

Apparent digestibility of protein and essential aminoacids from commonly used feed ingredients in Brazil for juvenile shrimp *Litopenaeus vannamei*

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ABSTRACT - This study determined the apparent digestibility for crude protein (ACPD) and essential aminoacids (AEAAD) of ingredients for *Litopenaeus vannamei* fed diets containing soy protein concentrate (SPC), corn gluten meal (CGM), poultry byproduct meal (PBM), meat and bone meal (MBM), hydrolyzed feather meal (HFM), spray-dried blood meal (DBM), tilapia byproduct meal (TBM), Brazilian marine fish meal (BFM), salmon byproduct meal (SLM), and krill meal (KRM). Digestibility was estimated using chromic oxide as a marker in a reference (REF) mixture. Shrimp of 6-8 g body weight were reared over three separate experimental stages lasting 29-30 days each. Shrimp survival exceeded 96% and was unaffected by test ingredient. The ACPD ranged from 66.7% for a diet containing DBM to 84.2% for the REF diet. Higher ACPD were observed for aquatic compared with plant and terrestrial animal byproducts. Aside from SPC (79.3%), ACPD for CGM was low at 47.5%. Among terrestrial animal byproducts, ACPD was higher for MBM compared with PBM, DBM, and HFM (71.2, 62.8, 48.6, and 45.9%, respectively). With the exception of BFM (59.7%), ACPD for all other aquatic proteins was high (KRM, 84.3%; TBM, 83.3%; SLM, 78.9%). Aquatic proteins have higher crude protein (CP) and essential aminoacid (EAA) digestibility for shrimp. Ingredients SLM, SPC, TBM, and KRM are preferable in feeds for the whiteleg shrimp since they carry a high CP and EAA content (>600 g kg⁻¹) combined with ADC near or in excess of 80%.

Keywords: available aminoacids, digestibility, digestible protein, whiteleg shrimp



1. Introduction

Global production of farm-reared crustaceans has almost doubled between 2010 and 2018, from 5 to 9.4 million metric tons. The Pacific whiteleg shrimp, *Litopenaeus vannamei*, accounts for more than half of this production (FAO, 2020). The species is reared with feeds containing high levels of digestible protein between 300-400 g kg⁻¹, on a fed basis (NRC, 2011). As a result, protein ingredients represent the main component and the highest cost driver in shrimp feeds.

The bulk of protein in industrially compounded shrimp feeds is in the process of moving from fish meal obtained from capture fisheries to cheaper plant and animal byproducts (Tacon and Metian, 2008). These include commodity ingredients from agriculture, such as meals and concentrates made from soybean, corn and wheat. Shrimp feeds may also contain proteins supplied by rendering facilities which convert inedible animal byproducts obtained from the slaughtering or processing of poultry, swine, cattle, and fish into meals. Meat and bone meal, meat meal, poultry meal, hydrolyzed feather meal, blood meal, and fish meals are the primary products resulting from the rendering process (Meeker, 2009).

Protein digestibility is an important factor that defines the quality and value of commercial raw materials (Glencross, 2020). It allows formulating on a digestible protein and aminoacid (AA) basis, while improving feed efficiency and shrimp growth performance (Lemos and Nunes, 2008) with direct implications to feed costs and water quality. A number of studies on the determination of the apparent digestibility coefficients (ADC) of feedstuffs for marine shrimp have been published (Brunson et al., 1997; Forster et al., 2003; Cruz-Suárez et al., 2007, 2008, 2009; Lemos et al., 2009; Yang et al., 2009; Liu et al., 2013; Molina-Poveda et al., 2015; Carvalho et al., 2016; Glencross et al., 2018; Qiu et al., 2018; Guo et al., 2020). However, their nutrient value, bioavailability, and utilization by shrimp tend to vary widely depending on origin, freshness, and processing conditions adopted. For example, ADC for protein in poultry byproduct meal has been reported to range from 58.3-72.4% for *Penaeus monodon* (Glencross et al., 2018) to 27.7-45.9 (Carvalho et al., 2016), 73.8 (Qiu et al., 2018), 78.7 (Lemos et al., 2009), and 83.9% (Liu et al., 2013) for *L. vannamei*. The present study aimed at assessing the ADC for protein and essential aminoacids (EAA) of conventional and alternative feed ingredients for juvenile *L. vannamei*.

2. Material and Methods

This study was carried out in Eusébio, State of Ceará, Brazil (3°50'01.55" S and 38°25'22.74" W). The following ten practical protein sources were evaluated: soy protein concentrate (SPC), corn gluten meal (CGM), poultry byproduct meal (PBM), meat and bone meal (MBM), hydrolyzed feather meal (HFM), spray-dried blood meal (DBM), tilapia byproduct meal (TBM), Brazilian marine fish meal (BFM), salmon byproduct meal (SLM), and full-fat krill meal (KRM). Their crude protein (CP) content ranged from 397.4 g kg⁻¹ (MBM) to a maximum of 863.6 g kg⁻¹ (DBM). Ingredient AA composition varied widely depending on protein type, plant versus animal, and origin, terrestrial versus aquatic (Table 1).

Table 1 - Proximate and essential aminoacid (EAA) composition of test ingredients

| Ingredient ¹ | Aminoacid composition (g of AA kg ⁻¹ of protein, as-is basis) | | | | | | | | | |
|-------------------------|--|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | SPC | CGM | PBM | MBM | HFM | DBM | TBM | BFM | SLM | KRM |
| DM | 943.3 | 933.4 | 936.2 | 921.2 | 916.7 | 891.5 | 928.9 | 922.0 | 914.1 | 915.5 |
| CP | 618.9 | 573.8 | 628.7 | 397.4 | 749.9 | 863.6 | 579.6 | 617.7 | 657.9 | 649.5 |
| Fat | 28.0 | NA | 136.3 | 100.7 | 69.4 | 4.0 | 68.8 | 78.0 | 100.3 | NA |
| Ash | 65.0 | NA | 144.1 | 418.2 | 56.4 | 53.6 | 231.2 | 322.3 | 153.8 | NA |
| Fiber | 42.0 | NA | 3.1 | 6.3 | 6.9 | 0.9 | 1.1 | 5.0 | 0.9 | NA |
| Arg | 44.0 | 19.3 | 41.2 | 29.2 | 50.3 | 38.2 | 37.5 | 40.1 | 37.9 | 39.7 |
| His | 16.0 | 12.7 | 10.0 | 5.0 | 7.8 | 53.7 | 14.5 | 9.1 | 15.7 | 15.2 |
| Ile | 27.8 | 22.9 | 25.3 | 8.6 | 36.1 | 12.6 | 16.4 | 25.4 | 24.0 | 31.6 |
| Leu | 47.0 | 92.2 | 44.6 | 17.9 | 62.3 | 109.9 | 31.1 | 45.5 | 42.9 | 49.7 |
| Lys | 36.2 | 10.1 | 26.5 | 17.0 | 18.9 | 73.8 | 32.4 | 25.0 | 43.4 | 46.3 |
| Met | 8.2 | 15.1 | 9.3 | 4.5 | 5.6 | 8.8 | 12.9 | 8.6 | 16.6 | 17.9 |
| Met+Cys ² | 16.3 | 25.7 | 27.3 | 6.5 | 43.5 | 16.6 | 16.9 | 27.0 | 21.6 | 22.5 |
| Phe | 30.9 | 35.0 | 25.5 | 10.9 | 37.3 | 58.6 | 18.4 | 27.1 | 23.6 | 37.1 |
| Thr | 24.0 | 19.4 | 25.2 | 10.0 | 34.7 | 35.9 | 21.2 | 26.2 | 25.5 | 27.5 |
| Try | 7.9 | 3.3 | 5.0 | - | 5.5 | 12.8 | 3.6 | - | 5.8 | 8.3 |
| Val | 29.1 | 26.8 | 33.2 | 12.8 | 55.0 | 73.9 | 21.3 | 36.7 | 29.5 | 32.3 |
| ΣEAA ³ | 271.1 | 256.8 | 245.8 | 115.9 | 313.5 | 478.2 | 209.3 | 243.7 | 264.9 | 305.6 |

DM - dry matter; CP - crude protein; NA - not available.

¹ SPC - soy protein concentrate (CJ Selecta S.A., Goiânia, Brazil); CGM - corn gluten meal (Protenose®, Ingredion Brazil, São Paulo, Brazil); PBM - poultry byproduct meal (courtesy of Guaraves Guarabira Aves Ltda., Guarabira, Brazil); MBM - meat and bone meal (Nordal Nordeste Derivados de Animais Ltda., Maracanaú, Brazil); HFM - hydrolyzed feather meal (Nordal Nordeste Derivados de Animais Ltda., Maracanaú, Brazil); DBM - spray-dried blood meal (courtesy of Neovia Nutrição e Saúde Ltda., Paulínia, Brazil); TBM - tilapia byproduct meal (courtesy of Neovia Nutrição e Saúde Ltda., Paulínia, Brazil); BFM - Brazilian marine fish meal (courtesy of Neovia Nutrição e Saúde Ltda., Paulínia, Brazil); SLM - salmon byproduct meal (Pesqueira Pacific Star, Puerto Montt, Chile); KRM - full-fat krill meal (QRILL™ Aqua, Aker BioMarine Antarctic AS, Lysaker, Norway).

² TSAA - total sulfur aminoacid.

³ ΣEAA - sum of essential aminoacids: Arg - arginine; His - histidine; Ile - isoleucine; Leu - leucine; Lys - lysine; Met - methionine; Phe - phenylalanine; Thr - threonine; Try - tryptophan; Val - valine.

For the *in vivo* digestibility assay, a reference diet (REF) was first formulated to fully meet juvenile *L. vannamei* nutrient requirements (NRC, 2011; Table 2). The apparent digestibility of test ingredients was evaluated following the conventional methodology (Cho et al., 1982), by mixing 70% of the REF with 30% of test ingredient. Chromium oxide III (Cr_2O_3) was included at 5 g kg^{-1} in all diets as an inert marker. Diets were manufactured with laboratory equipment as described by Nunes et al. (2011). Briefly, dried ingredients were ground, weighed, and mixed in a planetary mixer with freshwater. Feed additives (vitamin and mineral premix, binder, crystalline AA) were mixed separately with a 1-kg sample of all dried macro ingredients in a Y-mixer. Once a feed dough was formed, it was introduced into a laboratory extruder adjusted to operate at 95°C . Pellets of 2.0 mm in diameter by 5 mm in length were steam-cooked for 10 min and dried in a convection oven. Finished diets were stored in an air-conditioned room at 16°C .

The digestibility system used in this study was the same as described by Sabry Neto et al. (2015). Briefly, it consisted of 44 rectangular 61-L tanks, each equipped with its own water inlet and outlet, aeration system and feeding tray. All tanks were blue in color, made from polypropylene with $31.0 \times 35.5 \times 55.5 \text{ cm}$ (height \times width \times length) and a bottom area of 0.19 m^2 . Tanks were filled with sand-filtered and disinfected seawater. The rearing system operated under a continuous water recirculation regime at a rate between 15.65 and 21.81 L h^{-1} (26.1-36.4% of the tank volume h^{-1}). The recirculation system operated by draining culture water into a 10-m^3 reservoir. Drained water was sand-filtered and pumped into a 5-m^3 header tank that provided clean water to the rearing system. The water inlet system was adjusted to concentrate shrimp feces in one of the corners of the tank bottom for collection.

The study was carried out in three consecutive stages (1, 2, and 3) using lab-reared juvenile shrimp with a mean body weight (BW \pm standard deviation) of 6.95 ± 0.36 ($n = 440$, $P = 0.263$, One-Way ANOVA), 7.35 ± 0.63 ($n = 340$, $P = 0.649$), and $7.31 \pm 0.42 \text{ g}$ ($n = 340$, $P = 0.432$), respectively. During each stage, the REF diet and three or four test diets were evaluated simultaneously for 30, 30, and

Table 2 - Ingredient composition (g kg^{-1} of the diet, as-is basis) of the reference (REF) diet

| Ingredient | Dietary inclusion (g kg^{-1} , as-is) |
|---|---|
| Soybean meal ¹ | 349.6 |
| Wheat flour ² | 250.0 |
| Salmon meal ³ | 150.0 |
| Soy protein concentrate ⁴ | 80.0 |
| Poultry byproduct meal ⁵ | 60.0 |
| Soy lecithin | 25.0 |
| Wheat gluten meal | 20.0 |
| Krill meal ⁶ | 20.0 |
| Salmon oil | 20.0 |
| Vitamin and mineral premix ⁷ | 15.0 |
| Chromic oxide III ⁸ | 10.0 |
| Cholesterol ⁹ | 0.4 |

¹ Indústria e Comércio de Rações Dourado Ltda. (Eusébio, Brazil): 476 g kg^{-1} crude protein (CP), 32 g kg^{-1} lipids, 61 g kg^{-1} ash, 42 g kg^{-1} fiber, 103 g kg^{-1} moisture, 3,859 kcal kg^{-1} gross energy (GE), 14 g kg^{-1} methionine (Met), 35 g kg^{-1} lysine (Lys).

² Moinhos Cruzeiro do Sul S/A (Olinda, Brazil): 134 g kg^{-1} CP, 22 g kg^{-1} lipids, 12 g kg^{-1} ash, 8 g kg^{-1} fiber, 110 g kg^{-1} moisture, 4,043 kcal kg^{-1} GE, 2 g kg^{-1} Met, 3 g kg^{-1} Lys.

³ Pesequeira Pacific Star (Puerto Montt, Chile): 628 g kg^{-1} CP, 107 g kg^{-1} lipids, 160 g kg^{-1} ash, 1 g kg^{-1} fiber, 99 g kg^{-1} moisture, 4,559 kcal kg^{-1} GE, 17 g kg^{-1} Met, 46 g kg^{-1} Lys.

⁴ CJ Selecta S.A., Goiânia, Brazil: 626 g kg^{-1} CP, 8 g kg^{-1} lipids, 42 g kg^{-1} ash, 43 g kg^{-1} ash, 82 g kg^{-1} moisture, 4,017 kcal kg^{-1} GE, 13 g kg^{-1} Met, 59 g kg^{-1} Lys.

⁵ Kabisa S.A. (Porto Alegre, Brazil): 667 g kg^{-1} CP, 171 g kg^{-1} lipids, 97 g kg^{-1} ash, 24 g kg^{-1} moisture, 5,127 kcal kg^{-1} GE, 13 g kg^{-1} Met, 32 g kg^{-1} Lys.

⁶ QRILL™ Aqua (Aker BioMarine Antarctic AS, Lysaker, Norway): 590 g kg^{-1} CP, 180 g kg^{-1} lipids, 130 g kg^{-1} ash; 60 g kg^{-1} fiber, 71 g kg^{-1} moisture, 4,610 kcal kg^{-1} GE, 19 g kg^{-1} Met, 38 g kg^{-1} Lys.

⁷ Rovimix Camarões. DSM Produtos Nutricionais Brasil Ltda. (São Paulo, SP). See Sá et al. (2013) for composition.

⁸ Vetec Química Fina Ltda. (Rio de Janeiro, Brazil): Minimum of 990 g kg^{-1} of Cr_2O_3 .

⁹ Cholesterol XG, Dishman Netherlands B.V. (Veenendaal, Netherlands): 910 g kg^{-1} of active cholesterol.

29 days, respectively. The following test diets were evaluated: SPC, CGM, PBM, and TBM (stage 1); MBM, BFM, and SLM (stage 2); and, HFM, DBM, and KRM (stage 3). Ten replicate tanks were assigned for each test ingredient. The REF was evaluated in all stages always designating four replicate tanks. Shrimp were stocked at 10 animals per tank (53 shrimp m^{-2}) and first fed at 08:00 h, followed by 13:00 and 16:00 h for three days only with the REF diet. All rearing procedures were performed in compliance with relevant laws and institutional guidelines, including those related to animal welfare. Dissolved oxygen was kept saturated at $6.2 \pm 0.2 \text{ mg L}^{-1}$ ($n = 84$) during all experimental stages. Water pH, temperature, and salinity varied significantly ($P < 0.05$) between each experimental stage with means of 7.34 ± 0.1 ($n = 84$), $28.9 \pm 0.1 \text{ }^\circ\text{C}$ ($n = 84$), and $32 \pm 0.01 \text{ g L}^{-1}$ ($n = 84$) in stage 1, 7.66 ± 0.01 ($n = 84$), $28.6 \pm 0.1 \text{ }^\circ\text{C}$ ($n = 84$), and $35 \pm 0.1 \text{ g L}^{-1}$ ($n = 84$) in stage 2, and 8.01 ± 0.01 ($n = 84$), $28.5 \pm 0.1 \text{ }^\circ\text{C}$ ($n = 84$), and $35 \pm 0.01 \text{ g L}^{-1}$ ($n = 84$) in stage 3.

Feces collection was always carried out after the total withdrawal of feed remains from the previous meal. Shrimp started to be fed their respective test diets on the fourth day always in excess exclusively in feeding trays. Daily, starting at 07:20 h, each tank was cleaned to remove feces, feed remains, and shrimp exuviae. One hour after feeding, uneaten feed residues were collected from each tank to avoid contamination with feces. Feces collection took place four times daily, 1 h and 25 min (at 09:25 and 10:15 h) and 2 h and 15 min (14:25 and 15:15 pm), respectively, after feed delivery. In juvenile shrimp, nearly all foregut evacuation takes place within 2 h after feed intake, while the bulk of feces is produced within 1 h (*Farfantepenaeus subtilis*, Nunes and Parsons, 2000). No feces collection was carried out in tanks when molting was observed as feed intake is interrupted or reduced during this period. Collection of feces was done manually by syphoning, the most commonly employed method for *in vivo* digestibility studies with shrimp (Cruz-Suárez et al., 2008). Rations were adjusted according to the amount of feed leftovers collected from feeding trays. Feces samples were gently rinsed with distilled water for salt removal and stored at $-23 \text{ }^\circ\text{C}$. All samples were freeze-dried prior to chemical analysis.

Diets, ingredients, and feces were chemically analyzed according to the AOAC (2005) methods. Dry matter (DM) was determined by drying samples in a convection oven for 24 h at $105 \text{ }^\circ\text{C}$. The Dumas combustion method was applied to analyze CP (AOAC 968.06). The AA composition was determined using high-performance liquid chromatography (White et al., 1986; Hagen et al., 1989) as described by Figueiredo-Silva et al. (2015). Test ingredients were also analyzed for crude fat through acid hydrolysis (AOAC 954.02), ash content by burning samples in a muffle furnace at $600 \text{ }^\circ\text{C}$ for 2 h (AOAC 942.05), and crude fiber through enzymatic-gravimetric determination (AOAC 992.16). Chromium oxide content in shrimp feces and experimental diets was determined in triplicate using electrothermal atomic absorption spectrometry (ETAAS) according to Neves (2008).

The apparent digestibility of ingredients was estimated by the indirect method. Initially, the concentration of Cr_2O_3 in the finished diets and in shrimp feces was used to determine the apparent digestibility coefficient (ADC), according to the formula (Cho et al., 1982):

$$\text{ADC} = 100 - \left[100 \left(\frac{\% \text{Cr}_2\text{O}_3 d}{\% \text{Cr}_2\text{O}_3 f} \right) \times \left(\frac{\% \text{Nf}}{\% \text{Nd}} \right) \right], \quad (1)$$

in which ADC = apparent digestibility coefficient of CP (in %) and (or) EAA (in %), $\text{Cr}_2\text{O}_3 d$ = concentration (in g kg^{-1}) of chromic oxide in the diet, $\text{Cr}_2\text{O}_3 f$ = concentration (in g kg^{-1}) of chromic oxide in shrimp feces, Nd = concentration (in g kg^{-1}) of CP and EAA in diets, and Nf = concentration (in g kg^{-1}) of CP and AA in shrimp feces. Subsequently, the digestibility of CP and EAA of each individual test ingredient was calculated (Bureau and Hua, 2006):

$$\text{ADC}_{\text{test ing}} = \text{ADC}_{\text{test diet}} + \left[(\text{ADC}_{\text{test diet}} - \text{ADC}_{\text{ref. diet}}) \times \left(\frac{0.7 \times D_{\text{ref}}}{0.3 \times D_{\text{ing}}} \right) \right], \quad (2)$$

in which $\text{ADC}_{\text{test ing}}$ = apparent digestibility coefficient of CP and EAA of test ingredient (in %), $\text{DC}_{\text{test diet}}$ = apparent digestibility coefficient of CP and EAA of the test diet (in %), $\text{ADC}_{\text{ref. diet}}$ = apparent

digestibility coefficient of CP and EAA of the reference diet (in %), D_{ref} = concentration (in $g\ kg^{-1}$) of CP and EAA in the reference diet, and D_{ing} = concentration (in $g\ kg^{-1}$) of CP and EAA in the test ingredient.

To evaluate the need to adjust $ADC_{testing}$ for CP due to possible losses in water as a result of leaching, the following equation given by Cruz-Suárez et al. (2007, 2009) was applied:

$$\%NL = \frac{[N_{diet} \times 100 - N_{diw} (100 - \%DML)]}{N_{diet}}, \quad (3)$$

in which %NL = % leaching of CP in water prior to feed intake, N_{diet} = dietary nutrient concentration (CP, in $g\ kg^{-1}$ of the diet on a DM basis) after manufacture, N_{diw} = dietary nutrient concentration (CP, in $g\ kg^{-1}$ of the diet on a DM basis) after 1 h immersion in seawater ($35\ g\ L^{-1}$, $28\ ^\circ C$), and %DML = % dry matter leaching of the diet ($35\ g\ L^{-1}$, $28\ ^\circ C$) after 1 h immersion in seawater ($35\ g\ L^{-1}$, $28\ ^\circ C$).

At harvest, shrimp were counted and weighed individually in a 0.01-g precision scale to determine their final survival (%), final body weight (BW) (g), weekly growth (g), final biomass (g), total amount of feed delivered per stocked shrimp (FDS, $g\ shrimp^{-1}$), and feed conversion ratio (FCR).

One-way ANOVA followed by two-by-two comparisons with Tukey's HSD test was used to determine differences in shrimp performance between diets. The following mathematical model was adopted:

$$Y_{ij} = \mu + \tau_i + \epsilon_{ij}, \quad (4)$$

in which Y_{ij} is the j -th observation of diet i ; μ is the general mean response; τ_i is the non-random effect of diets, in which $\sum_{i=1}^k \tau_i = 0$; and ϵ_{ij} is the random diet error. Pearson's coefficient of correlation was applied to identify the effects of diet and ingredient composition on ADC. The statistical package SPSS 15.0 for Windows (SPSS Inc., Chicago, Illinois, USA) was used. The significant level of 5% was set in all statistical analyses.

3. Results

Experimental diets differed in regards to their CP content and AA composition (Table 3) as a result of the nutrient profile of each test ingredient. The DBM and MBM diets showed the highest and lowest CP content, and the sum of the total EAA and non-essential AA (NEAAs) content, respectively. Dietary CP varied from 430.5 (MBM, on a DM basis) to 593.2 $g\ kg^{-1}$ (DBM). The highest dietary content of EAA in DBM was driven by its high and unbalanced levels of histidine (His), leucine (Leu), and lysine (Lys). Although HFM contained a slightly lower dietary CP and EAA content, it resembled the AA profile of DBM. The HFM also contained the highest levels of cysteine (Cys) among all test diets. Both of these diets also showed high levels of glutamate (Glu). Total sulfur AA (TSAA, methionine+Cys) content in the experimental diets reached a mean of 16.3 $g\ kg^{-1}$. The TSAA values above average were observed in diets containing CGM (16.7 $g\ kg^{-1}$), SLM (16.7 $g\ kg^{-1}$), KRM (16.7 $g\ kg^{-1}$), PBM (17.7 $g\ kg^{-1}$), BFM (18.3 $g\ kg^{-1}$), and HFM (23.9 $g\ kg^{-1}$). On the other hand, Lys content was higher in diets containing DBM (42.2 $g\ kg^{-1}$), SLM (31.9 $g\ kg^{-1}$), KRM (31.4 $g\ kg^{-1}$), SPC (30.8 $g\ kg^{-1}$), and TBM (29.0 $g\ kg^{-1}$). The highest levels of methionine (Met) were found in diets containing marine test ingredients, including SLM and KRM.

Leaching of CP among all test diets was low, with an average of $1.32 \pm 0.81\%$. The ADC for CP (ACPD) of test diets varied from a minimum of 66.7 (DBM) to a maximum of 84.2% (REF and KRM; Figure 1). Overall, ACPD were higher in diets containing test ingredients from aquatic animals compared with plant or terrestrial animal byproducts. Among the aquatic animal feedstuffs, the highest ACPD were observed in diets with KRM (84.2%), TBM (83.8%), and SLM (82.0%). The diet containing BFM recorded an ACPD of 74.5%. Diets containing SPC (82.2%) and MBM (80.0%) also showed high ACPD. The lowest values were found for DBM (66.7%), HFM (66.8%), and CGM (69.9%). The diet with PBM achieved an ACPD of 75.4%.

The mean ADC for all EAA (AEAAD) of test ingredients reflected those of test diets (Table 4). Mean AEAAD was higher than 80% in test diets REF, SPC, MBM, TBM, and KRM. Comparatively, diets with

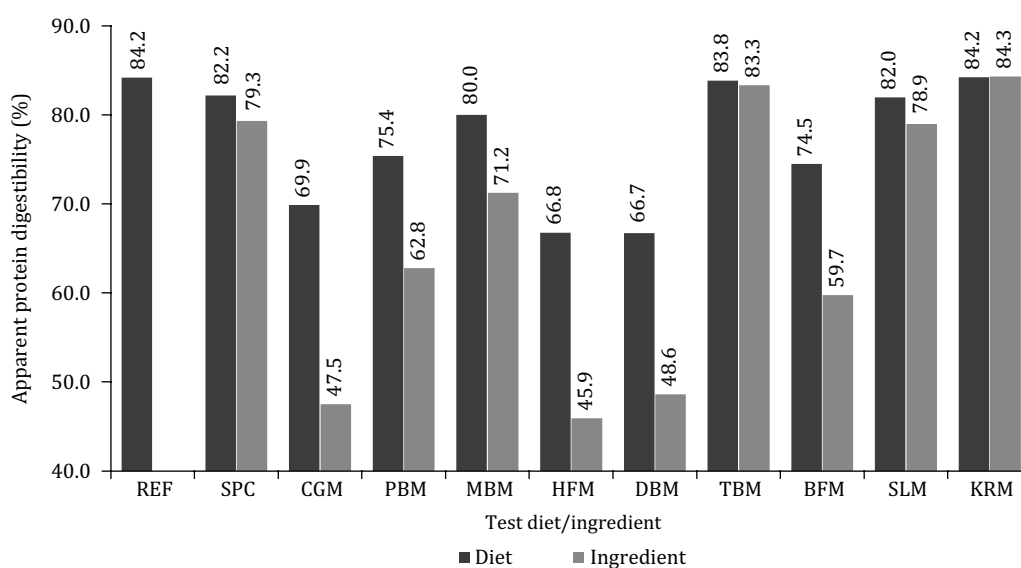
Table 3 - Crude protein (CP) content and aminoacid (AA) composition of test diets

| Test diet ¹ | Aminoacid composition (g of AA kg ⁻¹ of diet, dry matter basis) | | | | | | | | | | |
|------------------------|--|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | REF | SPC | CGM | PBM | MBM | HFM | DBM | TBM | BFM | SLM | KRM |
| CP | 437.5 | 509.8 | 492.3 | 513.4 | 430.5 | 553.4 | 593.2 | 512.9 | 505.0 | 526.9 | 494.1 |
| Arg | 29.1 | 35.8 | 26.0 | 34.8 | 29.6 | 37.4 | 32.6 | 34.1 | 33.4 | 33.2 | 31.8 |
| His | 9.8 | 12.2 | 10.6 | 10.0 | 8.3 | 9.1 | 24.0 | 10.4 | 9.6 | 11.0 | 11.4 |
| Ile | 18.3 | 22.2 | 19.8 | 20.9 | 15.3 | 24.7 | 16.0 | 18.8 | 21.0 | 20.3 | 22.7 |
| Leu | 31.5 | 37.9 | 52.4 | 36.9 | 27.4 | 42.2 | 58.0 | 33.0 | 36.6 | 35.3 | 36.7 |
| Lys | 25.0 | 30.8 | 20.0 | 27.2 | 23.0 | 24.3 | 42.2 | 29.0 | 25.8 | 31.9 | 31.4 |
| Met | 7.7 | 8.1 | 9.5 | 8.5 | 6.7 | 7.5 | 8.4 | 9.6 | 8.1 | 11.0 | 11.0 |
| Met+Cys | 13.7 | 15.0 | 16.7 | 17.7 | 11.4 | 23.9 | 14.5 | 14.8 | 18.3 | 16.7 | 16.7 |
| Phe | 20.5 | 25.0 | 25.7 | 23.0 | 17.5 | 26.4 | 34.0 | 20.7 | 23.4 | 22.1 | 25.6 |
| Thr | 16.6 | 19.6 | 17.6 | 20.0 | 14.6 | 23.1 | 23.4 | 18.7 | 20.0 | 19.8 | 19.9 |
| Try | 5.0 | 6.3 | 4.5 | 5.2 | 3.8 | 5.3 | 7.8 | 4.7 | 5.0 | 5.5 | 5.9 |
| Val | 20.3 | 23.8 | 22.4 | 25.3 | 18.1 | 31.9 | 38.3 | 21.7 | 25.9 | 23.3 | 24.2 |
| ΣEAA ² | 183.8 | 221.7 | 208.5 | 211.8 | 164.3 | 231.9 | 284.7 | 200.7 | 208.8 | 213.4 | 220.6 |
| Ala | 21.0 | 23.2 | 30.7 | 26.5 | 24.6 | 26.0 | 36.6 | 29.7 | 25.8 | 27.6 | 24.4 |
| Cys | 6.0 | 6.9 | 7.2 | 9.2 | 4.7 | 16.5 | 6.1 | 5.2 | 10.2 | 5.6 | 5.7 |
| Gly | 24.7 | 25.5 | 22.0 | 35.3 | 39.5 | 36.0 | 29.6 | 43.4 | 34.8 | 35.2 | 25.8 |
| Ser | 20.9 | 25.0 | 24.1 | 27.9 | 18.9 | 40.9 | 27.9 | 21.8 | 30.1 | 23.0 | 21.9 |
| Pro | 25.5 | 28.7 | 35.6 | 34.4 | 31.2 | 41.6 | 29.3 | 34.4 | 34.7 | 29.4 | 25.5 |
| Asp | 42.9 | 53.6 | 40.3 | 45.7 | 37.7 | 46.9 | 60.9 | 45.5 | 44.3 | 48.0 | 49.8 |
| Glu | 75.1 | 89.5 | 91.9 | 77.4 | 65.5 | 79.1 | 77.8 | 75.9 | 74.2 | 77.8 | 76.2 |
| ΣNEAA ³ | 216.1 | 252.4 | 251.8 | 256.4 | 222.1 | 287.0 | 268.2 | 255.9 | 254.1 | 246.6 | 229.3 |

¹ REF - reference diet (see Table 2); others are diets with 70% REF and 30% with the following ingredients: SPC - soy protein concentrate; CGM - corn gluten meal; PBM - poultry byproduct meal; MBM - meat and bone meal; HFM - hydrolyzed feather meal; DBM - spray-dried blood meal; TBM - tilapia byproduct meal; BFM - Brazilian marine fish meal; SLM - salmon byproduct meal; KRM - full-fat krill meal.

² ΣEAA - sum of essential aminoacids: Arg - arginine; His - histidine; Ile - isoleucine; Leu - leucine; Lys - lysine; Met - methionine; Phe - phenylalanine; Thr - threonine; Try - tryptophan; Val - valine.

³ ΣNEAA - sum of non-essential aminoacids: Ala - alanine; Cys - cysteine; Gly - glycine; Ser - serine; Pro - proline; Asp - aspartic acid; Glu - glutamic acid.



REF - reference diet; SPC - soy protein concentrate; CGM - corn gluten meal; PBM - poultry byproduct meal; MBM - meat and bone meal; HFM - hydrolyzed feather meal; DBM - spray-dried blood meal; TBM - tilapia byproduct meal; BFM - Brazilian marine fish meal; SLM - salmon byproduct meal; KRM - full-fat krill meal.

Figure 1 - Apparent crude protein digestibility (ACPD) of test diets and ingredients for juvenile *L. vannamei*.

SLM and PBM recorded an average AEAAD of 79.9±3.7 and 75.8±5.7%, followed by CGM and BFM with values of 72.1±9.2 and 70.2±5.3%, respectively. Mean AEAAD below 70% was detected in diets containing HFM and DBM (Table 4).

The ADC values for feed ingredients varied according to each individual EAA and feedstuff under evaluation (Table 4). There appeared to be no clear correlation between the ADC for individual EAA of the test diet and the test ingredient. For example, ADC for Met in the test diet with CGM reached 72.3%, but it was reduced to 57.9% when the test ingredient was evaluated alone. All other diets, showed ADC for Met in excess of 75%, except BFM with 66.0%. The ADC for Arg was higher than 80% for most diets, except those with PBM, DBM, and HFM. The ADC for Ile in diets containing CGM and HFM were lower than 70%. A similar response was recorded for TSSA (Met+Cys) with the lowest ADC values found in diets containing CGM and HFM.

Compared with the test diets, the effect of the ingredient source on the ADC was more pronounced with a wider variation within each protein type. Plant proteins reached ACPD varying from 47.5 (CGM) to 79.3% (SPC). The ACPD for terrestrial animal byproducts were: MBM (71.2), PBM (62.8), DBM (48.6), and HFM (45.9%). Compared with aquatic animal ingredients, plant proteins and terrestrial animal byproducts showed lower ACPD. Except for BFM (59.7%), all the evaluated aquatic animal feed ingredients reached ACPD higher than 80%.

The mean AEAAD showed a similar trend as the ACPD for the test ingredients (Table 4). The lowest AEAAD were found in raw-materials such as BFM (49.0±13.8%), HFM (49.1±9.3%), DBM (50.1±5.6%), and CGM (56.3±14.7%). Among the different protein ingredients, the highest AEAAD were observed in KRM (86.5±3.2%), TBM (83.2±5.0), and MBM (80.2±4.9). Ingredients SLM and SPC recorded AEAAD values of 74.6±5.7 and 79.3±5.1%, respectively.

The ADC for Met showed the highest values in KRM (93.3), TBM (85.5), MBM (82.6), SPC (82.4), and SLM (80.5%). The ADC for Met in the range of 65% was observed for HFM. On the other hand, Met was less digestible in CGM (57.9) and BFM (27.1%). The ADC for Lys showed a similar trend as Met, except for CGM which recorded an ADC of 83.3%.

Table 4 - AEAAD of test diets (Diet) and ingredients (Ing)

| Aminoacid | Apparent digestibility coefficient (ADC, %) | | | | | | | | | | | | | | | | | | | | | | |
|-----------|---|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|-----|
| | REF | | SPC | | CGM | | PBM | | MBM | | HFM | | DBM | | TBM | | BFM | | SLM | | KRM | | |
| | Diet | Diet | Ing | Diet | Ing | Diet | Ing | Diet | Ing | Diet | Ing | Diet | Ing | Diet | Ing | Diet | Ing | Diet | Ing | Diet | Ing | Diet | Ing |
| Arg | 87.8 | 88.5 | 89.4 | 83.4 | 69.6 | 78.6 | 65.3 | 81.2 | 68.6 | 68.2 | 44.9 | 73.8 | 51.9 | 89.0 | 91.0 | 81.0 | 70.4 | 85.9 | 82.8 | 87.7 | 84.3 | | |
| His | 84.8 | 83.1 | 80.8 | 75.0 | 59.5 | 81.4 | 74.5 | 84.7 | 84.4 | 78.3 | 61.5 | 56.4 | 45.8 | 84.4 | 83.7 | 69.3 | 33.2 | 80.2 | 74.3 | 84.6 | 84.2 | | |
| Ile | 82.4 | 79.6 | 75.9 | 67.4 | 42.7 | 72.3 | 57.3 | 82.7 | 84.2 | 64.0 | 44.8 | 77.4 | 62.7 | 82.1 | 81.4 | 68.3 | 46.5 | 78.1 | 71.3 | 84.7 | 87.6 | | |
| Leu | 82.7 | 80.0 | 76.3 | 56.3 | 37.7 | 72.8 | 58.5 | 82.3 | 80.9 | 63.2 | 43.0 | 60.0 | 46.7 | 83.0 | 83.6 | 72.2 | 56.6 | 79.6 | 75.1 | 83.7 | 85.1 | | |
| Lys | 87.5 | 86.9 | 85.9 | 86.8 | 83.3 | 84.4 | 78.3 | 84.8 | 77.1 | 80.1 | 59.9 | 64.8 | 48.9 | 88.5 | 90.1 | 74.8 | 47.4 | 84.9 | 81.7 | 88.7 | 90.1 | | |
| Met | 86.1 | 84.8 | 82.4 | 72.3 | 57.9 | 83.7 | 79.5 | 85.3 | 82.6 | 80.7 | 65.4 | 75.6 | 56.6 | 85.9 | 85.5 | 66.0 | 27.1 | 83.3 | 80.5 | 89.9 | 93.3 | | |
| Met+Cys | 79.3 | 77.6 | 74.6 | 69.3 | 58.3 | 66.4 | 53.1 | 80.1 | 83.3 | 55.3 | 39.7 | 70.9 | 56.6 | 79.5 | 79.8 | 71.8 | 63.5 | 76.7 | 73.2 | 81.5 | 84.3 | | |
| Phe | 81.2 | 79.3 | 76.6 | 63.4 | 41.9 | 72.7 | 58.5 | 81.6 | 83.0 | 64.1 | 44.7 | 61.9 | 47.9 | 80.8 | 79.8 | 66.4 | 42.3 | 77.4 | 70.5 | 83.3 | 85.7 | | |
| Thr | 80.3 | 78.5 | 76.0 | 69.3 | 50.0 | 71.2 | 58.9 | 80.5 | 80.9 | 61.4 | 42.9 | 64.1 | 48.6 | 80.6 | 80.9 | 69.9 | 55.6 | 77.6 | 74.0 | 81.8 | 83.6 | | |
| Try | 81.0 | 78.3 | 74.8 | 78.4 | 70.7 | 73.3 | 57.4 | 84.4 | - | 67.0 | 41.0 | 60.5 | 44.0 | 79.1 | 73.6 | 61.7 | - | 74.9 | 64.2 | 81.9 | 83.2 | | |
| Val | 80.3 | 78.0 | 74.8 | 68.2 | 49.4 | 68.0 | 52.7 | 80.2 | 80.1 | 59.0 | 42.9 | 59.7 | 48.1 | 80.9 | 82.1 | 72.0 | 62.1 | 76.9 | 72.1 | 82.0 | 84.4 | | |
| Mean ± | 83.0 | 81.7 | 79.3 | 72.1 | 56.3 | 75.8 | 64.1 | 82.8 | 80.2 | 68.6 | 49.1 | 65.4 | 50.1 | 83.4 | 83.2 | 70.2 | 49.0 | 79.9 | 74.6 | 84.8 | 86.5 | | |
| SD | 3.0 | 3.8 | 5.1 | 9.2 | 14.7 | 5.7 | 9.8 | 1.9 | 4.9 | 8.1 | 9.3 | 7.5 | 5.6 | 3.4 | 5.0 | 5.3 | 13.8 | 3.7 | 5.7 | 3.0 | 3.2 | | |

Arg - arginine; His - histidine; Ile - isoleucine; Leu - leucine; Lys - lysine; Met - methionine; Cys - cysteine; Phe - phenylalanine; Thr - threonine; Try - tryptophan; Val - valine; SD - standard error.

REF - reference diet; SPC - soy protein concentrate; CGM - corn gluten meal; PBM - poultry byproduct meal; MBM - meat and bone meal; HFM - hydrolyzed feather meal; DBM - spray-dried blood meal; TBM - tilapia byproduct meal; BFM - Brazilian marine fish meal; SLM - salmon byproduct meal; KRM - full-fat krill meal.

Shrimp survival exceeded 96% and was unaffected by test ingredient ($P>0.05$; Table 5). On the other hand, final BW, final biomass, weekly growth, FDS, and total FCR were significantly affected ($P<0.05$) by the test ingredients within each separate culture stage (Table 5). In the first experimental stage, final BW and growth of shrimp fed REF, SPC, and TBM were superior than those fed CGM and PBM ($P<0.05$). Shrimp fed PBM and TLP achieved a lower FCR compared with those fed REF, SPC, and CGM. On the other hand, FDS was statistically higher for shrimp fed SPC and REF, followed by TBM and GCM, and finally PBM. Final biomass was also lower for shrimp fed CGM and PBM compared with SPC, REF, and TBM ($P<0.05$).

In the second stage, final BW was superior in shrimp fed REF and SLM than in those fed MBM and BFM ($P<0.05$). Mean weekly growth varied significantly from a minimum of 0.58 (MBM) to a maximum of 0.76 g (SLM). No statistical differences in growth were detected between other diets ($P>0.05$). Final biomass and FDS were unaffected by the test ingredient with a mean of 95.7 ± 10.8 and 13.9 ± 0.5 g shrimp⁻¹ ($P>0.05$), respectively. Feed conversion ratio was highest for shrimp fed REF and MBM, but FCR achieved with the latter did not vary statistically when compared with BFM and SLM ($P<0.05$).

In the third stage, the highest final BW was achieved with KRM compared with REF, HFM, and DBM ($P<0.05$). Final BW did not differ between shrimp fed HFM and REF. On the other hand, there was no significant difference between HFM and DBM. Shrimp fed DBM achieved a lower BW than those fed REF ($P<0.05$). The lowest FCR was achieved with shrimp fed KRM followed by REF. There was no statistical difference in FCR between shrimp fed diets HFM and DBM, and between REF and DBM. The FDS for shrimp fed HFM and KRM were similar and highest among all test diets ($P>0.05$).

Pearson's coefficient of correlation indicated that dietary CP content had no significant effect over final shrimp BW ($P>0.05$; Table 6). Similarly, final BW was unaffected by dietary histidine (His), TSSA, phenylalanine (Phe), valine (Val), and total EAA content. Although Pearson's r was below 0.7, the highest levels of dietary Arg, Ile, Lys, Met, threonine (Thr), and tryptophan (Try) all affected final

Table 5 - Growth performance of *L. vannamei* fed diets with 30% of the test ingredients

| Stage/Diet | Final survival (%) | Final body weight (%) | Final biomass (g) | Weekly growth (g) | TFD (g shrimp ⁻¹) | FCR |
|------------|--------------------|-----------------------|-------------------|-------------------|-------------------------------|-------------|
| 1 | | | | | | |
| REF | 100.0±<0.1 | 10.38±1.01a | 103.8±1.7a | 0.80±0.03a | 14.6±0.1a | 1.41±0.03a |
| SPC | 99.0±3.16 | 10.24±1.01a | 101.4±4.8ab | 0.75±0.06a | 15.0±<0.1a | 1.47±0.04a |
| CGM | 100.0±<0.1 | 9.55±0.76b | 95.5±2.6c | 0.62±0.06b | 13.6±0.2b | 1.43±0.03a |
| PBM | 99.0±3.16 | 9.79±0.88b | 96.9±3.2bc | 0.66±0.06b | 13.0±0.5c | 1.33±0.06b |
| TBM | 99.0±3.16 | 10.42±0.90a | 103.1±5.5a | 0.81±0.09a | 13.7±0.3b | 1.32±0.05b |
| 2 | | | | | | |
| REF | 100.0±<0.1a | 10.30±1.32a | 97.9±9.7a | 0.66±0.07ab | 14.7±0.2a | 1.51±0.16a |
| MBM | 98.0±4.2a | 9.80±1.07b | 96.0±5.9a | 0.58±0.13a | 13.8±0.3a | 1.41±0.09ab |
| BFM | 98.0±4.2a | 10.05±1.10b | 98.5±7.1a | 0.64±0.10ab | 13.4±0.3a | 1.33±0.05b |
| SLM | 97.0±4.8a | 10.60±1.22a | 102.8±4.7a | 0.76±0.07b | 14.1±0.3a | 1.33±0.05b |
| 3 | | | | | | |
| REF | 100.0±<0.1 | 10.6±1.11ab | 105.6±6.7a | 0.81±0.16ab | 14.5±<0.1a | 1.38±0.09a |
| HFM | 96.0±7.0 | 10.2±1.12bc | 98.1±7.6a | 0.70±0.10b | 15.0±<0.1bc | 1.47±0.07b |
| DBM | 98.0±4.2 | 10.0±0.93c | 97.7±4.2a | 0.64±0.05b | 14.2±<0.1d | 1.42±0.03ab |
| KRM | 98.0±4.2 | 12.4±1.27d | 121.6±9.6b | 1.22±0.13c | 15.0±<0.1c | 1.35±0.11c |

TFD - total feed delivered; FCR - feed conversion ratio; REF - reference diet; SPC - soy protein concentrate; CGM - corn gluten meal; PBM - poultry byproduct meal; MBM - meat and bone meal; HFM - hydrolyzed feather meal; DBM - spray-dried blood meal; TBM - tilapia byproduct meal; BFM - Brazilian marine fish meal; SLM - salmon byproduct meal; KRM - full-fat krill meal.

Common letters in columns refer to non-statistically significant differences between diets within each experimental stage according to Tukey's HSD at $\alpha = 0.05$.

BW positively ($P < 0.05$). On the other hand, higher levels of dietary Leu had a negative effect over BW ($P < 0.05$). Final shrimp BW was also statistically affected by the ADC of test diets and test ingredients ($P < 0.05$). However, r values were below 0.7, which indicated that ADC were poorly correlated with shrimp growth performance (Table 6).

Table 6 - Pearson's coefficient of correlation (r) of final shrimp body weight (BW), nutrient composition, and apparent digestibility coefficient (ADC) of test diet and test ingredients

| Nutrient | Pearson's r | | |
|----------|------------------|----------|----------------|
| | Diet composition | ADC diet | ADC ingredient |
| CP | ns ¹ | 0.263** | 0.308** |
| Arg | 0.065* | 0.186** | 0.206** |
| His | ns | 0.162** | 0.221** |
| Ile | 0.213** | 0.264** | 0.304** |
| Leu | -0.138** | 0.263** | 0.291** |
| Lys | 0.163* | 0.148** | 0.193** |
| Met | 0.340** | 0.348** | 0.342** |
| Met+Cys | ns | 0.217** | 0.256** |
| Phe | ns | 0.270** | 0.299** |
| Thr | 0.070* | 0.234** | 0.275** |
| Try | 0.128** | 0.141** | 0.283** |
| Val | ns | 0.236** | 0.289** |
| ΣEAA | ns | 0.250** | 0.287** |

CP - crude protein; Arg - arginine; His - histidine; Ile - isoleucine; Leu - leucine; Lys - lysine; Met - methionine; Cys - cysteine; Phe - phenylalanine; Thr - threonine; Try - tryptophan; Val - valine.

Correlation was evaluated between each dietary nutrient and ADC values with 1,100 observations of shrimp BW obtained at harvest.

¹ Correlation is not statistically significant according to Pearson's coefficient.

* Correlation is significant at $\alpha = 0.05$ according to Pearson's coefficient.

** Correlation is significant at $\alpha = 0.01$ according to Pearson's coefficient.

4. Discussion

In the present study, ACPD (79.3%) and AEAAD (79.3%) for SPC were equivalent to SLM (78.9 and 79.9%, respectively). However, ADC for SPC were lower than that reported by Cruz-Suárez et al. (2009). The authors compared the protein and AA digestibility of SPC (710 g kg⁻¹ CP), soybean meal (SBM, 520 g kg⁻¹ CP), and soy protein isolate (SPI, 890 g kg⁻¹ CP) for *L. vannamei* with 6-g initial BW. They reported ADC of 93.0±1.5, 96.9±1.3, and 96.2±1.6%, respectively. The deviation between the ADC from our study and the value reported for SPC by Cruz-Suárez et al. (2009) may be related to differences in soybean production and processing conditions adopted. Galkanda-Arachchige et al. (2020) reported that the ACPD for 24 sources of solvent-extracted SBM fed to *L. vannamei* ranged from 87 to 98%. The ACPD for SPC from a similar source as used in the present study was recorded at 79.8% for juvenile *Penaeus monodon* (Glencross et al., 2018). Comparatively, Carvalho et al. (2016) reported ACPD between 82.9 and 89.5% for another commercial SPC sourced in Brazil. Regardless, SPC has proven as an effective protein source for the complete replacement of fish meal in diets for juvenile whiteleg shrimp (Sá et al., 2013). The differences between reported ADC may also be the result of nutrient leaching rates of test diets.

On the other hand, we recorded a low digestibility for CGM. Although this ingredient contained high levels of CP (573.8 g kg⁻¹) and TSSA (25.7 g kg⁻¹), their ADC were low at 47.5 and 58.3%, respectively. The ACPD values of CGM reported in the literature for farmed shrimp are conflicting. Values have varied from 55.7 (Liu et al., 2013), 59.1 (Lemos et al., 2009), and 59.2% (Carvalho et al., 2016) to 81.6% (Glencross et al., 2018) and 87.9% (Yang et al., 2009). Molina-Poveda et al. (2015) reported

ACPD ranging from 52.0 to 80.5% for *L. vannamei* diets containing CGM included from 86.4 to 345.6 g kg⁻¹ (g kg⁻¹ of the diet, as-is), respectively. The lowest ACPD reported by Molina-Poveda et al. (2015) corresponds to our findings (72.3%) obtained with the test diet containing 300 g kg⁻¹ of CGM. The authors found a depressed growth in juvenile shrimp and argued this was the result of a reduced palatability, and a low digestibility for dietary CP, Met, and Lys. Likewise, in the present study, ADC for Met was low (57.9%), although a value of 83.3% was observed for Lys. Future studies should consider microbial fermentation to enhance the nutrient value and digestibility of CGM when used in marine shrimp feeds. Jannathulla et al. (2018) applied fungal fermentation to SBM to significantly increase its ACPD from 89.46±0.30 to 95.49±0.63%.

The ACPD of shrimp fed the test diets with HFM and DBM were nearly the same (45.9 and 48.6%, respectively), but the poorest among all test ingredients. The ACPD for HFM was within the range of other studies conducted with *L. vannamei*, 34.4-43.8% (Carvalho et al., 2016), and 63.9% (Lemos et al., 2009), except when compared with an ACPD of 7.1% reported for *P. monodon* (Glencross et al., 2018). The literature reports ACPD for DBM in the range of 45.2 (Glencross et al., 2018), 69.1 (Liu et al., 2013), and 66.2-70.8% (Lemos et al., 2009). It has been demonstrated that HFM and DBM are poor feeding effectors for penaeid shrimp (Nunes et al., 2006; Suresh et al., 2011). The protein from these ingredients is predominantly composed of larger peptides with low levels of free AA and nucleotides (Suresh et al., 2011).

Conversely, ACPD and AEAAD for PBM was 62.8 and 64.1%, lower than MBM, but higher than HFM and DBM. Feed-grade PBM containing blood and (or) feather is more commonly used in feeds for marine shrimp and freshwater fish farmed in Brazil (Pastore et al., 2012). This type of commercially available PBM has been shown to exhibit a high variability in its nutrient composition (Nascimento et al., 2002) likely driven by the freshness and types of residual poultry slaughter content. As such, they are prone to exhibit varying ADC values depending on the source and type of PBM. For example, Glencross et al. (2018) reported that the ACPD for three types of PBM for *P. monodon* varied from 58.3 to 72.4% (with CP, total lipid, and ash range values of 659-722, 137-166, and 135-151 g kg⁻¹, respectively). For *L. vannamei*, Lemos et al. (2009), Liu et al. (2013), and Qiu et al. (2018) reported ACPD of 78.7, 83.9, and 73.8%, respectively. Comparatively, Carvalho et al. (2016) reported an ACPD of only 27.7-45.9% for a PBM sourced in Brazil containing 584 g kg⁻¹ CP, 131 g kg⁻¹ lipid, and 152 g kg⁻¹ ash. The authors also found that after an eight-week feeding trial, weekly growth of shrimp fed a test diet with 300 g kg⁻¹ SPC was superior to that of shrimp fed a similar diet containing 300 g kg⁻¹ PBM instead (1.40±0.04 vs. 1.24±0.13 g, respectively). As pointed out by Carvalho et al. (2016), the PBM used in their work, similar to the one sourced for the present study, probably contained blood since they both displayed high levels of Cys in their composition. Comparatively, for a pet-food grade PBM (663 g kg⁻¹ CP, 126 g kg⁻¹ lipid, and 120 g kg⁻¹ ash), Cruz-Suárez et al. (2007) reported an ACPD of 90.4±4.4% for *L. vannamei*. Chemical hydrolysis seems to improve the protein digestibility of PBM for *L. vannamei* as reported by Soares et al. (2020). Authors found that whiteleg shrimp is able to digest between 92.99 and 93.88% of the total CP content from protein hydrolysates made from PBM and PBM plus swine liver meal, respectively.

In our study, the test diet with MBM showed a relatively high CP and EAA digestibility. The ACPD (71.2%) and AEAAD (80.2%) for MBM were the highest among the test ingredients derived from terrestrial animal byproducts. Other studies have reported ACPD for MBM between 71.5 and 75.8% for *P. monodon* (513-532 g kg⁻¹ CP, Glencross et al., 2018), 58.6% for *Litopenaeus setiferus* (Brunson et al., 1997), and between 57.0 and 74.7% (Carvalho et al., 2016), 73.9% (515 g kg⁻¹, Yang et al., 2009), and 82.2% (565 g kg⁻¹ CP, Liu et al., 2013) for *L. vannamei*. Forster et al. (2003) evaluated the replacement of fish meal (723 g kg⁻¹ CP and 109 g kg⁻¹ lipid) for three types of MBM (533-556 g kg⁻¹ CP, 106-171 g kg⁻¹ total lipid, and 190-241 g kg⁻¹ ash) in diets for juvenile *L. vannamei*. They reported that MBM could replace as much as 75% of fish meal in a diet with 350 g kg⁻¹ CP. However, they noted a general decrease in shrimp growth above 25% replacement.

With the exception of BFM, all other aquatic animal proteins showed ACD values near or above 80%. However, recorded values for ACPD and AEAAD in TBM (83.3 and 83.2%) were higher than in SLM

(78.9 and 74.6%) and BFM (59.7 and 49.0%, respectively). These fish meals are all made from fish residues (trimmings and offal) obtained during processing of farmed or captured fish. The BFM, for example, consists of miscellaneous marine fish caught in the Southern part of Brazil. In this case, species, composition, storage, and processing conditions can vary widely. In comparison, these variables are kept more controlled and consistent for TBM and SLM, which are made from the processing residues of a single farmed fish species immediately after filleting. While the literature does not report ADC for TBM and BFM for marine shrimp, ACPD for SLM and hydrolyzed SLM for *L. vannamei* were reported at 85.8 and 90.9%, respectively (Guo et al., 2020). These values are higher than the ACPD from the present study for SLM and equivalent to ADC reported for fish meal made from whole fish. Lemos et al. (2009) reported the ACPD for eight different types of fish meal for *L. vannamei*: anchovy (87.9%), Peruvian anchovy (88.5%), herring (90.1%), hoki from New Zealand (88.1%), Chilean mackerel (88.8%), menhaden type a (89.0%), menhaden type b (83.7%), and miscellaneous fish meal from Peru (87.6%). Shrimp fed the test diet with 300 g kg⁻¹ of SLM achieved a larger final BW than those fed BFM. A similar result was observed for shrimp fed TBM within its experimental group.

The KRM exhibited the highest ACPD (84.3%) and AEAAD (86.5%) among all test ingredients. The ACPD for KRM have been reported between 80.5 and 89.4% for *L. vannamei* (587-680 g kg⁻¹ CP, 89-118 g kg⁻¹ lipid, and 97-102 g kg⁻¹ ash; Lemos et al., 2009) and at 95.1% for *P. monodon* (644 g kg⁻¹ CP, 211 g kg⁻¹ lipid, and 118 g kg⁻¹ ash; Glencross et al., 2018). A number of other studies have reported a positive effect on the feeding and growth of juvenile *L. vannamei* when fed low dietary inclusions of KRM (Nunes et al., 2011, 2019; Suresh et al., 2011; Sá et al., 2013; Derby et al., 2016).

For the most part, the lower ADC for CP and EAA of the ingredients evaluated in the present study resulted in a lower growth performance of *L. vannamei*. However, our data indicated that digestibility alone cannot be used as a single predictor of shrimp performance. Although shrimp performance also seemed to respond to the dietary CP content and AA composition, it was also not possible to establish a significant correlation between these variables. Some of the ingredients evaluated contained a high CP content, but an unbalanced EAA profile (e.g., CGM, HFM, and DBM) resulting in low ADC for CP and EAA. In these cases, ADC was a stronger indicator of ingredient performance for the whiteleg shrimp than their nutrient composition. These results contrast with the work of Lemos and Nunes (2008). Authors evaluated the growth performance of the whiteleg shrimp (3.28 g initial BW) fed six commercial diets for 56 days in a clear-water rearing system. They reported significant differences in weekly growth (0.56-0.98 g), final BW (9.07-12.96 g), yield (0.44-0.78 kg m⁻²), and FCR (2.05-2.80). Shrimp performance and *in vitro* degree of hydrolysis with pond-raised shrimp enzymes showed significant correlation ($P < 0.05$) for yield ($R^2 = 0.72$), growth rates ($R^2 = 0.72-0.80$), and FCR ($R^2 = 0.67$). However, as opposed to the work of Lemos and Nunes (2008), our test diets showed excessive levels of CP and some EAA, and shrimp were fed in excess making it difficult to correlate growth performance with diet composition or their digestibility. Carvalho et al. (2016) also reported slower growth rates in shrimp fed ingredients with lower ADC. They grouped test ingredients according to their digestible dietary protein: fish meal and SPC with the highest growth rates, followed by PBM, CGM, MBM, and HFM with the slowest growth rates. Their findings are in line with our results.

5. Conclusions

This study showed that the crude protein and essential aminoacid content in commercially available feed ingredients for the whiteleg shrimp should not be taken as the sole indicator of their quality. Instead, apparent digestibility coefficients for crude protein and essential aminoacid should be used along with other parameters to judge their quality and better predict shrimp culture performance. The spray-dried blood meal, hydrolyzed feather meal, and corn gluten meal carried a high level of crude protein and essential aminoacids, but their apparent digestibility coefficients were lower than 50%. As such, their dietary inclusion in whiteleg shrimp diets should be as low as possible. At the next level were feed ingredients prone to a high variation in composition and quality driven by the type of animal residues, storage, and processing conditions applied during manufacturing. These included poultry byproduct meal and Brazilian marine fish meal with apparent digestibility coefficients around 60%.

Their dietary inclusion should rely on additional criteria such as product freshness and consistency in composition over several batches. On the other side is meat and bone meal with a rather low crude protein and essential aminoacid content. The use of meat and bone meal as a protein source in shrimp feeds can be adopted as apparent digestibility coefficients were in excess of 70%. However, unless meat and bone meal with higher nutrient levels can be sourced, its dietary inclusion will be limited by its lower crude protein content and typically high ash levels. Finally, salmon byproduct meal, soy protein concentrate, tilapia byproduct meal, and full-fat krill meal are preferable ingredients in feeds for the whiteleg shrimp since they carried a high crude protein and essential aminoacid content ($>600 \text{ g kg}^{-1}$) combined with apparent digestibility coefficients near or in excess of 80%.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: A.J.P. Nunes. Data curation: C.C.F. Vieira and A.J.P. Nunes. Formal analysis: C.C.F. Vieira, R.C.C. Pinto and A.J.P. Nunes. Funding acquisition: A.J.P. Nunes. Investigation: C.C.F. Vieira and A.J.P. Nunes. Methodology: C.C.F. Vieira and A.J.P. Nunes. Project administration: C.C.F. Vieira and A.J.P. Nunes. Supervision: A.J.P. Nunes. Validation: A.J.P. Nunes. Visualization: A.J.P. Nunes. Writing-original draft: C.C.F. Vieira, R.C.C. Pinto, A.F. Diógenes and A.J.P. Nunes. Writing-review & editing: R.C.C. Pinto, A.F. Diógenes and A.J.P. Nunes.

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