Latin American Journal of Aquatic Research, 49(4): 608-619, 2021
DOI: 10.3856/vol49-issue4-fulltext-2678

Research Article

Digestible protein requirements for maintenance, growth, and efficiency of protein utilization in pacu (*Piaractus mesopotamicus*) juveniles: an exponential nitrogen utilization model

Cleber F.M. Mansano[1], Edney P. Silva[2], Kifayat U. Khan[3], Beatrice I. Macente[1]
Thiago M.T. Nascimento[3], Nilva K. Sakomura[2]
João B.K. Fernandes[3] & Leonardo S. Takahashi[4]
[1]Brazil University, Campus Fernandópolis, Fernandópolis, SP, Brazil
[2]Faculty of Agricultural and Veterinary Sciences (FCAV), São Paulo State University (UNESP) Jaboticabal, SP, Brazil
[3]Aquaculture Center, São Paulo State University (UNESP), Jaboticabal, SP, Brazil
[4]College of Agricultural and Technological Sciences (FCAT)
São Paulo State University (UNESP), Dracena, SP, Brazil
Corresponding author: Cleber F.M. Mansano (clebermansano@yahoo.com.br)

ABSTRACT. The present study aimed to estimate digestible protein (DP) requirements for maintenance and growth and assess the efficiency of protein utilization in pacu (*Piaractus mesopotamicus*) juveniles. A complete randomized design consisted of seven treatments, and five replicates (tanks) per treatment was used. After a one-week acclimation period, 350 pacu juveniles of 27.48 ± 1.8 g (initial average body weight - BW) were equally distributed among 35 tanks (10 fish per tank) of 450 L. Fish were fed with seven graded digestible protein levels (64.5, 111.8, 164.6, 217.1, 264.4, 316.2 and 369.5 g kg^{-1}) three times a day until apparent satiation for 120 days. The monomolecular model parameters including nitrogen maintenance requirement (NMR) (37.76 mg $BW^{0.67}$ kg^{-1} d^{-1}), daily nitrogen deposition (ND) (194.8 mg $BW^{0.67}$ kg^{-1} d^{-1}), and protein intakes (based on metabolic BW) of 1638, 1902, and 2241 mg $kg^{-1}BW^{0.67}$ d^{-1} for 75, 80 and 85% maximum theoretical nitrogen retention ($NR_{max}T$), respectively and the efficiency of protein utilization (529×10^{-6}) were estimated through the relationship between nitrogen intake (NI) and ND. The digestible protein requirements for 75, 80, and 85% of $NR_{max}T$ were estimated as 187, 217, and 256 g kg^{-1} respectively by the monomolecular model based on the daily feed intake of 2% of live BW of pacu juveniles.

Keywords: *Piaractus mesopotamicus*; feed intake; monomolecular model; maintenance requirement; nitrogen deposition; pacu nutrition

INTRODUCTION

Several feed formulations based on the feeding habit of fish have been developed, but still, there is a shortage of information regarding species-specific feeds. Pacu (*Piaractus mesopotamicus*) is one of the most farmed species in several countries of South America and East Asia (Food & Agriculture Organization 2020). Due to the omnivorous and herbivorous feeding habits, this species could efficiently utilize feeds with a wide range of nutritional compositions. The characteristics of better meat quality and easy adaptation to different types of rearing systems make it a suitable candidate for intensive, semi-intensive, and extensive production practices (Furlaneto et al. 2009, Silva et al. 2012, Rosa et al. 2016, Leonardo et al. 2018, Barros et al. 2020).

Aquafeeds contain optimum essential nutrients ratios required for normal muscle and body protein growth (NRC 2011). Several studies have been assessed the dietary protein requirements of fish, inclu-

Corresponding editor: Alvaro Bicudo

ding pacu (Fernandes et al. 2000, 2001, Carmo de Sá & Fracalossi 2002, Luo et al. 2004, Abimorad et al. 2007, Alam et al. 2008, Sá et al. 2008, Kim & Lee 2009, Bicudo et al. 2010, Signor et al. 2010, Zhang et al. 2010, Klein et al. 2014, Khan et al. 2020a,b). These studies have usually evaluated protein requirements through body weight gain, while little is known about establishing optimal dietary protein by considering the aspects of body nitrogen deposition and nitrogen retention and maintenance requirement, and efficiency of protein utilization (Liebert 2015). The latter approach is relatively practical as it may produce data that could be fitted later on to factorial models to estimate dietary nutrients requirements for obtaining any targeted performance (Silva et al. 2014, Fisher 2015, Sakomura & Rostagno 2016).

The monomolecular model considers three biologically important parameters, including maximum theoretical nitrogen retention ($NR_{max}T$), minimum nitrogen retention or nitrogen maintenance requirement (NMR) being genotype-specific (Samadi & Liebert 2006), and the efficiency of protein utilization which is expressed as "*b*" (Samadi & Liebert 2006, 2008). This model could be efficiently used to calculate protein requirements for maximum body protein retention by considering the daily protein intake needed for maintenance and body protein deposition. According to the monomolecular model, dietary factors do not affect the estimation of $NR_{max}T$ and NMR (Samadi & Liebert 2006, 2008) and the efficiency of protein utilization (Thong & Liebert 2004). Thus, using a factorial approach in pacu nutrition could be a suitable option for determining optimum dietary protein requirements due to the genetic variability among its commercial lots (Liebert et al. 2006, Samadi & Liebert 2006, Povh et al. 2009). In this context, the present study was conducted to estimate the digestible protein (DP) requirements for maintenance and growth by assessing protein utilization efficiency in pacu juveniles.

MATERIALS AND METHODS

The present 120-day experiment was conducted at the Aquaculture Center, São Paulo State University (UNESP), Jaboticabal, SP, Brazil. The experiment was done according to the Brazilian College of Animal Experimentation (COBEA) ethical guidelines. The materials and methods (protocol #009999/14) used in the present trial were approved by the Ethics Committee on Animal Use (CEUA) of the Faculty of Agricultural and Veterinary Sciences (FCAV), São Paulo State University (UNESP), Jaboticabal, SP, Brazil.

After the acclimation period of one week, 350 healthy juvenile fish were equally distributed among 35 polyethylene tanks of 450 L. The experiment was performed in an open flow-through system, provided with continuous aeration, supply, and water drainage with a renewal rate of 1 L min^{-1}. The freshwater water for experimental tanks was obtained from a tube well throughout the experiment. A completely randomized design which consisted of seven treatments and five replicates (as tanks) per treatment, was used. A total of 10 pacu juveniles of 27.48 ± 1.8 g (initial average body weight) were stocked per tank, maintaining the stocking density of 22.2 fish m^{-3}. During the 120 day feeding trial, fish were fed with experimental diets three times a day at 08:00, 12:00, and 17:00 h until apparent satiation. The photoperiod maintained during the experiment was: 12 h light and 12 h darkness.

The water temperature (average, 28.70 ± 0.86ºC), dissolved oxygen (average, 5.50 ± 0.42 mg L^{-1}), and pH (average, 7.10 ± 0.22) were measured daily throughout the experiment. The total ammonia (average, 118.36 ± 19.56 μg L^{-1}) was measured once a week using the colorimetric method (Golterman et al. 1978). The water quality data observed during this experiment were found within the range established by Kubitza (2017) and Khan et al. (2020a,b) for the successful farming of aquatic animals, including pacu, in intensive rearing systems.

Experimental diets

The experimental diets were formulated according to the diet dilution technique. Two basal diets, including a concentrated diet with 369.50 g kg^{-1} DP (higher protein concentration) and another protein-free diet (PFD), were formulated (Table 1). The concentrated diet had an excess of all essential amino acids - EAAs (1.5 times), but an ideal relationship among EAAs determined by Abimorad et al. (2010) was maintained. For the preparation of two basal diets, the ingredients required for each diet were weighed, mixed well in a twin v-shell blender of 100 kg capacity, and ground in a single centrifugal mill (Model MCS 280, Moinhos Viera, Tatuí, Brazil) equipped with a 0.03 mm sieve screen.

The experimental diets containing seven graded levels (64.5, 111.8, 164.6, 217.1, 264.4, 316.2, and 369.5 g kg^{-1}) of DP were obtained by the successive dilution of the concentrated diet with the PFD (Tables 1-2). The experimental diets were kept isocaloric according to the recommendation of Abimorad et al. (2007). Each experimental diet was extruded into 4 to 6 mm diameter pellets under identical extrusion conditions through a single screw extruder (Ex Micro Model, Exteec, Ribeirão Preto, Brazil) with a 20 kg h^{-1}

Table 1. Formulation of experimental diets and a protein-free diet (g kg^{-1}). [1]Digestibility according to Abimorad et al. (2008), Gonçalves & Cyrino (2014), Fabregat et al. (2008). [2]Protein-free diet. [3]Protein-rich diet. [4]Moisture (%) 2.0, ash (%) 71.6442, choline (mg kg^{-1}) 30,000, magnesium (%) 0.0085, sulfur (%) 1.1589, iron (mg kg^{-1}) 25,714, copper (mg kg^{-1}) 1.960, manganese (mg kg^{-1}) 13,345, zinc (mg kg^{-1}) 30,000, iodine (mg kg^{-1}) 939, selenium (mg kg^{-1}) 30, vitamin A (IU kg^{-1}) 600,000, vitamin D3 (IU kg^{-1}) 600,000, vitamin E (mg kg^{-1}) 12.000, vitamin K3 (mg kg^{-1}) 631, thiamine B1 (mg kg^{-1}) 1176, riboflavin B2 (mg kg^{-1}) 1536, pyridoxine B6 (mg kg^{-1}) 1274, vitamin B12 (mg kg^{-1}) 4000, niacin (mg kg^{-1}) 19,800, pantothenic acid B3 (mg kg^{-1}) 3920, folic acid (mg kg^{-1}) 192, biotin (mg kg^{-1}) 20, vitamin C (mg kg^{-1}) 40,250.

Ingredients	Digestible protein (DP)[1]							
	0.0[2]	64.5	111.8	164.6	217.1	264.4	316.2	369.5[3]
Soybean meal	-	42.5	88.1	133.8	179.4	225.0	270.7	316.5
Corn grain	-	36.0	74.8	113.5	152.2	190.9	229.6	268.5
Fish meal	-	20.1	41.8	63.4	85.0	106.6	128.3	150.0
Corn gluten	-	18.8	39.0	59.2	79.4	99.5	119.7	140.0
Wheat meal	-	13.4	27.8	42.3	56.7	71.1	85.5	100.0
Starch	862.0	746.3	622.0	497.7	373.4	249.1	124.8	-
Soybean oil	45.0	39.0	32.5	26.0	19.5	13.0	6.5	-
Cellulose	39.0	33.8	28.1	22.5	16.9	11.3	5.6	-
Dicalcium phosphate	21.5	18.6	15.5	12.4	9.3	6.2	3.1	-
Lysine	-	1.8	3.8	5.7	7.7	9.6	11.5	13.5
Valine	-	0.4	0.8	1.3	1.7	2.1	2.6	3.0
Sugar	13.0	11.3	9.4	7.5	5.6	3.8	1.9	-
Limestone	11.0	9.5	7.9	6.4	4.8	3.2	1.6	-
Mineral and vitamin supplement[4]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Antifungal	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
BHT	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Table 2. Dilution of the protein-rich diet with the protein-free diet to obtain seven experimental diets and nutritional composition. [1]Diets used for dilution. [2]Protein-free diet. [3]Concentrated diet. [4]Digestibility according to Abimorad et al. (2008), Gonçalves & Cyrino (2014), and Fabregat et al. (2008). [5]Etheridge et al. (1998). [6]Determined by bomb calorimetry (AOAC 2016). [7]Acid hydrolysis (AOAC 2016). [8]Acid hydrolysis and ion-exchange chromatography (High-Performance Liquid Chromatography - HPLC).

Digestible protein (DP)	Diets[1]	64.5	111.8	164.6	217.1	264.4	316.2	369.5
Dilution (%)	0.0[2]	86.57	72.15	57.73	43.32	28.90	14.48	0.0
	369.5[3]	13.43	27.85	42.27	56.68	71.10	85.52	100.0
Analyzed composition (dry matter basis)								
Crude protein (g kg^{-1})[5]	-	71.9	124.4	183.3	241.8	294.5	352.2	411.5
Digestible protein (g kg^{-1})[4]	-	64.5	111.8	164.6	217.1	264.4	316.2	369.5
Gross energy (KJ)[6]	15.55	15.41	15.57	15.47	15.63	15.49	15.66	15.72
Digestible energy (KJ)[4]	13.51	13.89	14.03	13.93	14.08	13.95	14.11	14.16
Crude ether extract (g kg^{-1})[7]	48.2	46.0	45.3	45.5	44.8	44.6	45.5	45.1
Essential amino acids digestible (dry matter basis) (g kg^{-1})[4,8]								
Arginine	-	3.0	6.2	9.4	12.6	16.0	18.5	22.3
Histidine	-	1.2	2.5	3.7	5.1	6.5	7.6	9.1
Isoleucine	-	2.0	4.1	6.2	8.5	10.7	12.7	15.0
Leucine	-	4.8	9.6	14.3	19.4	24.5	29.1	34.4
Lysine	-	3.6	7.3	11.1	14.9	18.6	21.5	25.7
Methionine	-	0.9	1.9	2.8	3.9	4.8	5.7	6.9
Phenylalanine	-	2.5	5.0	7.5	10.1	12.7	15.0	17.8
Threonine	-	1.9	4.0	5.9	8.0	10.1	11.8	14.1
Valine	-	2.8	5.6	8.5	11.6	14.6	16.8	19.9

average extrusion capacity. The temperature of the extruder was maintained above 90°C. After extrusion, the feed pellets were dried in an oven with forced air circulation at 55°C for 24 h. The dried pellets were removed from the oven, cooled at room temperature, and kept in a freezer at -20°C.

Growth and feeding parameters

The growth and feeding parameters assessed during the present experiment included: initial body weight (g) (BW_I); final body weight (g) (BW_F); feed intake (g) (FI) = (total quantity of feed offered to fish); feed conversion ratio (FCR) (g g^{-1}) = [(feed intake) / (live weight gain)]; and specific growth rate (SGR% per day) = [(ln final weight – ln initial weight) × 100/ (time (d)].

Estimation of nitrogen intake (NI), nitrogen deposition (ND), and nitrogen excretion (NEX)

A representative sample of 20 fish was collected at the start of the experiment to analyze the initial whole body composition. Again at the end of the trial, three fish per replicate (15 fish per treatment) were sampled to analyze the final whole body composition. Before each sampling procedure, fish were subjected to a 48 h fasting period and then anesthetized with benzocaine (ethyl-p-aminobenzoate) solution (60 mg L^{-1}) (Sigma-Aldrich, Brazil). The sampled fish were immediately frozen for the subsequent grinding process. After grinding in a meat grinder, the samples obtained were freeze-dried, lyophilized, and once again ground in a ball-grinder for proper homogenization. Subsequently, the dry matter, nitrogen (crude protein), lipid, and ash contents were determined in the homogenized samples.

Crude protein was determined according to the method described by Etheridge et al. (1998). The Soxhlet apparatus method was used to determine lipid content while ash content was determined in a muffle at 550°C by the incineration (AOAC 2016). Dry matter was obtained by putting the samples in an oven at 105ºC for 12 h, according to Silva & Queiroz (2002) method.

The following formulas calculated the performance parameters according to Liebert (2015) and Sakomura & Rostagno (2016):

Total protein intake (PI) per fish = protein quantity (%) in the diet × feed intake per fish / 100;

PI (mg $fish^{-1}$ d^{-1}) = [PI per fish / 120 × 1000];

Nitrogen intake (NI) (mg $fish^{-1}$ d^{-1}) = [PI (mg $fish^{-1}$ d^{-1}) / 6.25];

$BW^{0.67}$ kg^{-1} = [((average of initial and final body weight) / $1000)^{0.67}$];

NI mg $BW^{0.67}$ kg^{-1} d^{-1} = [NI (mg $fish^{-1}$ d^{-1}) / $BW^{0.67}$ kg^{-1}];

Nitrogen deposition (ND) (mg $fish^{-1}$ d^{-1}) = [(((final body (FB) weight (BW_F)) × (%CP in FB)) - (initial body (IB) weight (BW_I)) × (%CP in IB / 100))) / 6.25) × 1000) / 120];

ND mg $BW^{0.67}$ kg^{-1} = [ND (mg $fish^{-1}$ d^{-1}) / $BW^{0.67}$ kg^{-1}]; and

Nitrogen excretion (NEX) = [NI (mg $BW^{0.67}$ kg^{-1} d^{-1}) - ND (mg $BW^{0.67}$ kg^{-1})]

Estimation of maximum theoretical nitrogen retention ($NR_{max}T$), maximum theoretical nitrogen deposition ($ND_{max}T$), nitrogen maintenance requirement (NMR), and efficiency of protein utilization "*b*".

The monomolecular equation (Eq. 1) described by Liebert (2015) was used to determine the $NR_{max}T$ and NMR:

$$ND = NR_{max}T(1-e^{-b.NI}) - NMR \quad \text{(Eq. 1)}$$

where ND is the daily N deposition (mg $BW^{0.67}$ kg^{-1}), $NR_{max}T$ is the daily maximum theoretical N retention (mg $BW^{0.67}$ kg^{-1}), *b* is the slope of N retention curve, NI is the daily N intake (mg $BW^{0.67}$ kg^{-1}), and NMR is the daily N maintenance requirement (mg $BW^{0.67}$ kg^{-1}).

NMR was estimated through the relationship between NEX on Y-axis and NI on X-axis according to the following equation of Samadi & Liebert (2006);

$$NEX = NMRe^{b.NI} \quad \text{(Eq. 2)}$$

The NMR is a point of intersection on the Y-axis for NI = 0. It is based on a nitrogen-free feeding state simulation by the nonlinear approximation of the dose-response curve between NI and NEX. The exponent 0.67 was used to calculate the metabolic body weight according to Gebhardt (1980), and *"e"* is the Euler's number. The $NR_{max}T$, *b*, and NMR parameters were estimated using the modified Newton Gauss algorithm through the NLIN (nonlinear regression) procedure in SAS (2014).

The maximum daily protein deposition (mg $BW^{0.67}$ kg^{-1}) was calculated based on NMR, $NR_{max}T$, and $PD_{max}T$ ($ND_{max}T$ × 6.25).

The assumptions were made to predict daily feed intake of 1, 2, and 3% of live BW, a common observation at fish farms. The daily maximum theoretical nitrogen deposition (NDmaxT) was calculated at 75, 80, and 85% of NRmaxT, respectively. The NI, when multiplied by $BW^{0.67}$ kg^{-1}, was changed to mg d^{-1}. The concentration of dietary protein in percentage was calculated as it is a more practical approach.

Statistical analysis

The performance data obtained during the present experiment were evaluated through the one-way analysis of variance, and when the F test showed any statistical significance, the Duncan test ($P < 0.05$) was used for the multiple comparisons among treatment means. The statistical analysis was performed by using the SAS program (2014).

RESULTS

The growth performance and feeding parameters of pacu (*Piaractus mesopotamicus*) juveniles fed with six graded DP levels are shown (Table 3). Fish fed with 316.2 and 369.5 g kg^{-1} DP showed higher BW and FI. The SGR was higher in groups of fish fed with 264.4, 316.2, and 369.5 g kg^{-1} DP. The FCR was significantly ($P < 0.05$) decreased with the increase in DP level.

Pacu whole body composition fed with graded DP levels is shown in Table 4. The dry matter, ash, crude protein, and fat contents were significantly ($P < 0.05$) influenced by DP. According to the obtained results, dry matter, crude fat, and ash contents significantly ($P < 0.05$) decreased while crude protein content significantly ($P < 0.05$) increased with the increase in dietary protein level.

The NI, metabolic BW ($BW^{0.67}$ kg^{-1}), ND, NEX, and NR data are shown in Table 5. Fish consumed diet with 369.5 g kg^{-1} DP showed higher NI, metabolic BW, ND, NEX, and NR. The NMR was estimated as 37.76 mg $BW^{0.67}$ kg^{-1} $d^{-1,}$ as shown (Fig. 1). The values estimated for $NR_{max}T$ and $ND_{max}T$ are shown (Fig. 2). The $NR_{max}T$ and $ND_{max}T$ values tended to stabilize when they reached close to the maximum potential for nitrogen intake, exploring the behavior of maximum daily nitrogen deposition and maximum daily nitrogen retention (194.8 mg $BW^{0.67}$ kg^{-1} d^{-1}) over the test dietary protein range (Fig. 2).

The model used in the present study presented some peculiarities; thus, before calculating the $ND_{max}T$ threshold value, it is recommended to estimate the daily NMR through the relationship between NI and NEX as shown in Figure 1. NMR is not considered a true maintenance requirement in the monomolecular model but rather a part of the total nitrogen retention ($NR_{max}T = ND_{max}T + NMR$), indicating that ND > 0. Therefore, in this case, the influence of dietary factors on NMR cannot be completely excluded. The NMR data obtained by the monomolecular model are not used to derive maintenance requirements, but they provide a fixed point on the NMR curve (breakpoint on Y-axis = NMR). This process is iterative and facilitated when the Gauss-Newton algorithm is used.

The protein intakes based on 1.54% live BW per day (average value) observed in this experiment (Table 6) were found close to the dietary protein requirements calculated for pacu juveniles (Table 7), which may indicate that the concentration of protein in the diet could be decreased in response to the increased feeding performance in fish. By fitting the obtained data to the monomolecular equation, the protein concentration (mg d^{-1}) required for 80% $NR_{max}T$ based on the feed intake of 2% live BW was estimated as 356 mg d^{-1}, with the optimum DP concentration of 217 g kg^{-1} diet (Table 7). The monomolecular model was used in a linear way (Eq. 3) to estimate NI, and when this output was compared with the data presented in Table 6, it was revealed that the model has correctly estimated the nitrogen intake.

DISCUSSION

The body weight gain, feed intake, specific growth rate, and feed conversion ratio were significantly improved over the increased levels of DP. The diets with 264.4 to 369.5 g kg^{-1} DP resulted in similar growth and feeding parameters of fish, but these results were found better than those observed by Fernandes et al. (2001) in pacu juveniles. Fernandes et al. (2001) evaluated three crude protein levels (such as 180, 220, and 260 g kg^{-1}) with the 4200 kcal kg^{-1} crude energy and did not observe any significant difference in BWG among the treatments. However, the results of this study were found close to the findings of Fernandes et al. (2000) in pacu fingerlings, where three levels of crude protein, 220, 260, and 300 g kg^{-1}, were evaluated with 4200 kcal kg^{-1} crude energy and better BWG was observed in groups of fish fed with 260 and 300 g kg^{-1} CP. Bicudo et al. (2010) evaluated five levels of crude protein (220, 260, 300, 340, and 380 g kg^{-1}) and recommended 270 g kg^{-1} with an optimum crude protein to digestible energy (CP:DE) of 5305.92 kcal kg^{-1}. According to Abimorad & Carneiro (2007), being a species of tropical climate with frugivorous and herbivorous feeding behavior, pacu uses lipids and carbohydrates as effectively as protein and recommended 250 g kg^{-1} DP, 40 g kg^{-1} fat, and 460 g kg^{-1} carbohydrates for obtaining better growth of juvenile pacu.

The whole body crude protein content (Table 3) of pacu over the graded levels of DP showed the same behavior as that of the growth and feeding parameters. For optimum protein deposition, animals need a sufficient amount of dietary protein and energy with a favorable rearing environment to express their maximum genetic potential to uptake the limiting nutrient. Otherwise, the balance between synthesis and degradation of protein in the animal body will be disturbed, restricting the animals from expressing their maximum genetic potential regarding protein deposition (Ferguson 2006).

Higher body fat deposition was observed in fish fed with lower DP levels. Emmans (1981) introduced the concept of "desirable fat growth" to quantify the relationship among body protein, fat, and controlled feed intake. Kyriazakis & Emmans (1999) and Ferguson & Theeruth (2002) observed that excess body fat content could be reduced by avoiding inadequate

Table 3. Performance variables of pacu (*Piaractus mesopotamicus*) fed with graded levels of digestible protein (DP). [1]Mean ± standard deviation, n = 5. Superscripts letters differentiate averages in the same column by Duncan's test ($P < 0.05$).

DP ($g\ kg^{-1}$)	Average body weight (g)		Feed intake (g)	Feed conversion ratio ($g\ g^{-1}$)	Specific growth rate (%)
	Initial	Final			
64.5	27.39 ± 0.89[1]	42.7 ± 2.7^{e}	77.4 ± 6.4^{c}	5.26 ± 1.37^{e}	0.37 ± 0.07^{e}
111.8	27.73 ± 0.74	71.1 ± 5.6^{d}	109.8 ± 9.6^{b}	2.55 ± 0.22^{d}	0.78 ± 0.08^{d}
164.6	27.56 ± 0.46	93.1 ± 18.8^{c}	113.3 ± 18.1^{b}	1.77 ± 0.22^{c}	1.00 ± 0.05^{c}
217.1	27.38 ± 0.52	106.9 ± 12.5^{cb}	110.6 ± 11.2^{b}	1.40 ± 0.12b^{c}	1.13 ± 0.07^{b}
264.4	27.19 ± 0.51	120.4 ± 14.7^{b}	119.8 ± 18.5^{b}	1.29 ± 0.07^{a}	1.23 ± 0.11^{a}
316.2	27.72 ± 0.48	145.9 ± 19.1^{a}	144.5 ± 21.6^{a}	1.22 ± 0.07^{a}	1.24 ± 0.10^{a}
369.5	27.41 ± 0.23	145.7 ± 8.4^{a}	145.8 ± 7.05^{a}	1.22 ± 0.07^{a}	1.24 ± 0.04^{a}
P-value	0.829	<0.0001	<0.0001	<0.0001	<0.0001

Table 4. Body composition of pacu (*Piaractus mesopotamicus*) fed with graded levels of digestible protein (DP) for 120 days. [1]Mean ± standard deviation, n = 5. Superscripts letters differentiate averages in the same column by Duncan's test ($P < 0.05$).

Diet, DP ($g\ kg^{-1}$)	Whole-body composition ($g\ kg^{-1}$ of dry matter)			
	Dry matter	Crude protein	Crude fat	Ash
Initial	216.3	597.9	230.8	56.6
64.5	400.1 ± 3^{a}	309.5 ± 16^{f}	533.1 ± 20^{a}	58.3 ± 3^{ab}
111.8	372.6 ± 20^{b}	344.4 ± 8^{e}	519.9 ± 26^{a}	62.5 ± 2^{a}
164.6	345.2 ± 8^{c}	399.4 ± 5^{d}	450.9 ± 12^{b}	57.2 ± 3^{b}
217.1	310.9 ± 12^{d}	463.4 ± 7^{c}	374.1 ± 9^{c}	57.6 ± 2^{b}
264.4	297.3 ± 15de	509.2 ± 18^{b}	332.1 ± 21^{d}	53.9 ± 3^{b}
316.2	285.5 ± 14^{e}	530.7 ± 12^{a}	318.5 ± 20^{d}	53.5 ± 4^{b}
369.5	298.6 ± 5de	530.7 ± 8^{a}	22.1 ± 9^{d}	55.6 ± 3^{b}
P-value	<0.0001	<0.0001	<0.0001	<0.0044

Table 5. Nitrogen intake, nitrogen deposition, and nitrogen retention data for pacu (*Piaractus mesopotamicus*) fed with graded levels of digestible protein (DP) for 120 days. [1]Mean ± standard deviation, n = 5. Superscripts letters differentiate averages in the same column by Duncan's test ($P < 0.05$) NI: Nitrogen intake. ND: Nitrogen deposition. NEX: Total nitrogen excretion. NR: Nitrogen retention.

Diet, DP ($g\ kg^{-1}$)	NI ($mg\ d^{-1}$)	$mg\ BW^{0,67}\ kg^{-1}\ d^{-1}$	NI $mg\ BW^{0,67}\ kg^{-1}\ d^{-1}$	ND $mg\ BW^{0,67}\ kg^{-1}\ d^{-1}$	NEX $mg\ BW^{0,67}\ kg^{-1}\ d^{-1}$	NR $mg\ BW^{0,67}\ kg^{-1}\ d^{-1}$
64.5	6.66 ± 0.5^{g}	0.11 ± 0.002^{e}	62.89 ± 4.6^{g}	22.99 ± 1.8^{g}	39.91 ± 5.9^{f}	60.69 ± 1.8^{g}
111.8	16.37 ± 1.3^{f}	0.13 ± 0.004^{d}	122.71 ± 7.3^{f}	55.37 ± 6.9^{f}	67.35 ± 4.5^{e}	93.07 ± 6.9^{f}
164.6	24.88 ± 3.5^{e}	0.15 ± 0.014^{c}	162.94 ± 8.1^{e}	79.29 ± 11.7^{e}	83.64 ± 7.4^{e}	116.99 ± 11.7^{e}
217.1	32.04 ± 2.9^{d}	0.16 ± 0.009^{bc}	195.55 ± 9.7^{d}	93.90 ± 10.6^{d}	101.65 ± 8.8^{d}	31.60 ± 10.6^{d}
264.4	42.25 ± 5.9^{c}	0.17 ± 0.010^{b}	241.43 ± 21.3^{c}	108.58 ± 9.8^{c}	132.85 ± 13.8^{c}	146.28 ± 9.8^{c}
316.2	60.92 ± 8.2^{b}	0.19 ± 0.013^{a}	312.35 ± 22.5^{b}	121.81 ± 3.5^{b}	190.55 ± 20.5^{b}	159.51 ± 3.5^{b}
369.5	76.78 ± 3.1^{a}	0.20 ± 0.006^{a}	381.09 ± 13.1^{a}	135.19 ± 4.0^{a}	245.89 ± 15.6^{a}	172.89 ± 4.0^{a}
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

diets. When the animals are fed with a balanced diet controlled under favorable conditions, the relationship between feed intake, body protein, and fat deposition remains constant. Bicudo et al. (2010) stated that increasing energy content in the diet of pacu juveniles had no protein-sparing effect and contributed to an increased body fat content.

The daily NMR was calculated as 37.76 mg $BW^{0.67}$ kg^{-1} for pacu juveniles with 27 to 145 g live body weights. Like other animals, fish need some nitrogen (for maintenance purposes) to recover the continuous endogenous nitrogen losses (Liebert et al. 2006). The nitrogen excretion pathway was modeled by the exponential function, which showed that NMR in pacu

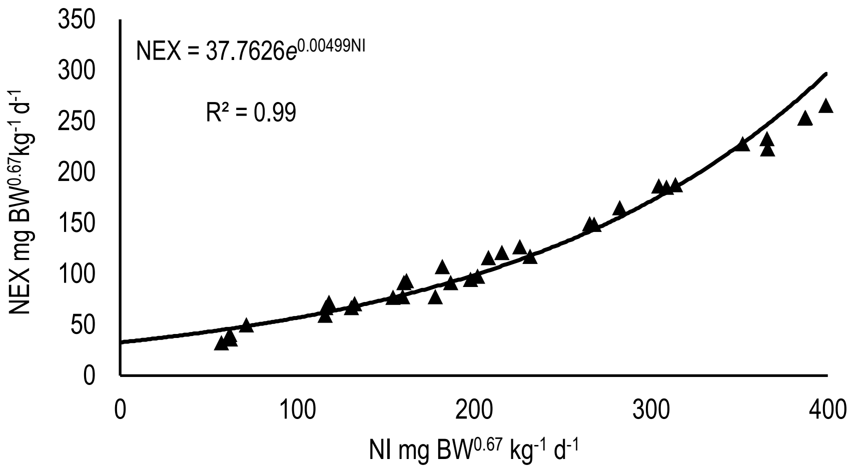

Figure 1. The nitrogen maintenance requirement (NMR) is estimated through the relationship between nitrogen excretion (NEX) at the Y-axis and NI at the X-axis in pacu (*Piaractus mesopotamicus*) juveniles, adjusted by the exponential function. The model's intercept was considered a nitrogen maintenance requirement (NMR = 38 mg $BW^{0.67}$ kg^{-1} d^{-1}).

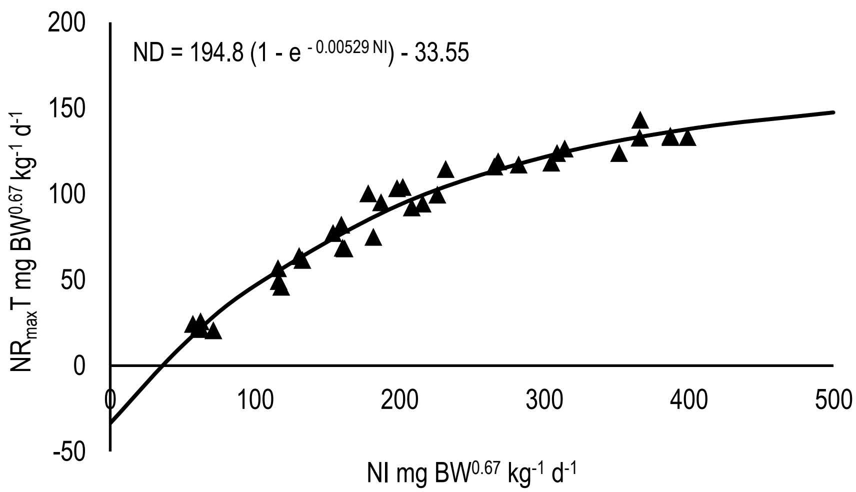

Figure 2. Estimation of the daily maximum theoretical nitrogen retention ($NR_{max}T$) in pacu juveniles fed with graded levels of digestible protein (64.5, 111.8, 164.6, 217.1, 264.4, 316.2, and 369.5 g kg^{-1}) through the monomolecular model of Liebert (2015).

is the NEX (Y-axis) intercept, so it provides a practical way of determining the NMR regardless of fish age or size. The minimum N retention (protein synthesis) in response to protein intake occurs in a nitrogen balance state. After fulfilling the requirements for maintenance (minimum N retention), animals try to deposit a sufficient amount of protein in their body over the fixed energy utilization in normal conditions (Reeds & Lobley 1980). This model was developed by Gebhardt (1966, 1980) and has already been used in several animal species such as poultry and pig (Thong & Liebert 2004, Samadi & Liebert 2008) as well as fish (Sünder & Liebert 2005, Liebert et al. 2006).

The NMR determined in this study for juvenile pacu (37.76 mg $BW^{0.67}$ kg^{-1}) was validated by replacing it with the NMR established for Nile tilapia (*Oreochromis niloticus*) (NMR = 70 mg N $BW^{0.67}$ kg^{-1} d^{-1}). As a result, the estimated values of optimal protein intakes increased from 12 to 19%, suggesting that NMR must be determined accurately in a given species; otherwise, the results will be adversely affected. Ogino & Chen (1973) determined the NMR of 152 mg N $BW^{0.67}$ kg^{-1} d^{-1} in common carp (*Cyprinus carpio*). Kaushik et al. (1981) concluded the NMR of 256 mg $BW^{0.67}$ kg^{-1} d^{-1} in rainbow trout (*Oncorhynchus mykiss*). Gatlin III et al. (1986) observed the NMR of 208 mg $BW^{0.67}$ kg^{-1} d^{-1} in catfish (*Ictalurus punctatus*).

Table 6. The observed feed intake (FI) and nitrogen intake (NI) and estimated nitrogen intake (NI) in response to nitrogen deposition (ND) in pacu (*Piaractus mesopotamicus*) fed with graded levels of digestible protein (DP) for 120 days. [1]Equation 3. [2]Mean ± standard deviation, n = 5.

Diet, DP ($g\ kg^{-1}$)	Feed intake % of BW	NI $BW^{0.67}\ kg^{-1}$ observed	NI $BW^{0.67}\ kg^{-1}$ monomolecular[1]
64.5	1.84 ± 0.14[2]	62.89 ± 4.6	64.74 ± 2.5
111.8	1.85 ± 0.10	122.71 ± 7.3	115.53 ± 12.2
164.6	1.57 ± 0.04	162.94 ± 8.1	165.64 ± 9.1
217.1	1.37 ± 0.06	195.55 ± 9.7	202.75 ± 8.2
264.4	1.35 ± 0.09	241.43 ± 21.3	250.12 ± 14.2
316.2	1.38 ± 0.07	312.35 ± 22.5	302.30 ± 16.8
369.5	1.42 ± 0.06	381.09 ± 13.1	382.36 ± 12.9

Table 7. Estimated protein requirements for pacu (*Piaractus mesopotamicus*) juveniles based on different daily feed intakes and protein deposition rates. [1]Considered to model protein requirements at different growth rates (75, 80, and 85%) for the asymptotic response of $NR_{max}T$ and $ND_{max}T$. [2]Predicted feed intake of 1% BW. [3]Predicted feed intake of 2% BW. [4]Predicted feed intake of 3% BW. $NR_{max}T$: maximum theoretical nitrogen retention. $ND_{max}T$: maximum theoretical nitrogen deposition. BW: body weight.

	Monomolecular model		
Level of performance (% $ND_{max}T$)[1]	75	80	85
Level of performance mg $BW^{0.67}\ kg^{-1}$	146	156	165
Daily protein deposition ($mg\ d^{-1}$) [BW = 82 g]	171	182	193
Dietary protein efficiency (*b*)	0.005	0.005	0.005
Protein requirements estimated as inputs for the monomolecular model			
mg $BW^{0.67}\ kg^{-1}\ d^{-1}$	1638	1902	2241
mg d^{-1} [BW = 82 g]	307	356	420
Optimal protein concentrations ($g\ kg^{-1}$) estimated for maximum growth			
$g\ kg^{-1}$ of feed[2]	374	434	512
$g\ kg^{-1}$ of feed[3]	187	217	256
$g\ kg^{-1}$ of feed[4]	125	145	171

Wilson (1989) proposed an NMR of 160 mg $BW^{0.67}\ kg^{-1}\ d^{-1}$. Zhu et al. (2009) determined the NMR in juvenile black porgy (*Acanthopagrus schlegeli*) at different body sizes, such as the NMR in 50 g fish as 172 mg, in fish of 80 g as 152 mg, in fish of 120 g as 136 mg, in fish of 160 g as 126.4 mg and in fish of 200 g as 120 mg $BW^{0.67}\ kg^{-1}\ d^{-1}$. Sadasivam & Teles (1985) in rainbow trout estimated the NMR as 94.4 mg $BW^{0.67}\ kg^{-1}\ d^{-1}$, Carter & Brafield (1992) estimated as 99.2 to 139.2 mg $BW^{0.67}\ kg^{-1}\ d^{-1}$ in grass carp (*Ctenopharyngodon idella*), and Xian & Zhu (2002) in bastard halibut (*Paralichthys olivaceus*) estimated as 59.2 mg $BW^{0.67}\ kg^{-1}\ d^{-1}$. Liebert et al. (2006) estimated the NMR in Nile tilapia of different genotypes as 70 mg $BW^{0.67}\ kg^{-1}\ d^{-1}$. The wide differences in the NMR among different fish species may be due to differences in species, feeding habits, size, or rearing environments.

The $NR_{max}T$ and $ND_{max}T$ obtained by the monomolecular model are shown (Fig. 2), and the results are summarized in Table 4. In this study, the $NR_{max}T$ and $ND_{max}T$ values tended to stabilize when they reached close to the maximum potential for nitrogen intake, indicating the response of maximum daily nitrogen deposition and maximum daily nitrogen retention (194.8 mg $BW^{0.67}\ kg^{-1}\ d^{-1}$) over the protein intakes (Fig. 2). $NR_{max}T$ is usually characterized by defining the physiological limit of a productive species. Thus, the monomolecular model (Liebert 2015) presents a level with an infinite threshold (Samadi & Liebert 2008), which shows that there will always be a limitation (either biological or physical) in the rearing environment.

In the present study, the initial growth phase of pacu was selected to evaluate its nitrogen retention capacity, as fish, including pacu in this phase, are usually more effective in utilizing the dietary nutrients (Moraes & Bidinotto 2008). Carter & Houlihan (2001) observed that protease activity in small fish's digestive system was higher than in old ones. The lower protein catabolism and higher protein synthesis in the muscle tissue of juvenile fish is another important influencing factor in protein requirement studies (Nemova et al. 2016).

Animals generally could not achieve their maximum theoretical nitrogen retention due to several factors such as genetics, nutrition, health, and the rearing environment. The present nitrogen metabolism data (NMR, $ND_{max}T$, and $NR_{max}T$) are important tools for future model application studies, particularly on amino acid requirements investigations that depend on protein deposition.

The protein requirements determined in the present study are presented in Table 6. For the viable performance of fish, protein intakes were estimated at 75, 80, and 85% of $NR_{max}T$, respectively, based on a daily feed intake of 2% of live body weight. The feed intake observed in this study was used to calculate optimum protein intake (in mg) per day. The dietary protein requirements decreased with the increase in feed intake and growth of fish. The protein requirements (based on metabolic BW) were estimated as 1638, 1902, and 2241 mg $BW^{0.67}$ kg^{-1} by the monomolecular model for pacu juveniles (Table 7). These data are important for the development of a balanced diet for the species under study.

Protein concentration in animal feed is influenced by several factors, including feed intake and rearing systems. So, it is very important from a commercial point of view to determine the protein requirements of animals in intensive, semi-intensive, and extensive rearing systems. The available data on optimal dietary protein intakes in pacu show great variation (180 to 360 g kg^{-1} of crude protein) (Fernandes et al. 2000, 2001, Abimorad & Carneiro 2007, Bicudo et al. 2010). In all previous studies, the conclusions were mostly based on weight gain, while the concept of controlled feed intake has been neglected. Although the present study was conducted in laboratory-based intensive rearing conditions, the investigation of protein requirements in semi-intensive and extensive conditions would be worthy. This study describes a direct approach to determine the maximum nitrogen (protein) deposition potential and uses these data to estimate protein requirements based on daily feed intake and body nitrogen (protein) deposition.

CONCLUSIONS

The prediction of feed intake per day based on the live body weight of an animal and daily protein deposition is a significant influencing factor in estimating optimal protein concentrations to be supplemented in practical diets. In the present study, the parameters of the monomolecular model, i.e. nitrogen maintenance requirement (37 mg $BW^{0.67}$ kg^{-1} d^{-1}), maximum theoretical nitrogen deposition (194.8 mg $BW^{0.67}$ kg^{-1} d^{-1}), protein intakes based on metabolic body weight (1.638, 1.902 and 2.241 mg $BW^{0.67}$ kg^{-1} d^{-1} at 75, 80 and 85% of $NR_{max}T$, respectively) and the efficiency of protein utilization "*b*" (529×10^{-6}) were estimated through the relationship between nitrogen (protein) intake and nitrogen (protein) deposition. The digestible protein requirements were estimated as 187, 217, and 256 g kg^{-1} for obtaining $NR_{max}T$ of 75, 80, and 85%, respectively, based on the daily feed intake of 2% live body weight of pacu juveniles.

ACKNOWLEDGMENTS

We thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for grants (1525577) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for financial support (2013/25761-4 and 2016/11016-3).

REFERENCES

Abimorad, E.G. & Carneiro, D.J. 2007. Digestibility and performance of pacu (*Piaractus mesopotamicus*) juveniles - fed diets containing different protein, lipid and carbohydrate levels. Aquaculture Nutrition, 13: 1-9. doi: 10.1111/j.1365-2095.2007.00438.x

Abimorad, E.G., Carneiro, D.J. & Urbinati, E.C. 2007. Growth and metabolism of pacu (*Piaractus mesopotamicus* Holmberg 1887) juveniles fed diets containing different protein, lipid, and carbohydrate levels. Aquaculture Research, 38: 36-44. doi: 10.1111/j.1365-2109.2006.01621.x

Abimorad, E.G., Favero, G.C., Squassoni, G.H. & Carneiro, D. 2010. Dietary digestibility lysine requirement and essential amino acid to lysine ratio for pacu (*Piaractus mesopotamicus*). Aquaculture Nutrition, 16: 370-377. doi: 10.1111/j.1365-2095.2009.00674.x

Alam, M.S., Watanabe, W.O. & Carroll, P.M. 2008. Dietary protein requirements of juvenile black sea bass, *Centropristis striata*. Journal of the World Aquaculture Society, 39: 656-663.

Association of Official Analytical Chemists (AOAC). 2016. Official methods of analysis. AOAC, Maryland.

Barros, A.F., Silva, A.C.C., Santo, P.R.J. & Barros, O.F. 2020. Investment and cost of production of native fish in polyculture and monoculture system-case study. Brazilian Journal of Development, 6: 16342-16359. doi: 10.34117/bjdv6n3-489

Bicudo, A.J.A., Sado, R.Y. & Cyrino, J.E.P. 2010. Growth performance and body composition of pacu (*Piaractus mesopotamicus*) (Holmberg 1887) in response to dietary protein and energy levels. Aquaculture Nutrition, 16: 213-222. doi: 10.1111/j.1365-2095. 2009.00653.x

Carmo de Sá, M.V.D. & Fracalossi, D.M. 2002. Exigência protéica e relação energia/ proteína para alevinos de piracanjuba (*Brycon orbignyanus*). Revista Brasileira de Zootecnia, 31: 1-10. doi: doi: 10.1590/S1516-35982002000100001

Carter, C.G. & Brafield, A.E. 1992. The bioenergetics of grass carp (*Ctenopharyngodon idella*): the influence of body weight, ration, and dietary composition on nitrogenous excretion. Journal of Fish Biology, 41: 533-543. doi: 10.1111/j.1095-8649.1992.tb02681.x

Carter, C.G. & Houlihan, D.F. 2001. Protein synthesis. Nitrogen excretion. In: Wright, P.A. & Anderson, P.M. (Eds.). Academic Press, California, pp. 31-69.

Emmans, G.C. 1981. A model of the growth and feed intake of ad libitum fed animals, particularly poultry. In: Hillyer, G.M., Wittemore, C.T. & Gunn, R.G. (Eds.). Computers in animal production. Animal Production Occasional Publication, 5, London, pp. 103-110.

Etheridge, R.D., Pesti, GM. & Foster, E.H. 1998. A comparison of nitrogen values obtained utilizing the Kjeldahl nitrogen and Dumas combustion methodologies (Leco, CNS 2000) on samples typical of an animal nutrition analytical laboratory. Animal Feed Science and Technology, 73: 21-28. doi: 10.1016/S0 377-8401(98)00136-9

Fabregat, T.E.H.P., Fernandes, J.B.K., Rodriguez, L.A., Borges, F.F., Pereira, T.S. & Nascimento, T.M.T. 2008. Digestibilidade aparente da energia e da proteína de ingredientes selecionados para juvenis de pacu *Piaractus mesopotamicus*. Ciência Animal, 6: 459-464. doi: 10.7213/cienciaanimal.v6i4.11606

Ferguson, N.S. 2006. Basic concepts describing animal growth and feed intake. In: Gous, R.M., Fisher, C. & Morris, T. (Eds.). Mechanistic modelling in pig and poultry production. CABI International, Wallingford, pp. 22-33.

Ferguson, N.S. & Theeruth, B.K. 2002. Protein and lipid deposition rates in growing pigs following a period of excess fattening. South African Journal of Animal Sciences, 32: 97-105. doi: 10.4314/sajas.v32i2.3751

Fernandes, J.B.K., Carneiro, D.J. & Sakomura, N.K. 2000. Sources and levels of crude protein in diets for pacu (*Piaractus mesopotamicus*) fingerlings. Revista Brasileira de Zootecnia, 29: 646-653. doi: 10.1590/S1516-35982000000300002

Fernandes, J.B.K., Carneiro, D.J. & Sakomura, N.K. 2001. Sources and levels of crude protein in diets for pacu (*Piaractus mesopotamicus*) fingerlings. Revista Brasileira de Zootecnia, 30: 617-626. doi: 10.1590/ S1516-35982001000300003

Fisher, C. 2015. An overview of poultry models. In: Sakomura, N.K., Gous, R.M., Kyriazakis, L. & Hauschild, L. (Eds.). Nutritional modelling for pigs and poultry. CABI, Wallingford, pp 1-22.

Food and Agriculture Organization (FAO). 2016. Fishery and aquaculture statistics. FAO, Rome.

Furlaneto, F.P.B., Esperancini, M.S.T., Bueno, O.C. & Ayroza, L.M.S. 2009. Eficiência econômica do bicultivo de peixes em viveiros escavados na região paulista do Médio Paranapanema. Boletim do Instituto de Pesca, 35: 191-199.

Gatlin III, D.M., Poe, W.E. & Wilson, R.P. 1986. Protein and energy requirement of fingerling channel catfish for maintenance and maximum growth. Journal of Nutrition, 116: 2121-2131. doi: 10.1093/jn/116.11. 2121

Gebhardt, G. 1966. Die bewertung der eiwißqualität von nahrungs- und futtermitteln mi hilfe des n-bilanzversuches. In: Hock, A. (Ed.). Vergleichende Ernährungslehre des Menschen und seiner Haustiere. Gustav Fisher Verlag, Jena, pp. 323-348.

Gebhardt, G. 1980. Eiweiß- und aminosäurenverwertung in beziehung zum stoffwechsel der limitierenden aminosäure. Archiv für Tierernaehrung, 30: 63-71. doi: 10.1080/17450398009441181

Golterman, H.L., Clymo, R.S. & Ohnstad, M.A.M. 1978. Methods for physical and chemical analysis of freshwaters. Blackwell Scientific, London.

Gonçalves, L.U. & Cyrino, J.E.P. 2014. Digestibility of energy, lipids and fatty acids of vegetable oils and poultry fat by pacu *Piaractus mesopotamicus* (Holmberg 1887). Aquaculture Nutrition, 20: 567-573. doi: 10.1111/anu.12108

Kaushik, S.J., Luquet, P. & Blanc, D. 1981. Usefulness of feeding protein and non-protein calories apart in studies on energy-protein interrelationships in rainbow trout. Annales de Zootechnie, 30: 3-11. doi: 10.1051/ animres:19810101

Khan, K.U., Gous, R.M., Mansano, C.F.M., Nascimento, T.M.T., Romaneli, R.S., Rodrigues, A.T., et al. 2020a. Response of juvenile pacu (*Piaractus mesopotamicus* Holmberg, 1887) to balanced digestible protein. Aquaculture Research, 51: 3729-3740. doi: 10.1111/ are.14722

Khan, K.U., Gous, R.M., Sakomura, N.K., Azevedo, J.M., Nascimento, T.M.T., Mansano, C.F.M., et al. 2020b. Modeling optimum levels of balanced digestible protein for adult pacu (*Piaractus mesopotamicus*) and the development of an economic optimization procedure for different marketing strategies of the final yield. Aquaculture, 526: 735368. doi: 10.1016/j. aquaculture.2020.735368

Kim, S.-S. & Lee, K.-J. 2009. Dietary protein requirement of juvenile tiger puffer (*Takifugu rubripes*). Aquaculture, 287: 219-222. doi: 10.1016/j.aquaculture.2008. 10.021

Klein, S., Lorenz, E.K., Bueno, G.W., Signor, A., Feiden, A. & Boscolo, W.R. 2014. Levels of crude protein in diets for pacu (*Piaractus mesopotamicus*) from 150 to 400g reared in cages. Archivos de Zootecnia, 63: 599-610. doi: 10.4321/S0004-05922014000400004

Kubitza, F. 2017. O impacto da amônia, do nitrito e do nitrato sobre o desempenho e a saúde dos peixes e camarões. A água na aquicultura, parte 3. Panorama da Aquicultura, 164: 1-25.

Kyriazakis, I. & Emmans, G.C. 1999. Voluntary food intake and diet selection. In: Kyriazakis, I. (Ed.). A quantitative biology of the pig. CABI Publishing, Wallingford, pp. 229-248.

Leonardo, A.F., Baccarin, A.E., Scorvo Filho, J.D. & Scorvo, C.M.D.F. 2018. Custo de produção da tilápia-do-Nilo (*Oreochromis niloticus*) e do pacu (*Piaractus mesopotamicus*) no vale do Ribeira, Estado de São Paulo. Informações Econômicas, 48: 21-33.

Liebert, F. 2015. Basics and applications of an exponential nitrogen utilization model ("Gottingen approach") for assessing amino acid requirements in growing pigs and meat type chickens based on dietary amino acid efficiency. In: Sakomura, N.K., Gous, R.M., Kyriazakis, I. & Hauschild, L. (Eds.). Nutritional modelling for pigs and poultry. CABI International, São Paulo, pp. 73-88.

Liebert, F., Sünder, A. & Mohamed, K. 2006. Assessment of nitrogen maintenance requirement and potential for protein deposition in juvenile tilapia genotypes by application of an exponential nitrogen utilization model. Aquaculture, 261: 1346-1355. doi: 10.1016/j.aquaculture.2006.05.029

Luo, Z., Liu, Y., Mai, K., Tian, L., Liu, D. & Tan, X. 2004. Optimal dietary protein requirement of grouper Epinephelus coioides juveniles fed isoenergetic diets in floating net cages. Aquaculture Nutrition, 10: 247-252. doi: 10.1111/j.1365-2095.2004.00296.x

Moraes, G. & Bidinotto, P.M. 2008. Digestive proteases of pacu (*Piaractus mesopotamicus*), fed on distinct protein-starch diets. Journal of Applied Aquaculture, 15: 197-207. doi: 10.1300/J028v15n03_17

National Research Council (NRC) 2011. Nutrient requirements of fish and shrimp. National Academic Press, Washington, DC.

Nemova, N.N., Lysenko, L.A. & Kantserova, N.P. 2016. Degradation of skeletal muscle protein during growth and development of salmonid fish. Russian Journal of Developmental Biology, 47: 161-172. doi: 10.1134/S1062360416040068

Ogino, C. & Chen, M.-S. 1973. Protein nutrition in fish. V. Relation between biological value of dietary proteins and their utilization in carp. Bulletin of the Japanese Society for the Science of Fish, 39: 955-959. doi: 10.2331/suisan.39.955

Povh, J.A., Ribeiro, R.P., Lopera-Barrero, N.M., Gomes, P.C., Blanck, D.V., Vargas, L., Jacometo, C.B. & Lopes, T.S. 2009. Monitoramento da variabilidade genética de pacu (*Piaractus mesopotamicus*), do programa de aumento de estoque do rio Paranapanema. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 61: 1191-1195. doi: 10.1590/S0102-0935200900050 0023

Reeds, P.J. & Lobley, G.E. 1980. Protein synthesis: are there real species differences? Proceedings of the Nutrition Society, 39: 43-52. doi: 10.1079/PNS198 00007

Rosa, P.D., Ortiz, J.C., Cáceres, A. de la C., Sebastián, S. & Roux, J.P. 2016. Desempeño del sábalo *Prochilodus lineatus* en policultivo con pacu *Piaractus mesopotamicus*. Latin American Journal of Aquatic Research, 44: 336-341. doi: 10.3856/vol44-issue2-fulltext-14

Sá, R., Pousão-Ferreira, P. & Oliva-Teles, A., 2008. Dietary protein requirement of white seabream (*Diplodus sargus*) juveniles. Aquaculture Nutrition, 14: 309-317. doi: 10.1111/j.1365-2095.2007.00532.x

Sadasivam, J.K. & Teles, A.O. 1985. Effect of digestible energy on nitrogen and energy balance in rainbow trout. Aquaculture, 50: 89-101. doi: 10.1016/0044-8486(85)90155-3

Sakomura, N.K. & Rostagno, H.S. 2016. Métodos de pesquisa em nutrição de monogástricos. FUNEP, São Paulo.

Samadi & Liebert, F. 2006. Estimation of nitrogen maintenance requirements and potential for nitrogen deposition in fast-growing chickens depending on age and sex. Poultry Science, 85: 1421-1429. doi: 10.1093/ps/85.8.1421

Samadi & Liebert, F. 2008. Modelling the optimal lysine to threonine ratio in growing chickens depending on age and efficiency of dietary amino acid utilization. British Poultry Science, 49: 45-54. doi: 10.1080/00071660701821667

Signor, A.A., Boscolo, W.R., Feiden, A., Bittencourt, F., Coldebella, A. & Reidel, A. 2010. Proteína e energia na alimentação de pacus criados em tanques-rede. Revista Brasileira de Zootecnia, 39: 2336-2341. doi: 10.1590/S1516- 35982010001100004

Silva, D.J. & Queiroz, A.C. 2002. Análise de alimentos (métodos químicos e biológicos). UFV, Viçosa.

Silva, E.P.D., Sakomura, N.K. & Hauschild, L. 2014. Modelos para estimar as exigências de aminoácidos para aves. In: Sakomura, N.K., Silva, J.H.V., Costa, F.G.P., Fernandes, J.B.K. & Hauschild, L. (Eds.). Nutrição de não ruminantes. Funep, Jaboticabal, pp. 218-223.

Silva, J.R., Rabenschlag, D.R., Feiden, A.A, Boscolo, W.R.B, Signor, A.A. & Bueno, G.W. 2012. Economic return from cage production of pacu in Itaipu reservoir, Brazil. Archivos de Zootecnia, 61: 245-254. doi: 10.21071/az.v61i234.2798

Statistical Analysis System (SAS) Institute. 2014. SAS/STAT 9.4. User's guide. SAS Institute Inc., North Carolina.

Sünder, A. & Liebert, F. 2005. Comparison of different methods for estimation of the N-maintenance requirement (NMR) in different species based on N-rise balance experiments. Proceedings of the Society of Nutrition Physiology, 14: 33 pp.

Thong, H.T. & Liebert, F. 2004. Amino acid requirement of growing pigs depending on efficiency of amino acid utilization and level of protein deposition. 1st Communication: lysine. Archives of Animal Nutrition, 58: 69-87. doi: 10.1080/0003942031000165669

Received: December 16, 2020; Accepted: May 17, 2021

Wilson, R.P. 1989. Amino acids and proteins. Fish nutrition. In: Halver, J.E. (Ed.). Academic Press, New York, pp. 111-151.

Xian, W. & Zhu, X. 2002. Effects of body weight and temperature on nitrogen budget of bastard halibut (*Paralichthys olivaceus*). Journal of Ocean University of China, 5: 200-205.

Zhang, J., Zhou, F., Wang, L., Shao, Q., Xu, Z. & Xu, J. 2010. Dietary protein requirement of juvenile black sea bream *Sparus macrocephalus*. Journal of the World Aquaculture Society, 41: 151-164. doi: 10.1111/j.1749-7345.2010.00356.x

Zhu, Q., Qian, G., Gao, Y. & Wang, C. 2009. Assessment of nitrogen maintenance requirement in juvenile black porgy (*Acanthopagrus schlegeli*). Chinese Journal of Oceanology and Limnology, 27: 63-68. doi: 10.1007/s00343-009-0063-6