Received: 1 July 2019 | Revised: 22 September 2019 | Accepted: 11 October 2019
DOI: 10.1111/are.14378

Aquaculture Research WILEY

ORIGINAL ARTICLE

Apparent energy, dry matter and amino acid digestibility of differently sourced soybean meal fed to Pacific white shrimp *Litopenaeus vannamei*

Harsha S. C. Galkanda-Arachchige[1,2] | **Jingping Guo**[1] | **Hans H. Stein**[3] | **Donald Allen Davis**[1]

[1]School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, Auburn, AL, USA

[2]Department of Aquaculture and Fisheries, Faculty of Livestock, Fisheries and Nutrition, Wayamba University of Sri Lanka, Makandura, Sri Lanka

[3]Department of Animal Sciences, University of Illinois, Urbana, IL, USA

Correspondence
Harsha S. C. Galkanda-Arachchige, School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, Auburn, AL, USA.
Email: hsg0009@auburn.edu

Funding information
Alabama Agricultural Experiment Station and the Hatch Program, Grant/Award Number: ALA016-08027; National Institute of Food and Agriculture, U.S. Department of Agriculture

Abstract

Due to the variations in nutrient quality of soybean meal (SBM) that is a result of differences in production location and processing specifications, a study was conducted to determine the fluctuations in apparent digestibility coefficients of differently sourced SBM fed to Pacific white shrimps (*Litopenaeus vannamei*). Twenty-four SBM-based diets were formulated by mixing a basal diet and test ingredients on a dry matter basis (70:30 ratio), while 1% chromic oxide was used as the inert marker. The digestibility trial was carried out in a semi-closed recirculation system with six replicate groups per treatment (mean shrimp weight of 10.2 g). Significant differences were observed for apparent dry matter, energy and protein digestibility coefficients ($p < .05$ was considered significant) among 24 sources of SBM and digestibility values ranged from 45% to 90%, 56% to 93% and 87% to 98%, respectively. Based on multivariate analysis, acid detergent fibre, neutral detergent fibre, lignin, raffinose and trypsin inhibitor were screened as the key chemical characteristics in SBM that influenced digestibility of nutrients in Pacific white shrimps. Variations in growth performances of shrimp were in line with the variations in apparent digestibility coefficients of SBM verifying the importance of digestibility data in shrimp feed formulations.

KEYWORDS
digestibility, growth, *Litopenaeus vannamei*, nutritional quality, soybean meal

1 | INTRODUCTION

World aquaculture feed production has been calculated to be between 50 and 60 million metric tons (MMT) and is expected to grow further in response to expansion of the industry. Historically, fishmeal has been the primary protein source used in aquaculture feed formulations consuming approximately 68% of fish meal production in world (Tacon & Metian, 2015) mainly due to its excellent amino acids profile, palatability and digestibility (Mallison, 2013; Tacon, Metian, & Hasan, 2009). However, average dietary inclusion levels of fishmeal have been steadily declining (from around 28% to 7%), because of static supply, higher cost and increased global use of alternative cheaper plant protein sources (Davis, Roy, & Sookying, 2008; Tacon & Metian, 2008). Among the wide variety of plant-based protein sources, solvent-extracted soybean meal (SBM) received the most attention (Amaya, Davis, & Rouse, 2007a, 2007b) mainly considering the comparable amino acid profile, worldwide availability, low price and consistent composition (Amaya et al., 2007a, 2007b; Davis & Arnold, 2000; Dersjant-Li, 2002; Gatlin et al., 2007; Swick, Akiyama, Boonyaratpalin, & Creswell, 1995). Based on industry estimates, average dietary inclusion levels of SBM have reached up to 30% (while fishmeal average only 9%) making it the dominant protein source in aquaculture feeds.

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Nutritional quality of SBM is influenced by production location attributed to its geographical features such as latitude, soil type and environmental conditions such as temperature, and the amount of precipitation (Maestri et al., 1998; Natarajan et al., 2016; Palmer, Hymowitz, & Nelson, 1996; van Kempen et al., 2002; Verma & Shoemaker, 1996). Furthermore, differences in processing methods and processing conditions such as temperature, time and moisture content also add variation to the final product quality (Balloun, 1980; van Kempen et al., 2002). One method of estimating nutrient availability of an ingredient/food is to determine apparent digestibility coefficients, which are primarily influenced by its chemical composition and the digestive characteristics of the species (Brunson, Romaire, & Reigh, 1997). However, most digestibility studies have been conducted to evaluate differences in digestibility parameters among ingredients rather than determining reasons for variability within different sources of the same ingredient. In most cases, the observed effects have been attributed to one chemical variable which is prominent in the particular ingredient used during the study without considering the effect of other chemical variables or interactions among them.

Pacific white shrimp, *Litopenaeus vannamei*, continues to be an important species in aquaculture accounting for 80% farmed shrimp production in the world (Li & Xiang, 2013; Panini et al., 2017). Shrimps were estimated to be the third largest consumer (6.18 million tonnes) of manufactured aquaculture feeds in 2015 (Tacon & Metian, 2015) while moved up to second in 2017 consuming 15% of total global aquaculture feed production (Alltech, 2018). Although Pacific white shrimp is one of the largest consumers of SBM, information explaining the association between growth/digestibility and its complete chemical variable matrix are yet to be discovered. With the objective of filling these research gaps, the current study investigated variations in digestibility of energy, dry matter and amino acids in SBM sourced from different geographical locations in the world when fed to Pacific white shrimps (*L. vannamei*). An effort was also made to identify the major chemical variables in SBM that are responsible for possible differences among sources in energy and nutrient digestibility.

2 | MATERIALS AND METHODS

2.1 | Experimental diets

Twenty-four sources of solvent-extracted SBM along with data for proximate composition, indispensable and dispensable amino acid profiles, sugars (fructose, sucrose, raffinose, stachyose, etc.), fibres (acid detergent fibre [ADF], neutral detergent fibre [NDF] and lignin), macro- and microminerals for each source were obtained from the Monogastric Nutrition Laboratory, Division of Nutritional Sciences, University of Illinois at Urban-Champaign, USA (Lagos & Stein, 2017). All soybean-based digestibility diets were formulated by mixing the basal diet and test ingredients on a dry matter basis using a 70:30 ratio, while 10 g/kg chromic oxide was used as the inert marker (Tables 1 and 2). Test diets were prepared in the feed laboratory at Auburn University, Auburn, AL, USA, using standard practices. Briefly, pre-ground dry ingredients and oil were weighted and mixed in a food mixer (Hobart Corporation)

TABLE 1 Codes for different soybean meal (SBM) used during the digestibility experiment

Diet	Ingredient code	Diet	Ingredient code
Basal	Local SBM[a]	13	45543
1	45531	14	45544
2	45532	15	45545
3	45533	16	45546
4	45534	17	45547
5	45535	18	45548
6	45536	19	45549
7	45537	20	45550
8	45538	21	45551
9	45539	22	45552
10	45540	23	45553
11	45541	24	45554
12	45542		

[a] De-hulled solvent-extracted soybean meal, Bunge Limited, Decatur, AL, USA.

TABLE 2 Composition of basal diet used in digestibility trial

Ingredient	g/kg as is
Soybean meal[a]	325.0
Fish meal[b]	100.0
Menhaden fish oil[b]	32.0
Corn Starch[c]	21.0
Whole wheat[d]	476.0
Mineral premix[e]	5.0
Vitamin premix[f]	18.0
Choline chloride[g]	2.0
Stay-C 35% active[h]	1.0
Lecithin[i]	10.0
Chromic oxide[h]	10.0

[a] De-hulled solvent-extracted soybean meal, Bunge Limited, Decatur, AL, USA.

[b] Omega Protein, Houston, TX, USA.

[c] MP Biomedicals, Solon, OH, USA.

[d] Bob's red mill, Milwaukie, OR, USA.

[e] Trace mineral premix (g/100 g premix): cobalt chloride, 0.004; cupric sulphate pentahydrate, 0.550; ferrous sulphate, 2.000; magnesium sulphate anhydrous, 13.862; manganese sulphate monohydrate, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulphate heptahydrate, 13.193; alpha cellulose, 69.664.

[f] Vitamin premix (g/kg premix): thiamine HCl, 4.95; riboflavin, 3.83; pyridoxine HCl, 4.00; Ca-Pantothenate, 10.00; nicotinic acid, 10.00; biotin, 0.50; folic acid, 4.00; cyanocobalamin, 0.05; inositol, 25.00; vitamin A acetate (500,000 IU/g), 0.32; vitamin D3 (1,000,000 IU/g), 80.00; menadione, 0.50; alpha cellulose, 856.81.

[g] VWR Amresco, Suwanee, GA, USA.

[h] Stay-C® (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins, Parsippany, NJ, USA.

[i] The Solae Company, St. Louis, MO, USA.

TABLE 3 Chemical analyses[a] (proximate composition and pepsin digestibility) of different digestibility diets formulated using 70:30 replacement technique

Composition	Crude protein	Moisture	Crude fat	Crude fibre	Ash	Pepsin digestibility
Diet 1	34.2	6.1	5.2	4.1	6.1	92.3
Diet 2	34.9	5.8	5.7	4.3	6.1	93.6
Diet 3	34.5	6.7	5.2	4.2	6.1	93.6
Diet 4	34.3	8.5	4.2	4.1	6.0	92.7
Diet 5	34.2	8.2	4.1	4.0	6.0	92.2
Diet 6	34.3	8.2	3.9	3.8	6.2	93.8
Diet 7	34.3	8.3	4.2	3.8	6.1	93.9
Diet 8	34.7	8.0	4.7	3.6	6.2	93.5
Diet 9	34.5	9.5	4.9	3.5	6.1	94.0
Diet 10	33.4	11.4	5.5	3.6	5.9	93.6
Diet 11	36.3	5.7	6.0	4.2	6.3	93.9
Diet 12	35.5	6.9	4.6	4.3	6.2	93.3
Diet 13	35.6	8.7	3.9	3.7	6.1	94.2
Diet 14	35.3	8.8	4.3	3.5	6.1	93.6
Diet 15	35.4	8.9	4.3	3.6	6.0	94.2
Diet 16	34.9	8.1	4.3	3.6	6.1	93.9
Diet 17	33.7	10.9	3.7	3.5	5.9	93.9
Diet 18	35.2	8.4	4.1	3.5	6.1	92.8
Diet 19	34.7	8.3	3.9	3.7	6.4	93.5
Diet 20	35.4	5.8	4.5	4.0	6.7	91.4
Diet 21	35.0	7.4	3.7	5.0	6.9	91.4
Diet 22	36.2	6.1	5.4	4.6	6.5	92.2
Diet 23	35.3	9.7	4.5	4.0	6.0	92.7
Diet 24	35.7	7.6	4.1	4.2	6.2	92.2

[a] Diets were analysed at the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA). Results are expressed on an 'as is' basis unless otherwise indicated.

for 15 min. Hot water (~30% by weight) was then blended into the mixture to attain a consistency appropriate for pelleting. Finally, all diets were pressure-pelleted using a meat grinder with a 3-mm die, dried in a forced air oven (50°C) to a moisture content of less than 10% and stored at 4°C. All diets were analysed for proximate composition, amino acid profile and pepsin digestibility at the University of Missouri Agricultural Experiment Station Chemical Laboratories, whereas chromium and energy were determined in house (Tables 3 and 4).

2.2 | Digestibility trial

The digestibility trial was carried out in a semi-closed recirculation system which was consisted of 36 aquaria (135 L, 0.52 × 0.52 × 0.48 m) connected to a common reservoir tank (800-L), vertical fluidized bed biological filter (600-L volume with 200-L of Kaldnes media), Aquadyne bead filter (0.2 m^2 media, 0.6 m × 1.1 m) and 0.25-hp recirculation pump. Mean water flow for an aquarium was 3 L/min with an average turnover of 20 min/tank. Saltwater used during the study was prepared by mixing artificial crystal sea salt (Crystal Sea Marinemix) with freshwater and maintained at around 6ppt during the digestibility trial.

The experiment was conducted in compliance with the Auburn University animal care policy. Eight Pacific white shrimp (mean individual weight of 10.2 g) were stocked per aquaria with six replicate groups per treatment. Shrimp were offered each diet, and the faeces from every two tanks were pooled into three replicate samples. Animals were allowed to acclimate to each experimental digestibility diet for at least 3 days before the faecal collection was initiated and given a resting period of 2 days with commercial shrimp diet (35% crude protein and 8% crude fat; Zeigler Bros) between two sets of digestibility diets. Animals were fed four times per day in slight excess, and all faecal samples were collected one hour after each feeding. All the uneaten diets were siphoned-out from each tank following the collection of faecal samples, to avoid possible ingestion of leached materials. Faeces were collected for 2–3 days period or until adequate samples were obtained. Each day, the first collection was discarded, and the samples from subsequent three collections were rinsed with distilled water, oven-dried (90°C) until a constant weight was obtained and stored in freezer at −20°C for further analysis.

Dry matter was determined by placing representative portions of each sample in an oven at 105°C until constant weight

TABLE 4 Amino acid (AA) profile[a] (as is basis) of different digestibility diets formulated using 70:30 replacement technique

Diet	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Alanine	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.4	1.6	1.5	1.6	1.5	1.5	1.5	1.4	1.5	1.5	1.5	1.5	1.5	1.5	1.6
Arginine	2.2	2.3	2.3	2.2	2.2	2.2	2.2	2.2	2.2	2.1	2.3	2.2	2.3	2.3	2.3	2.2	2.1	2.3	2.2	2.3	2.2	2.3	2.3	2.4
Aspartic acid	3.2	3.3	3.3	3.2	3.2	3.2	3.3	3.3	3.3	3.2	3.5	3.3	3.4	3.4	3.5	3.3	3.2	3.4	3.3	3.4	3.4	3.4	3.4	3.5
Cysteine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Glutamic acid	6.4	6.4	6.4	6.3	6.3	6.4	6.4	6.4	6.4	6.2	6.7	6.5	6.6	6.6	6.6	6.5	6.2	6.6	6.5	6.6	6.6	6.7	6.6	6.7
Glycine	1.6	1.6	1.6	1.5	1.5	1.6	1.6	1.6	1.5	1.5	1.7	1.6	1.6	1.6	1.7	1.6	1.5	1.7	1.6	1.6	1.7	1.6	1.6	1.7
Histidine	0.8	0.9	0.8	0.8	0.8	0.9	0.9	0.9	0.9	0.8	0.9	0.9	0.9	0.9	0.9	0.9	0.8	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Hydroxylysine	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Hydroxyproline	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Isoleucine	1.5	1.6	1.5	1.5	1.5	1.6	1.6	1.6	1.5	1.5	1.7	1.6	1.6	1.6	1.7	1.6	1.5	1.6	1.6	1.6	1.6	1.6	1.6	1.7
Leucine	2.5	2.5	2.5	2.5	2.4	2.5	2.5	2.5	2.5	2.4	2.7	2.6	2.6	2.6	2.6	2.6	2.5	2.6	2.5	2.6	2.6	2.6	2.6	2.6
Lysine	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	1.9	2.1	2.0	2.1	2.1	2.1	2.0	2.0	2.1	2.0	2.1	2.0	2.1	2.1	2.1
Methionine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Ornithine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Phenylalanine	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.6	1.8	1.8	1.8	1.8	1.8	1.7	1.7	1.7	1.7	1.7	1.7	1.8	1.7	1.8
Proline	2.0	2.0	2.1	2.0	2.0	2.0	2.0	2.1	2.0	1.9	2.2	2.1	2.1	2.0	2.1	2.1	2.0	2.1	2.1	2.1	2.1	2.2	2.2	2.1
Serine	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.3	1.5	1.4	1.5	1.4	1.4	1.4	1.4	1.5	1.4	1.4	1.4	1.5	1.5	1.4
Taurine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Threonine	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.3	1.2	1.3	1.2	1.2	1.2	1.2	1.3	1.2	1.2	1.2	1.3	1.3	1.3
Tryptophan	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.5	0.5	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Tyrosine	1.1	1.2	1.1	1.1	1.1	1.1	1.1	1.2	1.1	1.1	1.2	1.1	1.2	1.1	1.2	1.2	1.1	1.2	1.1	1.1	1.1	1.2	1.2	1.2
Valine	1.6	1.7	1.7	1.6	1.6	1.7	1.7	1.7	1.6	1.6	1.8	1.7	1.7	1.7	1.8	1.7	1.6	1.7	1.7	1.7	1.7	1.7	1.7	1.8
Total AA	32.6	33.1	32.9	32.5	32.3	32.7	32.7	33.2	32.6	31.8	34.7	33.3	34.3	33.6	34.1	33.2	32.1	34.0	33.3	33.7	33.6	34.1	33.8	34.6

[a] Analyses conducted by Agricultural Experiment Station Chemical Laboratories, University of Missouri, Columbia, Missouri, USA.

was obtained. Gross energy of diets and faecal samples was analysed with a semi micro-bomb calorimeter (Model 1425, Parr Instrument). Chromic oxide was determined as per the method described by McGinnis and Kasting (1964) in which, after a colorimetric reaction, absorbance was read on a spectrophotometer (Spectronic Genesys 5, Milton Roy) at 540 nm. Protein was determined by summing all dispensable and indispensable amino acids. The apparent digestibility coefficients for dry matter (ADMD) protein (APD) and energy (AED) of diets (D) were calculated according to Cho, Slinger, and Bayley (1982) as follows:

$$\mathrm{ADMD_D}\,(\%) = 100 - \left[100 \times \left(\frac{\%\ \mathrm{Cr_2O_3\ in\ feed}}{\%\ \mathrm{Cr_2O_3\ in\ faeces}}\right)\right]$$

$$\mathrm{APD_D\ and\ AED_D}\,(\%) = 100 - \left[100 \times \left(\frac{\%\ \mathrm{Cr_2O_3\ in\ feed}}{\%\ \mathrm{Cr_2O_3\ in\ faeces}} \times \frac{\%\ \mathrm{nutrients\ in\ faeces}}{\%\ \mathrm{nutrient\ in\ feeds}}\right)\right]$$

The apparent digestibility coefficients of dry matter ($\mathrm{ADMD_I}$), protein ($\mathrm{APD_I}$) and energy ($\mathrm{AED_I}$) of the test ingredients (I) were calculated according to Bureau and Hua (2006) as follows:

$$\mathrm{ADMD_I} = \mathrm{ADMD_D} + [(\mathrm{ADMD_D} - \mathrm{ADMD_{Dref}}) \times (0.7 \times D_{\mathrm{ref}}/0.3 \times D_{\mathrm{ingr}})]$$

$$\mathrm{ADMD_I} = \mathrm{ADMD_D} + [(\mathrm{ADMD_D} - \mathrm{ADMD_{Dref}}) \times (0.7 \times D_{\mathrm{ref}}/0.3 \times D_{\mathrm{ingr}})]$$

$$\mathrm{AED_I} = \mathrm{AED_D} + [(\mathrm{AED_D} - \mathrm{AED_{Dref}}) \times (0.7 \times D_{\mathrm{ref}}/0.3 \times D_{\mathrm{ingr}})\ ]$$

$$D_{\mathrm{ref}} = \%\ \mathrm{nutrient\ (or\ KJ/g\ gross\ energy)\ of\ basal\ diet\ (dry\ weight)}$$

$$D_{\mathrm{ingr}} = \%\ \mathrm{nutrients\ (or\ KJ/g\ gross\ energy)\ of\ test\ ingredient\ (dry\ weight)}$$

2.3 | Water quality monitoring

Dissolved oxygen (DO) was maintained near saturation using air stones in each culture tank and the sump tank using a common airline connected to a regenerative blower. Dissolved oxygen, salinity and water temperature in the sump tank were measured twice daily using a YSI-55 digital oxygen/temperature meter (YSI corporation). Total ammonia-N (TAN) and nitrite-N were measured twice per week according to the methods described by Solorzano (1969) and Spotte (1979), respectively. Water pH was measured twice weekly during the experimental period using the pHTestr30 (Oakton Instrument). During the growth trial, DO, temperature, salinity, pH, TAN and nitrite-N were maintained within acceptable ranges for *L. vannamei* at 6.4 ± 0.5 mg/L, 29.1 ± 0.9°C, 7.7 ± 0.4 ppt, 7.6 ± 0.5, 0.13 ± 0.05 mg/L and 0.15 ± 0.22 mg/L, respectively.

2.4 | Statistical analysis

All data were analysed using the statistical software packages of SAS (V9.3. SAS Institute) and R (R i386 3.5.1) where one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests was conducted using SAS while rest of statistical tests were conducted in R. Apparent digestibility coefficients were subjected to ANOVA followed by Tukey's multiple comparison test to evaluate significant differences among treatment means ($p < .05$). A principle component analysis (PCA) was used to explain the variability in digestibility data from the chemical characteristics of each SBM source. For PCA, entire chemical variable matrix of SBM was standardized by calculating *z* scores (*z* score or standard score = difference from mean/*SD*) to avoid different units and scales of measurements with the objective of placing them in an equal plain to compare variations. Furthermore, ingredient data for SBM were adjusted based on the inclusion ratio in the digestibility diets, since they were formulated on a dry matter basis and some of the variables such as protein and amino acids were excluded from the analysis considering their negligible variations in test diets assuming a neutral effect between treatments. Following the PCA, a multiple linear regression analysis was performed to identify the relationships between digestibility parameters ($\mathrm{ADMD_I}$, $\mathrm{AED_I}$ and $\mathrm{APD_I}$) and scores of each principle component of PCA. Based on regression outcomes, certain chemical variables were identified, which had major representation in principle components of interest due to their significant association with apparent digestibility coefficients. The identified chemical variables were subjected to liner regression analysis with apparent digestibility coefficients to identify their isolated individual effect on digestibility. Linear regression analyses were performed to determine the relationship between apparent digestibility coefficients and growth parameters of shrimp (thermal growth coefficient/TGC), while cluster analysis was used to identify the grouping patterns of SBM sources based on apparent digestibility coefficients and chemical characteristics.

3 | RESULTS

Significant differences were observed for apparent dry matter, protein and energy digestibility coefficients ($p < .05$) of test diets and ingredients used during the study (Table 5). Apparent dry matter digestibility ($\mathrm{ADMD_I}$) in SBM ranged from 45% to 90%, while apparent energy digestibility ($\mathrm{AED_I}$) and protein digestibility ($\mathrm{APD_I}$) values ranged from 56% to 93% and 87% to 98%, respectively. In general, SBM45531 (diet 1), SBM45536 (diet 6), SBM45541 (diet 11) and SBM45553 (diet 23) showed higher apparent digestibility of dry matter, energy and protein compared with SBM45542 (diet 12), SBM45544 (diet 14), SBM45546 (diet 16), SBM4550 (diet 20) and SBM4551 (diet 21). Apparent digestibility coefficients of individual and total amino acids in the 24 sources of SBM used in the study are presented in Table 6. In general, apparent digestibility coefficients of all individual amino acids followed the same trend as the protein and total amino acid digestibility with significant differences ($p < .05$) among sources of SBM.

Percentage variation in chemical characteristics of SBM explained by different principle components (PC) from PCA and respective loading values are presented in Tables 7 and 8. According to PCA, PC-1 explained the highest variation in SBM variable

TABLE 5 Apparent digestibility coefficients of dry matter (ADMD), protein (APD), energy (AED) of the diet (D) and ingredient (I) using 70:30 replacement technique offered to Pacific white shrimp, *Litopenaeus vannamei*

	$ADMD_D$	AED_D	APD_D	$ADMD_I$	AED_I	APD_I
Basal	78.52 ± 0.7^{abc}	83.78 ± 0.8^{abcd}	91.90 ± 0.6^{bcdefg}			
Diet 1	80.54 ± 0.1^{ab}	85.36 ± 0.3^{ab}	94.10 ± 0.4^{ab}	85.25 ± 0.4^{ab}	88.60 ± 1.0^{ab}	96.86 ± 0.9^{ab}
Diet 2	75.95 ± 0.8^{bcdefg}	81.92 ± 0.9^{abcdef}	92.50 ± 0.3^{abcdef}	69.95 ± 2.5^{abcde}	78.13 ± 2.6^{abcde}	93.24 ± 0.6^{abcdef}
Diet 3	77.85 ± 1.3^{abcd}	83.17 ± 1.0^{abcde}	93.42 ± 0.5^{abcd}	76.26 ± 4.3^{abc}	81.92 ± 3.0^{abcd}	95.32 ± 1.8^{abcd}
Diet 4	77.31 ± 1.6^{abcde}	81.98 ± 0.9^{abcdef}	92.88 ± 1.0^{abcde}	74.48 ± 5.4^{abcd}	78.31 ± 2.9^{abcde}	94.11 ± 2.1^{abcd}
Diet 5	75.41 ± 1.4^{bcdefg}	81.28 ± 1.6^{bcdef}	91.96 ± 0.8^{bcdefg}	68.13 ± 4.7^{bcdef}	76.17 ± 4.8^{bcde}	92.04 ± 1.7^{bcdefg}
Diet 6	80.83 ± 0.6^{ab}	85.39 ± 0.8^{ab}	93.78 ± 0.5^{abc}	86.21 ± 2.0^{ab}	88.68 ± 2.4^{ab}	96.13 ± 1.1^{abc}
Diet 7	77.05 ± 1.9^{abcdef}	82.35 ± 1.5^{abcdef}	92.57 ± 0.5^{abcdef}	73.60 ± 6.4^{abcd}	79.44 ± 4.6^{abcde}	93.40 ± 1.2^{abcdef}
Diet 8	71.79 ± 2.0^{efghi}	78.41 ± 1.7^{efgh}	89.71 ± 0.7^{g}	56.07 ± 6.7^{cdefg}	67.43 ± 5.2^{defg}	86.97 ± 1.6^{g}
Diet 9	75.26 ± 1.0^{bcdefgh}	81.60 ± 1.3^{abcdef}	92.26 ± 0.5^{abcdefg}	67.63 ± 3.2^{bcdefg}	77.15 ± 3.9^{abcde}	92.70 ± 1.1^{abcdefg}
Diet 10	75.87 ± 2.6^{bcdefg}	81.82 ± 1.8^{abcdef}	92.59 ± 1.0^{abcdef}	69.67 ± 8.7^{bcde}	77.82 ± 5.4^{abcde}	93.45 ± 2.2^{abcdef}
Diet 11	82.01 ± 1.0^{a}	86.69 ± 1.1^{a}	94.83 ± 0.1^{a}	90.14 ± 3.4^{a}	92.64 ± 3.5^{a}	98.48 ± 0.3^{a}
Diet 12	70.70 ± 0.2^{ghi}	77.68 ± 0.5^{fgh}	91.29 ± 0.2^{cdefg}	52.45 ± 0.5^{efg}	65.19 ± 1.6^{efg}	90.53 ± 0.5^{cdefg}
Diet 13	72.06 ± 2.6^{defghi}	78.90 ± 2.7^{defgh}	91.37 ± 0.7^{cdefg}	56.97 ± 8.6^{cdefg}	68.92 ± 8.3^{defg}	90.70 ± 1.5^{cdefg}
Diet 14	69.61 ± 4.1^{hi}	74.91 ± 4.1^{gh}	90.89 ± 1.6^{defg}	48.81 ± 13.6^{fg}	56.77 ± 12.6^{fg}	89.61 ± 3.6^{defg}
Diet 15	72.87 ± 1.1^{cdefghi}	79.09 ± 0.4^{defgh}	90.32 ± 0.7^{efg}	59.68 ± 3.8^{cdefg}	69.52 ± 1.3^{defg}	88.34 ± 1.5^{efg}
Diet 16	68.53 ± 3.6^{i}	74.53 ± 3.1^{h}	90.11 ± 1.2^{fg}	45.22 ± 12.1^{g}	55.63 ± 9.4^{g}	87.86 ± 2.7^{fg}
Diet 17	76.69 ± 2.1^{abcdefg}	81.95 ± 1.8^{abcdef}	92.67 ± 1.0^{abcdef}	72.41 ± 7.2^{abcde}	78.20 ± 4.2^{abcde}	93.64 ± 2.2^{abcdef}
Diet 18	74.39 ± 2.4^{cdefghi}	79.79 ± 1.6^{defgh}	91.32 ± 1.1^{cdefg}	64.73 ± 8.1^{cdefg}	71.64 ± 4.9^{cdefg}	90.58 ± 2.5^{cdefg}
Diet 19	73.42 ± 2.4^{cedfghi}	80.03 ± 1.8^{cdefg}	91.57 ± 1.8^{bcdefg}	61.51 ± 8.0^{cdefg}	72.38 ± 5.6^{bcdef}	91.14 ± 2.6^{bcdefg}
Diet 20	71.28 ± 0.7^{fghi}	77.77 ± 0.8^{fgh}	90.72 ± 0.4^{efg}	54.38 ± 2.4^{defg}	65.48 ± 2.3^{efg}	89.24 ± 0.9^{efg}
Diet 21	71.40 ± 2.8^{efghi}	78.27 ± 2.8^{efgh}	89.79 ± 1.3^{g}	54.76 ± 9.2^{defg}	66.99 ± 8.6^{defg}	87.13 ± 2.9^{g}
Diet 22	73.21 ± 1.6^{cdefghi}	80.51 ± 0.8^{bcdef}	91.33 ± 0.8^{cdefg}	60.81 ± 5.3^{cdefg}	73.82 ± 2.4^{bcde}	90.61 ± 1.7^{cdefg}
Diet 23	81.12 ± 0.7^{ab}	85.10 ± 0.8^{abc}	93.40 ± 1.2^{abcd}	87.17 ± 2.3^{ab}	87.81 ± 2.4^{abc}	95.26 ± 2.6^{abcd}
Diet 24	74.20 ± 1.0^{cdefghi}	78.69 ± 0.6^{defgh}	92.03 ± 0.3^{bcdefg}	64.09 ± 3.4^{cdefg}	68.29 ± 1.8^{defg}	92.18 ± 0.7^{bcdefg}

Note: See Table 1 for ingredient source in each diet.

Values from each diet/ingredient are means and *SD* of triplicate tanks. Values within column with different superscripts are significantly different ($p < .05$) based on one-way ANOVA followed by Tukey's multiple comparison test.

matrix, which is only 30%, while PC-2 and PC-3 explained 23% and 14% of sample variance, respectively. Multiple linear regression carried out among the scores of each PC and apparent digestibility coefficients yielded statistically significant impact of PC6 (<.05) on apparent digestibility coefficients, while strong association was observed between PC18, PC10, PC1 and apparent digestibility coefficients in SBM (Table 9). Based on the loading values, ADF, NDF, lignin, raffinose and trypsin inhibitor levels were identified as most influential chemical characteristics for SBM digestibility in Pacific white shrimps due to their higher representation in principle components. The cluster analysis carried out based on the chemical variable matrix of SBM segregated them in seven major groups (Figure 1). Verifying PCA outcomes, positive associations were observed between fibres: ADF ($\beta = 0.09$, $p = .38$, $r^2 = .04$), NDF ($\beta = 0.10$, $p = .45$, $r^2 = .03$) and lignin ($\beta = 0.02$, $p = .21$, $r^2 = .07$) and apparent digestibility coefficients, while negative effects on apparent digestibility were detected with raffinose ($\beta = -0.03$, $p = .18$, $r^2 = .08$) and trypsin inhibitor ($\beta = -0.05$, $p = .49$, $r^2 = .02$). However, these associations were not statistically significant at individual levels and might be due to the effect of swamping or interactions between several chemical variables.

Three major groups in SBM were identified (84% representation) using the scree pot of cluster analysis based on the apparent digestibility coefficients of diets and ingredients (Figure 2). Although it is not statistically significant (>.05), a strong positive association was observed between apparent digestibility coefficients and growth performances of Pacific white shrimp (Table 10), which was determined in a separate growth study using the same set of SBM (Galkanda Arachchige, Qiu, Stein, & Davis, 2019).

4 | DISCUSSION

Ingredient characterization and digestibility are two key strategies to determine the potential quality of any ingredient in aquaculture feed. Chemical composition and variability resulting from its place of origin and processing specifications is the first part of this evaluation, while the estimation of energy and nutrient availability in

TABLE 6 Apparent amino acid (AA) digestibility for the ingredient (I) using 70:30 replacement technique offered to Pacific white shrimp, *Litopenaeus vannamei*

SBM	Alanine	Arginine	Aspartic acid	Cysteine	Glutamic acid	Glycine	Histidine	Isoleucine	Leucine	Lysine
45531	95.3 ± 2.1^{ab}	97.5 ± 0.9^{ab}	96.6 ± 0.9^{ab}	89.1 ± 0.8^{abc}	97.9 ± 0.9^{ab}	93.9 ± 2.7^{ab}	96.3 ± 1.6^{ab}	96.5 ± 0.7^{ab}	96.0 ± 0.9^{a}	96.4 ± 0.7^{abc}
45532	87.7 ± 2.3^{bcdefg}	95.4 ± 0.9^{abcd}	93.1 ± 0.5^{abcdefg}	81.6 ± 1.9^{bcde}	95.2 ± 0.4^{abcd}	82.7 ± 2.1^{bcdef}	91.7 ± 0.9^{bcdef}	93.4 ± 0.8^{abcde}	92.0 ± 0.8^{abcde}	93.6 ± 0.2^{abcdef}
45533	92.6 ± 1.3^{abcd}	96.5 ± 1.1^{abcd}	95.2 ± 1.2^{abcd}	84.1 ± 2.6^{bcd}	96.7 ± 1.2^{abc}	90.2 ± 1.4^{abc}	93.7 ± 0.3^{abcd}	95.2 ± 1.2^{abcd}	94.2 ± 1.2^{ab}	94.9 ± 1.4^{abcde}
45534	91.5 ± 3.4^{abcde}	95.3 ± 1.8^{abcde}	93.9 ± 2.3^{abcdef}	82.4 ± 4.2^{bcde}	95.3 ± 2.3^{abcd}	89.9 ± 4.6^{abcd}	93.3 ± 2.3^{abcde}	93.5 ± 2.2^{abcde}	92.7 ± 2.5^{abcd}	93.5 ± 2.3^{abcdef}
45535	87.4 ± 3.4^{bcdefg}	93.2 ± 1.4^{bcdefgh}	91.9 ± 1.7^{bcdefghi}	79.7 ± 2.1^{cde}	93.4 ± 1.7^{abcde}	84.1 ± 5.3bc^{def}	91.0 ± 2.8^{bcdef}	91.8 ± 1.5^{abcdefg}	90.4 ± 1.9^{abcdefg}	90.9 ± 1.8^{bcdefg}
45536	95.0 ± 1.7^{ab}	96.8 ± 1.1^{abc}	95.9 ± 1.3^{abc}	88.8 ± 1.9^{abc}	96.7 ± 1.4^{abc}	94.1 ± 2.3^{ab}	96.0 ± 1.3^{ab}	95.6 ± 0.7^{abc}	95.1 ± 1.2^{ab}	96.7 ± 1.3^{ab}
45537	89.5 ± 1.8^{abcdefg}	94.8 ± 1.1^{abcdef}	93.1 ± 1.2^{abcdefg}	83.0 ± 1.3^{bcde}	94.7 ± 1.3^{abcd}	87.3 ± 2.6^{abcde}	93.3 ± 1.1^{abcde}	93.2 ± 1.4^{abcde}	91.7 ± 1.5^{abcdef}	94.3 ± 1.2^{abcde}
45538	79.5 ± 2.1^{fg}	89.2 ± 1.5^{gh}	86.4 ± 1.6^{i}	73.5 ± 2.6^{e}	88.6 ± 1.9^{ef}	75.4 ± 2.0^{ef}	86.6 ± 1.6^{f}	85.8 ± 1.7^{g}	84.3 ± 2.0^{g}	87.3 ± 1.9^{g}
45539	87.9 ± 1.7^{bcdefg}	93.9 ± 0.8^{abcdefg}	92.7 ± 1.1^{abcdefg}	82.8 ± 2.2^{bcde}	94.3 ± 1.1^{abcd}	84.1 ± 1.5^{bcdef}	92.4 ± 1.5^{abcdef}	91.9 ± 1.6^{abcdef}	90.7 ± 1.8^{abcdefg}	93.3 ± 1.5^{abcdefg}
45540	89.7 ± 4.0^{abcdef}	95.0 ± 2.0^{abcdef}	93.4 ± 2.2^{abcdefg}	81.3 ± 3.4^{bcde}	95.2 ± 2.2^{abcd}	87.2 ± 5.6^{abcde}	93.9 ± 2.7^{abc}	92.8 ± 2.1^{abcde}	91.4 ± 2.5^{abcdef}	93.7 ± 2.5^{abcdef}
45541	98.4 ± 0.7^{a}	98.6 ± 0.1^{a}	98.0 ± 0.1^{a}	94.9 ± 0.2^{a}	98.4 ± 0.2^{a}	98.7 ± 0.9^{a}	98.6 ± 0.2^{a}	97.6 ± 0.4^{a}	97.3 ± 0.5^{a}	98.1 ± 0.3^{a}
45542	84.2 ± 0.2^{cdefg}	92.7 ± 0.4^{bcdefgh}	91.0 ± 0.6^{bcdefghi}	80.8 ± 1.1^{bcde}	92.5 ± 0.8^{bcdef}	76.4 ± 2.4^{def}	90.1 ± 0.4^{bcdef}	90.6 ± 0.8^{bcdefg}	88.9 ± 0.8^{bcdefg}	90.5 ± 2.2^{cdefg}
45543	85.9 ± 2.9^{bcdefg}	92.6 ± 1.1^{bcdefgh}	90.9 ± 1.1^{cdefghi}	81.9 ± 2.5^{bcde}	92.0 ± 0.8^{cdef}	82.2 ± 4.2^{bcdef}	90.3 ± 2.5^{bcdef}	90.2 ± 1.6^{cdefg}	88.7 ± 1.6^{bcdefg}	91.3 ± 1.3^{bcdefg}
45544	82.6 ± 7.0^{defg}	91.7 ± 2.9^{defgh}	90.3 ± 3.4^{cdefghi}	80.1 ± 5.9^{cde}	91.7 ± 3.1^{cdef}	78.3 ± 8.8^{cdef}	89.4 ± 3.3^{cdef}	89.4 ± 3.8^{defg}	87.2 ± 4.1^{cdefg}	90.0 ± 3.5^{defg}
45545	81.7 ± 2.6^{efg}	90.1 ± 1.5^{fgh}	88.6 ± 1.5^{fghi}	77.7 ± 1.0^{de}	89.8 ± 1.4^{def}	78.3 ± 3.0^{cdef}	87.1 ± 2.1^{ef}	88.5 ± 1.8^{efg}	86.4 ± 1.8^{defg}	88.9 ± 1.8^{efg}
45546	79.2 ± 4.8^{g}	90.4 ± 2.0^{efgh}	88.1 ± 2.4^{ghi}	75.2 ± 4.2^{de}	90.2 ± 2.3^{def}	72.3 ± 7.2^{f}	87.0 ± 2.8^{ef}	88.0 ± 2.2^{efg}	85.5 ± 2.7^{efg}	89.0 ± 3.0^{efg}
45547	90.8 ± 3.8^{abcde}	94.7 ± 2.1^{abcdef}	93.4 ± 2.3^{abcdefg}	82.6 ± 4.4^{bcde}	94.3 ± 2.3^{abcd}	90.1 ± 5.3^{abc}	93.0 ± 2.6^{abcdef}	93.2 ± 2.3^{abcde}	91.8 ± 2.7^{abcdef}	94.2 ± 2.3^{abcde}
45548	86.4 ± 4.7^{bcdefg}	92.3 ± 1.9^{cdefgh}	90.0 ± 2.4^{defghi}	78.3 ± 5.3^{de}	91.4 ± 1.8^{cdef}	85.6 ± 4.9^{abcdef}	90.3 ± 2.5^{bcdef}	89.7 ± 2.9^{cdefg}	88.2 ± 3.2^{bcdefg}	92.0 ± 2.1^{bcdefg}
45549	86.1 ± 4.8^{bcdefg}	93.0 ± 2.3^{bcdefgh}	90.9 ± 2.4^{cdefghi}	80.8 ± 4.4^{bcde}	92.2 ± 2.3^{cdef}	83.2 ± 6.8^{bcdef}	90.5 ± 2.7^{bcdef}	90.5 ± 2.4^{bcdefg}	88.9 ± 3.2^{bcdefg}	92.9 ± 2.2^{abcdefg}
45550	82.8 ± 2.2^{defg}	91.5 ± 0.9^{defgh}	89.1 ± 0.8^{efghi}	80.0 ± 1.6^{cde}	90.6 ± 0.9^{def}	79.6 ± 3.4^{cdef}	88.9 ± 1.0^{cdef}	88.8 ± 0.9^{efg}	87.0 ± 0.8^{cdefg}	91.7 ± 0.8^{bcdefg}
45551	81.4 ± 4.6^{efg}	88.7 ± 2.5^{h}	86.6 ± 2.9^{hi}	77.8 ± 4.7^{de}	87.5 ± 2.6^{f}	78.4 ± 4.4^{cdef}	87.4 ± 3.0^{def}	86.7 ± 3.1^{fg}	85.0 ± 3.7^{fg}	88.0 ± 2.9^{fg}
45552	85.2 ± 3.5^{bcdefg}	92.5 ± 1.3^{bcdefgh}	90.4 ± 1.5^{cdefghi}	82.2 ± 2.7^{bcde}	91.6 ± 1.1^{cdef}	81.7 ± 5.5^{bcdef}	91.0 ± 1.1^{bcdef}	89.8 ± 1.5^{cdefg}	88.6 ± 1.6^{bcdefg}	91.6 ± 1.3^{bcdefg}
45553	93.7 ± 4.0^{abc}	95.9 ± 2.3^{abcd}	94.7 ± 2.5^{abcde}	89.8 ± 3.1^{ab}	94.8 ± 2.9^{abcd}	94.2 ± 4.2^{ab}	94.9 ± 2.5^{abc}	94.0 ± 3.0^{abcde}	93.5 ± 3.2^{abc}	95.9 ± 2.6^{abcd}
45554	88.9 ± 0.4^{abcdefg}	94.0 ± 0.7^{abcdefg}	92.2 ± 0.5^{bcdefgh}	83.4 ± 1.4^{bcd}	93.3 ± 0.8^{abcde}	87.3 ± 0.5^{abcde}	91.8 ± 0.3^{bcdef}	92.3 ± 0.7^{abcdef}	90.6 ± 1.1^{abcdefg}	93.9 ± 0.8^{abcdef}

SBM	Methionine	Phenylalanine	Proline	Serine	Threonine	Tryptophan	Tyrosine	Valine	Total amino acids
45531	95.2 ± 2.0^{ab}	96.5 ± 0.9^{a}	96.6 ± 0.9^{ab}	95.4 ± 0.7^{ab}	95.1 ± 0.8^{a}	98.0 ± 0.4^{a}	97.6 ± 1.1^{ab}	95.7 ± 1.4^{ab}	96.4 ± 1.0^{ab}
45532	89.5 ± 1.0^{abcde}	92.7 ± 1.1^{abcde}	91.9 ± 1.2^{abcdef}	91.1 ± 0.6^{abcdef}	89.3 ± 1.1^{abcde}	97.0 ± 0.2^{abc}	94.2 ± 0.3^{bcdefgh}	90.8 ± 1.3^{abcdef}	92.2 ± 0.7^{abcdef}
45533	93.4 ± 2.1^{abcd}	94.8 ± 1.2^{abc}	95.0 ± 1.1^{abc}	93.4 ± 1.2^{ab}	92.3 ± 1.8^{abc}	96.6 ± 1.3^{abc}	96.5 ± 1.1^{abcd}	93.6 ± 1.6^{abc}	94.6 ± 1.2^{abcd}
45534	88.9 ± 4.1^{bcdef}	93.6 ± 2.3^{abcd}	93.9 ± 2.7^{abcd}	92.4 ± 2.2^{abc}	90.5 ± 2.9^{abcd}	95.9 ± 1.6^{abcd}	94.3 ± 2.0^{bcdefgh}	92.4 ± 2.9^{abcde}	93.2 ± 2.5^{abcde}
45535	87.5 ± 2.6^{bcdefg}	91.6 ± 1.7^{abcdefg}	91.2 ± 1.7^{abcdef}	90.2 ± 2.0^{abcdef}	87.5 ± 2.2^{abcdef}	94.6 ± 1.8^{abcde}	92.9 ± 1.2^{cdefgh}	89.7 ± 2.4^{abcdefg}	90.8 ± 2.0^{bcdefg}
45536	94.6 ± 1.9^{ab}	95.5 ± 1.3^{ab}	95.0 ± 1.4^{abc}	95.0 ± 1.2^{ab}	93.6 ± 1.2^{ab}	96.8 ± 0.7^{abc}	97.2 ± 1.1^{abc}	93.6 ± 2.5^{abc}	95.5 ± 1.3^{abc}

(Continues)

TABLE 6 (Continued)

SBM	Methionine	Phenylalanine	Proline	Serine	Threonine	Tryptophan	Tyrosine	Valine	Total amino acids
45537	90.5 ± 1.0^{abcde}	92.1 ± 1.7^{abcdef}	91.7 ± 1.2^{abcdef}	91.5 ± 1.4^{abcde}	89.2 ± 1.6^{abcde}	96.1 ± 0.9^{abcd}	95.8 ± 1.1^{abcdef}	89.4 ± 2.7^{abcdefg}	92.4 ± 1.4^{abcdef}
45538	79.1 ± 1.6^{g}	85.6 ± 1.7^{g}	85.2 ± 1.8^{f}	84.4 ± 2.3^{f}	80.1 ± 2.1^{f}	92.4 ± 1.1^{def}	90.8 ± 1.3^{ghi}	80.8 ± 2.2^{h}	85.2 ± 1.8^{g}
45539	89.9 ± 2.2^{abcde}	91.6 ± 1.4^{abcdefg}	91.0 ± 1.3^{abcdef}	91.3 ± 0.9^{abcde}	88.4 ± 2.0^{abcdef}	95.0 ± 1.0^{abcde}	95.9 ± 0.6^{abcdef}	88.6 ± 1.2^{bcdefgh}	91.6 ± 1.3^{abcdefg}
45540	89.1 ± 2.2^{bcdef}	92.5 ± 2.5^{abcde}	91.8 ± 2.6^{abcdef}	90.9 ± 2.8^{abcdef}	87.8 ± 3.0^{abcdef}	94.9 ± 1.4^{abcde}	96.3 ± 1.5^{abcde}	90.5 ± 3.6^{abcdef}	92.5 ± 2.6^{abcdef}
45541	98.6 ± 0.5^{a}	97.6 ± 0.3^{a}	97.9 ± 0.5^{a}	96.8 ± 0.1^{a}	95.9 ± 0.5^{a}	97.3 ± 0.7^{ab}	99.3 ± 0.3^{a}	96.7 ± 0.5^{a}	97.8 ± 0.3a
45542	86.5 ± 2.2^{bcdefg}	90.1 ± 0.6^{bcdefg}	89.3 ± 0.6^{cdef}	88.8 ± 0.7^{bcdef}	84.8 ± 0.9^{cdef}	93.5 ± 0.6^{bcdef}	91.4 ± 0.7^{fghi}	86.5 ± 1.5^{cdefgh}	89.2 ± 0.6^{cdefg}
45543	86.4 ± 2.0^{bcdefg}	89.9 ± 1.5^{bcdefg}	89.8 ± 1.7^{bcdef}	89.0 ± 1.0^{bcdef}	85.3 ± 2.2^{bcdef}	93.4 ± 1.0^{cdef}	92.2 ± 1.4^{defgh}	85.9 ± 2.1^{cdefgh}	89.5 ± 1.6^{cdefg}
45544	83.5 ± 5.8^{efg}	89.1 ± 3.7^{cdefg}	87.6 ± 4.5^{def}	86.5 ± 3.6^{cdef}	82.8 ± 5.1^{def}	92.3 ± 2.9^{def}	91.8 ± 2.0^{efghi}	84.6 ± 5.1^{efgh}	88.3 ± 4.0^{defg}
45545	82.3 ± 3.6^{efg}	87.9 ± 1.5^{defg}	86.5 ± 1.5^{ef}	85.6 ± 0.8^{def}	81.3 ± 1.6^{ef}	92.1 ± 0.9^{ef}	91.1 ± 0.8^{ghi}	84.2 ± 1.2^{fgh}	87.0 ± 1.6^{efg}
45546	80.2 ± 3.9^{fg}	87.2 ± 2.3^{efg}	85.9 ± 3.9^{ef}	84.5 ± 3.8^{f}	79.9 ± 4.1^{f}	92.0 ± 0.8^{ef}	91.2 ± 1.5^{ghi}	82.3 ± 3.6^{gh}	86.2 ± 3.0^{fg}
45547	89.6 ± 4.0^{abcde}	93.3 ± 2.3^{abcde}	92.2 ± 2.8^{abcde}	92.1 ± 2.5^{abcd}	88.9 ± 2.9^{abcde}	95.2 ± 1.3^{abcde}	95.2 ± 1.6^{abcdefg}	90.9 ± 2.3^{abcdef}	92.7 ± 2.6^{abcdef}
45548	85.2 ± 4.8^{cdefg}	89.7 ± 2.6^{bcdefg}	88.9 ± 2.6^{cdef}	89.2 ± 2.3^{bcdef}	84.5 ± 4.4^{cdef}	93.5 ± 1.2^{bcdef}	92.3 ± 2.0^{defgh}	86.5 ± 2.8^{cdefgh}	89.4 ± 2.7^{cdefg}
45549	86.6 ± 3.8^{bcdefg}	90.4 ± 2.7^{bcdefg}	89.2 ± 3.2^{cdef}	89.4 ± 3.3^{bcdef}	84.7 ± 3.8^{cdef}	94.2 ± 1.5^{bcdef}	92.1 ± 2.6^{defgh}	87.5 ± 3.9^{cdefgh}	89.9 ± 3.0^{bcdefg}
45550	84.5 ± 0.8^{defg}	88.1 ± 0.7^{defg}	87.2 ± 1.1^{def}	86.6 ± 1.4^{cdef}	81.9 ± 1.6^{ef}	93.4 ± 0.3^{cdef}	90.4 ± 1.0^{hi}	85.1 ± 1.7^{defgh}	87.9 ± 1.0^{efg}
45551	81.6 ± 4.4^{efg}	86.3 ± 3.0^{fg}	85.3 ± 2.8^{f}	85.2 ± 3.4^{ef}	80.8 ± 4.4^{ef}	90.7 ± 1.5^{f}	87.4 ± 2.5^{i}	84.4 ± 3.6^{efgh}	85.5 ± 3.1^{g}
45552	85.3 ± 2.0^{cdefg}	90.0 ± 1.3^{bcdefg}	89.6 ± 2.3^{cdef}	89.0 ± 2.8^{bcdef}	84.9 ± 3.1^{cdef}	94.2 ± 0.5^{abcdef}	92.1 ± 1.0^{defgh}	87.8 ± 2.3^{bcdefgh}	89.4 ± 1.9^{cdefg}
45553	94.3 ± 2.6^{abc}	94.2 ± 2.8^{abc}	94.7 ± 2.8^{abc}	94.6 ± 2.4^{ab}	91.9 ± 3.0^{abc}	96.5 ± 1.4^{abc}	96.0 ± 2.3^{abcdef}	93.2 ± 3.1^{abcd}	94.5 ± 2.8^{abcd}
45554	89.7 ± 0.5^{abcde}	91.6 ± 0.6^{abcdefg}	91.3 ± 0.7^{abcdef}	89.9 ± 0.7^{bcdef}	85.6 ± 0.8b^{cdef}	94.8 ± 0.7^{abcde}	94.3 ± 0.6^{bcdefgh}	89.2 ± 0.7^{abcdefg}	91.4 ± 0.6^{abcdefg}

Note: Values for each amino acid digestibility are means and *SD* of triplicates. Values within column with different superscripts are significantly different ($p < .05$) based on one-way ANOVA followed by Tukey's multiple comparison test.

TABLE 7 Principle component analysis of chemical characteristics of soybean meal sources

Principle component	Standard deviation	Proportion of variance	Cumulative proportion
PC 1	2.584	0.303	0.303
PC 2	2.247	0.229	0.532
PC 3	1.738	0.137	0.669
PC 4	1.413	0.091	0.759
PC 5	1.215	0.067	0.826
PC 6	1.116	0.057	0.883
PC 7	0.913	0.038	0.921
PC 8	0.742	0.025	0.946
PC 9	0.670	0.020	0.966
PC 10	0.529	0.013	0.979
PC 11	0.373	0.006	0.985
PC 12	0.324	0.005	0.990
PC 13	0.299	0.004	0.994
PC 14	0.250	0.003	0.997
PC 15	0.182	0.002	0.998
PC 16	0.156	0.001	0.999
PC 17	0.084	0.000	1.000
PC 18	0.072	0.000	1.000
PC 19	0.055	0.000	1.000
PC 20	0.032	0.000	1.000
PC 21	0.023	0.000	1.000
PC 22	0.004	0.000	1.000
PC 23	0.000	0.000	1.000

particular ingredients when fed to an animal is also vital. Apparent digestibility coefficients provide indirect measurements of bioavailability of energy or nutrients in an ingredient or diet and are calculating from a ratio of an inert marker in feed and faeces (Glencross, Booth, & Allan, 2007). Soybean meal is the primary protein source used in most shrimp and fish diet formulations, due to its excellent nutrient profile, worldwide availability and comparatively cheaper price. Variations in nutrient quality among sources of SBM resulting from differences in production location and processing specifications are well documented (Balloun, 1980; Maestri et al., 1998; Natarajan et al., 2016; Palmer et al., 1996; van Kempen et al., 2002; Verma & Shoemaker, 1996). However, the effect of these variations on digestibility and growth performances of shrimps or fish is yet to be discovered.

Apparent dry matter, energy and protein digestibility of SBM observed during the current study ranged from 45% to 90%, 56% to 93% and 87% to 98%, respectively (Table 5), which are in agreement with previous findings (Akiyama, Coelho, Lawrence, & Robinson, 1989; Brunson et al., 1997; Cruz-Suárez et al., 2009; Divakaran, Velasco, Beyer, Forster, & Tacon, 2000; Fang, Yu, Buentello, Zeng, & Davis, 2016; Qiu, Nguyen, & Davis, 2018). However, as Smith, Tabrett, Glencross, Irvin, and Barclay (2007) and Zhu, Davis, Roy, Samocha, and Lazo (2013) pointed out, there is a possibility of having a larger variation in apparent digestibility coefficients for a nutrient in an ingredient, between different shrimp studies due to the potential error associated with limited consumption of feed per day and minimal production of faeces due to small intake. Direct excretion of faecal matter in water could complicate collections and accuracy of data due to possible problems such as leaching as well (Akiyama et al., 1989; Brunson et al., 1997). Nevertheless, significant differences in apparent digestibility coefficients of test diets and SBM (<.05) observed in the current study are likely not due to such differences, as experimental procedures between all digestibility diets were similar. In addition, numerous precautions were taken to minimize potential errors to improve consistency of data. All faecal samples were collected one hour after each feeding thus leaching of chromic oxide and nutrients would be negligible or constant through the collections. Furthermore, all the uneaten diet was siphoned-out from each tank following the collection of faecal samples to avoid possible ingestion of leached materials. Therefore, observed significant differences in apparent digestibility coefficients of test diets and SBM during the study were assumed to be a result of differences in chemical characteristics of SBM.

It is clear that multiple chemical variables in a feed ingredient may have different effects on biological processes such as growth or digestibility, demanding a multivariate statistical tool to capture these variations. Principle component analysis (PCA) was used during the study to identify the major chemical variables in SBM that were responsible for significant variations in digestibility, as it accounts for inherent collinearity among certain chemical variables (Tables 7 and 8). Multiple linear regressions carried out subsequent to PCA identified fibres (ADF, NDF and Lignin), raffinose and trypsin inhibitor level as having the greatest influence on SBM digestibility in Pacific white shrimps.

Plants often contain more carbohydrates than animal-based ingredients, which is also true for soybean that contains approximately 32% carbohydrates on a dry matter basis (Banaszkiewicz, 2011). Soluble carbohydrates in soybeans range from 12% to 15%, about half of which is sucrose and the remainder comprise low-molecular-weight oligosaccharides, which is 1%–2% raffinose and 5%–6% Stachyose (Dersjant-Li, 2002; Francis, Makkar, & Becker, 2001; Gatlin et al., 2007; Krogdahl, Penn, Thorsen, Refstie, & Bakke, 2010). The oligosaccharide component of SBM has been reported to reduce nutrient uptake and growth performances (Arnesen, Brattas, Olli, & Krogdahl, 1989; Refstie, Storebakken, & Roem, 1998) and SBM induced enteritis in several salmonid fish species (Gatlin et al., 2007; Krogdahl et al., 2010). Suggested causative reasons for negative effects of oligosaccharides may be due to either binding to bile acids or interfering with the uptake of nutrients via increasing the viscosity of the chime in the digestive tract (Refstie et al., 1998; Storebakken, Shearer, & Roem, 1998). However, the effect of soy oligosaccharides seems to be negligible on rainbow trout [*Salmo salar*] (Arnesen et al., 1989), tilapia [*Sarotherodon mossambicus*] (Jackson, Capper, & Matty, 1982) and carp [*Cyprinus carpio*] (Ufodike & Matty, 1983), while no information was found relevant to the enteritis inducing effect of isolated soybean oligosaccharides on fish (Gatlin et

TABLE 8 Loadings representing respective chemical variables for each principle component

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14	PC15	PC16	PC17	PC18	PC19	PC20
Trypsin inhibitor	0.221			0.329	0.154	0.297	0.313	0.233	0.403	0.475	0.109		0.322	0.132			0.18			
Fructose	0.185		0.286	−0.395	−0.162	0.161	−0.138	0.128	0.155	−0.128	0.284	−0.271		0.207		0.131		−0.202		0.455
Glucose	0.231		0.246	−0.335	−0.168	0.229	−0.102	−0.118	0.124	−0.11	0.213	0.15	0.146	−0.355	0.249	0.154	0.116	0.176		−0.0365
Sucrose	−0.304	−0.173		0.145	−0.208			−0.134	0.129		0.216	0.372	0.382				−0.313		−0.276	0.352
Raffinose	0.188		−0.316	0.181	0.141	0.13	0.432	−0.241	−0.241	−0.482	0.222	−0.155		0.158	0.292	0.193				
Stachyose	−0.166	−0.269		0.219	−0.219			−0.54	0.213	0.139	0.369	−0.261	−0.106	−0.221	−0.148			−0.188	0.167	−0.137
ADF	0.242	0.139	0.257	0.175		−0.363			−0.209	0.162	0.116			0.141	0.106	0.152	0.129	−0.599	−0.304	−0.145
NDF	0.212	0.125	0.267	0.118		−0.46			−0.291	0.155	0.37				−0.143			0.486	0.248	0.127
Lignin		0.124	−0.164	−0.259	0.133	−0.557	0.204	−0.207	0.653											
Ca	0.304	−0.204	−0.122		−0.142						0.123	−0.373			−0.207	−0.541			−0.229	
Phosphorus	−0.134	−0.305	0.311		0.124			0.148		−0.142		−0.234	0.178						−0.138	−0.42
P in phytic acid	−0.235	−0.105	0.374				0.351				−0.131								0.27	0.128
Total PA	−0.233		0.377				0.36				−0.104	−0.178	−0.109					0.277	−0.191	0.181
Nonphytate P	0.125	−0.353			0.249	−0.15	−0.215	0.133		−0.229		−0.104	0.429		−0.439	0.219	0.103	−0.102	0.164	
Cu	0.14	−0.21	−0.223		−0.516	−0.128	0.153	0.131				0.119			−0.113	0.23	0.26	0.191	−0.428	
Fe	0.335		0.141			0.16		−0.357			−0.233			0.236	−0.24	0.167	−0.633	0.102		−0.172
Mg	0.254	−0.211			0.405					0.155			−0.18	−0.643		0.109	−0.145		−0.2	0.36
Mn	0.259	−0.232		−0.168	−0.152		0.19	−0.292			−0.297	0.463		0.102		−0.291	0.302	−0.182	0.314	0.118
Mo	−0.215		−0.208	−0.409				−0.247	−0.302	0.527	−0.122	−0.247	0.338	0.104	0.239	0.109	0.125			0.101
K	−0.18	−0.15	−0.136	−0.417	0.191		0.326	0.171	−0.117	0.133	0.492	0.333	−0.223	0.138	−0.22		−0.155			−0.193
Na		−0.396	0.103		0.173	−0.142	−0.125						0.134	0.113	0.541	−0.4	−0.152		−0.105	−0.114
S		−0.373	0.107		0.176	0.111	−0.333	−0.135					−0.456	0.393		0.316	0.192	0.265		
Zn	0.146	−0.294	−0.126		−0.363	−0.21		0.336		0.128			−0.121		0.272	0.275	−0.334		0.423	

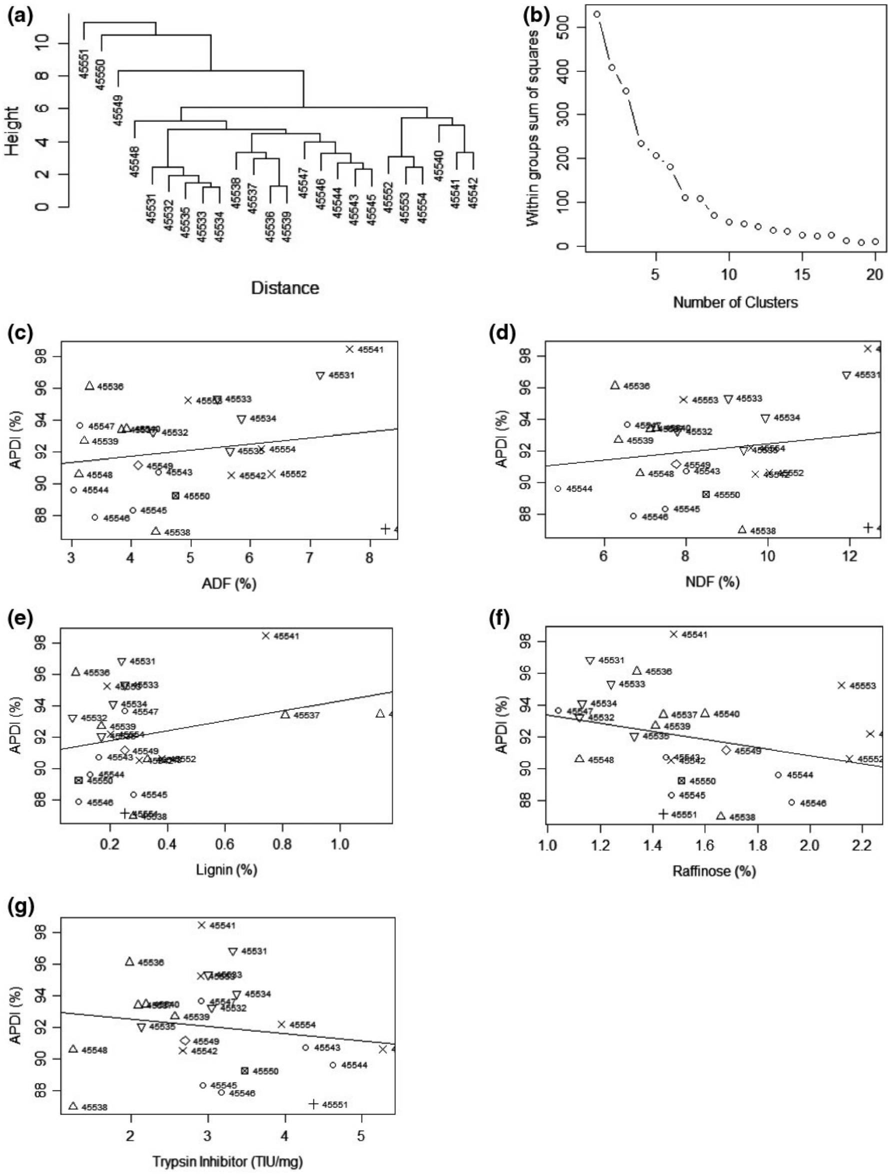
TABLE 9 Regression analysis between protein (APD$_{\text{I}}$), energy (AED$_{\text{I}}$) and dry matter (ADMD$_{\text{I}}$) digestibility coefficients of test ingredients and principle component scores

	APD$_{\text{I}}$		AED$_{\text{I}}$		ADMD$_{\text{I}}$	
Principle component	Estimate	*p*-value	Estimate	*p*-value	Estimate	*p*-value
PC 1	−0.406	.089	−0.931	.134	−1.394	.126
PC 2	0.323	.183	1.016	.149	1.346	.175
PC 3	0.107	.690	0.811	.319	0.841	.455
PC 4	0.547	.164	0.423	.647	0.972	.480
PC 5	−0.129	.734	−1.077	.348	−1.225	.447
PC 6	−1.193	.051	−4.084	.031	−4.685	.055
PC 7	0.417	.433	1.138	.443	1.196	.568
PC 8	−0.545	.408	−3.373	.124	−5.029	.117
PC 9	0.084	.902	−1.211	.542	−1.660	.561
PC 10	1.647	.131	5.554	.089	6.195	.151
PC 11	−1.225	.357	−4.796	.227	−5.650	.305
PC 12	2.831	.118	5.172	.251	7.322	.259
PC 13	−1.383	.399	−5.515	.257	−8.381	.239
PC 14	−0.464	.801	−9.855	.128	−10.824	.211
PC 15	1.926	.466	6.125	.415	11.482	.308
PC 16	0.645	.826	−5.030	.553	1.424	.905
PC 17	8.517	.187	24.487	.179	38.726	.152
PC 18	−17.157	.061	−42.218	.082	−58.493	.090
PC 19	−3.404	.688	13.055	.587	6.514	.848
PC 20	−4.118	.772	−13.342	.738	−3.118	.956
Multiple *R*-squared		.942		.952		.941
F-statistic		2.420		2.990		2.391
Model *p*-value		.255		.199		.258

al., 2007). Meanwhile, certain types and amounts of oligosaccharides such as mannose and fructose seem to stimulate the growth of certain microorganisms in the intestine, which may interact with the energy and nutrient digestibility, immune responses and growth performances of cultured fish or shrimp. Zhang et al. (2012) observed an improved growth performances of *L. vannamei* with dietary mannan oligosaccharide (MOS), which was optimum at 2%, while no statistical differences were noted between 2% and 8% addition to the diet. Even though it is not statistically significant, the tested growth and immune parameters seem to decline at higher rates of MOS additions, indicating a possible negative effect beyond the range they have tested. According to Krogdahl et al. (2010), effects of altered microbial population in gastrointestinal tract of fish due to oligosaccharides could be either positive or negative, which they attributed to variations in intestinal inflammations (enteritis) between studies and different durations of studies. The raffinose level of SBM used during the current study ranged from 1.04% to 2.23%, which is comparable to previous findings (Francis et al., 2001). Negative effects of raffinose in SBM on growth performances of Pacific white shrimp have been reported (Galkanda Arachchige et al., 2019; Zhou, Davis, & Buentello, 2015), and the current results reveal a negative correlation with digestibility (p = .18) albeit non-significant might be due to masking or interactions with other chemicals or simply the relatively small change of dietary level.

A positive association was observed between digestibility coefficients and ADF, NDF and lignin content of SBM sources (Figure 1), which are insoluble structural carbohydrates in plants. One possible explanation for the observed higher digestibility of energy and nutrients in SBM and ADF and NDF levels may be due to the regulatory ability of fibre on gut retention time of foods (Krogdahl et al., 2010; Lech & Reigh, 2012; Shiau, 1997). del Carmen González-Peña, Gomes, and Moreira (2002) reported significantly improved growth performance and protein efficiency in *Macrobrachium rosenbergii* with a diet containing 10% cellulose compared with those with lower levels. The observed outcomes were attributed to the gastric emptying time, which had a positive correlation with cellulose level in the diet assuming a consequent improvement in absorption of nutrients. However, Beseres, Lawrence, and Feller (2005) investigated a non-significant effect of fibre level (2.3%–11.3%) on gut passage time of food in three shrimp species: *Farfantepenaeus aztecus*, *Litopenaeus setiferus* and *L. vannamei*. Along with several other studies revealing the positive effect of fibre supplementation on growth and feed utilization of *M. rosenbergii* (Fair, Fortner, Millikin, & Sick, 1980; Ravishankar & Keshavanath, 1988), del Carmen González-Peña et al. (2002) observed a reduction in growth and production efficiencies due to 15% cellulose supplementation in diet. The observed cellulose levels in SBM used during the study were range from 2.95% to 7.16% (cellulose

FIGURE 1 Dendrogram of cluster analysis (grouping of soybean meal (SBM) based on chemical characteristics) (a), scree plot (b) and patterns of association between PCA selected chemical parameters of SBM (acid detergent fibre/ADF, neutral detergent fibre/NDF, lignin, raffinose and trypsin inhibitor) and apparent protein digestibility (APDI) of SBM in Pacific white shrimp, *Litopenaeus vannamei* (c, d, e. f & g). Twenty-four different SBM clustered in seven groups based on K-means clustering algorithm are represented in different symbols



% = ADF % − lignin %), which seems to be reasonable based on the studies conducted on freshwater prawns while not large enough to cause detrimental growth effects as well.

Negative effects of excess fibre could be due to its indigestibility, physical prevention of contact between other nutrients and absorptive surface of intestinal lumen, possible causation of diarrhoea in some fish reducing the gut retention time of feed, binding with protein and minerals thus reducing their availability (Krogdahl et al., 2010; Lech & Reigh, 2012; Shiau, 1997). In response, energy digestibility of aquatic animals found to be inversely related to the fibre content of the material fed to the animal (Brunson et al., 1997; Lech & Reigh, 2012). Fang et al. (2016) recorded a non-significant negative effect of fibre on energy digestibility in *L. vannamei* with a similar trend between fibre and mean final weight of shrimps ($r = -.061$ and p-value = .875). However, the fibre content of the soy sources utilized ranged from 2.1% to 3.9% which may not be sufficient to identify an effect. Effects of fibre on energy and nutrient digestibility in aquatic animals seem to be variable due to a number of possible impacts on calculated digestibility values. These different effects may depend on the type of dietary fibre ingested, animal species, duration of the study and variations in non-fibre components of the diet. However, the positive association observed during the growth study with fibre (Galkanda Arachchige et al., 2019) was repeated in this experiment with a positive effect of ADF (3.02%–8.29%), NDF (4.84%–12.58%) and lignin (0.07%–1.13%) on SBM digestibility in *L. vannamei.*

Based on PCA and Pearson correlation coefficients, the negative effect of trypsin inhibitor level on SBM digestibility by *L. vannamei* was confirmed. This has previously been described in the literature for numerous aquaculture species. (Dersjant-Li, 2002; Fang et al., 2016; Gatlin et al., 2007; Kaushik et al., 1995; Krogdahl et al., 2010; Lim & Akiyama, 1992; Olli & Krogdahi, 1994; Qiu, Buentello, et al., 2018; Zhou et al., 2015). Trypsin inhibitor level of SBM sources used during the study ranged from 1.25 to 5.27 mg/g which is comparable with the levels (2–6 mg/g) in commercial soybean products (Snyder & Kwon, 1987).

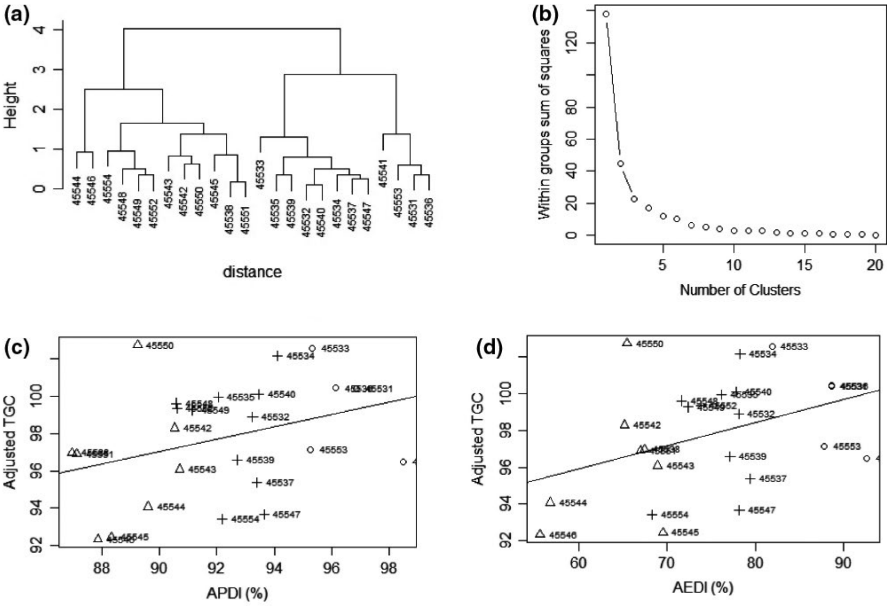


FIGURE 2 Dendrogram of cluster analysis (grouping of soybean meal based on digestibility characteristics) (a) scree plot (b) and patterns of association between ingredient (I) digestibility parameters (apparent digestibility coefficients for protein/APD and energy/AED) and standardized thermal growth coefficient of Pacific white shrimp, *Litopenaeus vannamei* (c & d) (twenty-four different SBM clustered in three groups based on K-means clustering algorithm are represented in different symbols)

It was unable to identify significant individual effects on digestibility for any individual chemical variable screened through PCA using simple linear regression, indicating that linear regression is less effective in capturing interactions, collinearity and possible swamping effects of multiple independent variables. Inconsistency among cluster groupings of SBM based on chemical characteristics and digestibility characteristics further proved the interactive augmented effect of multiple variables towards digestibility, which might shuffle the grouping pattern when it comes to digestibility being a function of several chemical variables (Figures 1 and 2). Thus, fairly bias conclusions are numerous in literature by attributing the observed outcome to a one chemical variable with moderate to higher richness in an ingredient. Francis et al. (2001) also emphasized the importance of considering interactions between chemical variables in an ingredient, highlighting reduced individual toxicity of several antinutrients due to the interactions such as saponin–tannin (Freeland, Calcott, & Anderson, 1985), tannin–lectin (Fish & Thompson, 1991) and tannin–cyanogen (Goldstein & Spencer, 1985).

Increased protein and energy digestibility of an ingredient could contribute to higher growth performance in shrimp, but greater digestibility is not a requisite to yield higher growth because the feed intake of shrimp or the balance of essential nutrients does not always depend on digestibility. Fang et al. (2016), Zhou et al. (2015) and Zhu et al. (2013) noted variable responses between nutrient digestibility in SBM and growth of *L. vannamei* which were assumed to be a result of differences in palatability or segregated effects of certain chemical variables on growth. However, a positive association was observed (not statistically significant) between apparent digestibility coefficients and growth performances of Pacific white shrimp during the current study (Figure 2), which might be due to the higher protein contribution from SBM (65% from total) to test diets.

TABLE 10 Association of dry matter (ADMD), energy (AED) and protein (APD) digestibility coefficients of test ingredients (I) and diets (D) with growth (standardized thermal growth coefficient) of Pacific white shrimp, *Litopenaeus vannamei*

Variable	Estimate/β	R^2	95% CI	*p*-value
$ADMD_D$	0.27	0.11	0.35	.12
AED_D	0.38	0.15	0.40	.06
APD_D	0.75	0.11	0.95	.12
$ADMD_I$	0.08	0.11	0.10	.12
AED_I	0.13	0.15	0.13	.06
APD_I	0.33	0.11	0.42	.12

5 | CONCLUSION

It is clear that the chemical characteristics of even reasonably similar sources of SBM generate significant different variations on apparent digestibility coefficients of energy and nutrients by Pacific white shrimp. However, it is difficult to make a firm conclusion about a specific culprit for the resulted fluctuations in digestibility and their threshold levels might be due to interactive positive and negative effects. Fibre, raffinose and trypsin inhibitor levels are vital chemical parameters for energy and nutrient digestibility in SBM, which may need to be further investigated before these parameters can be used as predictors for biological performances in shrimp. Variations in growth performances of shrimp were in line with variations in apparent digestibility coefficients of energy and nutrients verifying the importance of digestibility data in shrimp feed formulations.

ACKNOWLEDGMENTS

The authors would like to express our gratitude and appreciation to those who have taken time to critically review this manuscript as well as those who helped support this research at the E.W. Shell Research Station, School of Fisheries, Aquaculture and Aquatic Sciences, Auburn

University. Special thanks to students and staff who help maintain the daily management during the trials. This work was supported in part by the Alabama Agricultural Experiment Station and the Hatch Program (ALA016-08027) of the National Institute of Food and Agriculture, U.S. Department of Agriculture. Mention of trademark or proprietary product does not constitute an endorsement of the product by Auburn University and does not imply its approval to the exclusion of other products that may also be suitable.

DATA AVAILABILITY STATEMENT

I would like to confirm that the data associated with this paper is available at Dr. Allen Davis Laboratory, School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, AL, USA and could be access based on the permission of Dr D. Allen Davis.

ORCID

Harsha S. C. Galkanda-Arachchige https://orcid.org/0000-0002-5464-1458

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How to cite this article: Galkanda-Arachchige HSC, Guo J, Stein HH, Allen DavisD. Apparent energy, dry matter and amino acid digestibility of differently sourced soybean meal fed to Pacific white shrimp *Litopenaeus vannamei*. *Aquac Res*. 2020;51:326–340. https://doi.org/10.1111/are.14378