UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CENTRO DE AQUICULTURA DA UNESP

PERFIL IDEAL DE AMINOÁCIDOS ESSENCIAIS DIETÉTICOS PARA PACUS ADULTOS

Thaís da Silva Oliveira

Jaboticabal, São Paulo
2020

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Thaís da Silva Oliveira

Orientador: Prof. Dr. João Batista Kochenborger Fernandes

Dissertação apresentada ao Programa de Pós-graduação em Aquicultura do Centro de Aquicultura da UNESP - CAUNESP, como parte dos requisitos para obtenção do título de Mestre.

Jaboticabal, São Paulo
2020

Oliveira, Thaís da Silva
O48p Perfil ideal de aminoácidos essenciais dietéticos para pacus adultos / Thaís da Silva Oliveira. – – Jaboticabal, 2020
vi, 45 p. : il. ; 29 cm

Dissertação (mestrado) - Universidade Estadual Paulista, Centro de Aquicultura, 2020
Orientador: João Batista Kochenborger Fernandes
Banca examinadora: Danilo Cintra Proença, Dariane Beatriz Schoffen Enke
Bibliografia

1. Alimentação. 2. Nutrição pacu. 3. Proteína balanceada. 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

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Centro de Aqüicultura da Unesp - CAUNESP

CERTIFICADO DE APROVAÇÃO

Título: PERFIL IDEAL DE AMINOÁCIDOS ESSENCIAIS DIETÉTICOS PARA PACUS ADULTOS

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AGRADECIMENTOS

Primeiramente à Deus, por me guiar por todo este caminho percorrido, e permitir que fosse possível alcançar meus objetivos até o momento;

Aos meus pais por sempre me apoiarem em qualquer decisão;

Ao meu orientador, Dr. João Batista Kochenborger Fernandes, por me acolher em seu laboratório e grupo de pesquisa, além da oportunidade do desenvolvimento da presente pesquisa e por todos os conhecimentos adquiridos neste período;

À equipe de pesquisa do setor de Avicultura da UNESP,Campus de Jaboticabal, pelos auxílios nas análises dos dados e por todo conhecimento adquirido neste período;

Ao setor de Caprinocultura da UNESP, Campus de Jaboticabal, por disponibilizar o espaço para realizar parte dos processamentos das amostras;

A todos professores que contribuíram para minha formação até este momento, principalmente aos que me orientaram de perto: Dr.ª Dariane B. S. Enke, Dr. Eduardo A. Sanches, Dr. Guilherme W. Bueno e Dr. João B. K. Fernandes.

Aos grandes amigos conquistados no Caunesp, que de alguma forma me auxiliaram no desenvolvimento deste estudo ou pelos conselhos e momentos de distração, Valdecir (Val), Márcio (Perereca), Luiz, Elaine, Suely (em especial aos cafezinhos e chás), Eleuza, Lucia, entre outros que sempre estavam presentes nestes momentos;

Aos meus amigos e parceiros, Rafael Romaneli, Monique, Andressa, Manuel, Kifayat, Marcelo, Jéssica, Laíza, Magdiel, entre outros que tive oportunidade de conhecer neste tempo.

Aos amigos de longa data, que sempre estiveram presentes desde a graduação, principalmente Woshinghton (Zubu), Maicon Brandes e Letícia (Trutinha).

À FAPESP e ao CNPq pelo apoio financeiro para desenvolvimento da presente pesquisa;

À Evonik Industries AG, Alemanha, pela realização dos aminogramas.

APOIO FINANCEIRO

À Fundação de Amparo à Pesquisa no Estado de São Paulo (FAPESP), processo Nº 2013/25761-4 e Nº 2019/06343-3; e ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) processo Nº 130873/2019, pelo apoio financeiro para desenvolvimento da pesquisa.

À Evonik Industries AG Essen, North Rhine-Westphalia, Alemanha, pela realização dos aminogramas.

CAPÍTULO I – ASPECTOS GERAIS

RESUMO GERAL

Um balanço adequado de aminoácidos essenciais (AAEs) na dieta garante uma deposição de conteúdo proteico ideal no corpo animal, necessário para um crescimento de forma saudável. A falta de um aminoácido essencial ou em quantidade insuficiente na dieta ingerida, torna o peixe incapaz de depositar proteínas adequadamente, limitando seu crescimento e trazendo prejuízos econômicos e ambientais na produção. Para avaliar a resposta do animal, frente a essas deficiências dos AAEs na retenção de nitrogênio no corpo, dentre os métodos disponíveis, aplicou-se o método de deleção. Este método foi desenvolvido para validar o conceito de proteína ideal. para sua aplicação, realiza-se uma redução suficiente do aminoácido teste na dieta experimental e, pela técnica do abate comparativo, calcula-se a retenção de nitrogênio corporal. A partir destes resultados é possível determinar os níveis ideais de cada AAE e assim encontra-se o perfil ideal dos AAEs, servindo como base para a formulação de dietas para diferentes espécies em diferentes fases de crescimento. Informação nutricionais espécie-específico precisas e ajustadas ainda são escassas, ainda mais tratando-se de espécies de suma importância para a produção internacional. O pacu (*Piaractus mesopotamicus*) vem mostrando grande importância na produção mundial, endêmico da bacia do Prata, além de sua presença em países da América Latina, é produzido em países da América do Norte e Ásia. Portanto, pesquisas voltadas a nutrição desta espécie, poderão contribuir positivamente à produção internacional, principalmente para a indústria

de rações comerciais. O conhecimento disponível sobre as exigências nutricionais para as espécies de interesse comercial ainda é limitado, especialmente ao tratarmos sobre nutrição de peixes em fase de terminação. Considerando ainda, a atenção a fatores atrelados como otimizar o aproveitamento dos nutrientes oferecidos, reduzindo o conteúdo de proteína absoluta em dietas e redução da emissão de nitrogênio no ambiente, faz-se necessário o conhecimento dos níveis ótimos dos nutrientes essenciais para diferentes fases de crescimentos das espécies de importância na produção aquícola.

Palavras-chave: Alimentação, aquicultura sustentável,nutrição de pacu, proteína balanceada.

GENERAL ABSTRACT

Essential amino acid balance in the diet ensures the deposition of ideal protein content in animal body which is necessary for the healthy growth. However, the lack or insufficient ratios of essential amino acids in the diet do not allow the fish to deposit optimum protein contents. In fish nutrition, to assess the animal response to essential amino acid (EAA) deficiencies and their effect on the body nitrogen retention, several methods are used, including the deletion approach. In the deletion technique, the test amino acid levels are effectively reduced in the experimental diets, and by the comparative slaughter method, body nitrogen retention is determined. Using the body N retention data, the optimal ratios of essential amino acids are calculated, serving as a baseline for the formulation of practical and experimental diets for large size fish. This information is still scarce for several commercially cultivable fish species, including pacu. Pacu is a suitable fish for large-scale production, endemic to the freshwater bodies in Latin American

Countries as well as widely cultured in several other countries of the world as an exotic species. Therefore, research focused on the nutrition of this species may contribute positively to the global aquaculture production and supplies important data to the commercial feed industry. The available knowledge about amino acid requirements of adult pacu is still limited. The present study supplies an important outline for the optimization of amino acid use efficiency of adult pacu to reduce the absolute protein contents in the diets and minimize the excessive N emissions in aquatic environments.

Keywords: Feeding, Sustainable aquaculture, Pacu Nutrition, Balanced protein

INTRODUÇÃO GERAL

Sabe-se que a proteína está entre os nutrientes essenciais e mais onerosos de rações comerciais utilizadas na aquicultura (Li et al., 2009; Wilson, 2002). Portanto, estudos focados nos níveis deste nutriente e nos aminoácidos (AAs) que as compõem, tornam-se uma das ferramentas principais para a melhoria da eficiência de utilização proteica pelo animal, além de reduzir a carga do efluente, diminuindo o impacto no ambiente (Green e Hardy, 2002; Peres e Oliva-Teles, 2009).

Os AAs também são componentes importantes de controle das principais vias metabólicas (Li et al., 2009). Alguns AAs não podem ser sintetizados ou são sintetizados em quantidades insuficientes pelos animais, sendo estes chamados de aminoácidos essenciais (AAEs) (Sakomura e Rostagno, 2016). Já os aminoácidos que o organismo animal é capaz de sintetizar em quantidades suficientes para garantir um crescimento saudável, são chamados de aminoácidos não essenciais (AANEs), portanto não é necessário grandes

inclusões nas dietas como é o caso do AAEs. Os AAEs são os mais importantes a serem estudados, pois é necessário que sejam fornecidos em proporções adequadas aos animais através da dieta para que o organismo possa manter, de forma adequada, suas funções metabólicas e crescimento (Li et al., 2009; Wu, 2013; 2014).

Desta forma, entende-se o conceito de proteína ideal como um conjunto de AAs com total biodisponibilidade de digestão e metabolismo, presentes em quantidades e proporções que atendam, sem excessos, as exigências do animal para plena realização dos processos de mantença, além de atingir seu potencial genético de crescimento (Boisen, 2003; Furuya, 2010; Green e Hardy, 2002; Mitchell, 1964; Peres e Oliva-Teles, 2009).

Para formular uma dieta atendendo os conceitos de proteína ideal, torna-se necessário o conhecimento dos níveis adequados de inclusão dos AAs para a fase de crescimento da espécie estudada. A forma mais rápida de se encontrar esses níveis é a análise do perfil de AAs corporais dos animais, partindo do princípio de que a composição química corporal varia de acordo com o conteúdo nutricional ingerido. Embora este método apresente apenas valores aproximados, uma vez que não são considerados todos os gastos metabólicos do animal, somente a deposição dos AA nos tecidos corporais (Diógenes et al., 2015; Furuya, 2010; Green e Hardy, 2002; Peres e Oliva-Teles, 2009; Rollin et al., 2003), ele apresenta grande importância por ser rápido e servir como base para iniciar estudos de exigências nutricionais.

Outro método, conhecido como dose-resposta, é o mais utilizado para determinar os níveis ideais dos AAs. Através de tratamentos com diferentes níveis do AA estudado, é possível estimar um nível indicado dele. Todavia, para

encontrar o nível adequado de todos os AAEs, faz-se necessário que cada AAE seja estudado isoladamente, acarretando altos gastos financeiros e tempo (Diógenes et al., 2015; Green e Hardy, 2002; Peres e Oliva-Teles, 2009;Rollin et al., 2003). Além disso, cada experimento pode apresentar indesejáveis particularidades, pois utilizam lotes de peixes, dietas, período e condições ambientais diferentes (Green e Hardy, 2002; Rollin et al., 2003). Para eliminar estas fontes de variação, utiliza-se o método de deleção que tem por objetivo determinar o perfil ideal entre os aminoácidos essenciais e estimar proporções adequadas em um único experimento (Boisen et al., 2000; Diógenes et al., 2015; Green e Hardy, 2002; Peres e Oliva-Teles, 2009; Rollin et al., 2003). Isso permite maior grau de uniformidade e acurácia dos resultados e menor tempo de período experimental quando comparado com o método de dose-resposta.

Descrito por Wang e Fuller (1989), o método da deleção consiste na elaboração de tratamentos nos quais as dietas são isoenergéticas e isoproteicas, e apresentam apenas um AAE deficiente (reduzido). Este método foi desenvolvido para validação do conceito de proteína ideal. Desta forma, cada tratamento tem um nível de retenção de nitrogênio corporal específico, de acordo com o AAE deficiente na dieta. Com este perfil do AAE reduzido na dieta e a consequente retenção de nitrogênio corporal, podem ser estimados os níveis de AAEs para a espécie em estudo (Green e Hardy, 2002; Wang e Fuller, 1989).

Este método foi inicialmente desenvolvido para uso em suínos (Wang e Fuller, 1989), e vem sendo utilizada com ótimos resultados e aceitação para várias espécies de peixes, como salmonídeos (Green e Hardy, 2002; Rollin et al., 2003), dourada (Peres e Oliva-Teles, 2009), pargo (Marammazi et al., 2017) e tilápia-do-Nilo (Diógenes et al., 2015; Rodrigues, 2019). Este método foi aplicado

para determinar o perfil ideal de AAEs para juvenis de pacus (Boaratti et al., 2020). Contudo, para a fase de terminação ou pacus adultos ainda falta essa informação. O pacu vem mostrando grande importância na produção mundial. Trata-se de uma espécie nativa do Brasil, endêmica da Bacia do Prata, estando presente em outros países da América do Norte e Ásia (FAO, 2010; Flores Nava, 2007; Honglang, 2007). É um peixe que tolera baixas temperaturas em ambiente natural (Milstein et al., 2000). Além disso apresenta alto valor comercial, crescimento relativamente rápido (Jomori et al., 2005), alta prolificidade e fácil adaptação em cativeiro, favorecendo ainda mais sua produção e comercialização (Abimorad e Carneiro, 2004). Outra característica favorável é seu hábito alimentar onívoro e a fácil aceitação de alimentos com ampla composição nutricional (Abimorad e Carneiro, 2007; Abimorad et al., 2010; Fernandes et al., 2000; Fiod et al., 2010).

Neste contexto, este estudo foi delineado com os seguintes objetivos:

Objetivo geral:

Estabelecer um perfil ideal de aminoácidos essenciais (AAEs) dietéticos para adultos de pacu.

Objetivos específicos:

1: Determinar, pelo método de deleção, o perfil ideal dos aminoácidos essenciais (AAEs) dietéticos;

2: Avaliar aspectos de crescimento e alimentares dos animais;

3: Avaliar a deposição de nitrogênio em resposta a dieta controle e dietas com deficiência de aminoácidos essenciais.

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CAPÍTULO II – Artigo

Evaluation of an optimum dietary essential amino acid pattern for adult pacu (*Piaractus mesopotamicus*)

Submetido para Aquaculture Nutrition

ABSTRACT

The optimum levels of dietary essential amino acids (EAAs) are required for normal protein gain and physio-biochemical functions in fish. Thus, this 60-day study aimed to determine an optimum dietary EAA pattern for adult pacu (*Piaractus mesopotamicus*) by the amino acid (AA) deletion method. A total of 165 fish (1,109.83 ± 14.26 g) were equally distributed among 11 treatments, with three randomly-arranged replicates each, in a recirculation aquaculture system. Fish were fed with 11 diets including a control diet (CD) and 10 EAA limiting diets (LDs) two times daily until apparent satiation. The body nitrogen (N) retention and weight gain of fish fed with EAA LDs was lower ($p< 0.05$) than the CD. The feed efficiency of CD was similar ($p> 0.05$) to most EAA LDs, except arginine and histidine LDs. The specific growth rate of fish fed with tryptophan, arginine and histidine LDs was different ($p< 0.05$) when compared the CD. Based on the present N retention data obtained through the AA deletion method, the optimum pattern of dietary EAAs estimated in relation to lysine requirement of 100% was as follows: arginine 72.16%; phenylalanine 40.83%; histidine 30.15%; isoleucine 53.01%; leucine 81.71%; methionine 24.97%; threonine 58.20%; tryptophan 9.13% and valine 54.69%.

KEYWORDS

Final growing phase pacu, amino acid deletion method, feed efficiency, growth, nitrogen retention, sustainable nutrition

1 INTRODUCTION

Amino acids (AAs) are essential for normal muscle growth, protein gain and lipid and carbohydrate metabolism in animals including fish. They have a main role in the regulation of several functions at cellular or metabolic level as the enzymes made of AA residues work as catalysts for several biochemical reactions inside body (Tacon, 1982). The optimum proportions of AAs, particularly the essential ones are necessary to be maintained in fish feed to obtain optimum body growth and metabolism. Otherwise, their inadequate dietary proportions may adversely affect the growth and physio-biochemical functions in fish (Andriguetto et al., 2006; Kaushik, Fauconneau, Terrier, & Gras, 1988; Li, Mai, Trushenski, & Wu, 2009; Peres & Oliva-Teles, 2009; Portz & Furuya, 2012; Wilson, 2002; 2003). The growth stages, environmental variations and quality as well as quantity of dietary protein are common factors which influence the AA needs of animals (Boisen, Hvelplund & Weisbjerg, 2000; Cowey, 1994; Fuller, 1994). According to Wang & Fuller (1989) the quality of dietary protein is usually defined by its essential amino acid (EAA) pattern. In animals, the EAAs are either not synthesized completely or synthesized only in small amounts just to meet the maintenance or physiological needs while the non-EAAs are usually synthesized adequately (National Research Council - NRC, 2011; Sakomura & Rostagno, 2016). The EAA patterns that attend the total needs of animals may help to obtain optimum growth and health status of fish as well as to save the dietary protein which is one of the essential but most expensive nutrients of feed (Marammazi, Yaghoubi, Safari, Peres, & Mozanzadeh, 2017; Peres & Oliva-Teles, 2009). This strategy greatly contributes to improve the feed utilization and reduce the extra nitrogen excretion into water (Andriguetto et al., 2006; Boisen, Hvelplund, & Weisbjerg,2000; Furuya, 2010; Green & Hardy, 2002; Kaushik & Seiliez, 2010; Hu et al., 2008; Mitchell, 1964).

In aquaculture nutrition, several methods have been used for the estimation of optimum levels of dietary EAAs for different fish species which include the carcass deposition, carcass analysis and dose-response (Tacon, 1982). In the carcass deposition method, fish are fed with a whole protein source of high biological value and then the dietary EAA requirements are determined using the observed daily based deposition of

AAs in fish body (Arai, 1981; Ogino, 1980). However, according to Tacon & Cowey (1985) and Wilson & Poe (1985) no great variance exists between the relative amounts of individual EAAs necessary in fish feed and the relative amounts of the same ten EAAs in fish body. Thus, in the second method, carcass analysis method, the quantitative dietary EAAs are directly determined by the AA composition of whole body (Khan et al., 2020; Wilson & Cowey, 1985) or muscle tissue (Abimorad et al., 2010;Bicudo, Sado, & Cyrino, 2009; Machado & Sgarbieri, 1991) of fish without any specific feeding unlike the carcass deposition method. In the third method which is based on the dose-response approach, different diets containing several graded proportions of a test AA are fed to the fish to obtain the response curves. Then, the break point on the response curve is usually considered the optimum dietary level of the test AA (Cowey & Luquet, 1983; Ketola, 1982; Wilson, 1985).

Among the above mentioned methods, the third one is a standard method but it is an expensive and time consuming approach as well as at least 10 feeding trials are required to determine the requirement of each of the 10 EAAs for a given species (Diógenes et al., 2016; Mambrini & Kaushik, 1994; Rollin, Mambrini, Abboudi, Larondelle, & Kaushik, 2003; Wilson, 2003). The variations in fish age, genetic strain, the rate of feeding, rearing conditions and nutritional composition of the basal diet could also affect the estimation of AA requirements done through the dose-response experiments (Rollin et al., 2003). On the other hand, although the previous two methods which are based on the "ideal protein concept" allow determining the optimal ratios of all 10 EAAs in a single experiment (Furuya, 2010; Khan et al., 2020; Portz & Furuya, 2012), these methods sometimes could result in the over or under estimation of some EAAs (Rollin et al., 2003). In this regard, to avoid the limitations associated with the carcass deposition and carcass analysis methods, Fuller, McWilliam, Wang, & Giles, (1989) and Wang & Fuller (1989) developed a method in pigs which is relying on the principle that body nitrogen (N) gain is not influenced by the decrease in a non-limiting dietary AA. Contrariwise, the body N gain (protein synthesis) is directly associated with the limiting AA and the partial removal of a limiting AA may greatly decrease the N gain. The variations observed by Fuller et al. (1989) and Wang & Fuller (1989) in body N gain in response to the partial removal of each EAA were then used to estimate ideal dietary EAA profiles in which all EAAs were limiting to N gain. This procedure has been known as the AA deletion method which basically serves to validate the concept of ideal protein and is a good alternative to the above mentioned three

methods (Rollin et al., 2003).

Keeping in view the available literature, the AA deletion method provides reliable data regarding the estimation of dietary EAA requirements. This method has already been used successfully to determine the ideal dietary EAA profiles for several fish species including salmonids (Green & Hardy, 2002; Rollin et al., 2003), gilthead seabream (Peres & Oliva-Teles, 2009), juvenile Nile tilapia (Diógenes et al., 2016), adult Nile tilapia (Rodrigues et al., 2020), silvery-black porgy (Marammazi et al., 2017) and juvenile pacu (Boaratti et al., 2020). However, yet these data are unavailable for market-size (adult growth stage) pacu. Therefore, this work aimed to establish an optimum pattern of dietary EAAs for adult pacu through the AA deletion method.

2 MATERIAL AND METHODS

The materials and methods used in this trial accorded with the ethical guidelines of the Brazilian College of Animal Experimentation (COBEA). The design of this study was approved by the Ethics Committee on Animal Use, São Paulo State University (UNESP), School of Agricultural and Veterinarian Sciences, Jaboticabal through the protocol No. 9999/14.

2.1 Experimental design and rearing conditions

The present study was conducted at the Fish Nutrition Modelling Laboratory, Aquaculture Center, São Paulo State University (UNESP), Jaboticabal, São Paulo, Brazil. A completely randomized design which consisted of 11 treatments with three replicates each. After the acclimation period of one week, fish were equally distributed among 33 fiber tanks of 1,000-L, in a recirculation aquaculture system (RAS). The RAS was equipped with a mechanical and biological filter and a heat exchanger being programmed to maintain the system's water temperature at about 28ºC. A continuous aeration was provided to the experimental tanks by a radial compressor (blower).

At the start of the experiment, 24 hr after the fasting period, fish were anesthetized with benzocaine solution (50mg/L) (Ethyil-p-aminobenzoate) (Sigma-Aldrich, Brazil), weighed individually and 165 fish, originated from commercial fish farming in the region, with initial body weight of 1,109.83 ± 14.26 g (mean ± standard deviation - SD) were distributed per replicate/tank. During the 60-day experiment, fish were fed twice a day at 11:00 and 17:00 hr until apparent satiety, without any leftover food in the tank.

During the experimental period, it was necessary to realize a partial renovation (30%) of

the water in the recirculation system.

2.2 Experimental diets and analytical procedures

Eleven experimental diets including a control diet (CD) and 10 test essential amino acid limiting diets (EAA LDs) were formulated (SuperCrac®, Table 1) by taking into account the basal nutritional requirements of pacu described by Abimorad & Carneiro (2004; 2007), Abimorad, Squassoni, & Carneiro (2008), Abimorad, Favero, Squassoni, & Carneiro, (2010), Fernandes, Carneiro, & Sakomura (2000; 2001) and Khan et al. (2020). The CD consisted of 55% unpurified organic ingredients and 45% purified ingredients including a mix of crystalline amino acids – AAs (both essential and non- EAAs). The 10 test EAA LDs consisted of the same 55% unpurified organic ingredients and 45% purified ingredients including the AA mix (containing both essential and non- EAAs), however, each test EAA LD was kept in deficient up to 45% (average, fed basis; Table 1) in the respective test EAA. The partial deficiency of each test EAA was fulfilled by the three non-EAAs including glycine, glutamic acid and alanine. As a result, all the EAA LDs presented a nutritional composition 100% similar to that of the CD, as all the 11 experimental diets were maintained isoproteic and isoenergetic (Table 1).

The crystalline AA mix was coated with 1% agar-agar before being mixed with other feed ingredients of the CD or EAA LDs according to the method proposed by Mambrini & Kaushik (1994). The coating procedure was performed to improve the AA availability and efficiency of AA utilization (Mambrini & Kaushik, 1994; Peres & Oliva-Teles, 2009). An amount of water corresponding to 21% of the diet total weight was heated (100ºC), agar was diluted and the solution was cooled at a room temperature to approximately 45ºC before the addition of the crystalline AA mix. The coated AA mix was then mixed with other feed ingredients, properly homogenized and immediately extruded using an ExMicro extruder (Exteec Machines®, Brazil) into feed pellets with a granule dimension of about 4-6 mm. Subsequently, the diets were dried in a forced-ventilation-oven (55ºC for 24hr) and kept in a cold room at −12ºC until use.

The nutritional composition of feed ingredients and diets was analyzed according to the standard methods of Association of Official Analytical Chemists - AOAC International (2016). The AA profile of the ingredients and test diets (CD and EAA LDs) was determined by near infrared spectroscopy (NIRS - AMINONIRS®) and high performance liquid chromatography (HPLC) (Biochrom, Cambridge, UK), respectively at Evonik Industries AG, Essen, Germany.

The analyzed nutritional composition of the 11 experimental diets is shown in Table 2, which showed that the partial deletion of essential amino acids (EAAs) over the 10 EAA limiting diets (LDs) remained 41.71% (average, analyzed basis).

2.3 Nitrogen retention assessment

The comparative slaughter technique was used to determine the changes in body nitrogen (N) retention. A total of 10 fish were sampled from the initial population at the beginning of the experiment and 4 fish from each experimental unit (replicate/tank) were sampled at the end of the experimental period for the determination of initial and final body N deposition, respectively. The sampled fish were immediately processed in a meat grinder and the material obtained was lyophilized at −50°C and −80 kPa (VLP20, Thermo Fisher) for 72 hr. The body N content was determined by the Kjedhal method (Association of Official Analytical Chemists - AOAC International, 2016).

The whole body N content was used to determine the N deposition levels (N mg/$BW^{0.75}$ kg/day) for each of the three replicates per treatment using the following equation proposed by Rollin et al. (2003):

N Deposition = {(W_f * N_f) – (W_i * N_i)} / [0.5 * {(W_f/1000) ^ 0.75 + (W_i/1000) ^ 0.75} * Δt]

Where W_f and W_i are the final and initial body weights (g), respectively, Δt is the number of days of the experimental duration and N_f and N_i are the final and initial body N composition means in percentage, respectively.

2.4 Determination of the optimum pattern of dietary EAAs by the AA deletion method

Through the N deposition levels determined for each of the three replicates per treatment, the optimum dietary EAA pattern for adult pacu was determined using the following equation proposed by Rollin et al. (2003):

Optimum level = [EAA_{CD} * {2 – DEL – (ND_{EAA} / ND_{CD})}]

Where, “optimum level” is the estimated ideal proportion of the test EAA (g/kg, dry matter); EAA_{CD} is the concentration of the test EAA in CD (g/kg, dry matter); DEL is the partially deleted level (%) of the test EAA in the EAA LD, that is the test EAA concentration in EAA LD divided by the concentration of the same test EAA in CD; ND_{EAA} is the N deposition (N mg/$BW^{0.75}$ kg/day) in fish fed with test EAA LD; and ND_{CD} is the N deposition (N mg/$BW^{0.75}$ kg/day) in fish fed with CD.

Although Rollin et al. (2003) stated the outcome of the above mentioned equation as

"requirement" for each EAA, according to Wand & Fuller (1989) the basic principle of this method is the determination of the ideal relationship among EAAs and hence the estimation of ideal ratios of dietary EAAs (Wand & Fuller 1989). Thus, in this study the outcome of the equation proposed by Rollin et al. (2003) for the AA deletion method was named as "optimum level for each EAA" rather than "EAA requirement".

As the AA deletion method is based on the principle that body N retention is linearly associated with the dietary content of a limiting EAA while non-EAA has no impact on the body N retention. Thus, the relationship between AA intake and body N gain was determined in the present study. The ideal relationship among EAAs was obtained through dividing the estimated proportion of each EAA by the proportion of lysine (100%) estimated in this study (Green & Hardy, 2002; Rollin et al., 2003).

2.5 Statistical analysis

The data obtained during the present 60-day experiment were analyzed by one-way analysis of variance (ANOVA) using the Statistical Analysis System - SAS (2008). The differences among 11 treatment means were analyzed by Tukey test ($p< 0.05$) while the Dunnet test ($p< 0.05$) was used to compare the means of the EAA LDs with CD.

3 RESULTS

During the experimental period, no mortality or pathological signs were observed. In general, fish fed with essential amino acid limiting diets (EAA LDs) presented lower feed efficiency and growth than the fish fed control diet (CD) (Table 3). The final body weight (FBW) of fish fed by CD and lysine, methionine, threonine, valine and leucine LDs was found similar ($p> 0.05$). Among the EAA LDs, the FBW was similar among the 10 treatments, but arginine LD showed a significant difference ($p< 0.05$) with valine, methionine and leucine LDs. The body weight gain (BWG) of fish fed with CD and LD in methionine was similar ($p> 0.05$). Among the EAA LDs, the BWG of fish fed arginine LD showed a difference ($p< 0.05$) with lysine and methionine LDs. The feed efficiency (FE) of fish fed with CD was similar ($p> 0.05$) to the EAA LDs but the FE of fish fed histidine LD showed a difference ($p< 0.05$) with diets limiting in methionine and lysine. The specific growth rate (SGR) of fish fed with diets limiting in lysine, methionine, threonine, valine, isoleucine and leucine was similar ($p> 0.05$) to CD. Among the EAA LDs, the SGR of fish fed with histidine LD was different ($p< 0.05$) than the diet limiting in methionine.

All fish fed with EAA LDs showed significantly reduced ($p< 0.05$) body nitrogen (N)

deposition as compared to the fish fed with CD. Among EAA LDs, the fish feed with the arginine LD showed a significant body nitrogen reduction ($p< 0.05$) when compared with the threonine and histidine LDs (Table 4). The N retention efficiency (NRE) of fish fed CD was similar ($p> 0.05$) to lysine, methionine, arginine, leucine and phenylalanine LDs. Among EAA LDs, the NRE of fish fed arginine LD was different ($p< 0.05$) than threonine and histidine LDs.

The relationship between AA intake and body N deposition is presented in Fig. 1. The body N gain showed a linear response to all EAA LDs (Table 5). These results do indicate that the partial deletion of 41.71% (average, analyzed basis; Table 2) of any EAA has a deleterious impact on the body N deposition and thus, all EAA were found limiting to the growth of adult pacu. The slope values (coefficient b) obtained for the EAA LDs show the degree of the impact of partial deletion of EAAs on the body N deposition.

The optimum levels (mean ± standard deviation/SD; g/kg dry matter) of dietary EAAs obtained on the basis of the relationship between AA intake and body N deposition in adult pacu were as follows: lysine 21.34 ± 1.53; arginine 14.67 ± 1.52; phenylalanine 8.66 ± 0.71; histidine 6.43 ± 0.37; isoleucine 11.32 ± 0.10; leucine 17.55 ± 1.91; methionine 5.32 ± 0.55; threonine 12.40 ± 1.04; tryptophan 1.94 ± 0.33 and valine 11.70 ± 0.36. The optimum pattern of dietary EAAs estimated in relation to the lysine requirement (100%) was as follows: arginine 72.16%; phenylalanine 40.83%; histidine 30.15%; isoleucine 53.01%; leucine 81.71%; methionine 24.97%; threonine 58.20%; tryptophan 9.13% and valine 54.69%.

4 DISCUSSION

Amino acid (AA) deletion method is based on the basic principle that all EAAs are limiting to body nitrogen (N) gain in animals including fish (Baker, 2004; Boisen, 2003; Fuller et al., 1989; Peres & Oliva-Teles, 2009; Wang & Fuller, 1989). In the present study, the partial deletion (41.71% average, analyzed basis; Table 2) of dietary EAAs greatly declined the final body weight, body weight gain, specific growth rate, feed efficiency and N gain of adult pacu as compared to control diet (CD). The same pattern of productive performance and N gain has been obtained by Boaratti et al. (2020) in juvenile pacu (body weight 6.22g, feeding period 60-days) and Green & Hardy (2002), Marammazi et al. (2017), Peres & Oliva-Teles (2009), Rodrigues et al. (2020) and Rollin et al. (2003) in other fish species using the AA deletion method. The better growth, feed utilization (Table

3) and body N gain (Table 4) of fish over the CD is justifiable as CD contained a balanced EAA pattern unlike the unbalanced EAA LDs (Kaushik & Seiliez, 2010; Peres & Oliva-Teles, 2005; Wilson, 2003).

A firm AA composition of the diet is required in AA deletion method based experiments in order to avoid any irregularity in the animal response (Green & Hardy, 2002; Marammazi et al., 2017; Peres & Oliva-Teles, 2009; Wang & Fuller, 1989). The desired AA profile of the experimental diets in this study was obtained through the combination of crystalline AAs and intact protein which is relatively a practical approach (Ambardekar, Reigh, & Williams, 2009; Bodin et al., 2012). The crystalline AA mix (including both EAAs and non-EAAs) in the present experimental diets was used only to guarantee the required nutritional composition. Although the total amount of crystalline AA mix was low (about 16.42%, average), to avoid any problems of leaching and higher absorption rate (Alam et al., 2005; Fournier et al., 2002; Schuhmacher, Wax, & Gropp, 1997; Segovia-Quintero & Reigh, 2004), the AA mix was coated with 1% agar-agar before being mixed with other ingredients of a respective diet according to the previous recommendations (Mambrini & Kaushik, 1994; Peres & Oliva-Teles, 2009). The coating procedure performed was found efficient as the fish fed CD in this study showed optimum productive performance and N gain when compared with the EAAs LDs. The utilization efficacy of crystalline AAs or intact protein usually depends on the fish species, growth stage or nutritional composition of the diet but this topic is still under the discussion (Ambardekar et al., 2009; Dabrowski et al., 2010; Nunes, Sá, Browdy, & Vazquez-Anon, 2014; Peres & Oliva-Teles, 2005; Williams, Barlow, & Rodgers, 2001). In the case of achieving the required dietary AA profile (in AA deletion based studies) entirely or half by crystalline AAs, the assessment of AA availability and efficiency of AA utilization in fish fed diets containing the coated crystalline AAs in a given species would be worthy. The EAAs were limiting factors in this study and in order to avoid the limitation of dietary energy or protein, all experimental diets were kept isoenergetic and isoproteic according to the basic principle of the AA deletion method (Fuller et al., 1989; Peres & Oliva-Teles, 2009; Wang & Fuller, 1989).

The EAA LDs considerably reduced ($p< 0.05$) the body N deposition of fish as compared to CD but the degree of response over the EAA LDs varied. The difference in response (body N gain) of fish among the EAA LDs is described in Table 5. The lower slope values obtained for valine, lysine and arginine, respectively may indicate that their

partial deletion could severely decline the body N gain of fish. The other EAA LDs presented higher slope values, with the highest value obtained for the tryptophan LD (Table 5). This difference in N gain among the EAA LDs may be probably related to the body total needs, that is, first an animal may try to attend the basal level of an essential nutrient and then tend to deposit that nutrient in its body only when the ingested amount would exceed the requirements for maintenance and physiological functions (National Research Council - NRC, 2011; Sakomura & Rostagno, 2016). Among EAA LDs, histidine, methionine, threonine, isoleucine, leucine and phenylalanine LDs resulted in an intermediate N gain. Overall, this finding showed that dietary valine, lysine and arginine need proper attention during the preparation of diets for adult pacu. Although all EAAs are limiting to body N gain, the most limiting ones usually depend on the fish species or growth stage such as Boaratti et al. (2020) in juvenile pacu determined dietary lysine as the most limiting dietary EAA while in salmonids methionine has been reported as the most limiting EAA (Green & Hardy, 2002; Rollin et al., 2003). In juvenile Nile tilapia methionine, threonine and valine have been determined as the most limiting EAAs (Diógenes et al., 2016) while in adult Nile tilapia methionine, tryptophan and valine have been documented as the most limiting EAAs by Rodrigues et al. (2020). In gilthead seabream Peres & Oliva-Teles (2009) based on their CD have reported methionine as the most limiting EAA.

It is known that high levels of dietary arginine as compared to lysine could reduce lysine digestibility due to the competition for absorption sites in intestine (Berge, Sveier, & Lied, 2002; Kaushik et al., 1988), or competition even during post-absorptive assimilation and metabolism (Davies, Morris, & Baker, 1997). In the present study, although the diet limiting in lysine had a higher arginine to lysine ratio (137.43%, Table 2) as compared to CD (80.2%), there was no difference ($p > 0.05$) in gain of body N of fish fed lysine or arginine LD. This indicates that possibly the partial deletion of lysine from the lysine LD did not employ a deleterious effect on body N gain due to the antagonism with excess arginine in this diet. Fish fed leucine and isoleucine LDs showed a close ($p > 0.05$) N gain but different ($p < 0.05$) than the CD. Contrary to this finding, Chance, Mertz and Halver (1964) argue that the reduction of dietary leucine than the isoleucine severely affected the body N deposition in a negative way. This might have occurred due to the important role of leucine in myofibrillar proteolysis suppression, as this EAA acts as a protein degradation inhibitor (Nakashima, Ishida, Yamazaki, & Abe, 2005).

The production of mucin and maintenance of intestinal functions is highly dependent on the level of dietary threonine (Ball, Law, Bertolo, & Pencharz, 1999; Law, Bertolo, Adjiri-Awere, Pencharz, & Ball,2007). Therefore, its deficiency may affect the absorption of other EAAs (Specian & Oliver, 1991). Tryptophan can be converted into serotonin, which is an important neurotransmitter related to the wellbeing of animals. The ideal dietary ratio of this EAA is very important in pre-stress situations, since the prolonged rise in cortisol negatively affects growth, feed intake, protein gain and immunity (Vijayan, Mommsen, Glémet, & Moon, 1996). Histidine is related to muscle fatigue and buffering the effects of lactic acid and its deficiency in the diet could negatively affect maintenance, growth and physiological functions (Li et al., 2009). In this study, histidine LD resulted in an intermediate N gain like methionine, threonine, isoleucine, leucine and phenylalanine LDs as compared to valine, lysine and arginine LDs. Similar results for histidine were obtained by Rollin et al. (2003) in Atlantic salmon. These results show that in the case of partially deleted histidine from the histidine LD, fish body started to synthesize protein associated to reduced concentration of histidine such as keratin and collagen and as a result the protein synthesized in muscle tissue became sufficient to fulfill the animal nutritional needs for histidine (Heger & Frydrych, 1985). In general, all dietary EAAs were found limiting to body protein growth and the obtained data show that the inadequate proportion of any EAA in the diet may reduce the body N gain, growth performance and feed utilization of adult pacu. The linear response (Table 5) of body N gain to the partial deletion of all dietary EAAs illustrate that probably there exists a mechanism in fish that regulate the oxidation of the insufficient amount of a dietary EAA in the body (Jürss & Bastrop, 1995; Rollin et al., 2003).

The available data on dietary EAA requirements of pacu are mostly based on dose-response, carcass analysis or carcass deposition methods. Moreover, most of these previous data have been reported for juvenile pacu while still little is known for pacu in the adult growth stage. Abimorad et al. (2010) estimated the lysine requirement of juvenile pacu in a dose-response trial and then through the ideal protein concept (muscle AA composition) estimated an ideal profile of EAAs. Similarly, Bicudo, Sado, & Cyrino (2009) in another dose-response experiment determined the lysine requirement of juvenile pacu and then estimated the ideal profile of EAAs through the same concept of ideal protein (muscle AA composition) as used in coho salmon (*Oncorhynchus kisutch*) by Arai (1981). Similarly, Machado & Sargabieri (1991) determined the ideal profile of dietary EAAs for pacu

(*Colossoma mitrei*, Berg 1895) of 1.5 years through the AA composition of the fillet. Khan et al. (2020) through the whole-body AA composition calculated the ideal ratios of dietary EAAs for pacu during different growth stages. Mambrini & Kaushik (1995) described that the dietary EAA pattern determined on the basis of body tissue AA composition usually mirrors the actual EAA requirements. However, they also mention that the concept of ideal protein sometimes could cause an under or over estimation of some EAA requirements. Given that, the AA deletion method is a good alternative to the previous methods as this method has been efficiently used in previous researches to determine the EAA requirements of several animal and fish species (Fuller et al., 1989; Green & Hardy, 2002, Marammazi et al., 2017; Peres & Oliva-Teles, 2009; Rollin et al., 2003; Wang & Fuller, 1989). Boaratti et al. (2020) have efficiently used the AA deletion method to determine the ideal profile of dietary EAAs for pacu in the initial growth phase. Similarly, the same method was efficiently used in the present study to estimate the ideal ratios of dietary EAAs for pacu during the adult growth stage.

The studies of Abimorad et al. (2010), Boaratti et al. (2020), Bicudo et al. (2009) and Khan et al. (2020) on *P. mesopotamicus* as well as Machado & Sgarbieri (1991) on *C. mitrei* (Berg 1895) showed similarity in terms of some dietary EAA ratios to this study (Fig. 2), which may be probably due to the resemblance in fish species, growth stage or methodological approach. On the other hand, the optimum dietary EAA levels determined for pacu in the present study and those reported in previous studies for other fish species using the AA deletion method have been shown in Table 6. The resemblance of some dietary EAAs estimated in the present finding on pacu to previous studies on other fish species (Table 6) could be probably due to using the same methodological approach (AA deletion method) in these studies. Overall, in Nile tilapia, salmonids (salmon and trout) and gilthead seabream, relatively higher dietary ratios have been estimated for methionine (between 30 and 50%) and phenylalanine (greater than 100%), while in studies conducted with pacu including the present study these ratios remained below 30 and 50%, respectively (Fig. 2; Table 6). The dietary arginine ratios reported in the available literature show wide variation (Fig. 2; Table 6), with the minimum ratios determined for pacu (Machado & Sagarbieri, 1991) while maximum ratios (above 100%) for gilthead seabream (Peres & Oliva-teles, 2009) and Nile tilapia (Diógenes et al., 2016).

5 CONCLUSION

Based on the body nitrogen (N) retention data obtained in this work through the amino acid (AA) deletion method, the optimum pattern of dietary EAAs estimated in relation to lysine requirement of 100% was as follows: arginine 72.16%; phenylalanine 40.83%; histidine 30.15%; isoleucine 53.01%; leucine 81.71%; methionine 24.97%; threonine 58.20%; tryptophan 9.13% and valine 54.69%.

ACKNOWLEDGEMENTS

We highly acknowledge the FAPESP (Process No. 2019/06343-3 and Thematic Project No. 2013/25761-4) and CNPq (Process No. 130873/2019) for financially supporting the present study and Evonik Industries AG, Essen, Germany for providing the aminograms.

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TABLE 1 Formulation of experimental diets (Fed basis)

Ingredients (g/kg)	CD	LD-Arg	LD-Phe	LD-His	LD-Ile	LD-Leu	LD-Lys	LD-Met	LD-Thr	LD-Trp	LD-Val
Fish meal	72.05	72.05	72.05	72.05	72.05	72.05	72.05	72.05	72.05	72.05	72.05
Corn	204.60	204.60	204.60	204.60	204.60	204.60	204.60	204.60	204.60	204.60	204.60
Wheatbran	66.00	66.00	66.00	66.00	66.00	66.00	66.00	66.00	66.00	66.00	66.00
Soybean meal	42.35	42.35	42.35	42.35	42.35	42.35	42.35	42.35	42.35	42.35	42.35
Cornstarch	284.94	277.74	286.30	283.31	285.82	286.47	284.73	285.66	285.69	284.97	285.81
Purifiedcellulose	44.74	44.74	44.74	44.74	44.74	44.74	44.74	44.74	44.74	44.74	44.74
Fish oil	60.29	60.29	60.29	60.29	60.29	60.29	60.29	60.29	60.29	60.29	60.29
Limestone	10.88	10.88	10.88	10.88	10.88	10.88	10.88	10.88	10.88	10.88	10.88
Dicalciumphosphate	25.04	25.04	25.04	25.04	25.04	25.04	25.04	25.04	25.04	25.04	25.04
Agar-agar	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Premix of minerals and vitamins	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Salt	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Antifungal	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Antioxidant (BHT)	2.15	2.15	2.15	2.15	2.15	2.15	2.15	2.15	2.15	2.15	2.15
Essential amino acid (g/kg)											
L-Arginine	7.79	1.79†	7.79	7.79	7.79	7.79	7.79	7.79	7.79	7.79	7.79
L-Phenylalanine	3.87	3.87	0.59 †	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87
L-Histidine	2.25	2.25	2.25	0.34 †	2.25	2.25	2.25	2.25	2.25	2.25	2.25
L-Isoleucine	5.32	5.32	5.32	5.32	1.68 †	5.32	5.32	5.32	5.32	5.32	5.32
L-Leucine	6.26	6.26	6.26	6.26	6.26	0.53 †	6.26	6.26	6.26	6.26	6.26
L-Lysine	15.47	15.47	15.47	15.47	15.47	15.47	6.29 †	15.47	15.47	15.47	15.47
L-Methionine	2.98	2.98	2.98	2.98	2.98	2.98	2.98	0.98 †	2.98	2.98	2.98
L-Threonine	5.39	5.39	5.39	5.39	5.39	5.39	5.39	5.39	1.67 †	5.39	5.39
L-Tryptophan	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.00 †	0.59
L-Valine	5.59	5.59	5.59	5.59	5.59	5.59	5.59	5.59	5.59	5.59	1.49 †
Non-essential amino acids (g/kg)											
L-Acid glutamic	31.42	35.82	32.06	32.60	32.34	32.82	34.55	31.85	32.41	31.61	32.50
L-Alanine	39.44	43.84	40.08	40.62	40.36	40.84	42.57	39.87	40.43	39.63	40.52
L-Glycine	37.60	41.99	38.23	38.77	38.52	38.99	40.72	38.02	38.58	37.78	38.67

CD = Control diet; LD = Limiting diet; Arg = Arginine; Phe = Phenylalanine; His = Histidine; Ile = Isoleucine; Leu = Leucine; Lys = Lysine; Met = Methionine; Thr = Threonine; Trp = Tryptophan; Val = Valine.
BHT = Butylated Hydroxytoluene.
Premix of minerals and vitamins =Folic acid (1.25 g/kg); calcium pantothenate (1.20 g/kg); copper (2.50 g/kg); iron (15 g/kg); iodine (0.375 g/kg); manganese (12.5 g/kg); selenium (0.088 g/kg); zinc (0.0125 g/kg); cobalt (0.125 g/kg); Vit A (2,500 IU/kg); Vit B12 (4.00 g/kg); Thiamine B1 (4.00 g/kg); Riboflavin B2 (4.00 g/kg); Pyridoxine B6 (4.00 g/kg); Vit C (50.00 g/kg); Vit D3 (600,000 IU/kg); Vit E (37,500 IU/kg); Vit K3 (3.75 g/kg); niacin (122.50 g/kg); biotin (0.015 g/kg).
[†]Lower inclusion of the test essential amino acid to obtain the desired 45% (average) deficiency.

TABLE 2 Nutritional composition of experimental diets (dry matter, analyzed content)

Composition (g/kg)	CD	LD-Arg	LD-Phe	LD-His	LD-Ile	LD-Leu	LD-Lys	LD-Met	LD-Thr	LD-Trp	LD-Val
Dry matter[1]	968.6	951.8	970.3	957.8	956.4	943.8	958.3	962.9	960.5	963.5	954.1
Digestible protein[2]	258.2	259.5	261.6	262.9	257.3	263.3	261.4	264.3	263.4	254.9	265.2
Digestible ether extract[2,3]	70.7	72.7	71.5	72.2	72.4	72.0	72.7	72.0	73.3	72.7	70.6
Digestible energy (kcal/kg)[2]	3,780.11	3,847.92	3,796.38	3,815.13	3,775.72	3,848.62	3,831.85	3,911.66	3,887.65	3,788.98	3,760.57
Non-nitrogenextract[4]	534.0	527.1	530.6	527.7	522.6	522.7	524.3	524.5	526.3	534.5	525.5
Crude fiber[5]	51.6	52.6	51.6	52.2	52.3	53.0	52.2	52.0	52.1	51.9	52.4
Mineral matter	62.8	65.2	61.9	62.0	72.6	66.0	66.4	64.2	61.7	63.3	63.2
Digestible amino acids[1,2](g/kg)											
Arginine	14.0	7.9 †	13.9	14.0	13.5	13.9	13.6	13.7	13.8	13.4	14.0
Phenylalanine	8.0	8.1	4.6 †	8.4	8.0	8.2	8.1	8.1	8.1	8.0	8.2
Histidine	4.5	4.5	4.6	2.7 †	4.4	4.7	4.6	4.6	4.6	4.4	4.6
Isoleucine	8.6	8.6	8.7	9.0	4.7 †	8.9	8.7	8.8	8.8	8.4	8.8
Leucine	13.5	13.7	13.8	13.8	13.6	7.9 †	13.9	13.9	13.8	13.5	13.9
Lysine	17.4	17.7	17.7	17.8	17.6	17.9	9.9 †	17.9	17.7	17.4	17.9
Methionine	4.5	4.4	4.5	4.6	4.4	4.7	4.6	2.6 †	4.4	4.2	4.6
Threonine	8.6	8.7	8.8	8.8	8.6	8.8	8.7	8.7	5.2 †	8.6	8.8
Tryptophan	1.3	1.5	1.4	1.4	1.5	1.4	1.4	1.4	1.4	0.8 †	1.4
Valine	9.6	9.5	9.6	9.6	9.4	9.7	9.8	9.9	10.0	9.2	5.7 †
L-Acid glutamic	48.0	53.1	48.8	49.7	49.4	50.3	51.6	49.3	49.7	48.0	49.7
L-Alanine	48.1	53.4	49.1	50.5	49.5	50.8	52.1	48.8	49.1	48.3	49.7
L-Glycine	48.3	53.5	49.5	51.2	50.3	51.3	51.5	49.0	49.9	48.8	49.9
Deletion of each EAA (%)[6]	-	44.61	42.50	40.97	46.03	43.46	43.84	42.79	40.57	37.53	41.29

CD = Control diet; LD = Limiting diet; Arg = Arginine; Phe = Phenylalanine; His = Histidine; Ile = Isoleucine; Leu = Leucine; Lys = Lysine; Met = Methionine; Thr = Threonine; Trp = Tryptophan; Val = Valine.

[1]Aanalysis done by the high-performance liquid chromatography - HPLC technique (Biochrom, Cambridge, UK).

[2]Digestibility values calculated according to the digestibility coefficients determined by Abimorad & Carneiro (2004); Abimorad et al. (2008).

[3]Analysis of nutritional composition done according to the Association of Official Analytical Chemists - AOAC International (2016).

[4] Free nitrogen extract = {Dry matter - (Crude protein + Crude ethereal extract + Ash + Crude fiber)}.

[5] Calculated crude fiber.

[6] Deleted EAA ratios (EAA deletion) = 1 - (EAA from LD/EAA from CD).

TABLE 3 Mean values (± standard deviation - SD) of initial weight, final weight, body weight gain, feed efficiency and specific growth rate obtained during the 60 day-experiment over the 11 experimental diets

Treat.	Initial weight	Final weight	[1]Body weight gain	[2]FE	[3]SGR
CD	1,093.13 ± 6.73	1,230.61 ± 46.59^{a}	159.25 ± 17.45^{a}	0.31 ± 0.04$^{a. b}$	0.20 ± 0.06^{a}
LD-Lys	1,115.67 ± 8.31	1,177.42 ± 41.50$^{a. b. c}$	88.98 ± 15.68$^{b. c}$	0.40 ± 0.13^{a}	0.13 ± 0.02$^{a. b. c}$
LD-Met	1,110.07 ± 18.51	1,205.30 ± 52.00$^{a. b}$	128.09 ± 29.96$^{a. b}$	0.37 ± 0.05^{a}	0.18 ± 0.04$^{a. b}$
LD-Thr	1,103.53 ± 3.52	1,178.66 ± 61.95$^{a. b. c}$	42.35 ± 20.35$^{c. d}$	0.21 ± 0.20$^{a. b}$	0.11 ± 0.09$^{a. b. c}$
LD-Trp	1,109.27 ± 2.87	1,147.06 ± 14.59$^{b. c}$	37.79 ± 16.89$^{c. d}$	0.17 ± 0.05$^{a. b}$	0.06 ± 0.03^{c}
LD-Val	1,107.40 ± 11.61	1,194.05 ± 38.16$^{a. b}$	60.33 ± 19.00$^{c. d}$	0.28 ± 0.12$^{a. b}$	0.13 ± 0.07$^{a. b. c}$
LD-Arg	1,101.27 ± 13.39	1,110.44 ± 44.37^{c}	27.20 ± 0.00^{d}	0.11 ± 0.04^{b}	0.04 ± 0.00^{c}
LD-Ile	1,103.73 ± 8.06	1,152.42 ± 57.79$^{b. c}$	77.80 ± 13.70$^{b. c. d}$	0.35 ± 0.08$^{a. b}$	0.11 ± 0.02$^{a. b. c}$
LD-Leu	1,110.73 ± 4.35	1,116.18 ± 46.27^{a}	21.78 ± 40.43^{d}	0.30 ± 0.00$^{a. b}$	0.09 ± 0.00$^{a. b. c}$
LD-Phe	1,103.80 ± 10.57	1,138.78 ± 29.49$^{b. c}$	48.77 ± 13.37$^{c. d}$	0.18 ± 0.02$^{a. b}$	0.07 ± 0.02$^{b. c}$
LD-His	1,119.47 ± 8.37	1,134.82 ± 45.75$^{b. c}$	36.93 ± 0.88$^{c. d}$	0.10 ± 0.00^{b}	0.05 ± 0.00^{c}

Different letters in the same column show a significant difference ($p< 0.05$) obtained by the Tukey test while the lack of letters in the same column shows a non-significant difference ($p> 0.05$).
Treat. = Treatments.
[1]Body weight gain = ((final body weight – initial body weight) / total days of trial).
[2]FE: Feed efficiency (g/g) = body weight gain (g) / feed intake (g).
[3]SGR: Specific growth rate = ((ln (final body weight) – ln (initial body weight)) / time in days) × 100.

TABLE 4 Initial and final body nitrogen, the nitrogen deposition (ND) data (means ± standard errors) and the optimum levels of essential amino acids (EAAs) estimated according to [†]equation 1, with the respective ideal pattern of EAAs for adult pacu (*Piaractus mesopotamicus*)

Treat.	Body Nitrogen (N)		ND (g/WB$^{0.75}$/d)	[1]NRE	Optimum levels (g/Kg DM)	Ideal profile (%)
	Initial (g)	Final (g)				
CD	76.64 ± 0.47	86.67 ± 1.81^{a}	149.17 ± 17.58^{a}	0.63 ± 0.10^{a}		
LD-Lys	78.22 ± 0.58	81.36 ± 0.51^{b}	51.32 ± 13.07$^{b. c}$	0.32 ± 0.02$^{a. b. c}$	21.33	100.00
LD-Met	77.83 ± 1.30	81.90 ± 1.03^{b}	59.08 ± 18.09$^{b. c}$	0.54 ± 0.06$^{a. b}$	5.28	24.75
LD-Thr	77.37 ± 0.25	79.00 ± 1.20^{b}	23.53 ± 18.01^{c}	0.25 ± 0.24$^{b. c}$	12.37	58.01
LD-Trp	77.77 ± 0.20	79.59 ± 2.55^{b}	27.59 ± 30.3$^{b. c}$	0.25 ± 0.24$^{b. c}$	1.94	9.11
LD-Val	77.64 ± 0.81	81.29 ± 0.78^{b}	55.49 ± 5.57$^{b. c}$	0.24 ± 0.06$^{b. c}$	9.21	43.19
LD-Arg	77.21 ± 0.94	82.21 ± 1.80^{b}	77.12 ± 16.16^{b}	0.35 ± 0.14$^{a. b. c}$	14.67	68.80
LD-Ile	77.38 ± 0.56	79.70 ± 0.52^{b}	35.31 ± 1.77$^{b. c}$	0.14 ± 0.03^{c}	11.32	53.06
LD-Leu	77.87 ± 0.30	80.72 ± 1.23^{b}	43.08 ± 21.07$^{b. c}$	0.31 ± 0.16$^{a. b. c}$	17.48	81.94
LD-Phe	77.39 ± 0.74	82.22 ± 1.58^{b}	73.82 ± 13.16$^{b. c}$	0.44 ± 0.08$^{a. b. c}$	8.66	40.62
LD-His	78.49 ± 0.59	80.10 ± 1.37^{b}	24.46 ± 11.66^{c}	0.13 ± 0.03^{c}	6.43	30.16

Different letters in the same column show a difference ($p< 0.05$) between treatments.
Treat. = Treatments.
ND = Nitrogen deposition.
[1]NRE: Nitrogen retention efficiency = [{(Wf * Nf) – (Wi * Ni)} / (BWG$^{0.75}$ * Δd)] / N-Intake.
[†]Equation 1: Optimum level = [EAACD * {2 – DEL – (NDEAA / NDCD)}].

TABLE 5 Equation obtained for the response (body nitrogen gain) of adult pacu over the respective EAA limiting diet

Essential amino acid	Equation†
Tryptophan	ND = 44.029Trp – 29.700
Histidine	ND = 18.012His – 94.685
Methionine	ND = 12.668Met – 23.080
Threonine	ND = 10.000Thr – 110.280
Isoleucine	ND = 7.286Ile – 40.647
Phenylalanine	ND = 4.792Phe + 33.164
Leucine	ND = 4.085Leu – 17.543
Valine	ND = 3.192Val + 57.189
Lysine	ND = 2.823Lys + 0.754
Arginine	ND = 2.676Arg + 36.034

†The abbreviation of essential amino acid (EAA) in the equation column is the amount of EAA ingested and ND is the nitrogen deposition over the respective EAA intake.

TABLE 6 A comparison of the optimum ratios (%) of dietary essential amino acids determined for adult pacu (*Piaractus mesopotamicus*) in the present amino acid (AA) deletion based study to those estimated for other fish species by the same AA deletion method

Essential amino acid	Present estudy[1]	Green & Hardy, 2002[2]	Rollin et al., 2003[3]	Peres &Oliva-Teles, 2009[4]	Diógenes et al., 2015[5]	Marammazi et al, 2017[6]	Nascimento et al., 2020[7]	Rodrigues et al., 2020[8]
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Lys	72.16	86.60	76.00	108.3	125.00	87.00	85.78	41.18
Arg	40.83	124.80	105.00†	112.30	101.00	62.00	63.69	31.18
Phe	30.15	32.90	28.00	36.80	34.00	43.00	29.79	14.87
His	53.01	51.70	Not reported	49.70	57.00	77.00	56.05	42.07
Ile	81.71	89.90	Not reported	92.70	96.00	90.00	83.64	38.97
Leu	24.97	53.00	64.00	50.80	64.00	57.00	41.12	26.48
Met	58.20	63.10	51.00	58.10	93.00	87.00	103.42	54.94
Thr	9.13	11.40	14.00	14.60	24.00	16.00	15.49	8.58
Trp	54.69	57.70	59.00	62.60	76.00	77.00	60.25	45.55
Val	54.69	57.70	59.00	62.60	76.00	77.00	60.25	45.55

[1]Adult *Piaractus mesopotamicus*; [2]*Oncorhynchus mykiss*; [3]*Salmo salar*; [4]*Sparus aurata*; [5]Juvenile *Oreochromis niloticus*; [6]*Sparidentex hasta*;

[7]Growing *Oreochromis niloticus*; [8]Adult *Oreochromis niloticus*.

†Value obtained for phenylalanine + tyrosine.

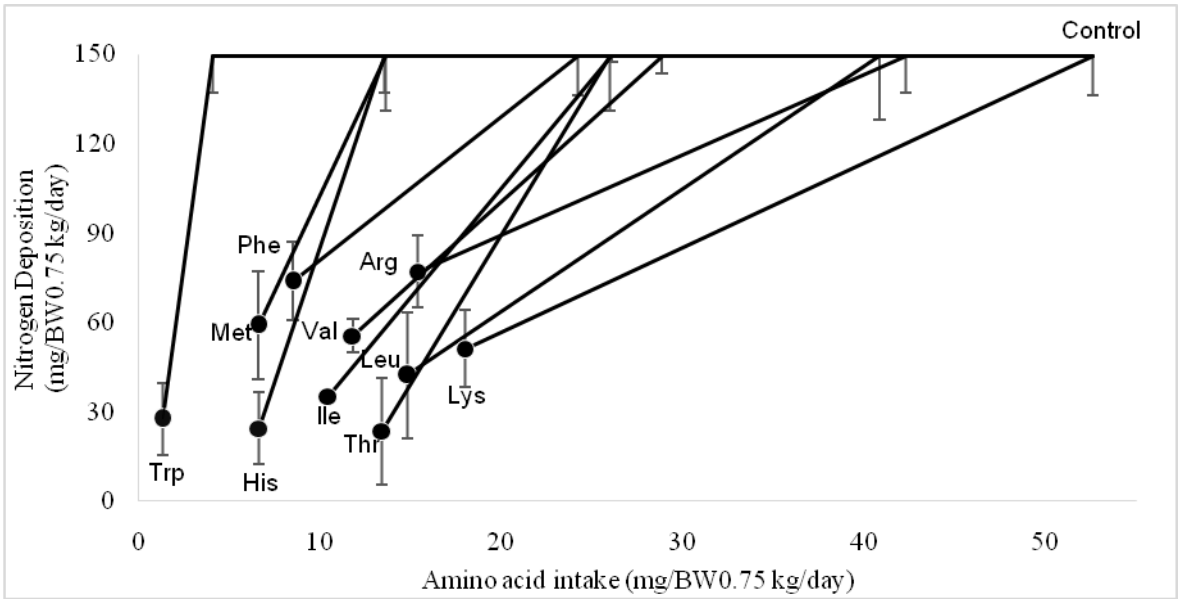

FIGURE 1 The body nitrogen retention of adult pacu when 41.72% (average) of eachessential amino acid was removed from the control diet

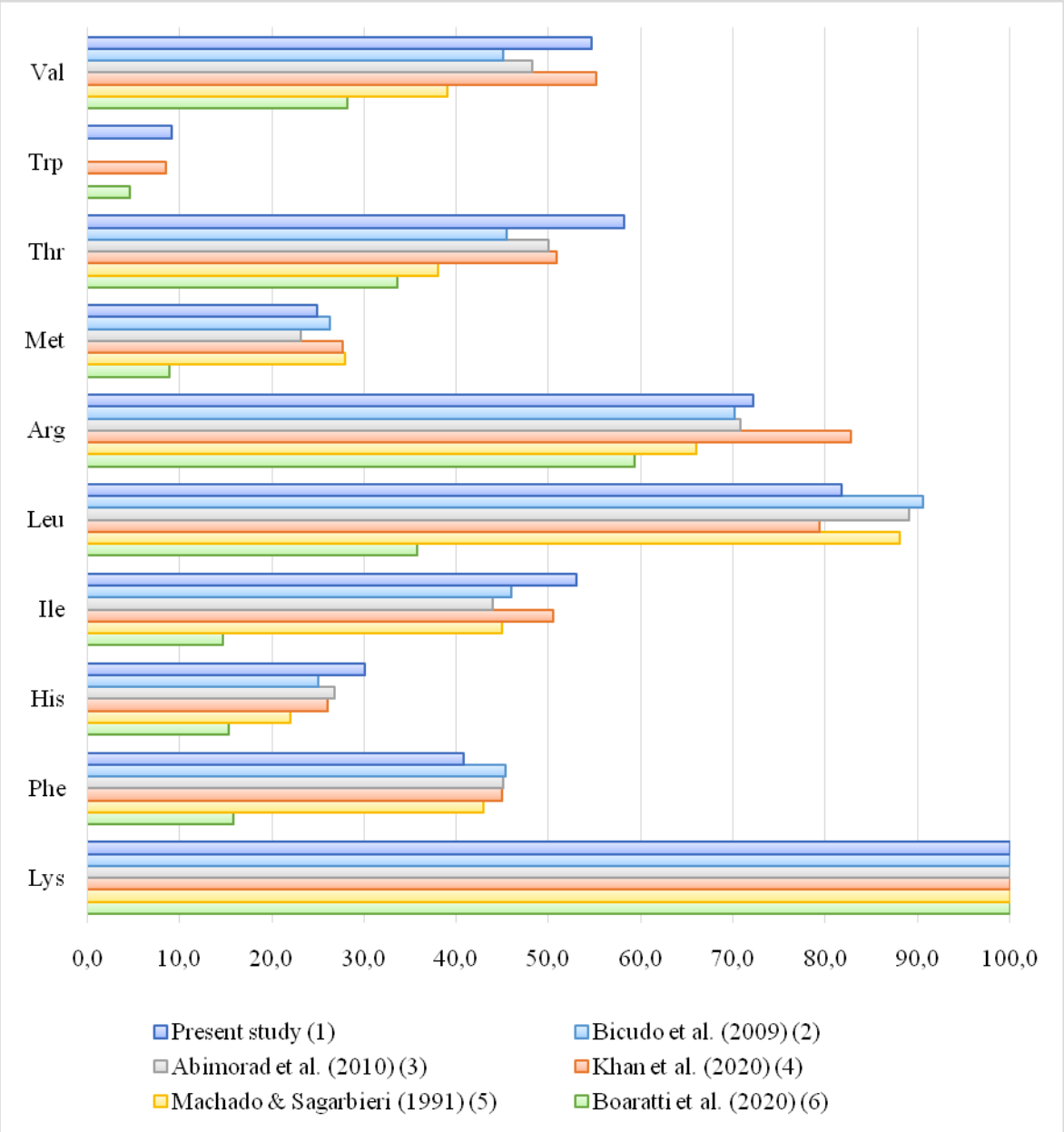

FIGURE 2 Ideal patterns of essential amino acids (EAAs) obtained in the present study and in other studies for pacu (*Piaractus mesopotamicus*): (1) the present amino acid (AA) deletion based study; (2) The EAA profile determined based on the relationship defined by Arai (1981); (3) The EAA profile determined by the muscle's amino acid composition; (4) The EAA profile determined by the amino acid composition of the whole body; (5) The EAA profile determined by the muscle's amino acid composition; (6) The EAA profile determined by the AA deletion method

CONSIDERAÇÕES FINAIS

Os resultados de retenção de nitrogênio obtidos mostram que a redução dos AAEs, em média 41,72%, afetou diretamente o desempenho zootécnico e a deposição de nitrogênio corporal no pacu adulto.

Para a formulação de dietas práticas e experimentais faz-se necessário que já tenha sido determinado a exigência de um dos aminoácidos essenciais, para ser utilizada o perfil determinado para cálculo de inclusão dos demais aminoácidos essenciais. O método de deleção, por ser uma ferramenta rápida, pode ser utilizada para espécies com potencial de produção que ainda não apresentam informações sobre suas exigências, principalmente peixes nativos, onde o conhecimento do nível de um único AAE permite a formulação de uma dieta balanceada.

O método apresenta vantagem de não ter influência de fatores internos ou externos, como pode-se observar em estudos de exigência, podendo ser aplicado para qualquer espécie. Portanto, o perfil encontrado no presente trabalho deve ser considerado para auxiliar a formulação de dietas para pacus adultos.

A padronização do método de deleção permitirá aos pesquisadores a realização de estudos com procedimentos rápidos e de baixo custo para estimar e avaliar a relação ideal entre os aminoácidos essenciais, não somente para o pacu nas diversas fases do crescimento, assim como para outras espécies de peixe.