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

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

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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

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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.

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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.