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Screening of selected feedstuffs by juvenile pacu, *Piaractus brachypomus* (Cuvier, 1818)

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Abstract

Apparent crude protein (ADC_{CP}), crude fat (ADC_{CF}), gross energy (ADC_{GE}) and phosphorus (ADC_{p}) digestibility coefficients of several typical and novel feedstuffs were determined to be utilized in formulated diets of pacu (20.3 ± 5.6 g). The tested feedstuffs included two fish meals, four terrestrial animal by-products, three plant protein concentrates, six high-protein plant meals and two low-protein plant meals. The values of ADC_{CP} varied in different fish meals ranging from 96.17% for Koli fish meal to 95.17% for Sardine fish meal. The values of ADC_{CP} for plant protein concentrates ranged from 92.17% for wheat gluten meal to 93.17% for corn gluten meal. The values of ADC_{CP} ranged from 50.17% for faba bean meal to 73.43% for spirulina meal. The ADC_{p} ranged from 51.77% for low-protein plant meals to 85.72% for fish meals. A significant ($p < 0.05$) linear regression ($r^2 = 0.96$) was observed among in vivo ADC_{CP} of five feedstuff classes fed to pacu and in vitro ADC_{CP}. Based on these observations, we conclude that pacu as an omnivorous fish tend to utilize nutrient-rich ingredients from high-protein plant meals to fish meals.

KEYWORDS

feedstuff, in vitro digestion, in vivo apparent digestibility coefficients, phosphorus digestibility, *Piaractus brachypomus*

1 | INTRODUCTION

The estimated production of the aquaculture industry for human consumption in 2014 was 73.8 million tons, where the United States with 4.5% of aquatic production was ranked second after Asia with 88.91% (FAO, 2016b). One of the objectives of the aquaculture industry was the domestication of wild species or the use of potential indigenous species with high growth rate, resistance to high stocking density and reproduction in captive conditions (Laure Begout, Kadri, Huntingford & Damsgard, 2012). In this regard, research is being conducted on the identification of bionormative indices of the candidate species, such as *Schizothorax pelzami*, *Barbus sharpeyi*, *Lutjanus johni*, *Rachycentron canadum*, *Pampus argenteus* and *Piaractus brachypomus* (Ebrahimi, Kamrani, Heydarnejad & Safari, 2017; FAO, 2016a).

Pacu from Characidae family and the Serrasalmidae subfamily known as the red pacu has high growth rate, dietary diversity, high resistance to stressful conditions in the breeding environment and the ability to withstand high storage densities (Fernandes, Lochmann & Bocanegra, 2004). In Latin America countries, the amount of pacu production is ranked second after tilapia cultivation (Suplicy, 2007). One of the challenges facing the aquaculture industry is to formulate the optimum diet and to determine the inclusion levels of feed ingredients in the diet (Glencross, Booth & Allan, 2007; Safari, Naserizadeh & Mohammadi Arani, 2016; Safari, Shahsavani, Paolucci & Atash, 2014). In this regard, the amount of palatability, apparent digestibility coefficient (ADC) of feed ingredients or diet, growth performance and the rate of nutrient retention, blood biochemistry and histopathologic studies are key indicators of feed ingredients assessment (Glencross et al., 2007).

Considering the strategy of the aquaculture industry to the development of fast-growing species, one of the approaches to produce economic aquafeed is the recognition of potential feed

ingredients that can be used. In this regard, the choice of the type of feed ingredient, the tolerable threshold of the antinutritional factors of the feed ingredients and the biological effects of these compounds on the physiological pathways and also the environmental effects of diets on aquatic systems should be carefully assessed. Moreover, attention should be paid to the method of feed processing (cold press, expansion and extrusion), basal formulation, adaptation period and the methods (Guelph, stripping and dissection) and the duration of faecal collection as key points in in vivo digestibility studies (Glencross et al., 2007).

There are several studies on the determination of in vivo ADC of gross energy and nutrients of feed ingredients in *P. brachypomus* (Fernandes et al., 2004); gross energy, crude fat and fatty acids of vegetable oils and poultry fat in feed ingredients of *P. mesopotamicus* (Gonçalves & Cyrino, 2014); crude protein, gross energy and amino acids of some feed ingredients in *P. mesopotamicus* (Abimorad, Squassoni & Carneiro, 2008); and gross energy and nutrients of crude and cooked Cassava and Palm in *Colosoma macropomus* and *P. brachypomus* (Chu-Koo, Camargo, Alvan-Aguilar, Trushenski & Kohler, 2016). According to the literature review, the limited feed ingredient classes with various chemical compositions were evaluated. Due to being technical, time-consuming and high cost of in vivo digestibility assays, in vitro digestibility tests (under constant pH conditions or with pH reduction) as an alternative method can be used (Dimes & Haard, 1994; Safari et al., 2014, 2016). Previous studies have confirmed that there is a good correlation between in vivo and in vitro digestibility coefficients (in constant pH conditions) to predict protein quality. Assessment of in vivo and in vitro ADCs leads to the classification of feed ingredients based on the aquaculture species and identifies potent nutritive sources (crude protein, crude fat, minerals, etc.) and gross energy, as well as formulates a balanced diet. Therefore, the aim of this study was to evaluate in vivo and in vitro ADCs of a wide range of commonly used feed ingredients in the practical diet of red pacu (*P. brachypomus*).

2 | MATERIALS AND METHODS

2.1 | Ingredients and experimental diets

Seventeen feedstuffs that were tested in this study were grouped into five classes based on their origin and utility in aquafeed formulation (Table 1). The five feedstuff classes were as follows: fish meals, terrestrial animal by-products, plant protein concentrates with <650 g/kg crude protein, high-protein plant meals with 250–600 g/kg crude protein and low-protein plant meals used as a source of carbohydrate that have protein concentrates of <200 g/kg of crude protein. Ytterbium oxide (Yb_2O_3; 0.1 g/kg) was used as inert marker (Safari et al., 2016). Briefly, a basal diet (326 g/kg, crude protein; 74 g/kg, crude fat; and 17.6 MJ/kg, gross energy) was formulated that met or exceeded all known nutritional requirements (Vásquez-Torres & Arias-Castellanos, 2013) for pacu (Table 2). Test diets were then formulated for each of the test feedstuffs using a 70:30 ratio (dry weight basis) of basal diet to test an ingredient with WinFeed software (WinFeed Limited, Cambridge, UK). After feedstuffs were ground to a particle size of <250 μm (Glencross et al., 2007), the mash was processed by extrusion cooking technology (Fardan Machine Shargh CO, Khorasan Razavi, Iran) with mesh size of 3 mm. Then, fish oil was coated over the pellet after extruding the diets and decreasing pellet temperature in mixer, dried at 30°C, packed in three-layer waterproof nylon bags and maintained at −20°C (Hardy and Barrows, 2002) until use.

2.2 | Fish and sample collection

Juvenile pacu *P. brachypomus* (20.3 ± 5.6 g) were randomly distributed in 51 experimental aquaria (150 L; 15 fish/aquarium) with L:D 14:10 hr. Water temperature was maintained at 27°C throughout the feeding trial. DO (7.5 ± 0.59 mg/L), pH (7.19 ± 0.31), hardness (141 ± 6.2 mg/L as $CaCo_3$), unionized ammonia (0.051 ± 0.04 mg/L) and nitrite contents (<0.2 mg/L) were evaluated every 3 days. Fish were fed daily at 8:00, 13:00 and 18:00 hr until apparent satiation for 21 days. Unconsumed feed was collected 3 hr after feeding and weighed. Faecal samples were obtained through collection of voided faeces (settlement faecal collection). The collection of voided faeces was accomplished by siphoning 20 min before the next feeding and continued for 21 days (Glencross et al., 2007; Safari et al., 2014, 2016). Faecal samples for a given tank were dried overnight at 50°C and stored at −20°C until chemical analyses were carried out.

2.3 | Calculation of in vivo apparent nutrient digestibility

In vivo ADCs of organic matter (ADC_{OM}), crude protein (ADC_{CP}), crude fat (ADC_{CF}), gross energy (ADC_{GE}) and phosphorus (ADC_P) of experimental diets and feedstuffs were calculated according to the following equations (Glencross et al., 2007):

$$ADC_{test} = 100 \times \left[1 - \frac{Marker_{test} \times Nutrient_{feces}}{Marker_{feces} \times Nutrient_{test}}\right] \quad (1)$$

$$\text{Nutrient AD ingredient} = \frac{(70\% \times Nutrient_{basal} + Nutrient_{ingredient} \times 30\%) \times AD_{test} - (70\% Nutrient_{basal} \times AD_{basal})}{Nutrient_{ingredient} \times 30\%} \quad (2)$$

In Equation 1, the terms $Marker_{test}$ and $Marker_{feces}$ represent the marker contents (%) of the diet and faeces, respectively, and $Nutrient_{test}$ and $Marker_{feces}$ represent the nutritional parameters (e.g., protein [g/kg] or energy [MJ/kg]) in the diet and faeces, respectively. In Equation 2, apparent digestibility (AD) of an ingredient (%) is the digestibility of a given nutrient from the test feedstuffs included in the diet at 30%. AD_{test} is the apparent digestibility of the nutrient interest (%) in the test diet. AD_{basal} is the apparent digestibility of the basal diet (%), which makes up 70% of the test diet. $Nutrient_{ingredient}$, $Nutrient_{test}$ and $Nutrient_{basal}$ are the levels (g/kg) of the nutrient of interest in the feedstuffs, test diet and basal diet, respectively.

TABLE 1 Composition of five classes of feedstuffs fed to pacu (g/kg, dry weight basis)

Ingredient	Class[a]	Dry matter	Crude protein	Crude fat	Crude fibre	Ash	NFE	Phosphorus	Gross energy (MJ/kg)
Koli fish meal[b]	1	940	515.2	204.4	8.6	126.1	85.7	31.7	21.2
Sardine fish meal[b]	1	954	531.8	213.6	7.3	114.9	86.4	32.8	21.9
Blood meal[c]	2	921	796.7	33.1	9	62.9	19.3	5.2	20
Feather meal[c]	2	951	769.4	67.5	8.3	30	75.8	6.1	21.6
Meat meal[c]	2	952	494.1	181.8	7.4	267	1.7	15.7	18.4
Poultry by-products[b]	2	952	547.5	352.2	9	27.1	16.2	11.8	26.4
Corn gluten meal[b]	3	937	670	30	7	8	222	5.5	20.1
Soy protein concentrate[b,d]	3	964	790.5	17.9	63.3	53.7	38.6	8.4	20.5
Wheat gluten meal[b]	3	927	622	28.7	6.1	7.8	224.4	6.1	19.9
Canola meal[e]	4	927	299.4	41.3	130.1	60.5	395.7	9.3	17.4
Faba bean flour[e]	4	931	260.7	38.8	17.3	29.5	584.7	3.1	17.8
Full-fat canola[e]	4	958	258.6	327.2	155.6	37.4	179.2	6.9	23.9
Full-fat soybean[e]	4	957	362.7	187.5	110.6	50.5	245.7	6.7	21.6
Soybean meal[b,f]	4	907	351.9	44.8	54.8	52.2	403.3	14.3	17.6
Spirulina meal[g]	4	973	598.9	19.5	13.2	68.9	272.5	10.3	19.6
Corn flour[b]	5	907	63.5	62.6	16.5	11	753.4	3.2	17.1
Wheat flour[b]	5	936	111.4	34.6	32	14.7	743.3	3.8	17.2

Note. [a]The five feedstuff classes were as follows: 1: fish meals; 2: terrestrial animal by-products; 3: plant protein concentrates with >650 g/kg crude protein; 4: high-protein plant meals with 250–600 g/kg crude protein; and 5: low-protein plant meals used as a source of carbohydrate that have protein concentrations <200 g/kg of crude protein. [b]Behparvar Aquafeed Co, Iran. [c]Ghezelalaye Abharood Co, Iran. [d]Alcohol-extracted soy protein concentrate. [e]Behpak Oilseed & Meal Co, Iran. [f]Dehulled and defatted soybean meal. [g]Sinamicroalgae Co, Iran.

2.4 | Calculation of voluntary feed intake

Voluntary feed intake (VFI; % body weight [BW] per day) of pacu fed with the experimental diets was calculated according to the following equation (Glencross et al., 2007).

$$\text{Voluntary feed intake (VFI)} = \frac{\text{Feed}_{\text{consumed}}(\text{DM})}{W_{\text{mean}} \times t} \times 100. \quad (3)$$

In Equation 3, $\text{Feed}_{\text{consumed}}$ and W_{mean} represent the consumed feed (g) and the mean weight (g), respectively.

2.5 | Measuring in vitro digestibility of the selected feedstuffs

Fifty-one pacu (20 ± 5 g) were independent of in vivo digestibility study but, with similar fish source, fed with basal diet for 21 days and killed 24 hr after the last meal according to protocols described earlier (Safari et al., 2014, 2016). Briefly, the pyloric caeca and intestine were quickly removed, rinsed with distilled water, dried with paper towel and frozen immediately at liquid nitrogen. The caeca and intestine were homogenized (25 g/75 ml distilled water) using a homogenizer (DI 18 Disperser), and the homogenate was then centrifuged at 10,000 *g* at 4°C for 25 min. The supernatant was collected and stored at −80°C until further use (Safari et al., 2016). Total proteins were evaluated with the Bradford method using bovine serum albumin (BSA) as standard. Specific activity of digestive enzymes was reported based on the soluble protein in extracts with a UV/VS spectrophotometer (Ultro Spec 2000 Pharmacia Biotech, Stockholm, Sweden). Trypsin activity was measured according to the method of Chong, Hashim, Chow-Yang, and Ali (2002) using BAPNA (a-N-benzoyl- D, L-arginine-p-nitroanilide). One unit of activity was defined as 1 μmole of p-nitroaniline released per min at final reading of 410 nm. Chymotrypsin activity was measured using BTEE (N-benzoyl-L-tyrosine ethyl ester) as substrate (Hummel, 1959). One unit of activity was defined as 1 μmole of N-benzoyl-DL-tyrosine released per min at final reading of 256 nm. The digestion protocol was adjusted based on the pH-stat method via two digitally analog sensors with sensing time of 10 s (Carter, Bransden, van Barneveld & Clarke, 1999; Safari, 2011). The extracted supernatant obtained before was added to buffered Trizma-Base at pH 8.0. Ground feedstuffs were added to the buffered enzyme solution (25 ml) at a concentration of 1 mg nitrogen per ml of enzyme solution in a 100-ml

TABLE 2 Composition of the basal diet

Ingredient	g/kg (dry weight basis)
Anchovy fish meal[a]	620
Corn gluten[a]	70
Wheat flour[a]	100
Dextrin[b]	40
Fish oil[a]	100
Carboxymethyl cellulose[b]	20
Choline chlorid[c]	5.9
Vitamin C (stay)[c]	9
Vitamin premix[c,d]	20
Mineral premix[c,e]	15
Ytterbium oxide[b]	0.1
Chemical composition	**g/kg (dry weight basis)**
Dry matter	901
Crude protein	326
Crude fat	74
Crude fibre	140
Nitrogen-free extract	408
Ash	52
Phosphorus	12
Gross energy (MJ/kg)	17.6
Crude fat/Crude protein	0.23

Note. [a]Behparvar Aquafeed Co, Iran. [b]Sigma, Germany. [c]KIMIAROSHD Pharmaceutical Co., Iran. [d]Mineral premix contains (mg/kg) Mg, 100; Zn, 60; Fe, 40; Cu, 5; Co, 0.1; I, 0.1; antioxidant (BHT), 100. [e]Vitamin premix contains (mg/kg) E, 30; K, 3; thiamine, 2; riboflavin, 7; pyridoxine, 3; pantothenic acid, 18; niacin, 40; folacin, 1.5; choline, 600; biotin, 0.7; and cyanocobalamin, 0.02.

conical flask and then incubated in a shaking water bath at 25°C for 12 hr. The proteins and peptides were precipitated by the addition of 14% sulfosalicylic acid, and the solution was shaken for further 20 min. After centrifugation (20,000 *g*, 4°C, 6 min), the supernatant was discarded, 50 ml of distilled water was added, and the resulting mixture was recentrifuged (the procedure was repeated five times; Hardy & Barrows, 2002). The mixture was then filtered through a 0.7-mm millipore filter, air-dried, weighed to measure the indigestible dry matter and analysed for nitrogen Kjeldahl. The insoluble nitrogen fraction was assumed to equate to the nondigestible nitrogen, and in vitro ADC_{CP} was calculated as $100 \times (N_{diet} - N_{insoluble}/N_{diet})$. Dry matter digestibility was calculated in the same way using the weight of dry matter that remained on the filter. Measuring of in vitro ADCs of nitrogen and dry matter for each feed ingredient was carried out in three replicates.

2.6 | Chemical analysis

Analysis of dry matter (oven drying, 105°C), crude protein (N × 6.25, Kjeldahl system: Buchi Labortechnik AG, Flawil, Switzerland), crude fat (Soxtec System HT 1043: Foss Tecator, AB), ash (muffle furnace, 550°C), gross energy (Parr bomb calorimetry model 1266, Parr Instrument Co., Moline, IL) and crude fibre (after digestion with H_2SO_4 and NaOH) in feedstuffs, diets and faeces were performed according to standard methods (AOAC, 2005). Nitrogen-free extract (NFE) was calculated by subtraction (dry matter minus crude protein, crude fat, crude fibre and ash content). Organic matter (OM) was calculated by subtraction (dry matter minus ash content). Ytterbium oxide and total phosphorus were determined in diets and faeces by inductively coupled plasma atomic absorption spectrophotometry (ICP; GBC Integra XL, Australia).

2.7 | Statistical analysis

All percentage data were transformed using arcsine method. Normality of the data and the homogeneity of variance were confirmed using Leaven and Kolmogorov–Smirnov tests (Zar, 1999), respectively. One-way ANOVA was used to compare the treatments in a completely randomized design. All diets were studied at the same time. Duncan test was applied to compare significant differences among the treatments ($p < 0.05$) with SPSS™ version 19. In addition, the relationship between in vivo ADC_{CP} of pacu fed with different feedstuff classes and in vitro ADC_{CP} was estimated using linear regression analysis with XLSTAT 2012. All measurements were carried out in triplicate, and results were given as mean ± *SEM*.

3 | RESULTS

3.1 | In vivo ADCs of feed ingredients and VFI

3.1.1 | Fish meals

The values of in vivo ADC_{OM}, ADC_{CP}, ADC_{CF}, ADC_{GE} and ADC_P of fish fed Anchovy fish meal were significantly ($p < 0.05$) higher than those of other fish meals (Table 3). The comparison of the average values for in vivo ADC_{OM} of fish meals (90.92%) did not show a significant difference with that of plant protein concentrates (96.13%; Table 4). Nevertheless, the value of ADC_{OM} of fish meals was significantly ($p < 0.05$) higher than those of animal by-products (75.68%), high-protein plant meals (80.56%) and low-protein plant meals (72.26%; Table 4). The average values for ADC_{CP} in fish meals (95.67%) did not have a significant difference with those of plant protein concentrates (92.56%). The significantly ($p < 0.05$) highest average values of ADC_{CF} (96.82%), ADC_{GE} (96.78%) and ADC_P (85.72%) were for fish meal class (Table 4).

3.1.2 | Animal by-products

In vivo ADC_{OM} and ADC_{CP} values of animal by-products were in the range of 67.63%–82.23% and 78.23%–90.63%, respectively (Table 3). The value of in vivo ADC_{OM} poultry by-products (82.23%) was significantly ($p < 0.05$) higher than those of meat meal (80.53%), feather meal (72.30%) and blood meal (67.63%; Table 3). The significantly ($p < 0.05$) highest in vivo ADC_{OM}, ADC_{CP}, ADC_{GE} and ADC_P are associated with poultry by-products, and the smallest amount

TABLE 3 In vivo ADCs (±*SEM*[1]) for organic matter (ADC_{OM}), crude protein (ADC_{CP}), crude fat

Ingredients	ADC_{OM}	ADC_{CP}	ADC_{CF}	ADC_{GE}	ADC_{P}	VFI
Basal diet	98.41	96.68	98.01	97.34	88.49	1.69
Fish meals						
Koli fish meal[2]	89.50^{a}	96.17^{b}	97.50^{b}	97.27^{b}	87.20^{b}	1.53^{b}
Sardine fish meal[2]	92.33^{b}	95.17^{a}	96.13^{a}	96.30^{a}	84.23^{a}	1.27^{a}
SEM	0.41	0.18	0.54	0.50	0.26	0.58
Animal by-products						
Blood meal[3]	67.63^{a}	78.23^{a}	76.27^{a}	78.43^{a}	63.43^{b}	1.03^{bc}
Feather meal[3]	72.30^{b}	81.37^{b}	79.30^{b}	80.27^{b}	60.47^{a}	0.67^{a}
Meat meal[3]	80.53^{c}	85.17^{c}	89.30^{d}	85.43^{c}	80.83^{c}	0.93^{b}
Poultry by-products[3]	82.23^{d}	90.63^{d}	85.33^{c}	91.40^{d}	85.27^{d}	1.07^{c}
SEM	0.45	0.31	0.28	0.38	0.33	0.58
Plant protein concentrates						
Corn gluten meal[2]	96.20^{ab}	93.17^{b}	77.97^{b}	86.30^{a}	49.20^{a}	2.37^{a}
Soy protein concentrate[2]	95.60^{a}	92.33^{a}	74.43^{a}	93.43^{c}	61.30^{c}	2.63^{b}
Wheat gluten meal[2]	96.60^{b}	92.17^{a}	79.27^{c}	87.57^{b}	52.43^{b}	2.40^{a}
SEM	0.36	0.20	0.34	0.39	0.31	0.42
High-protein plant meals						
Canola meal[4]	78.27^{b}	68.67^{e}	71.37^{c}	85.10^{f}	69.20^{d}	2.10^{c}
Faba bean flour[2]	67.53^{a}	50.17^{a}	63.50^{a}	59.33^{a}	44.13^{a}	1.30^{a}
Full-fat canola[4]	78.70^{b}	64.33^{d}	89.23^{f}	83.47^{e}	73.43^{e}	2.87^{e}
Full-fat soybean[4]	79.40^{b}	57.87^{c}	73.30^{d}	73.30^{b}	62.43^{c}	2.63^{d}
Soybean meal[4]	81.10^{c}	53.33^{b}	65.23^{b}	73.57^{b}	60.43^{b}	1.80^{b}
Spirulina meal[5]	98.33^{d}	73.43^{f}	81.97^{e}	82.33^{c}	89.30^{f}	3.03^{f}
SEM	0.57	0.48	0.26	0.35	0.35	0.35
Low-protein plant meals						
Corn flour[2]	72.97^{b}	44.83^{b}	63.63^{a}	69.20^{b}	44.40^{a}	1.00^{b}
Wheat flour[2]	71.60^{a}	40.43^{a}	65.43^{b}	65.37^{a}	59.13^{b}	0.90^{a}
SEM	0.73	0.31	0.43	0.28	0.25	0.10
Average value for all animal ingredients	80.75^{a}	87.79^{b}	87.31^{b}	88.18^{b}	76.91^{b}	1.08^{a}
Average value for all plant ingredients	83.3^{a}	66.43^{a}	73.21^{a}	78.09^{a}	60.49^{a}	2.09^{b}

Notes. Different superscript (a-e) within a column for a feedstuff class indicates significant differences at $p < 0.05$. (ADC_{CF}), gross energy (ADC_{GE}), phosphorus (ADC_{P}) and voluntary feed intake (VFI; % body weight per day) of basal diet and selected feedstuffs fed to pacu obtained from the settlement faecal collection method ($n = 3$).

[1]Pooled standard error of mean. [2]Behparvar Aquafeed Co, Iran. [3]Ghezelalaye Abharood Co, Iran. [4]Behpak Oilseed & Meal Co, Iran. [5]Sinamicroalgae Co, Iran.

is related to blood meal (Table 3). The highest ($p < 0.05$) in vivo ADC_{CF} was obtained from meat meal (89.30%), and the smallest one ($p < 0.05$) was for blood meal (76.27%; Table 3). The value of VFI in pacu fish fed with poultry by-products (1.07% BW per day) did not show a significant difference with blood meal (1.03% BW per day; Table 3), while the VFI value in pacu fish fed with poultry by-products had a significant difference ($p < 0.05$) with meat meal (0.93% BW per day) and feather meal (0.67% BW per day; Table 3). The comparison of the average values of feed ingredients found in each class showed that in vivo ADC_{OM} value of animal by-products (75.68%) did not have a significant difference with those of plant meals with low protein content and high protein content (Table 4). Nonetheless, the values of ADC_{OM} of two mentioned feed classes were significantly ($p < 0.05$) lower than those of fish meals (90.92%) and plant protein concentrates (96.13%; Table 4). Comparing the average values of feed ingredients in each class revealed that in vivo ADC_{CF} and ADC_{GE} values of animal by-products were not significantly different from the amount of ADC_{CF} and ADC_{GE} of plant protein concentrates (Table 4). Moreover, the average values for in vivo ADC_{P} of animal by-products (72.5%) did not have a significant difference with those of ADC_{P} of high-protein plant meals (66.49%; Table 4).

TABLE 4 Mean In vivo ADCs (±*SEM*[1]) for organic matter (ADC_{OM}), crude protein (ADC_{CP}), crude fat (ADC_{CF}) and gross energy (ADC_{GE}) from five feedstuff classes fed to pacu fish (n = 3)

Ingredients	ADC_{OM}	ADC_{CP}	ADC_{CF}	ADC_{GE}	ADC_{P}
Fish meals	90.92^{c}	95.67^{d}	96.82^{d}	96.78^{d}	85.72^{c}
Animal by-products	75.68^{ab}	83.85^{c}	82.55^{c}	83.88^{c}	72.5^{b}
Pant protein concentrates	96.13^{c}	92.56^{d}	77.22^{bc}	89.1^{c}	54.31^{a}
High-protein plant meals	80.56^{b}	61.3^{b}	74.1^{b}	76.18^{b}	66.49^{b}
Low-protein plant meals	72.26^{a}	42.63^{a}	64.53^{a}	67.28^{a}	51.77^{a}
SEM	3.77	3.38	3.76	3.08	8.11

Notes. Different superscript (a–d) within a column for a feedstuff class indicates significant differences at $p < 0.05$.
[1]Pooled standard error of mean.

3.1.3 | Plant protein concentrates

The in vivo ADC_{OM} and ADC_{CP} of plant protein concentrates ranged from 95.60% to 96.60%, and 92.17% to 93.17%, respectively (Table 3). The lowest in vivo ADC_{OM}, ADC_{CP} and ADC_{CF} were associated with soy protein concentrate (Table 3). There was a significant difference in ADCOM values of soy protein concentrate and wheat gluten meal (Table 3). In vivo ADC_{OM} of corn gluten meal (96.2%) demonstrated no significant difference with that of soy protein concentrate (95.60%; Table 3). Also, the in vivo ADC_{OM} of corn gluten meal (96.2%) did not have a significant difference with that of wheat gluten meal (96.6%; Table 3). The in vivo ADC_{CP} of the wheat gluten meal (92.17%) did not have a significant difference with that of soybean protein concentrate (92.33%; Table 3). Voluntary feed intake in fish fed soy protein concentrate was higher than fish fed on other plant protein concentrates (Table 4).

3.1.4 | High-protein plant meals

In vivo ADC_{OM} value of high-protein plant meals ranged from 67.53% to 81.10% (Table 3). The highest and lowest values of in vivo ADC_{OM}, ADC_{CP}, ADC_{P} as well as VFI were significantly ($p < 0.05$) related to spirulina meal and faba bean flour, respectively (Table 3). In this regard, the highest ($p < 0.05$) in vivo ADC_{GE} was associated with canola meal (85.10%; Table 3). In vivo ADC_{OM} of full-fat canola (78.7%) did not show significant difference with those of full-fat soybean (79.4%) and canola meals (78.27%; Table 3). Average values for in vivo ADC_{OM} of high-protein plant meals (80.56%) did not show a significant difference with that of animal by-products (75.68%; Table 4). Average values of in vivo ADC_{CF} of high-protein plant meals (74.1%) did not have a significant difference with that of plant protein concentrates (77.22%; Table 4).

3.1.5 | Low-protein plant meals

The value of in vivo ADC_{OM} in corn flour (72.97%) was significantly ($p < 0.05$) higher than that of wheat flour (71.60%; Table 3). There were significant differences in ADC_{CP} and ADC_{GE} values of corn flour and wheat flour (Table 3). The value of VFI in pacu fish fed with corn flour (1.00% BW per day) was significantly ($p < 0.05$) higher than that of wheat flour (0.90% BW per day; Table 3). Although ADC_{OM} values of the two feed classes were significantly different ($p < 0.05$) with those of plant protein concentrates (96.13%), fish meals (90.92%) and high-protein plant meals (80.56%; Table 4). The average values for ADC_{P} of low-protein plant meals (51.77%) did not have a significant difference with those of plant protein concentrates (54.31%; Table 4). The lowest average values for ADC_{OM} (72.26%), ADC_{CP} (42.63%), ADC_{CF} (64.53%), ADC_{GE} (67.28%) and ADC_{P} (51.77%; $p < 0.05$) were for low-protein plant meal class (Table 4).

3.1.6 | Comparison of average values for in vivo ADCs of feed ingredients with animal and plant origin

As shown in Table 3, average values for in vivo ADC_{OM} feed ingredient with plant origin (83.3%) did not have significant differences with that of ADC_{OM} with animal origin (80.75%). The average values for ADC_{CP}, ADC_{CF}, ADC_{GE} and ADC_{P} of feed ingredients with animal origin were significantly higher than those of plant meals. However, the value of VFI in pacu fish fed with plant-origin feed ingredients (2.09% BW per day) was significantly higher than that of animal-origin ingredients (1.08% BW per day; Table 3).

3.1.7 | Relationships between in vivo ADC_{CP}, ADC_{CF} and ADC_{GE} with the chemical composition of test feed ingredients

Significant positive correlations ($p < 0.05$) between in vivo ADC_{CP} and crude protein content of feed ingredients ($R = 0.96$), in vivo ADC_{P} with crude fat content of feed ingredients ($R = 0.928$) and in vivo ADC_{GE} with crude protein content of feed ingredients ($R = 0.879$) were observed (data not shown). Also, a significant negative correlation was found between the in vivo ADC_{CP} and NFE content ($R = -0.918$).

3.1.8 | Relationship between in vivo and in vitro ADC_{CP} of test feed ingredients

A significant linear regression relationship ($p < 0.05$) between in vitro ADC_{CP} of five feed ingredient classes and in vitro ADC_{CP} ($r^2 = 0.96$) was obtained (Figure 1).

4 | DISCUSSION

4.1 | The apparent digestibility of the selected food in in vivo conditions

Piaractus brachypomus is an omnivorous species, and it tends to be herbivore. It should be noted that the present study is the first research to compare commonly used feed ingredient classes. Plant protein products as low-cost sources have a wide range of antinutritional factors (ANFs; phytate, trypsin inhibitors, glucosinolates, tannins, soluble fibres, nonstarch polysaccharides, etc.) as well as the deficiency of some essential amino acids (lysine and methionine; Francis, Makkar & Becker, 2001). ANFs or their metabolites depending on the chemical composition, the type of species (herbivorous, omnivorous and carnivorous), life cycle and the technology used in preparing the diet can affect the mechanism of digestion and absorption of the gastrointestinal tract (Safari et al., 2014, 2016). Further research is required to determine the type of appropriate marker, the method of diet processing, the period and faecal sampling method and the gastrointestinal evacuation rate. According to the present results and other studies, the use of plant-origin or predigested sources (silage) can help to produce balanced diets.

The ADC_{OM} values of soybean meal, fish meal, corn meal and wheat bran in pacu fish, *P. brachypomus* (with an initial weight of 370.21 g, using cold press pellet, 1% chromium oxide for 6 days and siphoning faecal collection method), was reported in the range of 82%–90% (Fernandes et al., 2004). They reported the values of ADC_{CP} of soybean meal (75.88%), fish meal (90.49%), corn (85.06%) and wheat bran (61.62%) and ADC_{CP} of soybean meal (63.03%), fish meal (77%), corn (83.1%) and wheat bran (82.45%). In another study, the values of ADC_{CP} and ADC_{GE} in *P. mesopotamicus* (with an initial weight of 34.3 g, using extruded feed, 1% chromium oxide for 7 days and Guelph faecal collection method) were studied on some feed ingredients (Abimorad et al., 2008). The ADC_{CP} value in fish meal (84.6%), soybean meal (90.6%), alcoholic beverage (81.5%), corn gluten meal (95.6%), wheat bran (87.7%) and corn flour (85.8%) was reported. Moreover, the ADC_{GE} value of fish meal (74.5%), soybean meal (78.1%), alcoholic beverage (73%), corn gluten meal (86%), wheat bran (74.4%) and corn flour (75.8%) was obtained.

The values of ADCs of dry matter of three crude and processed carbohydrate sources (peach palm, plantain and cassava) in *P. brachupomus* (with an initial weight of 3.6 g, using a cold press pellet for 10 days with 1% chromium oxide and siphoning faecal collection method) were reported in the range of 82.1%–87.1% (Chu-Koo et al., 2016). The ADC_{GE} of *P. brachypomus* fed with cooked (81.4%) and crude (74.2%) peach palm, cooked (42.4%) and crude (39.7%) plantain and cooked (40.9%) and raw (32.7%) cassava in the diet using 1% chromium oxide and siphoning faecal collection method was investigated by Chu-Koo et al. (2016). The ADC_{CP} values of the abovementioned three feeds were 85.6%, 57.5% and 53.1%, respectively, for raw feed ingredients, and 86.8%, 62.9% and 55.7%, respectively, for cooked ingredients. In this study, the cooking method did not significantly improve the ADC_{DM}, ADC_{CP}, ADC_{CF} and ADC_{GE} values. In this regard, ADC_{CF} and ADC_{GE} values of materials were reported as 54.9%–92.2% and 32.7%–81.4%, respectively. Perhaps one of the reasons for the failure of the cooking method in this study is to improve the apparent digestibility of the nutrients or the chemical composition (matrix) of the feed ingredients. In this regard, all three carbohydrate sources were high in crude fibre (69.5%–93.7%). In the present study, the carbohydrate sources used (wheat flour and corn flour) showed ADC_{CP} value of about 42.63% and ADC_{GE} value was 67.28%. These sources had a lower amount of crude fibre (16.5%–32%) than those of the previous study. Moreover, in another report, feeding *P. mesopotamicus* (with an initial weight of 146 g, for 5 days using 0.5% chromium oxide and stripping faecal collection method) with the silage of fermented by-products (saline and freshwater fishes and tilapia) with *Lactobacillus plantarum* improved ADC_{CP} values (72.5%–80%; Vidotti, Carneiro & Viegas, 2002).

In the present study, the lowest value of ADC_P in plant protein concentrates (corn gluten meal, wheat gluten meal and soy protein concentrate) and low-protein plant meal (wheat flour and corn flour) was 54.31% and 51.77%, respectively. The highest ADC_P value was measured in fish meals (85.72%). In this regard, animal by-products and high-protein plant meals were in the next ranks. As the crude protein content increases, the ADC_{CP} also increases, and conversely, with an increment in NFE content, the ADC_{CP} will decrease. The ADC_P is considered as an important indicator of aquafeed industry to reduce the eutrophication of the aquatic environment. The amount of phosphorus digestion (ADC_P) depends on the type of fish species (with or without stomach), the chemical matrix of feed ingredients, the type of processing technology of the feed ingredient

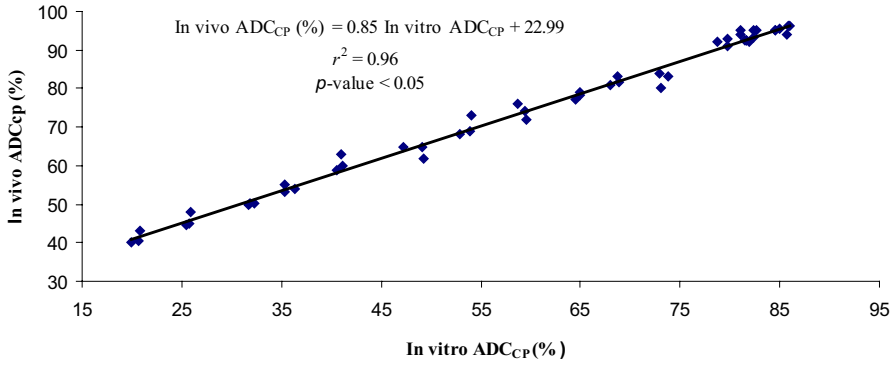

FIGURE 1 The relation between in vitro ADC_{CP} (%) and in vivo ADC_{CP} (%) obtained from settlement faecal collection method in the juvenile pacu fed to different feedstuff classes (fish meals, animal by-products, plant protein concentrates, high-protein plant meals and low-protein plant meals) with three replicates ($p < 0.05$) [Colour figure can be viewed at wileyonlinelibrary.com]

(organic or inorganic), the method of aquafeed production and the amount of phytate as an ANF (Chu-Koo et al., 2016; Safari et al., 2014, 2016). ADC_P in salmon with true stomach was measured from 40% to 60%. ADC_P of white fish meal fed with common carp and rainbow trout was reported as 10%–26% and 60%–72%, respectively (Vielma & Lall, 1998). It seems that the particle size of feed ingredients and the use of enzyme treatments can increase ADC_P. During diet formulation of pacu, care should be taken to use plant-origin feed ingredients with low protein content in order to maintain water quality (Bureau, Halver & Hardy, 2002; Ogino, Takeushi, Takeda & Watanabe, 1979; Vielma & Lall, 1998).

The basic diet formulation for *P. brachypomus* has been optimized in our laboratory for the past 4 years. The mean values of ADC_{CP} of 61% or more (83%–95%) in the present study (except low-protein plant meal) revealed that low-protein plant meal can be used efficiently in the formulation of diets, if the nutrient requirements are sufficiently provided. However, the interactions of feed ingredients (positive or negative) and some biological factors (e.g., age and digestive enzyme profiles) are considered critical to interpret results (Abimorad et al., 2008; Glencross et al., 2007; Safari et al., 2014, 2016). In this regard, digestive enzyme secretion and their activity ratios play key roles in determining ADC of nutrients (Corrêa, de Aguiar, Lundstedt, Cia & Moraes, 2007). To the best of our knowledge, there is no information on the profile of digestive enzymes in *P. brachypomus*. In this regard, the increased dietary levels of corn meal (40%–50%) increased the activities of amylase and maltase in *Clossoma macropomum*. The activities of trypsin and chymotrypsin in this fish did not increase with an increase in starch level. Increasing dietary starch in *Cyprinus carpio*, *Oreochromis mossambicus*, *Clarias batrachus*, *B. melanopterus* and *P. mesopotamicus* increases the activity of amylase. Besides the chemical composition of the basal diet, the composition of experimental feed ingredients is necessary to standardize the results (Kawai & Ikeda, 1971; Morales & Bidinotto, 2000; Mukhopadhyay, 1977; Nagase, 1964; Reimer, 1982). Further studies need to focus on the effects of ANFs on ADC values of nutrients and GER, and especially, growth performance of pacu fish.

4.2 | In vitro ADCs of feed ingredients

In the current study, a significant linear regression was observed between in vitro and in vivo ADC_{CP}. These results are in line with the study of rainbow trout (*Oncorhynchus mykiss*) (Farhangi, 2003), beluga (*Huso huso*) (Safari et al., 2016) and narrow-clawed crayfish (*Astacus leptodactylus leptodactylus*) (Safari et al., 2014). Therefore, it is possible to estimate in vivo ADC_{CP} of feed ingredients during the shortest time in an economical way. Although the final answer must be confirmed in an in vivo condition. Factors such as species type, diet, age, chemical composition, type of products (processed or raw) and the method of assay (under constant or reduced pH conditions) can have effect on the determination of in vitro ADCs. In the present study, the constant pH method was used. This method is recommended because of monitoring initial response of crude juice gastrointestinal tract enzyme extract during hydrolysis (Sales, 2009). To identify *P. brachypomus* digestive enzyme profile and set the hydrolysis conditions for determining in vitro digestibility can help in the technical knowledge necessary to produce a balanced environmentally friendly diet. However, considering the nature of the herbivores diet of this species, the determination of the tolerance level for ANFs in the diet can be effective to reduce the error in determining the ADCs of nutrients and energy. However, optimization of emerging techniques of in vitro digestion determination (e.g., membrane technique; Carter, Bransden, van Barneveld, & Clarke, 1999; Morales & Moyano, 2010; Safari et al., 2014, 2016) and the hydrolysis conditions can be considered in future studies.

5 | CONCLUSION

In summary, ADC_{CP} values of fish meals (95.67%) and plant protein concentrates (92.56%) were high for pacu (20 g). Fish meals exhibited higher ADC_{GE} value (96.78%) compared to those of plant protein concentrates (89.1%), animal by-products (83.88%) and high- and low-protein plant meals (76.18% and 67.28%, respectively), so it is recommended in pacu feed formulation. In addition, a good regression coefficient (r^2 = 96%) between in vitro ADC_{CP} (pH-stat approach) and in vivo ADC_{CP} can be used as a new avenue to evaluate further feed ingredients in pacu-rearing industry.

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