Apparent amino acid digestibility of feed ingredients for juvenile shrimp (Litopenaeus vannamei): a new method of determination using soybean meal as an example

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Abstract

Literature information on apparent digestibility coefficients (ADC) of dietary nutrients in feedstuff for shrimp species dates to 1980´s though the nutritional consistency of ADC values determined in individual feed ingredients continues under scrutiny. This may be attributed to: a. the large variety of ingredients tested under a single standard methodology (i.e. the partial replacement of a reference diet mix by a fixed proportion of the target ingredient), b. the complex effects of this dietary strategy upon palatability, digestion/digestibility, and consequently feeding rates, and c. nutrient leaching of diets during experiments. While the biological phenomenon behind ADC, the so-called “nutrient disappearance” between diet and feces through animal´s digestive system, is measured by the difference of nutrient and inert marker content in diet and feces, ADC of a feed ingredients has been mathematically calculated considering the difference in ADC from test (e.g. 30% replacement of a reference diet mash by the target ingredient) *versus* reference diet ADC and their nutrient content. The present study proposes the determination of individual amino acid ADC of soybean meal (SBM) based on the effect of inclusion of SBM in increasing dietary levels in four practical test diets (5, 10, 15 and 20% SBM, named diets T4, T5, T6 and T7, respectively) upon ADC of test diets. For comparison, the study also included three diets formulated under the standard replacement method (i.e. 10, 20 and 30% replacement of the reference diet mash by SBM, named diets T1, T2 and T3). The feeding trial was carried out under high shrimp performance with automated feeding (20-22h pellet delivery/day, minimized leaching), and daily feces collection over the trial period (55 days). The estimation of ADC of amino acids in SBM was based on ADC determined in test diets: $ADC_{AA\ SBM}$ = Mean [($ADC_{AA\ diet\ (T4,\ T5,\ T6\ or\ T7)}$/ $ADC_{protein\ diet\ (T4,\ T5,\ T6\ or\ T7)}$) x $ADC_{protein\ SBM}$], diet ADC experimentally determined and an assigned ADC value of SBM true protein ($ADC_{protein\ SBM}$) estimated by three criteria: effect of increasing inclusion of SBM upon true protein ADC of test diet; additivity of digestible protein supplied by proteinaceous ingredients composing test diets T4 to T7, literature values and author’s experience. Results by the new proposed method showed SBM ADC values between 76 and 88% for indispensable amino acids and contrasted with values obtained by applying the conventional replacement method at 30% inclusion of SBM into a reference diet (ADC_{AA}: 87–96%). It was also checked either the improvement or reduction of certain individual amino acid ADC with increasing SBM inclusion level in diets. In conclusion, the new proposed methodology produced reduced and more realistic ADC values of amino acids compared the conventional method of ingredient replacement into a reference diet at one fixed level. This new methodology for ADC determination is not intended to become an unquestionable reference but rather to offer an alternative view for more realistic values of ADC of feed ingredients for farmed shrimp.

Introduction

In farmed shrimp, nutrients are supplied either by natural food (plankton, benthos, biofilm, ions) and compound feeds; depending on stocking density and management feeds may become the main source in some periods of the growing cycle (Akiyama and Yukasano, 2022). Shrimp feed continuously upon food and pellets, and a rather short food transit time and simple digestive system offer surface and enzymes for nutrient absorption over a limited time span for processing the ingesta (Ceccaldi, 1989). Proper amounts of pellets delivered into the system (Jory, 2018), including physical characteristics such as suitable particle size of feed ingredients (Bortone and Kipfer, 2016), facilitate shrimp digestion and nutrient assimilation, and if combined with suitable water quality may result in more efficient feed use (growth, FCR). Studies on apparent digestibility in shrimp have aimed to consensually assign quantitative values (ADC: apparent digestibility coefficient) in a single ingredient by comparing reference *versus* test diets containing the target feed ingredient replacing the reference diet mix at a fixed inclusion level (Cho et al., 1982; Akiyama et al., 1989; Davis et al., 2002; Cruz-Suarez et al., 2009; Jannathulla et al., 2018; Shi and Lee, 2021). However, most cited procedures to (indirectly) determine ADC in feed ingredients, including dietary strategies and calculations, have been under questioning regarding potential methodological weaknesses affecting the accuracy of the ADC value obtained, as e.g., lack of an ideal dietary level of target ingredient in test diet applicable to all ingredients, poor attention to standardize physical properties including particle size of prepared pellets, deficient feed management including leaching of pellets and feces samples, and poor performance of shrimp in digestibility trials (Tacon, 1996; Smith and Tabrett, 2004; Lemos et al., 2009, Carvalho et al., 2016).

Characterization of feed attributes including feed ingredients is constantly targeted by the aquafeed industry and quality control may be ideally reached through in-house R&D. Research database support development of predictive tools, including nutrient digestibility, allowing sampling of a batch of raw material and the prompt access to its nutritional features so that nutritional information becomes available within short periods (Moughan, 1999). Values of ADC may be further combined with available *in vitro* methodologies in the development of NIRs predictive models build upon consistent data sets (Raggi, 2016; Simon et al., 2022). Outputs of ADC should be ideally accompanied by suitable shrimp performance as a complimentary validation of digestibility data, i.e., ADC representing the efficiency of nutrient use and residue delivery as a consequence of intense biomass accumulation in a representative trial period (Carvalho and Phan, 1998).

The aim of the present study is to offer an alternative view on determining ADC of nutrients in feedstuffs for juvenile farmed shrimp (*Litopenaeus vannamei*) in a trial carried out under proper shrimp performance, with experimental period representative of the growing cycle of the species, focusing on soybean meal (SBM) tested at different levels in practical diets. The present calculation of ADC for individual amino acids was based on contrasting the effect of SBM inclusion level into practical diets (5, 10, 15 and to 20%) in terms of the relationship between true protein and individual amino acid ADC in test diets.

Materials And Methods

Methodological context

The determination of apparent digestibility coefficient (ADC) of amino acids was assessed by testing the effect of increasing inclusion levels of solvent-extracted soybean meal (SBM) upon digestibility of juvenile shrimp (Table 1). The dietary inclusion levels chosen are within the recommended range for use of SBM in compound shrimp feeds (Tacon et al., 2009). The four test diets with increasing levels of SBM (5, 10, 15 and 20%) were formulated to have similar levels of crude protein, ether extract and gross energy. For comparison, three other test diets were prepared by the conventionally applied replacement method for determination of ADC, when the target feed ingredient replaces the mash of a certain reference diet at a fixed proportion (test diet), and ADC of feed ingredient is calculated by the difference or not in ADC between test and reference diets (Cho et al., 1982; Bureau and Hua, 2006; NRC, 2011). Presently, SBM was tested replacing 10, 20 and 30% of a practical reference diet mash under this methodology. Therefore, the study focused on the comparison between a currently applied methodology (fixed replacement by target ingredient) and a new approach for the determination of ADC of amino acids in SBM for farmed shrimp.

Table 1

Proximate composition (%, as-is) of feed ingredients[1] used in formulation of test diets for juvenile shrimp (*Litopenaeus vannamei*).

	Moisture	Crude protein	Lipid	Energy (cal/g)	Ash
Wheat[2]	11.5	12.1	2.14	3,872	1.54
Soybean meal[3]	8.95	46.5	2.94	4,313	6.79
Fish meal (local)[3]	7.52	54.8	8.0	3,917	29.0
Poultry by-product meal[3]	5.55	59.5	14.4	4,919	16.5
Squid meal[4]	6.58	84.7	3.33	5,413	4.0
Fish hydrolysate[5]	-	24.5	10.0	-	-
Blood meal[6]	5.57	90.9	1.05	5,669	1.55
Dried yeast[7]	7.20	36.2	2.69	4,313	7.20
Fish oil[3]	-	0.32	99.4	-	-
Soy lecithin oil[3]	-	3.55	88.4	-	7.71

(-): not determined.

[1] Mean of duplicate analysis.

[2] Coamo, Campo Mourão, Brazil

[3] Guabi, Campinas, Brazil

[4] Polinutri, Osasco, Brazil

[5] Aquativ, Elven, France

[6] A&R, Maringá, Brazil

[7] ICC, São Paulo, Brazil

Feed ingredients and experimental diets

Feed ingredients used in the present study were supplied by the local feed industry and are commonly employed in shrimp feeds. The composition of the feed ingredients was determined (Tables 1 and 2) and results followed typical values (Hertrampf and Piedad-Pascual, 2000; Tacon et al., 2009; NRC, 2011). Prior to pellet preparation, feed ingredients were milled to < 250 μm particle size in a hammer mill (MCS 350 Moinhos Vieira, Tatuí, Brazil), recommended for feeding juvenile shrimp (Terrazas-Fierro et al., 2010; Ye et al., 2012).

Table 2
Amino acid composition of soybean meal (%, as is) used in formulation of test diets for juvenile shrimp (*Litopenaeus vannamei*).

Arg	**His**	**Iso**	**Leu**	**Lys**	**Met**	**Phe**	**Thr**	**Try**	**Val**	**Tau**
3.43	1.13	2.30	3.88	2.83	0.57	2.41	2.11	0.45	2.37	0.03
Asp	Glu	Ser	Gly	Ala	Pro	Tyr	Cys			
2.88	7.30	2.25	2.23	2.37	2.61	1.67	0.85			

A practical diet formulation (named T6), suitable in attending species nutrient requirements and previously proved efficient for juvenile *L. vannamei*, was chosen as the reference for preparation of diets designed for determination of ADC under the conventional methodology of replacing part of the reference diet mash by the target feed ingredient (Cho et al., 1982) (Table 3). Though the replacement methodology has been mostly applied to feed ingredients at 30:70 ingredient:reference diet ratio (presently diet T3), for further comparison, additional inclusion levels of SBM were also tested, at 10% (T1) and 20% (T2), as previously assessed with some feed ingredients assessed in shrimp diets (Merican and Shim, 1995; Divakaran et al., 2000; Smith et al., 2007; Rivas-Vega et al., 2009; Carvalho et al., 2016; Panini et al., 2017).

Table 3
Formulation (%) of test diets[1] for juvenile shrimp (*Litopenaeus vannamei*).

	T1 (10:90)[2]	**T2 (20:80)**[2]	**T3 (30:70)**[2]	**T4**	**T5**	**T6 (reference)**	**T7**
Soybean meal	23.5	32.0	40.5	5.0	10.0	15.0	20.0
Wheat	32.3	28.6	24.9	42.0	39.5	36.0	34.0
Fish meal	18.0	16.0	14.0	20.0	20.0	20.0	20.0
Poultry by-product meal	9.0	8.0	7.0	11.5	10.5	10.0	9.0
Blood meal	1.80	1.60	1.40	4.50	3.0	2.0	0.0
Squid meal	4.50	4.00	3.50	5.00	5.00	5.00	5.00
Fish hydrolysate	3.60	3.20	2.80	4.00	4.00	4.00	4.00
Dried yeast	2.70	2.40	2.10	3.00	3.00	3.00	3.00
Fish oil	1.80	1.60	1.40	2.00	2.00	2.00	2.00
Soy lecithin oil	0.90	0.80	0.70	1.00	1.00	1.00	1.00
Vit. and Min. premix[3]	0.90	0.80	0.70	1.00	1.00	1.00	1.00
Binder[4]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Chromic oxide[5]	0.50	0.50	0.50	0.50	0.50	0.50	0.50

[1] Further details in Material and methods.

[2] Conventional replacement method, SBM inclusion at 10, 20 and 30% into the reference diet mash (T6).

[3] DSM, Mairinque, São Paulo, Brazil

[4] Guabi, Campinas, Brazil

[5] Dinâmica, Indaiatuba, Brazil

A novel strategy to estimate ADC of nutrients in individual feed ingredients was presently assessed by testing diets containing SBM at 5, 10, 15 and 20%, corresponding to diets T4, T5, T6 and T7, respectively (Table 3). Thus, diet T6 played a double role as reference and test diet (at 15% SBM) under present methodological approach. To keep nutrient content, attractivity, palatability and nutritional consistency, dietary levels of fish meal, squid meal, fish hydrolysate, yeast, fish oil, lecithin, vitamin and mineral premix were kept constant in diets T4 to T7. Similar crude protein, lipid and gross energy levels among test diets T4 to T7 was intended by varying dietary levels of poultry by-product meal, blood meal and wheat in formulation of test diets. Chromic oxide was included at 0.5% in all diets as inert marker for determination of apparent digestibility.

Diet preparation started with mixing the dry ingredients in a planetary mixer (ES-600, Hobart) for 10 min. Next, liquid ingredients, including boiled distilled water, were added to the ingredient mix to form the dough prior to pelleting. The resulting dough was cold pressed (ca. 45°C) and the pellets (3.0–5.0 mm length, 2.0 mm diameter) were dried in a forced air dryer overnight (35–45°C for 18 h). Pellets were stored in zip plastic bags at -15°C until used. The proximate and amino acid composition of the test diets are shown in Table 4. True protein levels refer to the sum of analyzed amino acids.

Table 4
Composition of experimental diets (%, as-is) for juvenile shrimp (*Litopenaeus vannamei*). Data expressed as mean for proximate composition (n = 2).

	T1 (10/90)[1]	T2 (20/80)[1]	T3 (30/70)[1]	T4	T5	T6 (reference)	T7
Moisture	5.12	5.43	4.22	6.16	5.12	5.86	5.99
Crude protein	41.2	42.1	43.2	39.4	39.7	39.9	37.3
True protein[2]	37.2	38.7	39.3	35.5	36.4	35.9	34.0
Ether extract	6.42	6.76	5.97	7.55	7.66	8.41	7.27
Gross energy	4,581	4,557	4,603	4,552	4,573	4,508	4,646
Ash	10.1	9.64	9.39	10.1	10.4	10.2	7.71
Cr	0.320	0.328	0.314	0.305	0.314	0.323	0.353
Amino acids							
Arg	2.50	2.65	2.77	2.25	2.37	2.37	2.32
His	0.90	0.95	0.97	0.89	0.89	0.86	0.81
Iso	1.54	1.63	1.75	1.31	1.39	1.44	1.56
Leu	2.79	3.01	3.19	2.81	2.82	2.76	2.65
Lys	2.21	2.33	2.43	2.14	2.13	2.16	1.94
Met	0.73	0.69	0.70	0.75	0.76	0.73	0.65
Phe	1.68	1.79	1.90	1.59	1.60	1.60	1.53
Thr	1.62	1.70	1.77	1.59	1.60	1.55	1.42
Try	0.27	0.29	0.30	0.19	0.21	0.22	0.24
Val	1.79	1.88	2.00	1.80	1.81	1.76	1.65
Tau	0.13	0.12	0.11	0.15	0.15	0.14	0.11
Asp	3.44	3.58	3.18	3.02	3.19	3.19	3.09
Glu	6.15	6.39	6.45	5.56	5.83	5.82	5.97
Ser	1.83	1.88	1.95	1.74	1.77	1.74	1.58
Gly	2.96	2.90	2.84	3.06	3.10	3.00	2.54
Ala	2.31	2.34	2.38	2.37	2.39	2.29	1.99
Pro	2.51	2.54	2.59	2.50	2.56	2.48	2.42
Tyr	1.13	1.20	1.29	1.01	1.07	1.07	1.03
Cys	0.71	0.84	0.76	0.74	0.71	0.69	0.49

T4, T5, T6 and T7: practical test diets with SBM inclusion at 5, 10, 15 and 20%, respectively

[1] Conventional replacement method, SBM inclusion at 10, 20 and 30% into the reference diet mash (T6). More details in Material and Methods.

[2] Sum of analyzed amino acids.

Experimental shrimp

The study was carried out at the coastal Aquaculture Laboratory (LAM) from the University of São Paulo (USP) (Oceanographic Institute, Ubatuba, Brazil). Postlarval *L. vannamei* (PL 15, Speedline strain) was supplied by Aquatec hatchery (Barra do Cunhaú, Brazil) and was reared in clear water tank nursery system (2,000 L), including daily cleaning and partial water renewal until reaching approximately 0.2 g ind weight. Postlarvae were fed commercial crumbled feed (FlashShrimp, Polinutri, Brazil) continuously delivered by belt feeders (24h Baby belt feeder, Pentair, Brookfield, USA). The seawater (35 ppt salinity) was pumped from the sea (the sheltered Flamengo Cove, Ubatuba) following filtration through 25 µm and 5 µm cartridge filters. The nursery population was further stocked into a 12,000 L tank and raised in biofloc system, fed commercial pellets (Policamarão, Polinutri, Brazil) until stocking into the recirculated trial system at ca. 3.4 g ind shrimp weight. Water quality in the biofloc nursery system: dissolved oxygen (> 70% saturation), total ammonia-N (< 0.05 mg/L), nitrite (< 0.1 mg/L), pH (7.5–8.2) and alkalinity (> 120 mg $CaCO_3$/L).

Shrimp trial system for simultaneous determination of performance and digestibility

The trial was conducted in a clear water system composed of 21 x 500 L semi-conical tanks connected in full recirculation including mechanical and biological filtration (Sweetwater bead filter with UV treatment 4 cubic feet, model 930087, Pentair, Brookfield, USA), and a flow heater (10 kW, Globalmar, São Paulo, Brazil). Feeding was provided by belt feeders (Baby belt feeder, Pentair, Brookfield, USA) individually installed upon experimental tanks that continuously delivered feed pellets over the trial (20–22 h/day). Each tank contained a settling column for feces and solids accumulation and removal with trans lucid Falcon tubes threaded to receive residual solids (feces and eventual pellet leftovers), allowing tube cleaning and exchange. The layout and operational efficiency of the recirculated system and settling columns were previously detailed in Carvalho et al. (2013).

Seawater was filtered (25 and 5 µm cartridge filters) and disinfected by UV before entering the recirculated tank system. A flow rate of 4 L min^{-1} into each tank allowed water vortex for feces removal. The trial system continually delivered small amounts of pellets so that pellet apprehension time by shrimp did not exceed one minute. An air stone installed at 30 cm above each tank bottom further sustained dissolved oxygen level and avoided particle suspension.

Shrimp was stocked in tanks after individual weighing in seawater at 34 shrimp per tank that correspond to 85 individuals/m^3. Shrimp was acclimated to test diets five days prior starting collection of feces samples. Feed ration was initially set based on tank population biomass, mean individual weight and temperature (Forster et al., 2003). During the trial, daily ration was adjusted according to presence or not of pellet leftovers in the Falcon tube attached to the settling columns overnight. Eighty percent of daily feed ration was continuously delivered by belt feeders and the remaining 20% were divided in three hand-fed meals at 9 am, 11 am and 2 pm. Under this continuous feeding regime, nutrient leaching is minimized once average time for pellet apprehension by shrimp was 30s and 5 min for total pellet intake (visual and camera observations). The trial comprised 55 days and freshly released feces samples were collected 5 days per week, 4 to 6 times per day, morning and afternoon. Daily sample collection started 1h after cleaning of settling tubes and columns (shrimp feces settled during the night were discarded). Maximum exposure time of sampled feces in water (Falcon tube collector) was 1.5h. Collected feces samples were gently rinsed with distilled water during vacuum filtration to remove excess salt. Pooled feces were accumulated during the trial and kept frozen (-15 ^{o}C). Feces samples were lyophilized, milled, and stored at -18°C until analysis. Daily monitored water parameters were dissolved oxygen, salinity, and temperature (YSI Pro 2030, Yellow Springs, USA), weekly determination for total ammonia, nitrite, and alkalinity (Alfakit, Florianópolis, Brazil). During the trial, water quality parameters were [mean (s.d.)]: dissolved oxygen 5.60 (0.18) mg/L, 86.5 (3.22) % of saturation; temperature 29.1 (1.10) ^{o}C; salinity 35.2 (0.41) ppt; total ammonia 0.30 (0.46) mg/L; nitrite 0.10 (0.08) mg/L; pH 8.0, and alkalinity 106.7 (10.3) mg/L $CaCO_3$.

Experimental diets were tested in triplicate tanks. At trial finishing, harvested shrimp was counted and individually weighted for determination of survival, growth and feed conversion ratio considering total feed delivered during trial.

Feed Conversion Rate (FCR) = total feed consumed (g) divided by total weight gain (g);

Daily feed intake (g/ind) = total feed consumed divided by stock number considering survival rate and days of culture.

Ingredient and diet analysis

Moisture, crude protein, lipid and ash contents of diets, feces and shrimp were determined following AOAC (2005). Moisture was analyzed after oven drying at 105°C until constant weight - dried samples were used for further analysis. Crude protein was analyzed by Kjeldahl (N x 6.25) using copper sulphate as catalyst in the acid digestion. Lipid was determined by ether extraction (ST255, Soxtec^{tm}, FOSS). Gross energy content of samples was analyzed with a calorimetric bomb (IKA 5000, Staufen, Germany). Amino acids were determined by HPLC (White et al., 1986; Hagen et al., 1989) after acid and alkaline digestion by the ionic exchange method (Kwanyuen and Burton, 2010), with tryptophan analyzed separately (Lucas and Sotelo, 1980). Feces amino acid content was the only analysis performed in samples sourced from pools of replicate tanks. Chromium in diets and feces samples was determined by atomic absorption spectrometry according to standard method no. 0968.08 (AOAC, 2005).

Calculation of apparent digestibility coefficients: conventional and novel approaches for feed ingredients

Apparent digestibility coefficients (ADC) of dry matter, energy and nutrients (%) in test diets were calculated as follows:

ADC (dry matter, %) = 100–100 (% Cr in diet/% Cr in feces)

ADC (nutrient or energy) = 100–100 [(% Cr in diet/% Cr in feces) x (Nutrient or energy in feces/Nutrient or energy in diet)]

The partial replacement of a reference diet mix by the target ingredient as dietary strategy, following the comparison of the ADC of this test diet with ADC of the reference diet using mathematical calculations (formula), has been the most used method in digestibility studies assessing feed ingredients for shrimp species (Akiyama et al., 1989; Davis and Arnold, 1993; Ezquerra et al., 1997; Cruz-Suárez et al., 2000; Nieto-López et al., 2011; Davis et al., 2002; Cruz-Suárez et al., 2007; Lemos et al., 2009; Niu et al., 2011; Liu et al., 2013; Carvalho et al., 2016). The present study aimed to compare this rather consensual method for ADC determination in a feed ingredient with a novel approach, considering the target ingredient (presently SBM) at different inclusion levels in practical test diets and using the determined ADC of these test diets as reference for estimation of ingredient ADC (Hernández et al., 2008; Yang et al., 2015).

The calculation for ADC of energy and nutrients (%) of SBM under the conventional methodology (SBM presently tested at 10, 20, and 30% inclusion into the reference diet - diets T1, T2 and T3) followed the mathematical approach of weighing ADC of test and reference diets with nutrient or energy content in diet and ingredient:

$ADC_{test\ ingredient} = ADC_{test\ diet} + [(ADC_{test\ diet} - ADC_{ref.\ diet}) \times (0.7 \text{ or } 0.8 \text{ or } 0.9 \times D_{ref}/0.3 \text{ or } 0.2 \text{ or } 0.1 \text{ X } D_{ingr})]$

Where, D_{ref} is % nutrient or energy in reference diet mash and D_{ingr} is % nutrient or energy in test ingredient (Bureau and Hua, 2006; Terrazas-Fierro et al., 2010; Hernández et al., 2011; NRC, 2011; Carvalho et al., 2016; Panini et al., 2017; Qiu et al., 2018).

The presently proposed new approach for estimating amino acid ADC in individual feed ingredients (presently focused on SBM) is based on the premise that the value of apparent true protein digestibility (ATPD) of a certain diet represents the average of each individual amino acid digestibilities composing this diet (NRC, 2011). Accordingly, the apparent digestibility value of individual amino acids in the diet would range above and below the mean value found for diet ATPD. Under the present new proposal and dietary experimental strategy, the calculation (mathematical) of apparent individual amino acid digestibility (AIAAD) in a feed ingredient requires: 1. Estimation of the ATPD value of the target ingredient included in test diets (presently SBM); and 2. Comparison of ATPD and AIAAD in each test diet (here composed by different inclusion levels of SBM, diets T4, T5 T6 and T7) that represent the effect of increasing inclusion levels of the target ingredient upon AIAAD of test diet.

The estimation of the ATPD value to be assigned for SBM was based on the combination of different criteria: a. the additive effect of increasing dietary inclusion level of SBM upon ATPD of test diets (presently between 5 and 20%) - i.e., if higher dietary inclusion levels of SBM results in increasing ATPD of test diets, the ATPD value of SBM as feed ingredient is therefore higher than the ATPD of teste diets – b. literature values of ADC in SBM (Akiyama et al., 1989; Divakaran et al., 2000; Yang et al., 2009; Zhu et al., 2013; Fang et al., 2016; Qiu et al., 2018) and author´s experience under currently tested SBM dietary inclusion levels (Lemos et al., 2004; Lemos et al., 2009; Carvalho et al., 2016); and c. the consistency of total dietary protein supplied in test diets (5–20% SBM), i.e. the sum of digestible protein sourced from each relevant proteinaceous feed ingredient in formulation was equivalent to determined (*in vivo*) ATPD value in the diet, considering the additivity of ingredient protein supply and the estimated ATPD of each feed ingredient using literature data (Ezquerra et al., 1997; Nieto-López et al., 2011; Yang et al., 2009; Ye et al., 2012; Liu et al., 2013; Villarreal-Cavazos et al., 2014; Zhao et al., 2017) and author´s experience for the ingredients fish meal, wheat, poultry by-product meal, blood meal, squid meal, fish hydrolysate, dried yeast and soy lecithin. It was also considered ATPD of test diets may be affected by rather elevated inclusion level of the feed ingredients blood meal (diets T4 and T5) and poultry by-product meal (T4, T5 and T6) used in the present study (Tacon et al., 2009).

The true protein ADC value of SBM assigned for test diets was then used for calculation of individual amino acid digestibility of SBM, so that:

$ADC_{AA\ SBM} = \text{Mean } [(ADC_{AA\ diet\ (T4,\ T5,\ T6\ or\ T7)} / ADC_{protein\ diet\ (T4,\ T5,\ T6\ or\ T7)}) \times ADC_{protein\ SBM}]$

Where:

$ADC_{AA\ SBM}$: apparent digestibility coefficient of individual amino acid;

$ADC_{AA\ diet\ (T4,\ T5,\ T6\ or\ T7)}$: apparent digestibility coefficient of individual amino acid in the test diet;

$ADC_{protein\ diet\ T4,\ T5,\ T6\ or\ T7}$: apparent digestibility coefficient of true protein in test diet;

$ADC_{protein\ SBM}$: apparent digestibility coefficient of true protein assigned to SBM (as above described).

Example

- ADC of arginine in test diets = 72, 76, 77 and 80% with SBM inclusion at 5, 10, 15 and 20%, respectively;
- ADC of true protein in test diets = 69, 73, 74 and 77% with SBM inclusion at 5, 10, 15 and 20%, respectively;
- ADC of true protein assigned to SBM = 85%;

Thus, ADC of arginine in SBM (%) = Mean [(72/69) x 85]; [(76/73) x 85]; [(77/74) x 85]; [(80/77)] x 85 = 88.5%.

Statistical analysis

Data was submitted to normality and homoscedasticity test prior to application of analysis of variance (ANOVA) or Kruskall-Wallis ANOVA to compare diet performance. Differences between means were analyzed by post-hoc Tukey HSD or Dunn tests. Pearson analysis was applied to test for the statistical significance of the correlations and coefficient of determination (R^2). Differences and correlations were considered significant at $P < 0.05$ (Zar, 1984).

Results

The feeding trial resulted in significant different performance of juvenile *L. vannamei* depending on the dietary treatment ($P < 0.05$, Table 5). Individual final weight was highest with diet T1 while growth rate was reduced in diets with 5 and 10% SBM inclusion (T4 and T5, respectively) compared to higher SBM level diets (15 and 23.5%, corresponding to diets T6 and T1, respectively). Accordingly, FCR was lower in diets with 23.5, 40.5 and 15% SBM (T1, T3 and T6, respectively) compared to 5% SBM inclusion diet (T4). Individual daily feed intake was found reduced only in 40.5% SBM inclusion diet (T3), a treatment of highest protein content. In practical terms, the performance of juvenile *L. vannamei* in clear water (growth rate and FCR) in most dietary treatments may be considered acceptable and support the validation of the digestibility data obtained (Tacon, 1996; Carvalho et al., 2016).

Table 5

Performance of juvenile shrimp (*Litopenaeus vannamei*) after 55 days stocked at 85 ind/m^3; 29 ^oC and 35 ppt salinity. Values expressed as mean (s.d.). Means in the same line bearing different superscripts are significantly different ($P < 0.05$).

	T1 (10/90)[1]	**T2** (20/80)[1]	**T3** (30/70)[1]	**T4**	**T5**	**T6** (reference)	**T7**
Initial ind wt (g)	3.42 (0.03)	3.33 (0.16)	3.37 (0.13)	3.39 (0.27)	3.35 (0.08)	3.36 (0.16)	3.44 (0.12)
Final ind wt (g)	15.4^a (0.51)	14.2^{ab} (0.52)	14.4^{ab} (0.08)	13.2^b (0.28)	13.7^b (0.72)	14.9^{ab} (0.28)	14.0^{ab} (0.68)
Growth (g/week)	1.52^a (0.07)	1.38^{ab} (0.12)	1.40^{ab} (0.02)	1.24^b (0.04)	1.32^b (0.09)	1.47^a (0.04)	1.35^{ab} (0.07)
FCR	1.61^a (0.14)	1.75^{ab} (0.11)	1.48^a (0.11)	1.95^b (0.13)	1.75^{ab} (0.07)	1.47^a (0.12)	1.75^{ab} (0.19)
Survival (%)	87.3 (7.40)	89.2 (8.49)	98.0 (3.40)	88.2 (2.94)	91.2 (5.09)	96.1 (4.49)	89.2 (6.12)
Daily feed intake (g/individual)	0.321^a (0.006)	0.318^a (0.010)	0.290^b (0.009)	0.305^{ab} (0.014)	0.304^{ab} (0.005)	0.299^{ab} (0.009)	0.311^{ab} (0.011)
FCR: feed conversion ratio.							
T4, T5, T6 and T7: practical test diets with SBM inclusion at 5, 10, 15 and 20%, respectively							
[1] Conventional replacement method, SBM inclusion at 10, 20 ad 30% into the reference diet mash (T6). More details in Material and Methods.							

The apparent digestibility of test diets also resulted in significant differences ($P < 0.05$) among dietary treatments for most assessed nutrients (Table 6). Apparent dry matter digestibility ranged from 57.3–63.1% and showed higher value in T7 (20% SBM), only comparable to diet T3 (40.5% SBM). Though showing higher values compared to apparent crude protein digestibility (ACPD), apparent true protein digestibility (ATPD) was significantly correlated with ACPD ($P < 0.05$; $R^2 = 0.99$), digestibility values ranging at 64.5–76.8% for ACPD and 69.4–81.7% for ATPD. Diets with elevated SBM inclusion (T1, T2 and T3) showed increased ACPD and ATPD compared to other test diets with SBM inclusion between 5 and 20%, though significant higher digestibility ($P < 0.05$) was found in T3 (40.5% SBM) followed by T1, T2 and T7 (23.5, 32.0 and 20.0% SBM, respectively). Reduced ACPD was checked in diets T4 and T5 ($P < 0.05$). In contrast to protein, dietary treatments did not result in significant different in apparent digestibility of ether extract ($P > 0.05$). Reduced apparent gross energy digestibility was found in diet T2 (32.0% SBM) that was significantly lower than diet T7 (20% SBM, $P < 0.05$). As previously observed for shrimp species reared in marine water, apparent ash digestibility displayed negative results (-32.3 to -17.0%) and no significant difference was found among dietary treatments ($P > 0.05$).

Table 6
Apparent digestibility (%) of test diets for juvenile *Litopenaeus vananmei*. Values expressed as mean (s.d.). Different superscripts in the same row are significantly different (P < 0.05).

	T1 (10/90)[1]	T2 (20/80)[1]	T3 (30/70)[1]	T4	T5	T6 (reference)	T7
Dry matter	59.5^{ab} (1.42)	57.3^{a} (2.49)	61.1^{ab} (0.91)	58.1^{a} (0.52)	58.5^{a} (1.40)	58.0^{a} (1.60)	63.1^{b} (1.61)
Crude protein	72.5^{a} (1.09)	71.2^{ac} (1.96)	76.8^{b} (1.44)	64.5^{d} (0.77)	68.0^{cd} (0.91)	69.3^{ac} (2.27)	72.5^{a} (1.01)
True protein	77.6	76.1	81.7	69.4	72.7	74.5	77.6
Ether extract	77.7 (2.81)	82.3 (8.16)	81.8 (3.86)	76.8 (1.78)	81.2 (3.31)	81.1 (2.96)	77.9 (4.46)
Gross energy	71.3^{ab} (0.86)	69.2^{b} (1.77)	72.3^{ab} (1.52)	70.1^{ab} (0.73)	71.0^{ab} (0.38)	70.1^{ab} (2.25)	73.4^{a} (1.26)
Ash	-23.0 (8.57)	-26.8 (10.5)	-17.0 (4.10)	-32.1 (2.42)	-29.2 (3.98)	-29.7 (7.14)	-32.3 (3.54)
Amino acids							
Arg	79.5	79.0	84.6	72.9	75.8	76.7	79.6
His	71.4	70.2	77.1	59.7	64.6	67.2	73.8
Iso	76.5	74.4	81.5	68.6	71.1	72.3	77.5
Leu	73.3	72.1	79.0	63.5	67.9	69.6	75.9
Lys	77.8	76.1	82.4	68.3	71.9	74.1	78.9
Met	77.8	75.3	80.0	72.0	73.6	74.4	76.3
Phe	74.8	72.6	79.0	62.3	66.7	68.8	75.1
Thr	73.3	70.4	77.7	63.4	65.0	67.2	70.7
Try	76.9	73.7	79.5	60.8	68.8	68.4	72.7
Val	70.5	68.5	77.2	60.3	65.2	67.2	73.1
Tau	87.2	85.0	88.8	85.4	85.4	87.6	86.0
Asp	84.7	84.9	88.0	78.3	81.0	84.9	86.4
Glu	84.5	83.1	88.0	78.3	80.7	83.0	85.1
Ser	72.1	70.4	77.0	62.5	64.9	67.1	68.3
Gly	74.4	72.0	76.3	68.1	71.0	71.5	70.1
Ala	74.8	72.4	78.4	66.7	70.6	71.4	74.2
Pro	76.5	74.5	79.7	69.9	72.9	73.7	75.8
Tyr	76.1	74.2	80.9	65.7	69.4	71.2	74.6
Cys	78.9	82.4	85.9	75.1	79.1	81.1	77.2

[1] Conventionally applied replacement method for determination of digestibility, SBM inclusion at 10, 20 and 30% into the reference diet mash (T6).

Apparent individual amino acid digestibility of test diets (AIAAD) showed higher values in T3 (40.5% SBM) followed in most cases by diets T1 (23.5% SBM) and T7 (20% SBM), whilst diet T4 (5% SBM) registered the lowest AIAAD values (Table 6). Exception was checked for taurine where ADC was > 85% for all test diets. Among required/indispensable amino acids for *L. vannamei* (taurine included: Yue et al., 2013, To and Liou, 2021), digestibility of test diets showed wider variation for tryptophan, histidine, phenylalanine and valine whilst the narrowest variation was found for AIAAD of taurine and methionine. Apparent digestibility ranged among diets as: 75.8–84.6% for arginine; 59.7–77.1% for histidine; 68.6–81.5% for isoleucine; 63.5–79.0% for leucine; 68.3–82.4% for lysine; 72.0–80.0 for methionine; 62.3–79.0% for phenylalanine; 63.4–77.7% for threonine; 60.8–79.5% for tryptophan; 60.3–77.2% for valine; and 85.4–88.5% for taurine. Present ADC results confirmed the premise that ATPD value of the diet represent the average of AIAAD composing the diet (NRC, 2011) since these results correlated significantly ($P < 0.05$, $R^2 = 0.99$). The increased amino acid digestibility with higher inclusion of SBM (20–30% and 40.5%) suggests ATPD of this ingredient was superior to ADC of the reference diet (T6). As protein was the main nutrient showing differences in ADC, feces true protein content was found negatively correlated with ATPD (Table 7; $P < 0.05$, $R^2 = 0.98$). The same trend was not observed when regressing feces amino acid content with AIAAD, either for indispensable or non-indispensable amino acids.

Table 7
Feces composition (%) expressed as mean (s.d.), dry matter basis. Different superscripts in the same row are significantly different ($P < 0.05$).

	T1 (10/90)[1]	**T2 (20/80)**[1]	**T3 (30/70)**[1]	**T4**	**T5**	**T6 (reference)**	**T7**
Crude protein	29.5^{a} (0.18)	30.0^{a} (0.30)	26.9^{b} (1.06)	35.6^{c} (0.45)	32.3^{d} (0.19)	31.0^{ad} (1.17)	29.7^{a} (0.38)
True protein	21.7	22.9	19.3	27.6	25.2	23.1	21.9
Ether extract	3.73 (0.35)	2.95 (1.35)	2.91 (0.60)	4.45 (0.30)	3.67 (0.64)	4.02 (0.58)	4.63 (0.83)
Gross energy (cal/g)	3,421 (74.5)	3,470 (51.4)	3,416 (107.8)	3,457 (42.3)	3,732 (71.9)	3,403 (129.5)	3,569 (49.7)
Ash	32.5 (1.17)	30.2 (0.76)	29.5 (1.68)	34.0 (1.01)	34.0 (1.77)	33.6 (3.16)	29.5 (1.86)
Cr	0.83 (0.030)	0.81 (0.046)	0.84 (0.020)	0.78 (0.010)	0.80 (0.027)	0.82 (0.032)	1.02 (0.046)
Amino acids							
Arg	1.33	1.38	1.15	1.55	1.45	1.40	1.37
His	0.67	0.70	0.59	0.91	0.80	0.71	0.61
Iso	0.94	1.03	0.87	1.05	1.02	1.01	1.01
Leu	1.94	2.08	1.80	2.61	2.30	2.12	1.85
Lys	1.28	1.38	1.15	1.73	1.52	1.42	1.18
Met	0.42	0.42	0.37	0.53	0.51	0.47	0.44
Phe	1.10	1.21	1.07	1.53	1.36	1.26	1.10
Thr	1.13	1.25	1.06	1.48	1.42	1.29	1.20
Try	0.16	0.19	0.17	0.19	0.17	0.18	0.19
Val	1.37	1.47	1.22	1.82	1.60	1.46	1.28
Tau	0.04	0.04	0.03	0.06	0.06	0.04	0.04
Asp	1.37	1.33	1.02	1.67	1.54	1.22	1.21
Glu	2.49	2.67	2.07	3.07	2.87	2.51	2.57
Ser	1.33	1.38	1.20	1.66	1.58	1.45	1.45
Gly	1.97	2.01	1.81	2.48	2.29	2.17	2.19
Ala	1.51	1.60	1.38	2.00	1.79	1.66	1.48
Pro	1.54	1.60	1.41	1.92	1.77	1.65	1.69
Tyr	0.70	0.77	0.66	0.88	0.83	0.78	0.76
Cys	0.39	0.37	0.29	0.47	0.38	0.33	0.32

[1] Conventionally applied replacement method for determination of digestibility, SBM inclusion at 10, 20 and 30% into the reference diet mash (T6).

Since a practical value of ATPD for SBM was pre-requisite for the application of the new methodology for estimation of amino acid digestibility (as described in Material and Methods), it was initially checked, considering ADC values of test diets with increasing SBM inclusion into the reference diet mix (10, 20 and 30%) and additivity of SBM dietary inclusion in diet digestibility, ATPD of SBM to be higher than 74.5%, i.e. the experimentally determined ATPD value in reference diet T6 (Table 6). Based on this evidence, the value of 85% was assigned for SBM ATPD that is further supported by literature data and author´s experience (Table 8). The assigned SBM ATPD value was assumed stable in test diets (i.e. not affected by SBM level) under tested inclusion range (5 to 20%), also because SBM levels in test diets may be considered practical for shrimp feeds (Hertrampf and Piedad-Pascual, 2000; Tacon et al., 2009). Though the ATPD of SBM may be enough for calculation of amino acid digestibility under the present proposal, the expected ATPD of other protein sourcing ingredients was also assigned for complimentary analysis in order to compare the sum of expected digestible protein from feed ingredients, considering additive nutrient supply, with experimentally determined ATPD in test diets (Table 8). From the nine protein-supplying ingredients used in test diets, only blood meal and poultry by-product meal were considered to have decreasing ATPD with higher dietary inclusion levels, and thus assigned ATPD value of these ingredients varied among test diets, as previously reported with juvenile *L. vannamei* (Cruz-Suárez et al., 2007; Carvalho et al., 2016). The assigned values of ATPD and consequent digestible protein from feed ingredients (the sum of protein supplies from each ingredient) showed similar values compared to experimentally determined ATPD (Table 6; Table 8).

Table 8
Additivity of protein supply: total and digestible true protein of feed ingredients used in test diets with different inclusion levels of soybean meal (SBM, T4 to T7) for juvenile *Litopenaeus vannamei*. Apparent digestibility coefficient of true protein (ADC tp) assigned according to literature[1] and author´s experience, considering ingredient type and potential effect of dietary inclusion level (in parenthesis) upon digestibility. Total true protein content of diets considering ingredient composition and respective inclusion level in diets (Prot, %), and digestible protein from diet ingredients (DP, %) considering ingredient true protein and ADC tp; the sum of DP from feed ingredients represents additively predicted DP in diets. Predicted ADC tp (= total DP/ total Prot) is compared to experimentally determined ADC tp (as in Table 6). More details in Material and Methods.

Feed ingredient	**T4 (5% SBM)**			**T5 (10% SBM)**			**T6 (15% SBM)**			**T7 (20% SBM)**		
	ADC tp (%)[1] (inclusion, %)	Prot (%)	DP (%)	ADC tp (%)[1] (inclusion, %)	Prot (%)	DP (%)	ADC tp (%)[1] (inclusion, %)	Prot (%)	DP (%)	ADC tp (%)[1] (inclusion, %)	Prot (%)	DP (%)
SBM	85 (5)	2.19	1.86	85 (10)	4.37	3.72	85 (15)	6.56	5.57	85 (20)	8.74	7.43
Wheat	85 (42)	4.78	4.06	85 (39.5)	4.49	3.82	85 (36)	4.10	3.48	85 (36)	3.86	3.28
Fish meal	70 (20)	10.30	7.21	70 (20)	10.3	7.21	70 (20)	10.3	7.21	70 (20)	10.3	7.21
PBM	60 (11.5)	6.43	3.86	65 (10.5)	5.88	3.82	70 (10)	5.59	3.92	80 (9)	5.03	4.02
Blood meal	55 (4.5)	3.84	2.11	60 (3)	2.57	1.54	60 (2)	1.71	1.03	60 (0)	0.00	0.00
Squid	70 (5)	3.98	2.78	70 (5)	3.98	2.78	70 (5)	3.98	2.78	70 (5)	3.98	2.78
Fish hydrolysate	70 (4)	0.92	0.64	70 (4)	0.92	0.64	70 (4)	0.92	0.64	70 (4)	0.92	0.64
Dried yeast	70 (3)	1.02	0.72	70 (3)	1.02	0.72	70 (3)	1.02	0.72	70 (3.0)	1.02	0.72
Soy lecithin oil	85 (1)	0.03	0.02	85 (1)	0.03	0.02	85 (1)	0.03	0.02	85 (1.0)	0.03	0.02
Total		33.5	23.3		33.6	24.3		34.2	25.4		33.9	26.1
Diet ADC tp (Predicted: totalDP/totalProt)			69.5			72.3			74.2			77.1
Diet ADC tp (Determined: Table 6)			69.4			72.7			74.5			77.6

[1] Akiyama et al., 1989; Ezquerra et al., 1997; Divakaran et al., 2000; Nieto-López et al., 2001; Lemos et al., 2004; Lemos et al., 2009; Yang et al., 2009; Ye et al., 2012; Liu et al., 2013; Zhu et al., 2013; Villarreal-Cavazos et al., 2014; Carvalho et al., 2016; Fang et al., 2016; Zhao et al., 2017; Qiu et al., 2018.

Amino acid ADC (AIAAD) of SBM showed contrasting values between the different strategies for digestibility determination (Table 9). Apparent digestibility coefficients determined by the conventional replacement method varied depending on the replacement level of SBM into the reference diet and ATPD was 99.5, 81.2 and 95.0% at 10, 20 and 30% replacement, respectively. Since ATPD represent the average of individual amino acid digestibility, AIAAD values found by replacement method at 10 and 30% SBM may be considered very high, and the great majority of values found in these dietary treatments (T1 and T3) were much higher than determined by the presently proposed approach for all amino acids. Digestibility varied according to SBM inclusion in the replacement method ranging from 63.4 to 129.7%, 42.4 to 88.7% and 87.0 to 108.4% in replacement levels of 10, 20 and 30%, respectively. Results of ADC under the replacement method at 20% SBM were lower compared to findings at 10 and 30% but still differed from the presently proposed new calculation. Accordingly, with exception to leucine, ADCs differed from 2 to 57 percent points in assessed amino acids. Higher AIAAD at 20% replacement compared to the presently proposed new approach was checked for histidine, phenylalanine, threonine, tryptophan, serine and tyrosine, and most important differences were found in taurine and isoleucine (55 and 21 percent points, respectively).

Table 9
Apparent digestibility of amino acids in soybean meal (SBM) feeding juvenile shrimp *Litopenaeus vananmei*. Traditional calculation (SBM/Reference diet) was applied as diet strategy in T1, T2 and T3, and presently proposed alternative calculation (different levels of target ingredient into practical diets). Values expressed as mean (s.d.) of different dietary inclusions of SBM (5 to 20%).

	Dietary strategy			
	T1 (10/90)[1]	T2 (20/80)[1]	T3 (30/70)[1]	Proposed method (5 to 20% inclusion)[2]
True protein	99.5	81.2	95.0	85.0[3]
Arg	96.5	85.0	96.8	88.2 (0.97)**
His	99.0	79.1	94.2	76.5 (3.23)*
Iso	71.0	66.7	87.0	83.6 (1.05)
Leu	96.2	78.9	94.1	79.9 (2.27)*
Lys	102.7	82.1	96.8	84.7 (1.22)*
Met	115.3	79.4	96.3	85.7 (1.96)**
Phe	109.3	82.6	94.4	78.8 (2.51)*
Thr	112.2	79.4	95.1	76.9 (0.76)
Try	112.7	83.5	91.5	78.1 (2.65)*
Val	92.0	72.1	94.0	76.7 (2.56)*
Tau	72.8	42.4	100.5	99.7 (4.26)**
Asp	82.4	85.2	95.7	95.5 (1.06)
Glu	94.6	83.6	97.1	94.5 (1.11)
Ser	105.7	80.3	94.4	75.9 (0.82)
Gly	109.1	74.6	90.8	81.2 (3.04)**
Ala	103.7	76.0	93.9	81.7 (0.56)
Pro	99.2	77.5	92.5	84.5 (1.17)**
Tyr	129.7	88.7	108.4	81.1 (0.51)
Cys	63.4	86.2	94.8	90.4 (3.89)**

[1] Conventional replacement method, SBM inclusion at 10, 20 ad 30% into the reference diet mash (T6) (references in Material and Methods).

[2] Based on live animal output fed diets with SBM inclusion at 5 to 20%.

[3] Assigned values, as explained in Material and Methods and Results.

Asterisks: * Increase or ** decrease checked in diet ADC with rising inclusion of SBM (5 to 20% inclusion in the present study).

The proposed new method showed AIAAD between 75.9 and 99.7%, and variation (s.d.) was related to the effect of variability SBM inclusion level produced in diet ADC. Under this methodology, highest ADC among indispensable amino acids was found for taurine (99.7%) followed by arginine (88.2%), methionine (85.7%), lysine (84.7%) and isoleucine (83.6%) whilst lower values (ADC < 80%) were checked for histidine

(76.5%), valine (76.7%), threonine (76.9%), tryptophan (78.1%), phenylalanine (78.8%) and leucine (79.9%). Among non-indispensable amino acids higher ADC was verified for aspartic acid (95.5%), glutamic acid (94.5%) and cysteine (90.4%), followed by proline (84.5%), alanine (81.7%), glycine (81.2%), tyrosine (81.1%) and serine (75.9%). Increasing inclusion level of SBM in test diets used in the new methodology resulted in increased, reduced or no effect upon diet digestibility (as indicated by asterisk in Table 9). Among indispensable amino acids, increasing dietary SBM resulted in reduced ADC in arginine, methionine and taurine, whereas it was checked higher for histidine, leucine, lysine, phenylalanine and valine. Glycine, proline and cysteine ADCs were also negatively affected by SBM increasing level.

Discussion

Shrimp requires 46 to 50 individual nutrients to be provided in diets at adequate amount and in digestible form for proper health and growth; dietary requirement includes eleven amino acids (Yue et al., 2013; Tacon, 2015; To and Liou, 2021). Therefore, data on digestibility of feedstuffs is needed according to required nutrients to be sourced in diets through feed ingredients. A single feed ingredient has its own digestibility features, but also the combination of feed ingredients and feed preparation method may affect nutrient digestibility (Molina and Espinoza, 2020). Determining apparent nutrient digestibility has been a criterion for quality control of feedstuffs (Hertrampf and Piedad-Pascual, 2000) and this have been assessed for over thirty years in shrimp species (Akiyama et al., 1989). However, the most used methodology in assessing digestibility in shrimp may present some limitation. Important points on methodologies for determination of digestibility in shrimp species include: a. technical limitation in the application of a fixed inclusion level of target ingredient replacing a reference diet mix for a large variety of raw materials (for different reasons some feed ingredients are not recommended to be included in diets at levels as high as 30 or even 15% - see: Tacon et al., 2009 for review), i.e. some raw materials may produce reduced palatability and impaired digestibility if included at elevated levels (Lemos et al., 2009; Carvalho et al., 2016); b. lack of standardization on particle size of feed ingredients tested (critical for digestibility in shrimp, Ye et al., 2012, Wade et al., 2018); c. poor attention for animal performance potential in tank/aquaria systems, including feces collection strategies not suitable to sustain growth and FCR consistent with practical farming (ADC values should be ideally validated by proper shrimp performance) ; d. potentially significant leaching nutrient losses from pellets prior to complete feed intake, largely related to inappropriate feeding management, as well as leaching of fecal material, leading to potential overestimation of ADC (Smith and Tabrett, 2004; Cruz-Suarez et al., 2009; Bortone and Kipfer, 2016); e. short experimental period of digestibility trials in terms of individual weight gain, sometimes poorly representative of the farming cycle (Tacon; 1996); and f. limitation in applying a conventional mathematical calculation in determining ADC in which equation terms compare digestibility of test and reference diets for determination of ADC value for a target ingredient (Forster, 1999; Bureau and Hua, 2006; Carvalho et al., 2016). These methodological limitations suggest the need for improved techniques to determine the ADC value in a single feed ingredient under practical conditions in order to be widely applicable in the industry. Accordingly, assessing the digestibility of experimental diets including the target ingredient at different practical/recommended inclusion levels enables checking the effect of ingredient inclusion upon diet ADC and therefore the estimation of a reasonable ADC value of the feed ingredient (Tacon and Akiyama, 1997; Divakaran et al., 2000).

Shrimp growth rate and FCR in the present trial were acceptable for juvenile *L. vannamei* in clear water and further validate digestibility data (Carvalho et al., 2016; Lemos et al., 2021). The presented ADC values are thus representative of shrimp growing cycle in the trial, while collected feces samples denote the type of residue of this culture operation, which may be complimentary to performance data. The suitable performance may be attributed to key factors including proper water quality (mainly dissolved oxygen > 85% saturation) and automatic feeding regime (continuous feed supply and minimized leaching) over the course of the trial, composing the system for simultaneous determination of performance and digestibility in juvenile shrimp (Smith and Tabrett, 2004; Carvalho et al., 2013; Yang et al., 2009; Zainuddin and Aslamyah, 2014; Akiyama and Yukasano, 2022).

The present dietary strategy for determination of digestibility in a single feed ingredient, that contrasted ADCs of test diets including different percentages of the target ingredient, showed increasing SBM inclusion favored protein and amino acid digestibility in practical diets. It is well known a great variety of aquafeed ingredients are not suitable/recommended to be included above 10% in diet formulation in shrimp, and elevated levels may reduce ADC due to, e.g., decreased palatability, reduced feed intake, endogenous nitrogen losses (Columbus and Lange, 2012). In early times of digestibility determination in aquafeeds (starting with fish species), raw materials such as fish meal and soy products have composed most tested ingredients and have been often assayed at conventional 30% inclusion into reference diets, with minor focus on alternative animal and plant feedstuff (Cho et al., 1982; Dimes et al., 1994). With further advancement on the topic, it became clear these ingredients could eventually be used at this high established level with not so serious effects upon digestibility compared to several others currently used or potentially valuable in shrimp diets (Divakaran et al., 2000; Carvalho et al., 2016). As feed represents a major cost in farming, feed formulation requires continuous development and diversification of feed ingredients supported by characterization of raw materials including values of nutrient digestibility.

A common issue checked in literature reporting AIAAD values in some individual feed ingredients such as SBM for shrimp species have been very high ADC values (sometimes 95–99%, obtained by application of mathematical calculation) leading to uncertainty for use in practical feed formulation. Beyond potential leaching effect in trials leading to overestimated ADC (Smith and Tabbrett, 2004; Dias et al., 2009), limitation in formula for calculation of the effect of a fixed replacement level of target ingredient into a reference diet mix should also be analyzed. A bias in the digestibility calculation formula for apparent digestibility of feed ingredients was previously reported as at lower inclusion levels, the apparent digestibility coefficient of nutrients resulting from target ingredients with a high biological value are magnified and may reach values over 100% (Carvalho et al., 2016). The opposite has been checked for ingredients with a lower biological value.

A starting point for interpretation of ADC values of a feed ingredient may be checking apparent digestibility of true protein and individual amino acids determined in test diets under different inclusion of target ingredient, which represent more closely the biological process of nutrient absorption by determining diet and feces content with intermediation of an inert marker included in diets. Presently proposed alternative methodology for ADC determination has also limitations (e.g. the precision of estimation in true protein ADC for SBM) and is not intended to become an unquestionable reference, but rather offer and alternative view and more realistic values of ADC, to be continuously improved through studies on potential and limitation of inclusion of feed ingredients into practical diets. Thus, beyond the uncertainty derived from variability and elevated values of ADC values obtained by the conventional methodology (fixed inclusion level of target ingredient), the proposed assessment resulted in more reasonable ADC values, to be considered as starting point for further assessment of SBM and other valuable raw materials for shrimp feeds. As important competitors in the aquafeed sector, R&D based companies develop in-house solutions for continuous assessment of feed ingredients including several different techniques. Determination of nutrient digestibility is a crucial topic under development by the aquafeed industry for farmed shrimp.

Declarations

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