influenced by the dietary protein and reduced with increasing the level of digestible protein. Abimorad et al. (2007), Bicudo et al. (2010) and Klein et al. (2014) have reported that dietary protein significantly improves the body composition in pacu. In concordance to the present results Ye et al. (2017) in juvenile and pre-adult giber carp (*Carassius auratus gibelio* var. CAS III), and Cortes-Jacinto et al. (2003) in

juvenile crayfish (*Cherax quadricarinatus* (Decapoda: Parastacidae) have noticed the same behaviour and these authors documented that body composition was improved with dietary protein. In agreement to the present results, in our previous trials with juvenile and pre-adult pacu, dietary protein significantly improved the body composition of fish. Kim et al. (2004) and Catacutan et al. (2001) reported similar results in other fish species.

Several scenarios were hypothesized by changing the selling price of the whole body, eviscerated and sliced adult pacu and those of the fish meal and soybean meal. Fluctuations in the selling price of fish meal and soybean considerably influenced the optimum economic protein levels. When the selling price of the ingredients was increased by 10 percent, the economic protein level dropped but in the case of decreasing the price by 10 percent, the optimum economic protein levels slightly increased allowing getting extra profit. On the other hand when the selling price of the whole body, eviscerated and sliced pacu was increased by 10 percent, the optimum economic level increased in order to get extra profit while decreasing the selling price by 10 percent, the optimum economic protein level was slightly reduced. These scenarios are describing that how optimum economic protein requirements change in relation to the market prices of the ingredients and whole fish and cut-up parts.

The optimum digestible protein requirement determined in the present trial was lower than the determined levels in our two previous feeding trials with juvenile and pre-adult pacu. The present estimated economic protein level of adult pacu was found lower than the present optimum biological protein level. The biological model estimated the optimum biological balanced digestible protein requirement of 245 g kg^{-1} (DM basis), with the optimum DP: DE ratio of 16.78 g MJ^{-1}. On the other hand for whole-body, eviscerated and sliced adult pacu, the economic model estimated the optimum economic balanced digestible protein requirement of 215, 217, and 222 g kg^{-1} (DM basis), with the optimum DP: DE ratio of 14.65, 14.79, and 15.13 g MJ^{-1}, respectively. These results indicate that the formulation of diets for adult pacu according to the present economic protein levels considerably increases the profitability without any negative impact on growth. Moreover, it is strongly recommended that the economic protein levels should be adjusted to the variations in the actual selling price of protein rich ingredients and whole fish and cut-up parts, accordingly. The present study suggests conducting trials with other fish species using the same experimental design, diet formulation methodology and

biological and economic models in order to help feed industries to formulate nutritionally balanced, economically viable and environment-friendly diets for different fish species in order to further increase the aquaculture production.

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Final considerations

Results of the present study could efficiently contribute to ptimize the production of pacu by the establishment of nutritionally balanced and economically viable diets. It is a common practice in fish nutrition that fish nutritionists favour to add high protein content in the diets for gaining maximum performance. However, in the case of high selling prices of protein rich ingredients, the supplementation of biological levels would further decline the gross margin. The present study showed that using biological protein to achive maximum performance may lead to a considerable decline in the profitability. On the other hand, the supplementation of optimum economic levels may result in higher gross margin and better growth response. The scenarios which were hypothesized in the present study better explained the efficiency of the present economic model. The results showed that optimum economic balanced protein requirements are subjected to the fluctuations in the selling prices of protein-rich ingredients and whole body, eviscerated and sliced pacu. The present study suggests that optimum economic protein levels should be adjusted according to the changes in the selling prices of protein rich ingredients and whole body, eviscerated and sliced fish. Thus, keeping in view the importance of balanced protein approach, future studies should be focused on the evaluation of the optimum biological and economic dietary protein requirements through the present models in different fish species. Such efforts may considerably improve the efficiency of the aquaculture production systems.