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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

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ABSTRACT

Marketing strategies and feeding costs are key factors affecting the sustainability of intensive aquaculture systems. The present 44-day feeding trial aimed to model the optimum levels of balanced digestible protein (BDP) for adult pacu (*Piaractus mesopotamicus*) and develop an economic optimization procedure for the final yield. The experiment was conducted in a freshwater recirculation aquaculture system (RAS). A randomized block design with six treatments and three replicates was used. A total of 180 pacu adults with initial body weight of 1158.50 ± 21.59 g (± standard deviation) were distributed (10 fish per tank) among 18 concrete-tanks of 2000 L. Six consecutive isoenergetic experimental diets containing 163, 201, 238, 272, 315 and 348 g BDP kg^{-1} (dry matter basis – DM basis) were prepared through the diet dilution procedure. The triplicate groups of fish were hand-fed three times a day at 09:00, 12:00 and 17:00 h until apparent satiation. At the end of the trial, the productive and economic responses were evaluated through appropriate mathematical models and statistical analyses. The optimum levels of BDP were modeled as 232 and 245 g kg^{-1} (DM basis) by the quadratic model based on maximum final body weight (FBW) and body weight gain (BWG) of adult pacu, respectively. Using an individual based economic model, the economically optimized levels of BDP were modeled as 215, 217 and 222 g kg^{-1} (DM basis) for obtaining maximum profitability from the fish that are marketed in the form of whole-body, eviscerated and sliced adult pacu, respectively. The optimum levels of BDP modeled for maximum profitability were found considerably lower than those modeled for maximum FBW and BWG. The hypothesized scenarios confirmed that profitability and economically optimized levels of dietary protein were greatly affected by the fluctuations in feed costs and marketing strategy of the final yield.

1. Introduction

In intensive aquaculture systems, optimum levels of protein are required to be supplemented in fish feeds to obtain optimum performance and maximum profitability (National Research Council, 2011). The under or over supplementations of this important and expensive nutrient have negative impacts on the productive performance and profitability of the final yield (Webb Jr and Gatlin III, 2003; Wu and Gatlin III, 2014). When over supplemented, its amino acid pattern breaks down either to serve as dietary energy which may affect the quality of the final yield (Grisdale-Helland et al., 2008) or excrete into the aquatic environment in the form of excess nitrogen (Davis and Arnold, 1997; Kaushik and Médale, 1994; Thoman et al., 1999). The supplementation of high levels of absolute protein contents in fish feeds without providing the amino acid balance (Khan et al., 2019) considerably declines the productive performance and economic returns (Halver and Hardy, 2002), and may increase the aquatic nitrogen loads (National Research Council, 2011).

In fish farming, feed costs have a great impact on the total production costs and profit of the final yield (El-Sayed, 2013; Lall and

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Tibbetts, 2009; Nguyen et al., 2009; Nguyen, 2013; Shipton and Hasan, 2013; Uchoi et al., 2015). In intensive production systems, feed costs account for approximately 40–60% of total production costs probably due to the protein-rich ingredients (Lovell, 2002). The previous researches have been usually focused on the use of absolute protein contents in fish feeds (National Research Council, 2011). The formulation of diets based on essential amino acid balance is somewhat neglected in fish nutrition (Guroy et al., 2017; Khan et al., 2019; National Research Council, 2011). Aquatic species usually need protein diets provided with optimum amino acid ratios rather than absolute dietary protein contents (Broberger, 2005; Houlihan et al., 2001). The diets based on amino acid balance have positive impacts on the feed utilization efficiency and normal growth of animals (Emmans, 1981; Gous and Morris, 1985). This strategy helps to fix the supplementation of protein content in fish feeds in order to avoid unsustainable increase in feed costs and get optimum growth with maximum economic profits (Khan et al., 2019; Li et al., 2009; Wilson, 2002). The diet dilution procedure allows the formulation of animal diets with protein-bound amino acid balance (Fisher and Morris, 1970), and has been widely used in poultry and pig nutrition (D'Mello, 1993). In fish nutrition, the graded supplementation method has been widely used for the formulation of practical and experimental diets (National Research Council, 2011). Although Abboudi et al. (2006, 2007) in Atlantic salmon (*Salmo salar*) and Liebert and Benkendorff (2007) in Nile tilapia (*Oreochromis niloticus*) have used the diet dilution procedure for the evaluation of amino acid requirements. But still little is known about the use of diet dilution procedure for the formulation of diets to determine the dietary protein requirements of fish (Khan et al., 2019). Feeds that are prepared according to the diet-dilution procedure being provided with amino acid balance are usually called balanced protein feeds (Clark et al., 1982; Eits et al., 2005a, 2005b; Gous and Morris, 1985; Mack et al., 1999). According to the balanced protein concept, the ideal relationship among all EAAs is maintained constant, and only EAAs proportions vary according to the dietary protein levels (Gous and Morris, 1985). Lysine is used as reference amino acid to calculate the ideal ratios of other essential amino acids (Eits et al., 2005b). The experimental diets are achieved through the serial dilutions of a concentrated protein diet either with a low protein diet or protein free diet. The dietary energy and other necessary purified ingredients including vitamin and mineral premix, antifungal and antioxidants are maintained at a constant proportion in the basal diets, meeting the optimum requirements of the animal (Fisher and Morris, 1970; Mack et al., 1999).

The establishment of optimum levels of dietary protein by taking into account productive performance and economic outputs is a sustainable way to avoid the over supplementation this important and costly nutrient (Food and Agriculture Organization, 2013; Kaushik, 2000; Liu et al., 2013; Shearer, 2000; White, 2013; Zeitoun et al., 1976). The development of cost-effective protein feeds (Duong et al., 2011; Sealey et al., 2013) for different marketing strategies of the final yield (Eits et al., 2005a, 2005b) could help to better cope with the increasing feed costs and environmental concerns (De Silva and Hasan, 2007). The body size and feed formulation procedures considerably affect the dietary protein requirements of fish (National Research Council, 2011; Ye et al., 2015, 2017). Most of the previous studies have evaluated the dietary protein requirements of juvenile fish including pacu (*Piaractus mesopotamicus*) (Abimorad et al., 2007; Alam et al., 2008; Bicudo et al., 2010; Carmo de Sá and Fracalossi, 2002; Caseras et al., 2000; Fernandes et al., 2000, 2001; Kim and Lee, 2009; Klein et al., 2014; Lundstedt et al., 2004; Luo et al., 2004; Melo et al., 2006; Sá et al., 2008; Signor et al., 2010; Zhang et al., 2010). The dietary protein requirements of fish usually decline with the increase in body size (National Research Council, 2011). The evaluation of protein requirements of fish in different growth phases may help to obtain maximum biological and economic performance at sustainable feed costs (Craig, 2017; Ye et al., 2015, 2017). This strategy could considerably improve the efficiency and sustainability of intensive aquaculture production systems (Guy et al., 2018; Shipton and Hecht, 2013). On the other hand, feeding large size fish with dietary protein being established for juvenile ones may considerably decline both production and economic profit (Cho and Bureau, 1998, 2001; Kaushik, 2013; Shiau and Lan, 1996; Yang et al., 2002, 2003).

Pacu (*P. mesopotamicus*) is an important freshwater fish species endemic to the water bodies of Latin American countries (Borghetti and Canzi, 1993; Machado and Sgarbieri, 1991). It has been successfully introduced as an exotic species in several other countries (Cagauan, 2007; Pullela, 1997; Uchoi et al., 2015). Being an omnivorous fish, it can efficiently utilize feeds with wide range of compositions (Froese and Pauly, 2000; Merola, 1988). The characteristics of fast growing and good fillet quality (Jomori et al., 2003) are making it suitable for production in intensive, semi-intensive and extensive aquaculture systems (Uchoi et al., 2015). In previous studies, the protein requirements of pacu have been usually evaluated in the juvenile growth phase but to our knowledge still no such information is available for adult pacu. The available dietary protein levels have been reported for feeding pacu that are marketed only in the form of whole body fish and no such data are available for pacu that are marketed in the form of processed parts. Thus, this study aimed to model the optimum levels of balanced digestible protein for adult pacu (*P. mesopotamicus*) and develop an economic optimization procedure for different marketing strategies of the final yield.

2. Materials and methods

2.1. Feed formulation

Two basal diets, a concentrated protein diet containing 366 g balanced protein kg^{-1} (DM basis) and another low protein diet with 128.30 g balanced protein kg^{-1} (DM basis) (Table 1), were prepared according to the diet dilution procedure of Fisher and Morris (1970). The basal diets were in line with previous nutritional recommendations

Table 1
Formulation of basal protein diets (fed basis).

Ingredients	Basal protein diets (g kg^{-1})	
	Concentrated-protein diet	Low-protein diet
Fish meal	176.40	47.10
Soybean meal	441.00	124.30
Corn gluten	37.20	11.30
Corn	258.70	151.50
Broken rice	0.00	162.66
Corn starch	0.00	255.00
Rice husk	0.00	75.00
Soybean oil	41.40	100.00
L-lysine HCL	10.80	3.70
DL-methionine	1.90	0.44
L-threonine	1.50	0.50
Di-calcium phosphate	17.20	36.00
Limestone	5.40	24.00
Antifungal Euromold®	3.00	3.00
Antioxidant (BHT)	0.50	0.50
Vitamin-mineral premix*	5.00	5.00
Total	1000.00	1000.00
Feed cost (US$ kg^{-1} feed)	0.70	0.48

Vitamin-mineral premix* contained: vitamin A 258.00 mg kg^{-1}; Vitamin D3 6.00 mg kg^{-1}; Vitamin E 7047 mg kg^{-1}; Vitamin K3 1400 mg kg^{-1}; Vitamin B1 2100 mg kg^{-1}; Vitamin B2 2150 mg kg^{-1}; Vitamin B6 2100 mg kg^{-1}; Vitamin B12 2.2 mg kg^{-1}; Vitamin C 25,000.00 mg kg^{-1}; Niacin 10,000.00 mg kg^{-1}; Calcium pantothenate 5600 mg kg^{-1}; Folic acid 580 mg kg^{-1}; Biotin 17.00 mg kg^{-1}; Choline chloride 60,000.00 mg kg^{-1}; Inositol 3570 mg kg^{-1}; Copper 1800 mg kg^{-1}; Manganese 5000 mg kg^{-1}; Zinc 8000 mg kg^{-1}; Iodine 90 mg kg^{-1}; Selenium 36 mg kg^{-1}; Cobalt 55 mg kg^{-1}.
US$, USA dollar.

for pacu (Abimorad and Carneiro, 2004; Abimorad et al., 2007; Abimorad et al., 2010; Bicudo et al., 2010; Carneiro, 1983; Fernandes et al., 2000, 2001; Khan et al., 2020; Klein et al., 2014; Signor et al., 2010). The digestible values used in the formulation of basal diets were based on the apparent digestibility coefficients determined by Abimorad et al. (2008), Fabregat et al. (2008), and Gonçalves and Cyrino (2014) in pacu while those of the crystalline amino acids were considered as 100% (Nunes et al., 2014).

According to the principle of the diet dilution procedure, an optimum protein-bound EAA balance was provided to both basal diets and the digestible energy was kept at a constant level in order to achieve isoenergetic experimental diets with six increased levels of balanced digestible protein. The vitamin and mineral premix, antifungal and antioxidant (BHT) were added at the same proportion to both basal diets. The EAA ratios provided to the concentrated protein diet were 1.6% in excess and those in the low protein diet were reduced by 0.5% than those reported by Khan et al. (2020) for pacu. An ideal relationship was maintained among EAAs of the basal and experimental diets, and only the EAAs proportions varied according to the dietary protein level. Due to the use of some plant ingredients it was not possible to attain a pure (100%) protein-bound EAA balance, thus small amounts of crystalline L-lysine, L-threonine and DL-methionine were added just to get the desired EAA balance.

2.2. Feed preparation and extrusion

The unpurified feed ingredients of the two basal diets (concentrated protein and low protein) were ground in a feed hammer mill (Moinho Vieira MCS 280 (5cv) Tatuí, SP, Brazil), and then each basal diet along with the respective purified ingredients was properly mixed in a feed mixer. The concentrated protein diet was diluted with the low protein diet at proper proportions and six consecutive isoenergetic experimental diets containing 163, 201, 238, 272, 315, and 348 g BDP kg^{-1} (DM basis) were achieved (Table 2). Before extrusion, 22% water was added to each experimental diet and again mixed in the feed mixer. The homogenized feed samples were extruded into feed pellets of 4 mm diameter through a single screw micro-laboratory extruder (Ex Laboratório, Exteec Máquinas, Ribeirão Preto, SP, Brazil). The extruded feed pellets of each diet were dried in a forced-air-circulation oven at 55 °C for 24 h, cooled at room temperature, putted into separate plastic bags and then kept in a cold chamber at −12 °C until use.

2.3. Experimentation ethics

The materials and procedures used in the present experiment were approved by the Ethics Committee on Animal Use of the Faculty of Agricultural and Veterinary Sciences, São Paulo State University (UNESP), Jaboticabal, Brazil (Protocol no: 009999/14).

Table 2
The sequential dilutions of the concentrated protein diet with the low protein diet (fed basis) obtaining the six experimental diets.

BDP (g kg^{-1}) (Dry matter basis)	Basal balanced protein diets (g kg^{-1} fed basis)		Total cost (US$ kg^{-1} feed)
	Concentrated protein diet	Low protein diet	
163	193.32	806.68	0.52
201	352.64	647.36	0.56
238	511.97	488.03	0.59
272	671.29	328.71	0.63
315	830.61	169.39	0.67
348	989.94	10.06	0.70

Note: The total feed cost (US$ kg^{-1} feed) for each experimental diet was calculated on the basis of the price of the proportions obtained from the concentrated protein and low protein diets.
BDP = Balanced digestible protein.

2.4. Fish, acclimation and trial design

Pacu adults purchased from a local fish farm were immediately transferred to the Laboratory of Modeling in Fish Nutrition, Aquaculture Center, Sao Paulo State University (UNESP), Jaboticabal, SP, Brazil. Before purchasing, fish were genetically confirmed as pure *P. mesopotamicus* species through the molecular analyses performed at the Fish Genetics Laboratory (LAGENPE), Department of Biological Sciences (DCB), Faculty of Sciences, UNESP, Bauru – SP, Brazil. Fish were acclimated to the laboratory conditions for 7 days before the trial and fed with a commercial feed once a day. After acclimation, a total of 180 healthy adult pacu with initial average body weight of 1158.50 ± 21.59 g (± SD) were distributed (10 fish per tank) among 18-concrete tanks of 2000-L. A randomized block design with six treatments and three replicates was used. The triplicate groups of fish were hand-fed three times a day at 09:00, 12:00 and 17:00 h until apparent satiation.

2.5. Fish rearing system

The present 44-day experiment was conducted in an indoor facility provided with closed freshwater based intensive recirculation aquaculture system (RAS). A controlled photoperiod 12: 12 h (light: dark) was maintained during the entire experiment. The recirculation aquaculture system (RAS) was equipped with a biological and mechanical filter, a heat exchange system (Model no: SD60S & Serial no: SD6D-97-0383; Sodaramar Industriae Commercio Ltda., Brazil) and aeration. The biological and mechanical filter was receiving water from the outlets of the experimental tanks for filtration. An electric water pump (Model no: TH – 16NR – TRIF. IP 21/AR; Thebe Bombas Hydraulicas Ltda., Brazil) was continuously supplying the filtered water to the experimental setup. The heat exchange system helped in maintaining a constant water temperature. The freshwater used were obtained from a tube well and aeration was produced by an electric radial oxygen compressor (Model no: JKW002 - Trifásico Liscomex; JKW Compressores, Brazil). The aeration was equally distributed among experimental tanks through small plastic pipes provided with air stones. The experimental tanks were siphoned once a week and the water removed was again refilled with the freshwater of the tube well.

2.6. Water quality assessment

The water quality parameters were regularly measured throughout the trial. The water temperature (average 28.6 ± 0.5 °C) and dissolved oxygen (average 5.74 ± 0.10 mg L^{-1}) were measured by Digital Thermometer LCD HC–520 and Digital Dissolved Oxygen Meter YSI EcoSense® DO200A, respectively on daily basis while the water pH (average 7.3 ± 0.9) was measured by Digital pH Meter YSI EcoSense® pH100A once a week. The total ammonia concentration (average 0.14 ± 0.4 mg L^{-1}) was measured once a week according to the phenol-hypochlorite method of Solorzano (1969).

2.7. Sample collection and analytical procedures

At the beginning of the trial, fish were subjected to a 24 h fasting, anesthetized with a low dose (50 mg L^{-1}) of benzocaine solution (Sigma-Aldrich, Brazil) (Bicudo et al., 2010) and immediately weighed. A total of 10 adult pacu en masse were sampled from the initial fish population, anesthetized with the overdose (500 mg L^{-1}) of benzocaine solution (Bicudo et al., 2010), weighed and frozen. The frozen fish were then subjected to the grinding and analytical procedures for the determination of initial whole body proximate composition of adult pacu. Additional 8 adult pacu were collected, anesthetized with the overdose (500 mg L^{-1}) of benzocaine solution, weighed and processed, obtaining the initial weight data for the whole body, eviscerated and sliced adult pacu (Table 5). Similarly, at the end of the trial, fish were again fasted for 24 h, anesthetized with a low dose (50 mg L^{-1}) of benzocaine solution and immediately weighed. A total of 3 adult pacu en masse were sampled from each experimental tank, anesthetized with the overdose (500 mg L^{-1}) of benzocaine solution, weighted and frozen. The frozen fish were then subjected to the subsequent grinding and analytical processes for the evaluation of final whole body proximate composition of adult pacu. Another 4 adult pacu en masse per experimental tank were collected, anesthetized with the overdose (500 mg L^{-1}) of benzocaine solution, weighed and immediately processed individually by a well-trained operator into scales, fins, visceral content, head and main body trunk (Klein et al., 2014). The processed parts for each fish were immediately weighted and these data were used to obtain: the whole body adult pacu; eviscerated adult pacu (whole body adult pacu – visceral content); and sliced adult pacu (whole body adult pacu – visceral content – head – scales – fins) (Table 5).

The two basal and six experimental diets were analyzed according to the standard methods of Association of Official Analytical Chemists – AOAC International (2016) for the quantification of dry matter, total lipid, crude protein and ash content as well as gross energy (Table 3). The amino acid compositions of the feed ingredients and two basal protein diets (concentrated protein and low protein) were analyzed by high performance liquid chromatography – HPLC technique (Biochrom Ltd., Cambridge, UK) at Evonik industries AG - Essen, Germany (Table 4).

Table 4
The amino acid composition of the concentrated protein and low protein diets (g kg^{-1} dry matter basis).

Essential amino acids (EAAs)	CP-diet Digestible[a] EAAs	CP-diet Total[b] EAAs	LP-diet Digestible[a] EAAs	LP-diet Total[b] EAAs
Methionine	7.80	8.50	2.50	2.80
Lysine	28.00	30.20	9.10	9.90
Threonine	14.70	16.60	4.80	5.60
Arginine	24.30	25.50	8.40	8.80
Isoleucine	14.20	16.00	4.80	5.60
Leucine	27.80	29.90	8.80	9.70
Valine	15.30	17.40	5.20	6.20
Histidine	8.20	8.80	2.80	3.00
Phenylalanine	16.80	18.00	5.80	6.30
Tryptophan	3.50	3.90	1.30	1.50

Non-essential amino acids (NEAAs)	CP-diet Total[b] NEAAs	LP-diet Total[b] NEAAs
Cysteine	5.30	2.30
Glycine	23.40	8.60
Serine	18.30	7.00
Proline	24.10	9.40
Alanine	21.60	8.50
Aspartic acid	38.00	14.30
Glutamate	63.50	25.00
Methionine + Cysteine	13.80	5.60

Note: CP-diet Digestible EAAs = Digestible essential amino acids contents in concentrated protein diet; LP-diet Digestible EAAs = Digestible essential amino acids contents in low-protein diet; CP-diet Total EAAs = Total essential amino acids contents in concentrated protein diet; LP-diet Total EAAs = Total essential amino acids contents in low-protein diet.
CP-diet Total NEAAs = Total non-essential amino acids contents in concentrated protein diet; LP-diet Total NEAAs = Total non-essential amino acids contents in low-protein diet.

[a] Calculated values.

[b] Analyzed values.

Similarly, the same standard procedures of Association of Official Analytical Chemists – AOAC International (2016) were adapted for the evaluation the initial and final whole body moisture, crude protein, total lipid and ash content of adult pacu (Table 7).

2.8. Evaluation of productive and economic responses of adult pacu

At the end of the present feeding trial, the productive responses of adult pacu fed increased levels of balanced digestible protein were calculated according to the following formulas:

$$\text{Body weight gain g (BWG)} = \text{final body weight} - \text{initial body weight}$$

$$\text{Feed intake g (FI) fed basis} = \text{total feed consumed per fish}$$

$$\text{Protein intake g (PI)} = \text{feed intake per fish} \times \text{amount of protein in the feed}$$

$$\text{Feed conversion ratio g g}^{-1}\text{(FCR)} = \text{total feed intake} \div \text{body weight gain}$$

$$\text{Feed efficiency g g}^{-1}\text{ (FE)} = \text{body weight gain} \div \text{total feed intake}$$

$$\text{Specific growth rate (SGR\%day}^{-1}\text{)} = [\{(\ln\ \text{final body weight} - \ln\ \text{initial body weight}) \div \text{days}\} \times 100]\ \text{and}$$

$$\text{Protein efficiency ratio g g}^{-1}\text{ (PER)} = \text{body weight gain} \div \text{protein intake}$$

The following economic responses (inputs) were calculated to be used in the individual based economic model of Eits et al. (2005b) for modeling optimum levels of economic BDP for pacu adults.

1. Feed price (US\$ kg^{-1} feed) = b × BDP + a ÷ 1000; where, BDP is the level of balanced digestible protein (DM basis) and "a" and "b" are the parameters of the linear equation obtained for the feed price (Fig. 1). Feed price (US\$ kg^{-1} feed), including the feed processing costs, for each diet of this work was calculated according to the local market prices of the unpurified and purified ingredients (US\$ kg^{-1} ingredient) used in the present formulations.
2. Feed intake (Fig. 3) and final whole body weight (Fig. 4) responses

Table 3
Proximate composition of the two basal and six experimental diets (dry matter basis).

	Basal and experimental balanced protein diets (g kg^{-1})							
Proximate composition	CP-diet	LP-diet	163	201	238	272	315	348
Digestible protein* g kg^{-1}	366.00	128.30	163.00	201.00	238.00	272.00	315.00	348.00
Crude protein g kg^{-1}	409.80	147.00	185.00	227.10	268.00	305.60	353.20	389.70
Digestible lipid* g kg^{-1}	73.10	106.40	98.30	93.40	87.60	81.90	77.20	71.90
Total lipid g kg^{-1}	90.10	126.80	117.80	112.40	106.00	99.60	94.50	88.60
Ash g kg^{-1}	72.90	91.50	85.80	82.80	80.10	77.00	74.60	71.70
Dry matter g kg^{-1}	928.92	935.29	954.23	950.56	951.16	955.31	946.18	946.67
Digestible energy* (MJ kg^{-1})	15.04	15.06	14.63	14.68	14.67	14.60	14.73	14.72
Gross energy (MJ kg^{-1})	19.25	17.56	17.38	17.71	17.95	18.13	18.57	18.82
DP: DE ratios* (g MJ^{-1})	0.00		11.14	13.69	16.22	18.63	21.38	23.64

Note: CP-diet = Concentrated protein diet; LP-diet = Low-protein diet; DP: DE ratios = Digestible protein to energy ratios; * = Calculated values.

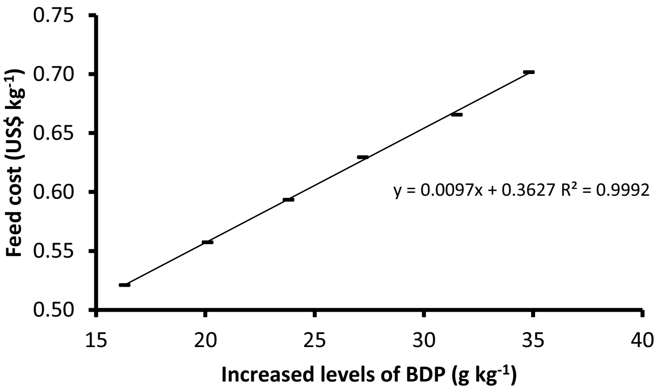

Fig. 1. Effect of the increased levels of balanced digestible protein on the total cost of different experimental diets.

were evaluated by the quadratic regression model; $y = a + bx + cx^2$, where "x" is the protein level, "y" is the response variable, "a" is the curve intercept, "b" and "c" are representing the slopes, and $\{x = -b \div 2c\}$ gives the optimum level of balanced digestible protein.

3. The final weights of the whole body, eviscerated and sliced adult pacu (Table 5) fed increased levels of BDP were calculated in natural log (ln), and then the allometric relationship (Fig. 2) between final weight of the processed fish part "Y" and final body weight "X" were predicted through the equation of Danisman and Gous (2011); Ln Y = ln a + b ln X, where, the exponent "a" is the constant term (y-intercept) and "b" represents the slope of the linear regression. In the present study, final body weight was used for the calculation of the economically optimized levels of BDP and the prediction of allometric relationships. Fish farmers can easily collect the final body weight data which can be used to quickly calculate the economically efficient levels of dietary protein in the case of an abrupt change in feed costs or marketing strategy of the yield in to avoid any substantial decline in profitability.
4. The selling prices (US$ kg^{-1} final yield), including the processing costs in the case of the processed fish parts, were obtained from the local consumer market for different marketing strategies of the final fish yield including whole body, eviscerated and sliced adult pacu.
5. The six levels of balanced digestible protein (163, 201, 238, 272, 315 and 348 g kg^{-1}; DM basis). In the economic calculations of the present study, all values obtained in kg were converted into g.

The above 5 economic inputs were provided to the economic model of Eits et al. (2005b) which generated the following three outputs:

1. Feeding costs (US$ g^{-1} whole body/processed fish part^{-1}) = {(Feed price × feed intake) ÷ 1000}.
2. Revenue (US$ g^{-1} whole body/processed fish part^{-1}) = {Final weight of whole body/processed fish part (including the processing costs) × selling price per kg whole body/processed fish part (including the processing costs)}; and.
3. Gross margin (US$ g^{-1} whole body/processed fish part^{-1}) = {Revenue (US$ g^{-1} whole body/processed fish part^{-1}) - feeding costs (US$ g^{-1} whole body/processed fish part^{-1})}.

2.9. Modeling optimum levels of balanced digestible protein for maximum performance

The optimum levels of balanced digestible for maximum performance were modeled through the quadratic regression model fitted to the final body weight (Fig. 4) and body weight gain (Fig. 5) responses of adult pacu fed increased protein levels.

2.10. Modeling optimum economic levels of balanced digestible protein for maximum profitability of the final yield

The optimum economic levels of BDP for adult pacu in different marketing strategies such as whole body (Fig. 6; Part-A), eviscerated (Fig. 6; Part-B) and sliced (Fig. 6; Part-C) fish were modeled based on the maximum gross margins through the following individual based economic model;

Table 5
Growth and feeding responses of pacu adults fed increased levels of balanced digestible protein (BDP) (g kg^{-1}).

BDP (g kg^{-1} DM basis)	FCR (g g^{-1})	FE (g g^{-1})	SGR (% fish^{-1} day^{-1})	PER (g g^{-1})
163	2.48 ± 0.19^{b}	0.40 ± 0.03^{c}	0.22 ± 0.02^{c}	2.48 ± 0.19^{a}
201	2.09 ± 0.11^{cd}	0.48 ± 0.02^{b}	0.27 ± 0.02^{b}	2.38 ± 0.12^{ab}
238	1.90 ± 0.06^{d}	0.53 ± 0.02^{a}	0.30 ± 0.01^{a}	2.21 ± 0.07^{b}
272	1.87 ± 0.08^{d}	0.53 ± 0.02^{a}	0.31 ± 0.02^{a}	1.97 ± 0.08^{c}
315	2.30 ± 0.18^{bc}	0.44 ± 0.03^{bc}	0.24 ± 0.02^{c}	1.38 ± 0.10^{d}
348	3.11 ± 0.30^{a}	0.32 ± 0.03^{d}	0.18 ± 0.02^{d}	0.93 ± 0.09^{e}
P-value	0.001	0.001	0.001	0.001

Initial and final mean weights (g) of the whole body, eviscerated and sliced adult pacu.

	Initial WBW (g)	Final WBW (g)	Evisc. pacu FW (g)	Sliced pacu FW (g)
Initial body weights (g) of Eviscerated and Sliced pacu			1046 ± 117.2	805.13 ± 97.2
163	1178.2 ± 28.0	1300.4 ± 21.1^{a}	1087 ± 63.5^{b}	836 ± 49.2^{b}
201	1165.3 ± 18.6	1312.0 ± 16.3^{a}	1147 ± 6.9^{ab}	833 ± 9.8^{ab}
238	1161.1 ± 20.0	1325.3 ± 18.2^{a}	1159 ± 69.0^{ab}	891 ± 54.0^{ab}
272	1139.8 ± 21.1	1306.6 ± 21.0^{a}	1210 ± 33.5^{a}	941 ± 41.5^{a}
315	1169.1 ± 28.0	1298.4 ± 19.5^{a}	1143 ± 16.6^{ab}	885 ± 26.3^{ab}
348	1150.8 ± 7.7	1246.0 ± 16.9^{b}	1080 ± 54.7^{b}	824 ± 42.4^{b}
P-value	0.3705	0.0045	0.0451	0.0403

Note: BDP = Balanced digestible protein; FCR = Feed conversion ratio; FE = Feed efficiency; SGR = Specific growth rate; PER = Protein efficiency ratio.
Initial WBW = Initial whole body weight; Final WBW = Final whole body weight; Evisc. pacu FW = Final weight of eviscerated adult pacu; Sliced pacu FW = Final weight of sliced adult pacu.
Values are presented as means of three replicates ± standard deviation, numbers in the same column with different superscripts are significantly ($P < 0.05$) different.

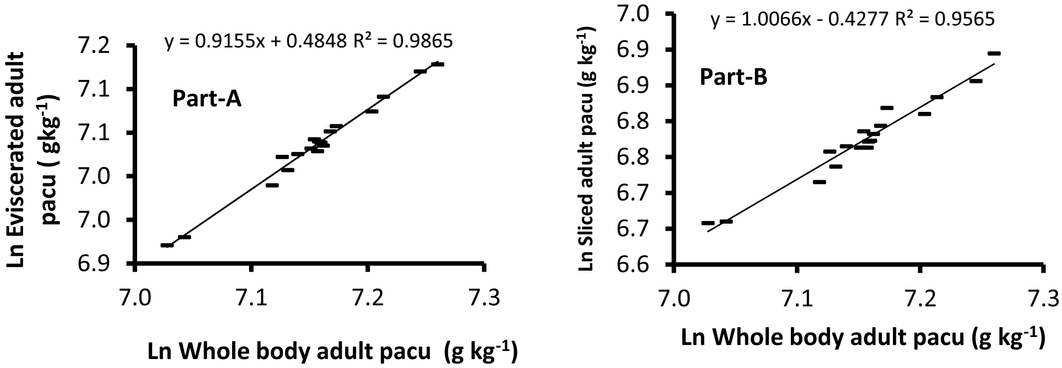

Fig. 2. Allometric relationships between the Ln whole body adult pacu and Ln eviscerated (Part-B) and Ln sliced (Part-A) adult pacu fed increased levels of balanced digestible protein.

Gross margin (GM) = Revenue–Feeding cost

The above model can be algebraically expressed as follows;

Gross margin (GM) = {(a × (d)) – (c° × b)}, where, GM is the maximum profit obtained on the basis of the two outputs of the economic model (revenue and feeding costs). The functions of the above equation can be elaborated as follow;

$d = c_0 + c_1x + c_2x^2$, estimates of the quadratic regression model obtained for the final whole body weight, and in the case of cut-up portions $d = c_0 + c_1x$, estimates of the linear allometric regression derived between final body weight and cut-up portion

$c^\circ = y_0 + y_1x$, estimates of the linear equation obtained for feed price

$b = f_0 + f_1x + f_2x^2$, estimates of the quadratic regression model obtained for the feed intake; or

If we expand the above model as follows;

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

Where, a, c_0, c_1, c_2, y_0, y_1, f_0, f_1, and f_2 are constants while “x” is unknown.

Maximum gross margin occurs when $\frac{\partial GM}{\partial X} = 0$, which could also be expressed as follow;

$$\frac{\partial GM}{\partial X} = (ac_1 + 2\,ac_{2x}) - (y_0f_1 + 2\,y_0f_2x + y_1f_0 + 2\,y_1f_1x + 3\,y_1f_2x^2)$$

$$= (ac_1 - y_0f_1 - y_1f_0) + (2\,ac_2 - 2\,y_0f_2 - 2\,y_1f_1)\,x - (3\,y_1f_2)\,x^2$$

Let $k_a = -3\,y_1f_2$

$k_b = 2\,ac_2 - 2\,y_0f_2 - 2\,y_1f_1$

$k_c = ac_1 - y_0f_1 - y_1f_0$

Then maximum gross margin (GM) occurs when *x* takes the value of

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

Where, $x^\circ$is the maximum gross margin (US\$ g^{-1} $fish^{-1}$/cut–up $portion^{-1}$) at a given dietary protein level. According to the economic model, the level of the dietary protein where maximum gross margin occurs would be the optimum economic level.

2.11. Hypothesized alternate scenarios

Beside the normal feed costs and selling prices of the final yield evaluated in the present study, some alternate scenarios (Table 6) were hypothesized to check the effect of fluctuations in feed costs and selling prices of whole body, eviscerated and sliced adult pacu on the total feeding costs, revenues and gross margins. The protein ingredients considerably affect the total aquafeed costs, thus, the alternate scenarios were designed on the basis of fluctuations in the prices of two protein-rich ingredients including fish meal and soybean meal which were used in the present formulations. The selling prices of the fish meal and soybean meal and those of the final yields were increased and decreased by 20% as compared to the normal prices of this study (Table 6).

2.12. Statistical analysis

The growth, feeding and body composition data were evaluated by Statistical Package for Social Sciences (SPSS Inc., 2007) software. One-way analysis of variance (ANOVA) was used to evaluate the significant differences. When a significant ($P < 0.05$) difference was observed, the Least Significant Test (LST) was used to compare the means. The final body weight, body weight gain and feed intake responses were evaluated by Statistical Analysis system (SAS Institute Inc., 2014) using the non-linear programming procedure (PROC NLP).

3. Results

3.1. Growth performance and feeding responses

No mortality was observed during the trial and the survival rate was 100% in all the treatments. The supplementation of increased levels of balanced digestible protein significantly ($P < .05$) affected the growth and feeding responses of adult pacu (Table 5). The specific growth rate (SGR%), feed conversion ratio (FCR) and feed efficiency (FE) were significantly ($P < .05$) associated with digestible protein in a parabolic manner. The maximization of SGR (0.31 ± 0.02 g g^{-1}) and FE (0.53 ± 0.02 g g^{-1}) and minimization of FCR (1.87 ± 0.08 g g^{-1}) occurred at 272 g balanced digestible protein kg^{-1} (DM basis). Protein efficiency ratio (PER) was significantly ($P < .05$) influenced by BDP in a linear and descending way, with the minimum PER observed at 348 g BDP kg^{-1} (DM basis). Similarly, the supplementation of balanced digestible protein also significantly ($P < .05$) affected the final weights of the whole body, eviscerated and sliced adult pacu (Table 5). The maximum final weight of whole-body adult pacu (1325.3 ± 18.2 g) was obtained at 238 g BDP kg^{-1}. The supplementation of 272 g BDP kg^{-1} resulted in obtaining maximum final weight of the eviscerated (1210 ± 33.5) and sliced fish (941 ± 41.5) (Table 5).

Based on the quadratic regression analysis, feed intake (FI) (Fig. 3), final body weight (FBW) (Fig. 4) and body weight gain (BWG) (Fig. 5) responses were significantly ($P < .05$) increased up to the plateau and thereafter significantly ($P < .05$) decreased.

Table 6

Effect of variations in marketing strategy and feed costs on the gross margins of the final yield and the corresponding economically optimized levels of balanced digestible protein (BDP) of adult pacu.

Scenarios		BDP	Feeding costs	Gross margins
a. Variations in marketing strategy				
WBP Normal selling price	Normal feeding costs	216	0.177	2.496
(− 20) Alternate selling price	Normal feeding costs	211	0.176	1.961
(+ 20) Alternate selling price	Normal feeding costs	219	0.178	3.031
Evisc. P Normal selling price	Normal feeding costs	220	0.179	3.579
(− 20) Alternate selling price	Normal feeding costs	216	0.177	2.828
(+ 20) Alternate selling price	Normal feeding costs	222	0.179	4.331
Sliced P Normal selling price	Normal feeding costs	222	0.179	4.124
(− 20) Alternate selling price	Normal feeding costs	220	0.179	3.263
(+ 20) Alternate selling price	Normal feeding costs	224	0.180	4.984
b. Variations in feeding costs				
WBP Normal selling price	Normal feeding costs	216	0.177	2.496
WBP Normal selling price	(− 20) alternate FC	218	0.170	2.504
WBP Normal selling price	(+ 20) alternate FC	218	0.171	2.503
Evisc. P Normal selling price	Normal feeding cost	220	0.179	3.579
Evisc. Normal selling price	(− 20) alternate FC	221	0.171	3.587
Evisc. Normal selling price	(+ 20) alternate FC	221	0.172	3.586
Sliced P Normal selling price	Normal feeding cost	222	0.179	4.124
Sliced P Normal selling price	(− 20) alternate FC	224	0.172	4.132
Sliced P Normal selling price	(+ 20) alternate FC	225	0.173	5.396
WBP Normal selling price	Normal feeding cost	216	0.177	2.496
WBP Normal selling price	(− 20) alternate FC	218	0.171	2.503
WBP Normal selling price	(+ 20) alternate FC	216	0.176	2.497
Evisc. P Normal selling price	Normal feeding cost	220	0.179	3.579
Evisc. P Normal selling price	(− 20) alternate FC	221	0.172	3.586
Evisc. P Normal selling price	(+ 20) alternate FC	220	0.178	3.580
Sliced P Normal selling price	Normal feeding cost	222	0.179	4.124
Sliced P Normal selling price	(− 20) alternate FC	223	0.172	4.131
Sliced P Normal selling price	(+ 20) alternate FC	222	0.178	4.125

Note: BDP = Balanced digestible protein; WBP = Whole body adult pacu; Evis. P = Eviscerated adult pacu; Sliced P = Sliced adult pacu; FC = Feeding costs (US$ g^{-1} feed); GM = Gross margins (US$ g^{-1} whole body/eviscerated/sliced adult pacu^{-1}).

(− 20) and (+ 20) alternate selling price indicate the 20% decrease and increase in the selling prices of the final yields as compared to the normal selling prices of those yields obtained for the calculations of the present study.

(− 20) and (+ 20) alternate FC indicate the total feeding costs based on a 20% decrease and increase in the selling price of fish meal and soybean meal, respectively as compared to the normal selling prices of those ingredients obtained for the calculations of the present study.

i. Effect of fluctuations in the selling price of fish meal on the gross margins and optimum economic levels of balanced digestible protein.

ii. Effect of fluctuations in the selling price of soybean meal the gross margins and optimum economic levels of balanced digestible protein.

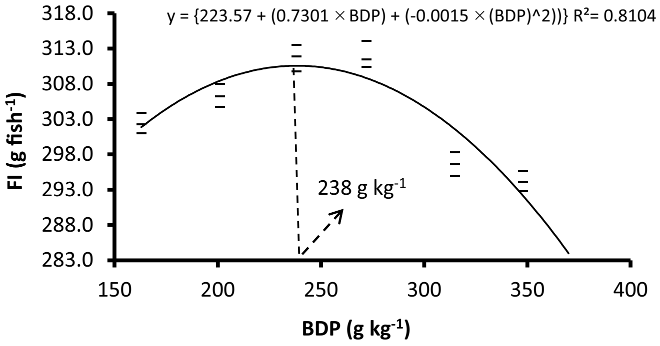

Fig. 3. Quadratic regression analysis of the feed intake of adult pacu fed increased levels of balanced digestible protein.

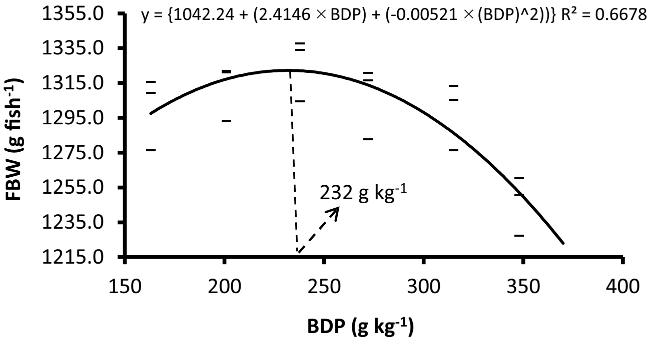

Fig. 4. Quadratic regression analysis of the final body weight of adult pacu fed increased levels of balanced digestible protein.

3.2. Optimum levels of balanced digestible protein for maximum performance

Based on maximum final body weight and body weight gain data, the optimum levels of balanced digestible protein were modeled as 232 (Fig. 4) and 245 g kg^{-1} (DM basis) (Fig. 5), respectively by the quadratic regression model. The corresponding optimum DP: DE feed ratios were calculated as 15.46 and 16.70 g MJ^{-1} (DM basis) for maximum final body weight and body weight gain, respectively of adult pacu.

3.3. Economic model outputs

The economic analyses performed in this study revealed that the supplementation of increased levels of balanced digestible protein considerably affected the feeding costs, revenues and gross margins of the final yield (Fig. 6: Part-A, B and C). The feed costs per experimental diet were linearly associated with the increased dietary protein contents (Fig. 1).

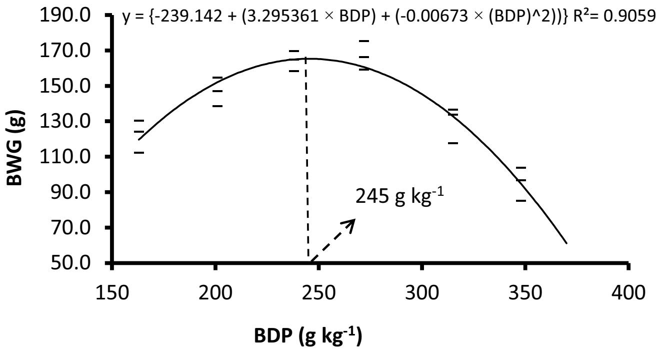

Fig. 5. Optimum level of balanced digestible protein for maximum body weight gain of adult pacu modeled by the quadratic regression model.

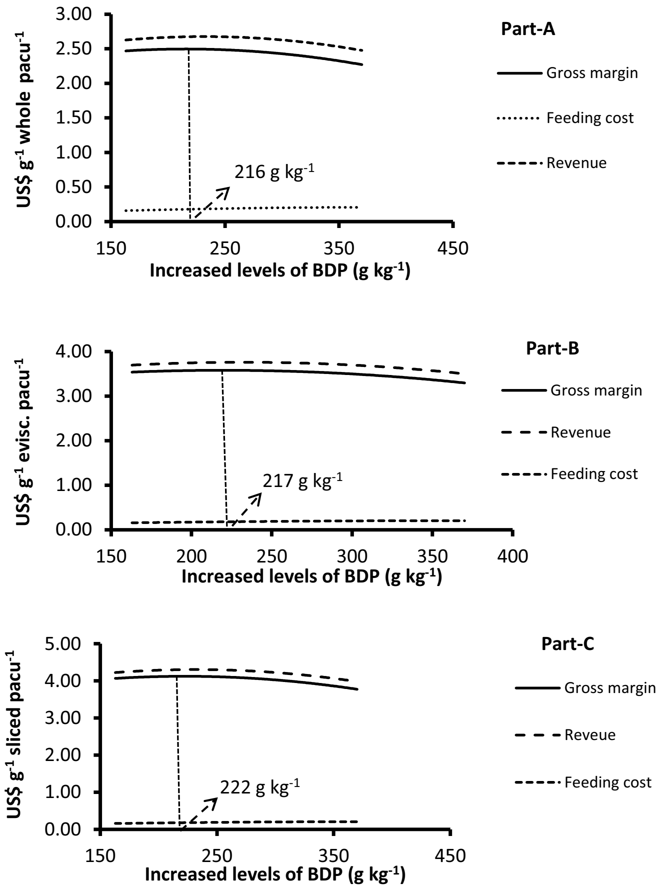

Fig. 6. Economically optimized levels of balanced digestible protein modeled by the individual-based economic model for maximum gross margins of the final yields (Part-A, B and C) of adult pacu.

3.4. Optimum economic levels of balanced digestible protein for maximum profitability

According to the individual based economic model (Fig. 6; Part-A, B and C), with the feeding costs of 0.177 US$ g^{-1} whole body adult $pacu^{-1}$, the maximum gross margin of 2.496 US$ g^{-1} whole body adult $pacu^{-1}$ was achieved at the optimum economic level of 216 g BDP kg^{-1} (DM basis) for pacu that are marketed as whole body adult fish (Fig. 6; Part-A). Similarly, with the current normal feeding costs of 0.179 US$ g^{-1} processed adult pacu $part^{-1}$, the maximum gross margins (GMs) of 3.579 and 4.124 US$ g^{-1} processed fish $part^{-1}$ were achieved at the optimum economic levels of 220 and 222 g BDP kg^{-1} (DM basis) for fish that are marketed in the form of eviscerated (Fig. 6; Part-B) and sliced (Fig. 6; Part-C) adult pacu, respectively. The corresponding optimum economic DP: DE feed ratios were calculated as 14.72, 14.79, and 15.13 g MJ^{-1} (DM basis) for whole body, eviscerated and sliced adult pacu, respectively.

3.5. Hypothesized alternate scenarios

The hypothesized alternate scenarios (Table 6) confirmed that the 20% increase and decrease in the selling price of fish meal and soybean meal and that of the final yield product of fish considerably affected the total feeding costs, revenues and gross margins. These scenarios indicated that the optimum economic levels of balanced digestible protein were subjected to the feed costs and selling strategy of final yield (Table 6).

3.6. Proximate composition of whole body pacu

The proximate composition of whole body adult pacu fed increased levels of balanced digestible protein is presented in Table 7. According to the ANOVA done, the crude protein and lipid content of the whole body adult pacu were significantly ($P < .05$) affected by balanced digestible protein. The whole body ash content was also significantly ($P < .05$) associated with BDP but the overall differences for the ash content among different treatments were not promising. On the other hand, the whole body moisture did not show any significant ($P > .05$) association with balanced digestible protein (Table 7).

4. Discussion

The supplementation of optimum levels of balanced dietary protein in fish feeds is needed to obtain maximum growth performance and feed efficiency as well as optimum physiological aspects of fish (Guroy et al., 2017; Li et al., 2009; Ma et al., 2019; National Research Council, 2011; Sa et al., 2014; Wilson, 2002). The use of diets containing imbalanced protein (that lack amino acid balance) could adversely affect the growth and feeding responses of the animal and may lead into inaccurate estimation of dietary protein (Eits et al., 2005a, 2005b; Gous and Morris, 1985; Khan et al., 2019). In this study, adult pacu (*P. mesopotamicus*) fed with increased levels of balanced digestible protein showed significantly ($P < .05$) improved performance and feeding aspects. Based on the analysis of variance (ANOVA), the feed efficiency (FE), specific growth rate (SGR) and feed conversion ratio (FCR) of adult pacu were significantly ($P < .05$) associated with balanced digestible protein in a parabolic manner while protein efficiency ratio (PER) was significantly ($P < .05$) affected in a linear and descending way by digestible protein (Table 5). Based on the quadratic regression model, the feed intake (FI) (Fig. 3), final body weight (FBW) (Fig. 4) and body weight gain (BWG) (Fig. 5) of pacu fed different levels of BDP were significantly ($P < .05$) increased up to a plateau and beyond significantly ($P < .05$) decreased. These results showed that animals tried to attain maximum growth through enhanced feed efficiency (Emmans, 1981; Gous et al., 2018). Abimoard et al. (2007) in juvenile pacu reported that feed intake aspect was decreased with the increased dietary protein content. The reason for the restricted feed intake with increased dietary protein content might be the lack of amino acid balance (Gous and Morris, 1985). In concordance to the present study, a similar behavior of FCR and FE has been reported in juvenile pacu in response to dietary protein (Bicudo et al., 2010; Klein et al., 2014; Signor et al., 2010). The protein efficiency ratio (PER) was significantly ($P > .05$) affected by the BDP in a linear and descending way (Kim et al., 2003; Wilson, 2002). Similar results for PER have been reported by Bicudo et al. (2010) in juvenile pacu (*P. mesopotamicus*), Kim et al. (2005) in Olive flounder (*Paralichthys olivaceus*) and Ma et al. (2019) in sub-adult triploid rainbow trout. Some studies report that increasing the energy level of the diet could improve the protein utilization

Table 7
Whole body proximate composition (g kg^{-1} wet weight basis) of pacu adults fed increased levels of balanced digestible protein (g kg^{-1} DM basis).

Treatments	Moisture	Crude protein	Total lipid	Ash
Initial	580.00	165.00	201.90	46.80
163	560.03 ± 15.47^{b}	147.83 ± 1.29^{c}	225.90 ± 3.62^{a}	55.97 ± 1.03^{a}
201	574.53 ± 20.66^{ab}	151.00 ± 2.17^{b}	211.97 ± 4.85^{b}	50.87 ± 4.67^{ab}
238	585.13 ± 3.84^{a}	153.13 ± 0.32^{b}	203.30 ± 1.95^{c}	46.43 ± 4.71^{b}
272	584.50 ± 6.07^{a}	156.23 ± 0.72^{a}	198.03 ± 5.20^{bc}	46.77 ± 2.80^{b}
315	573.03 ± 8.85^{ab}	157.17 ± 1.36^{a}	190.07 ± 0.90^{bc}	51.03 ± 1.70^{ab}
348	$579.33 \pm 6.61a^{b}$	158.17 ± 0.35^{a}	182.77 ± 1.89^{c}	50.90 ± 1.81^{ab}
P-value	0.1764	0.0000	0.0000	0.0294

Note: Values are presented as means of three replicates ± standard deviation, numbers in the same column with different superscripts are significantly ($P < 0.05$) different.

efficiency of fish (Nguyen, 2017). However, the increasing levels of dietary energy besides improving the PER may negatively affect the quality of the final yield (Bicudo et al., 2010), with the increase in aquatic nitrogen load (Thoman et al., 1999). Like the present study, Bai et al. (1999) in juvenile yellow puffer (*Takifugu obscyrus*) and Kim et al. (2002, 2005) in juvenile olive flounder (*Paralichthys olivaceus*) (Temminck et Schlegel) and Olive flounder (*Paralichthys olivaceus*), respectively reported that PER showed a linear and downstream response to the increased dietary protein. Dabrowski (1979) stated that the response of PER to dietary protein depends on several internal and external factors. PER usually considers lipid deposition as protein deposition and does not provide any information about the amount of dietary protein being used for the maintenance purpose (Bicudo et al., 2010). This drawback probably makes it less important in fish nutrition studies (De Silva and Anderson, 1995; Kim et al., 2016).

In fish nutrition, several models such as broken-line, quadratic regression and second order quadratic regression and exponential models have been used for the estimation of nutritional requirements (Shearer, 2000; National Research Council, 2011). Although the selection of model depends on several factors but quadratic regression analysis could be more accurate than the other methods in the case of wide intervals between dietary levels of the test nutrients and particularly, when the objectives are economic and dose-response evaluations (Zeitoun et al., 1976). The quadratic regression model relates growth to the dose and provides more valid information about the behavior of response at each dietary level of a test nutrient (Corte´s-Jacintoet al. (2003); Gurure et al., 1995; Ye et al., 2017; Zeitoun et al., 1976). Keeping in view the economic and dose-response aims, wide intervals between the dietary protein levels and the behavior of obtained responses, quadratic regression model was exercised in this study (Zeitoun et al., 1976). The present 44-day feeding trial was found enough to develop appropriate equations for the feed intake, final body weight and body weight gain responses of adult pacu. These equations were used to calculate the economically optimized protein levels for obtaining different growth rates and economic gains during different economic scenarios and marketing strategies (Clark et al., 1982; Eits et al., 2005a, 2005b). Based on maximum final body weight and body weight gain of adult pacu, the optimum levels of BDP were modeled as 232 and 245 g kg^{-1} (DM basis), respectively by the quadratic regression model. These values were found considerably different than those reported by Bicudo et al. (2010) 270 g crude protein - CP kg^{-1}, Fernandes et al. (2000) 260 g CP kg^{-1}, Klein et al. (2014) 260 g CP kg^{-1}, and Signor et al. (2010) 250 g CP kg^{-1} for juvenile pacu (with average body weight lower than 300 g). Abimorad et al. (2007) recommended 230 g DP kg^{-1} for juvenile pacu of about 11 g in intensive rearing conditions. The reason might be that Abimorad et al. (2007) evaluated two levels (200 and 230 g kg^{-1}) of dietary protein where only the high protein level showed better results. They reported that feed intake aspect was declined at the 23 g DP kg^{-1}, indicating that the lack of amino acid balance probably affected the feed intake (Gous and Morris, 1985). In the present study, the FI, FBW and BWG of fish were significantly increased up to the plateau and beyond declined (Gous et al., 2018). In the diet-dilution method, animals fed with optimum levels of dietary protein show maximum feeding and growth responses than the dietary protein beyond their requirements (Burnham et al., 1992; Clark et al., 1982; Freeman, 1979).

In fish nutrition, marketing strategy of the final yield is somewhat neglected during the establishment of optimum levels of dietary protein (National Research Council, 2011). In other animals such as poultry, it is already known that optimum levels of dietary protein vary in different marketing strategies of the final yield (Eits et al., 2005b; Pack and Schutte, 1995). The present finding showed that variation in marketing strategy of the final yield results in different gross margins and the corresponding economically optimized levels of BDP also vary accordingly. Using the individual based economic model of Eits et al. (2005b), the economically optimized levels of balanced digestible were modeled as 216, 220, and 222 g kg^{-1} (DM basis) for adult pacu that are marketed in the form of whole body (Fig. 6; Part-A), eviscerated (Fig. 6; Part-B) and sliced (Fig. 6; Part-C) fish, respectively. The optimum economic levels of BDP modeled for obtaining maximum economic benefits were found considerably lower than those estimated for obtaining maximum final body weight (232 g kg^{-1}; DM basis) and body weight gain (245 g kg^{-1}; DM basis) responses of adult pacu. The results obtained for the three marketing strategies of the final yield of adult pacu showed that comparatively high dietary protein was needed for feeding fish that are marketed in the form of sliced fish than the eviscerated and whole body fish. The reason for the high dietary protein required for the rearing of sliced fish might be the higher prices of the sliced fish in consumer market. In the case of sliced fish, the economic model allowed the use of high dietary protein to obtain better growth and maximum profitability without any increase in feeding costs (Pack and Schutte, 1995). On the other hand, due to the low demand and selling prices of the eviscerated and whole body fish as compared to the sliced fish, the economic model calculated comparatively low dietary protein to maintain the sustainability of production (Eits et al., 2005b).

The hypothesized alternate scenarios (Table 6) produced in the present study confirmed that fluctuations in feed costs and selling prices of the final yield considerably influence the gross margins and economically optimized levels of balanced digestible protein (Eits et al., 2005b; Pack and Schutte, 1995). The increase of 20% in the selling prices of fish meal and soybean meal considerably increased the total feed costs and as a result the gross margins were declined during all marketing strategies. In the case of high (20%) feed costs, the economic model calculated low dietary protein than the normal situations because decreasing the dietary protein was necessary to cope with the increased feed costs. However, when the prices of fish meal and soybean meal were decreased by 20%, then the economic model allowed increasing the dietary protein to increase the productivity and profitability without any increase in the feeding costs. On the other hand, the 20% increase in the selling prices of the final fish yield in the consumer market permitted the economic model calculating comparatively high dietary protein to increase the growth rate and get higher profitability but in the case of the 20% decrease in the selling prices of the final fish yield, the calculation of high dietary protein content was not permitted by the economic model because it would have only increased the feeding costs rather than increasing the economic profit. These alternate scenarios confirmed that feeding fish with optimum dietary protein established for achieving maximum performance may considerably decline the profitability of the final yield, particularly in the circumstances of high feed prices (Abboudi et al., 2006; Árnason et al., 2009; Khan et al., 2019; Lee et al., 2003; Ye et al., 2017). Also, these scenarios indicated that feeding fish that are marketed in the form of sliced and eviscerated fish with dietary protein being established for fish that are marketed only in the form of whole body fish would considerably decline the sustainability of production in intensive aquaculture systems (Eits et al., 2005b; Pack and Schutte, 1995).

The use of proper dietary protein to energy ratios in fish feeds avoids the utilization of dietary protein as an energy source (National Research Council, 2011). In the present study, based on maximum final body weight (FBW) and body weight gain (BWG) of adult pacu, the corresponding optimum DP: DE feed ratios were calculated as 15.47 and 16.70 g MJ^{-1} (DM basis), respectively. While for obtaining maximum profitability from the marketing of whole body, eviscerated and sliced adult pacu, the corresponding optimum economic DP: DE feed ratios were calculated as 14.72, 14.79, and 15.13 g MJ^{-1} (DM basis), respectively. The establishment of optimum DP: DE feed ratios for fish are important for sustainable production (Shiau and Lan, 1996) as well as for obtaining the lowest fatness in an animal at a given weight (Gous et al., 1990). In the present study, the optimum DP: DE feed ratios calculated for maximum biological performance (FBW and BWG) were found different than those calculated for obtaining maximum economic profit in different marketing strategies of the final yield. The DP: DE feed ratios obtained in this study for maximum performance of adult pacu were found different than the finding (CP: DE; 22.2 g MJ^{-1}) of Bicudo et al. (2010) for maximum performance of juvenile pacu. Bicudo et al. (2010) suggest that the use of optimum protein to energy feed ratios calculated for maximum performance responses could not be appropriate when the objective of fish farming is to get good quality of the final yield with maximum economic profits. Sweilum et al. (2005) evaluated the economic returns of Nile tilapia (*Oreochromis niloticus*) production and reported that maximum economic profit with better performance was gained when the fish fed diets provided with adjusted and intermediary protein to energy ratios.

In the present study, whole body proximate composition of adult pacu, except the moisture content, was significantly ($P < .05$) affected by the balanced digestible protein. Similar results have been reported by Abimorad et al. (2007), Bicudo et al. (2010) and Klein et al. (2014) for juvenile pacu in response to dietary protein. The whole body crude protein and total lipid contents were significantly ($P < .05$) affected by BDP in ascending and descending manners, respectively (Abimorad et al., 2007; Bicudo et al., 2010; Gous et al., 1990). The whole body ash content was significantly ($P < .05$) associated with digestible protein in a parabolic way but the overall difference for ash among the treatments was not promising. The whole body moisture content did not show any significant ($P > .05$) difference among the treatments. In concordance to the present study, Catacutan et al. (2001) in mangrove red snapper (*Lutjanus argentimaculatus* Forsskal 1775), Corte ́s-Jacintoet al. (2003) in juvenile crayfish (*Cherax quadricarinatus* (Decapoda: Parastacidae), Kim et al. (2004) in Korean rockfish (*Sebastes schlegeli*), and Ye et al. (2017) in juvenile and pre-adult giber carp (*Carassius auratus gibelio* var. CAS III) have reported a similar association between the whole body proximate composition of fish and dietary protein.

In conclusion, the results obtained for the two different objectives of this work revealed that the optimum levels of balanced digestible protein modeled for obtaining maximum biological performance were considerably higher than those modeled for obtaining maximum profitability of the final yield of adult pacu. Furthermore, it was confirmed that the economically optimized levels of BDP were greatly affected by the marketing strategy of the final yield. The hypothesized scenarios helped to better explain the practicality and flexibility of the individual based economic model exercised in this study. Thus, this study suggests that optimum levels of dietary protein must be adjusted in the case of any variation in feed costs or marketing strategy of the final yield to maintain the sustainability of production in intensive aquaculture systems.

Author contributions

1. Dr. Kifayat Ullah Khan: Designed the study, conducted the experiment, collected the experimental data, performed the biological and economic analyses, statistically analyzed the experimental data, drafted and wrote the paper and performed revisions
2. Prof. Dr. Robert Mervyn Gous: Helped in designing the study, economic evaluations, statistical analysis of the experimental data and paper drafting, writing and revisions
3. Prof. Dr. Nilva Kazue Sakomura: Helped in designing the study, economic evaluations, statistical analysis of the experimental data and paper drafting, writing and revisions
4. Dr. Jefferson Moraes Azevedo: Helped in the statistical analysis of the experimental data and economic evaluations
5. Dr. Thiago Matias T. Nascimento: Helped in the collection of the experimental data and biological analyses
6. Dr. Cleber Fernando M. Mansano: Helped in the collection of the experimental data and biological analyses
7. Mr. Rafael de Souza Romaneli: Helped in the collection of the experimental data and biological analyses
8. Ms. Thaís da Silva Oliveira: Helped in the collection of the experimental data and biological analyses
9. Mr. André Zuffo Boaratti: Helped in the collection of the experimental data and biological analyses
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Declaration of Competing Interest

All authors of this work confirm no potential conflict of interests.

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