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Evaluation of soybean meal from different sources as an ingredient in practical diets for Pacific white shrimp *Litopenaeus vannamei*

Harsha Sameera Chathuranga Galkanda Arachchige[1,2] | Xuan Qiu[3] | Hans H. Stein[4] | D. Allen Davis[1]

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

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

[3]Feed Technology Function Line, Chia Tai Group Agro-Industry and Food Business, Beijing, China

[4]Department of Animal Sciences, University of Illinois, Urbana, Illinois

Correspondence
Harsha Sameera Chathuranga Galkanda Arachchige, School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, Auburn, Alabama.
Email: hsg0009@auburn.edu

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Abstract

Solvent extracted soybean meal (SBM) is generated using different varieties of soybeans grown under a range of conditions and then processed at different crushing plants. Due to its competitive cost and availability, it is a popular plant-based protein source for shrimp feed formulations. However, there is limited information about effects of variations in the nutritional composition of soybean meal have on performances of shrimp. Hence, the present study was designed to determine the effects of different soybean sources on the growth performances of *Litopenaeus vannamei*. Two growth trials were conducted with iso-nitrogenous and iso-lipidic (350 g/kg protein and 80 g/kg lipid) test diets formulated with 25 sources of soybean meal. Trial one incorporated 14 treatments including a soy-based diet containing 517 g/kg SBM (eight replicates) and this soy source was then replaced with 13 different soybean sources (four replicates per treatment). The second trial used the same basal diet and 11 different sources of soybean meal (Total 12 diets) with five replicates per treatment. Both growth trials were conducted with a stocking density of 10 shrimps/aquarium in a semi-closed recirculating system and the initial weight of shrimps for trials 1 and 2 were 0.23 g ± 0.02 and 0.67 g ± 0.02 respectively. During the two trials, shrimp were fed four times/day assuming a FCR of 1.8, over 42 days for trial 1 and 35 days for trial 2. Results indicated that there are differences among sources of soybean meal for standardized percentage TGC. Diet 21 that contained SBM4550 had the largest value for TGC whereas the lowest value for TGC was observed for shrimp fed diet 17 that contained SBM45536. According to the statistical analysis that was used to interpret the growth performance data from the complete chemical profile of the SBM, phosphorous, phytate-phosphorous and total phytic acid levels had positive correlations ($p < 0.005$) with TGC whereas raffinose ($p = 0.086$) had a negative correlation with TGC. Results of this work indicates phosphorous, phosphorous in phytic acid and total phytic acid and raffinose are important components in SBM that may have significant effects on the growth performances of pacific white shrimp.

KEYWORDS
nutritional quality, production location, shrimp growth, soybean meal

1 | INTRODUCTION

The Pacific white shrimp, *Litopenaeus vannamei*, continues to be an important species for world aquaculture. This species accounts for 85% of total shrimp production in China (Li & Xiang, 2013) and accounts for 80% of the farmed shrimp production in the world (Panini et al., 2017). The aquaculture production of shrimp depends on the provision of nutrients in the form of industrially produced compound feed. As this industry continues to expand so does the demand for key feed ingredients. Fishmeal was the main protein source used in aquaculture feed consuming approximately 68% of fish meal production in the world (Mallison, 2013; Tacon & Metian, 2015). This is not only due to its excellent amino acids profile, palatability and digestibility, but also because fish meal is a source of nucleotides, essential fatty acids, phospholipids, minerals, and fat-soluble and water-soluble vitamins (Tacon, Metian & Hasan, 2009). Because of static supply, increasing demand, price and ethical issues, average dietary fish meal inclusion levels within compound feed for shrimp has been steadily declining (from around 28% to 7%) and it is expected that total usage will decrease by 37.7% from 2006 to 2020 (Tacon & Metian, 2008). Fish meal is no longer the primary protein source, but more of a strategic ingredient used in less price-sensitive phases in the culture cycle (Jackson, 2012). Of protein sources, solvent extracted soybean meal (SBM) received the most attention of terrestrial plant sources (Amaya, Davis & Rouse, 2007a) considering its well-balanced amino acid profile, advantage of being resistant to oxidation and spoilage, worldwide availability, low price and consistent composition (Amaya, Davis & Rouse, 2007b; Davis & Arnold, 2000; Dersjant-Li, 2002; Gatlin et al., 2007; Swick, Akiyama, Boonyaratpalin & Creswell, 1995).

However, the inclusion level of SBM in practical shrimp diet is restricted due to the presence of anti-nutritional factors (ANFs) (trypsin inhibitors, antigens, lectins, saponins and oligosaccharides), insufficient levels of essential amino acids (EAA) (methionine and lysine) and poor palatability, which negatively affects digestion and nutrient availability to shrimp (Dersjant-Li, 2002; Gatlin et al., 2007;

TABLE 1 Chemical analysis[a] (proximate composition, gross energy and trypsin inhibitors) of the different Soybean meal used in diets of Pacific white shrimp, *Litopenaeus vannamei*

Soybean meal sample key	g/100g as is					GE, kcal/kg	Trypsin inhibitors/mg (TIU)
	Dry matter	Moisture	Ash	Crude protein	Fat		
AU Soy	88.14	11.86	5.78	43.7	1.03	4,394	
45531	89.37	10.63	6.44	45.85	1.25	4,191	3.32
45532	89.77	10.23	6.58	46.40	1.53	4,213	3.05
45533	89.42	10.58	6.42	45.35	1.39	4,194	3.00
45534	89.70	10.30	6.36	45.78	1.10	4,204	3.37
45535	89.40	10.60	6.48	45.92	1.07	4,185	2.13
45536	88.93	11.07	6.99	47.50	0.86	4,168	1.98
45537	88.85	11.15	6.96	46.62	1.40	4,190	2.09
45538	89.51	10.49	7.06	47.87	1.37	4,210	1.25
45539	89.01	10.99	7.01	47.16	1.38	4,209	2.57
45540	89.43	10.57	6.90	47.43	3.47	4,238	2.19
45541	88.19	11.81	6.77	47.31	1.45	4,163	2.92
45542	88.26	11.74	6.39	48.02	2.13	4,232	2.67
45543	90.01	9.99	7.45	51.08	0.83	4,241	4.27
45544	88.08	11.92	6.42	50.29	2.55	4,302	4.62
45545	87.55	12.45	6.46	51.02	1.55	4,231	2.93
45546	88.59	11.41	6.45	47.70	1.55	4,173	3.17
45547	88.66	11.34	6.12	47.79	1.88	4,190	2.91
45548	89.68	10.32	6.41	49.94	2.00	4,254	1.25
45549	87.83	12.17	7.34	47.02	1.44	4,075	2.70
45550	87.77	12.23	7.43	45.48	1.51	4,042	3.47
45551	88.53	11.47	8.60	48.06	1.47	4,113	4.37
45552	88.82	11.18	6.84	49.07	1.83	4,189	5.27
45553	87.23	12.77	5.60	50.96	0.87	4,146	2.9
45554	88.72	11.28	6.59	50.63	0.63	4,175	3.95

[a]Monogastric Nutrition Laboratory, Division of Nutritional Sciences, University of Illinois at Urban-Champaign. Results are expressed on an "as is" basis unless otherwise indicated (Lagos & Stein, 2017).

TABLE 2 Indispensable Amino acid profile[a] (as is basis) of the Soybean meal used diets of Pacific white shrimp, *Litopenaeus vannamei*

Soybean meal sample key	Indispensable amino acids (%)										
	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophan	Valine	Total
AU[2]	3.39	1.23	2.19	3.60	2.94	0.68	2.37	1.79	0.69	2.39	21.27
45531	3.31	1.26	2.07	3.33	2.86	0.61	2.22	1.58	0.65	2.13	20.02
45532	3.38	1.30	2.15	3.45	2.94	0.63	2.30	1.67	0.66	2.23	20.71
45533	3.33	1.28	2.11	3.40	2.88	0.61	2.25	1.63	0.64	2.17	20.30
45534	3.24	1.28	2.09	3.36	2.91	0.63	2.20	1.66	0.65	2.16	20.18
45535	3.36	1.24	2.08	3.39	2.91	0.64	2.23	1.68	0.68	2.16	20.37
45536	3.31	1.35	2.19	3.62	3.04	0.65	2.41	1.82	0.70	2.29	21.38
45537	3.23	1.33	2.18	3.61	2.97	0.63	2.40	1.78	0.69	2.27	21.09
45538	3.32	1.34	2.13	3.56	2.88	0.62	2.39	1.76	0.69	2.23	20.92
45539	3.33	1.36	2.16	3.64	3.04	0.65	2.42	1.83	0.71	2.24	21.38
45540	3.36	1.36	2.26	3.66	3.04	0.64	2.43	1.78	0.70	2.36	21.59
45541	3.22	1.30	2.23	3.59	2.91	0.61	2.41	1.72	0.66	2.30	20.95
45542	3.30	1.34	2.25	3.61	2.97	0.63	2.42	1.74	0.69	2.32	21.27
45543	3.56	1.41	2.39	3.83	3.14	0.66	2.60	1.86	0.68	2.48	22.61
45544	3.52	1.36	2.41	3.89	3.14	0.65	2.57	1.87	0.73	2.47	22.61
45545	3.55	1.41	2.46	3.96	3.15	0.68	2.68	1.87	0.72	2.51	22.99
45546	3.45	1.40	2.32	3.75	3.15	0.67	2.49	1.84	0.72	2.41	22.20
45547	3.40	1.38	2.24	3.68	3.06	0.64	2.42	1.77	0.70	2.34	21.63
45548	3.63	1.44	2.30	3.79	3.21	0.69	2.51	1.85	0.76	2.40	22.58
45549	3.40	1.38	2.26	3.68	3.05	0.66	2.43	1.78	0.68	2.31	21.63
45550	3.30	1.32	2.14	3.52	2.96	0.62	2.31	1.72	0.68	2.24	20.81
45551	3.42	1.39	2.29	3.73	3.08	0.66	2.42	1.82	0.65	2.38	21.84
45552	3.42	1.38	2.21	3.58	3.03	0.62	2.39	1.73	0.66	2.28	21.30
45553	3.71	1.46	2.41	3.92	3.25	0.68	2.62	1.90	0.70	2.49	23.14
45554	3.63	1.44	2.35	3.82	3.18	0.67	2.55	1.85	0.69	2.44	22.62

[a]Monogastric Nutrition Laboratory, Division of Nutritional Sciences, University of Illinois at Urban-Champaign. Results are expressed on an "as is" basis unless otherwise indicated (Lagos & Stein, 2017).

TABLE 3 Dispensable Amino acid profile[a] (as is basis) of the different Soybean meal used in diets of Pacific white shrimp, *Litopenaeus vannamei*

Soybean meal Sample key	Dispensable amino acids (%)									Sum of amino acids (%)
	Alanine	Aspartic acid	Cysteine	Glutamic acid	Glycine	Proline	Serine	Tyrosine	Total	
AU[2]	2.03	5.33	0.63	8.53	1.98	2.40	2.00	1.59	24.49	45.76
45531	1.79	4.78	0.62	7.77	1.75	2.06	1.86	1.57	22.2	42.22
45532	1.91	4.96	0.65	8.01	1.87	2.16	1.95	1.6	23.11	43.82
45533	1.86	4.86	0.63	7.87	1.82	2.07	1.95	1.55	22.61	42.91
45534	1.90	4.94	0.63	7.95	1.87	2.10	2.01	1.26	22.66	42.84
45535	1.91	4.96	0.65	8.02	1.9	2.13	2.06	1.57	23.20	43.57
45536	2.05	5.12	0.62	8.21	1.97	2.26	2.15	1.72	24.10	45.48
45537	2.00	5.02	0.60	8.07	1.92	2.20	2.07	1.67	23.55	44.64
45538	1.99	5.03	0.61	8.05	1.95	2.24	2.09	1.69	23.65	44.57
45539	2.03	5.15	0.62	8.30	1.93	2.26	2.2	1.73	24.22	45.60
45540	2.04	5.16	0.60	8.30	1.99	2.19	2.08	1.72	24.08	45.67
45541	1.98	5.11	0.59	8.16	1.98	2.22	2.08	1.62	23.74	44.69
45542	2.02	5.17	0.62	8.20	2.00	2.24	2.04	1.68	23.97	45.24
45543	2.17	5.50	0.65	8.78	2.10	2.37	2.18	1.80	25.55	48.16
45544	2.15	5.50	0.61	9.00	2.07	2.36	2.35	1.76	25.80	48.41
45545	2.16	5.50	0.66	8.98	2.10	2.44	2.26	1.82	25.92	48.91
45546	2.09	5.35	0.64	8.60	2.04	2.39	2.13	1.78	25.02	47.22
45547	2.02	5.19	0.62	8.34	1.97	2.30	2.05	1.67	24.16	45.79
45548	2.11	5.43	0.66	8.92	2.04	2.40	2.17	1.74	25.47	48.05
45549	2.02	5.24	0.61	8.46	1.98	2.21	2.09	1.62	24.23	45.86
45550	1.95	5.03	0.61	8.10	1.92	2.19	2.03	1.62	23.45	44.26
45551	2.05	5.33	0.64	8.61	2.07	2.36	2.21	1.66	24.93	46.77
45552	1.98	5.22	0.61	8.31	1.99	2.27	2.06	1.69	24.13	45.43
45553	2.14	5.66	0.64	9.11	2.12	2.51	2.27	1.74	26.19	49.33
45554	2.10	5.53	0.64	8.88	2.08	2.45	2.21	1.75	25.64	48.26

[a]Monogastric Nutrition Laboratory, Division of Nutritional Sciences, University of Illinois at Urban-Champaign. Results are expressed on an "as is" basis unless otherwise indicated (Lagos & Stein, 2017).

Qiu et al., 2018). Although SBM is available worldwide and widely used in shrimp and fish diet formulations, information on the complete nutritional profile of SBM sourced from different locations is limited and effects of differences in nutritional profile on production performances of shrimps or fish is not known. Palmer, Hymowitz and Nelson (1996), Verma and Shoemaker (1996) and Van Kempen et al. (2002) indicated that the location of production could affect the growth characteristics, yield and nutritional value of SBM because of genetic variability among soybeans, which are used to make the meal. Furthermore, it is clear that the processing methods and conditions such as processing temperature, time and moisture content may add variation to the final nutritional quality of SBM (Balloun, 1980; Van Kempen et al., 2002).

In practical applications, a clear understanding about effects of variation among sources of SBM on growth of shrimps is needed. With the objective of filling research gaps, the current study, investigated the effect of different SBM sourced from different geographical locations in the world on the growth performance of pacific white shrimps (*L. vannamei*).

2 | MATERIALS AND METHODS

2.1 | Diet preparation

Twenty-four sources of solvent extracted soybean meal (SBM) along with data for proximate composition, indispensable and dispensable amino acid profiles, sugars (fructose, sucrose, raffinose, stachyose, etc.), fibres (acid detergent fiber (ADF), neutral detergent fibre (NDF) and lignin), macro minerals and micro minerals (Tables 1–5) for each source were obtained from the Monogastric Nutrition Laboratory, Division of Nutritional Sciences, University of Illinois at Urban-Champaign (Lagos & Stein, 2017). Each source of SBM was fed to Pacific white shrimp and the hypothesis that the growth performances of Pacific white shrimp could be predicted

TABLE 4 Percentage composition of sugars & fibre[a] of the different Soybean meal used in diets of Pacific white shrimp, *Litopenaeus vannamei*

Soybean meal sample key	Sugars, %						Fibre, %		
	Fructose	Glucose	Sucrose	Maltose	Raffinose	Stachyose	ADF	NDF	Lignin
AU Soy									
45531	0.07	0.00	8.87	0.00	1.16	5.51	7.17	11.92	0.24
45532	0.07	0.00	9.54	0.00	1.12	5.75	4.37	7.79	0.07
45533	0.07	0.00	9.07	0.00	1.24	5.59	5.44	9.03	0.25
45534	0.07	0.00	8.97	0.00	1.13	5.66	5.85	9.94	0.21
45535	0.07	0.00	8.90	0.00	1.33	5.72	5.65	9.41	0.17
45536	0.06	0.00	8.05	0.00	1.34	5.50	3.3	6.27	0.08
45537	0.07	0.00	7.87	0.00	1.44	5.66	3.84	7.12	0.81
45538	0.12	0.07	7.50	0.00	1.66	4.77	4.41	9.37	0.28
45539	0.06	0.00	8.12	0.00	1.41	5.58	3.21	6.36	0.17
45540	0.07	0.00	6.77	0.00	1.60	4.96	3.92	7.28	1.14
45541	0.07	0.00	4.86	0.00	1.48	4.08	7.66	12.44	0.74
45542	0.08	0.00	4.81	0.00	1.47	3.58	5.68	9.69	0.30
45543	0.06	0.00	6.32	0.00	1.45	4.90	4.45	8	0.16
45544	0.07	0.00	6.20	0.00	1.88	4.69	3.04	4.88	0.13
45545	0.08	0.00	5.53	0.00	1.47	5.19	4.02	7.49	0.28
45546	0.08	0.00	8.29	0.00	1.93	6.46	3.39	6.72	0.09
45547	0.10	0.08	9.52	0.00	1.04	6.32	3.14	6.56	0.25
45548	0.07	0.00	8.52	0.00	1.12	6.69	3.12	6.88	0.33
45549	0.07	0.00	8.18	0.00	1.68	6.34	4.12	7.76	0.25
45550	0.06	0.00	8.71	0.00	1.51	6.72	4.74	8.49	0.09
45551	0.42	0.31	1.80	0.00	1.44	3.28	8.26	12.45	0.25
45552	0.00	0.00	5.09	0.00	2.15	5.66	6.35	10.04	0.38
45553	0.00	0.00	5.81	0.00	2.12	6.05	4.95	7.94	0.19
45554	0.00	0.00	6.10	0.00	2.23	5.43	6.18	9.58	0.20

[a]Monogastric Nutrition Laboratory, Division of Nutritional Sciences, University of Illinois at Urban-ChampaignResults are expressed on an "as is" basis unless otherwise indicated (Lagos & Stein, 2017).

from the nutrition profile of SBM was tested. Twenty-five soy-based grow-out diets were formulated to be iso-nitrogenous and iso-lipidic (350 g/kg protein and 80 g/kg lipid). Twenty-four of the diets contained the aforementioned SBM from Illinois and a reference diet (Diet 1) was prepared using a local SBM (Tables 6–8). The test diets were prepared in the feed laboratory at Auburn University, Auburn, AL, USA, using standard practices. Pre-ground dry ingredients and oil were weighted and mixed in a food mixer (Hobart Corporation, Troy, OH, USA) 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 were analysed for proximate composition, amino acid profile, pepsin digestibility and trypsin inhibitor levels at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA) (Tables 9–11).

2.2 | Culture system

The semi-closed recirculation system used for growth trials consisted of a series of 60-L aquaria connected to a common reservoir tank (800-L). Water quality was maintained by recirculation through an Aquadine bead filter (0.2 m^2 media, 0.6 m × 1.1 m) and a vertical fluidized bed biological filter (600-L volume with 200-L of Kaldnes media) using a 0.25-hp centrifugal pump. Mean water flow for an aquarium was 3 L/min with an average turnover of 20 min/tank. Salt water was prepared by mixing artificial crystal sea salt (Crystal Sea Marinemix, Baltimore, MD, USA) with freshwater and maintained at around 7 ppt during the each growth trial. Aquariums were covered with styrofoam sheets during the each growth trial (except during the weekly counting) to avoid any possible variation could cause due to different light conditions. Dissolved oxygen 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 was measured twice daily using a YSI-55 digital oxygen/temperature meter (YSI corporation, Yellow Springs, Ohio, USA), and total ammonia N (TAN) and nitrite-N were measured twice per week according to the methods described by Solorzano (1969) and Spotte (1979) respectively. The pH of the water was measured two times per week during the experimental period using the pHTestr30 (Oakton Instrument, Vernon Hills, IL, USA). All water quality parameters measured during the study are presented in Table 12.

2.3 | Growth trials

Dietary treatments were randomly assigned to tanks and each trial was conducted using a double-blind experimental design. Growth trials were conducted in two phases. The first growth trial was conducted with 14 treatments and 4 replicates for diets 2–14, whereas 8 replicates were assigned to the control diet (Diet 1). Twelve treatments were tested during the second growth trial, each with five replicates including the control diet and diets 15–25. In each trial, 10 shrimp were stocked per tank with an average initial weight of 0.23 ± 0.02 g in trial one and 0.67 ± 0.02 g in trial two. Shrimp were offered test diets four times daily. The daily ration of feed was calculated based on an estimated weight gain from previous trials and expected feed conversion ratio (FCR) of 1.8. Shrimp were counted weekly and the feed was adjusted each week based on survival and observations of feeding responses of shrimp. Growth trial-1 was conducted for 6-weeks, whereas trial-2 was conducted for 5 weeks. At the conclusion, shrimp were counted and group-weighed. The average final weight, final biomass, percent survival and feed conversion ratio were determined.

2.4 | Statistical analysis

All data were analysed using SAS (V9.3. SAS Institute, Cary, NC, USA). Data from individual growth trials were analyzed separately using one-way ANOVA followed by Tukey 5 pairwise comparison test to evaluate significant differences ($p < 0.05$) among treatment means (Tables 13 and 14). The thermal Growth Coefficient (TGC) for the shrimp was calculated with the objective of combining the growth data from trial 1 and 2. The TGC values of different SBM were standardized by calculating the "percentage TGC" reference to the TGC of the control diet for that trail. Standardized TGC values were analysed using one-way ANOVA followed by Tukey pairwise comparison test to evaluate significant differences among treatment means (Table 15; Figure 1). With the objective of reducing the dimensions and grouping different SBM sources, Principle component analysis (PCA) and a Cluster analysis was performed using the chemical characteristics of SBM (Table 16; Figure 2). For the PCA and Cluster analysis, the entire data set was standardized by calculating *z* scores (standard scores) to avoid the different units and scales of measurements while some of the variables, which were balanced during the formulations (such as protein and its direct relatives), were excluded from the analysis. Furthermore, ingredient data of SBM were adjusted based on the inclusion ratio as the diets were formulated to be iso-nitrogenous by adjusting the SBM inclusion in the diet. Multiple linear regression was performed to identify the relationships between TGC with principle components selected from PCA (Table 17). A correlation coefficient analysis was conducted to identify the relationships between TGC and major variables representing the principle components, which had a significant impact on TGC (Table 18).

3 | RESULTS

3.1 | Growth performances

At the conclusion of the culture period of trial one, no significant differences were detected in final average weight, weight gain, percentage weight gain and TGC among shrimp fed the different diets, whereas FCR differed ($p < 0.05$) among diets (Table 13). Diet-8, which contained SBM45537 resulted in the numerically largest FCR (1.97), whereas the lowest FCR was recorded from diets 4 and 5 with FCR values of 1.60 and 1.64 respectively. Survival, final weight and weight gain ranged from 80% to 98%, 5.1 to 5.9 g, and 4.8 to 5.7 g respectively. At the end of trial two, differences ($p < 0.05$) were detected for final average weight, weight gain, percentage weight gain, survival and TGC for shrimp fed experimental diets (Table 14). Diet-21, which contained SBM45550 resulted in the largest numerical values for final average growth, weight gain, and percentage weight gain respectively, with 6.33 g, 5.66 g and 851%. According to the statistical analysis among percentage TGC values of all the experimental SBM, differences ($p < 0.05$) were observed among the sources of SBM (Table 15, Figure 1). Diet 21, which contained SBM45550 resulted in the largest value for TGC, whereas the lowest value for TGC was noted from diet 17, which contained SBM45536.

3.2 | Grouping information based on Cluster analysis

According to the dendrogram generated through the cluster analysis, the 24 sources of SBM were separated in to five major groups, which were clearly observed in the score plot of PCA as well (Figure 2). The SBM used in diets 2–11 and in diets 14–19 were grouped together, whereas SBM used in diets 12, 13, 23, 24, 25 were clustered into another group. Three individual points were observed for the SBM used in diets 20, 21 and 22.

3.3 | Principle component analysis

The PCA of chemical characteristics of SBM sources and their loadings are presented in Table 16. Collectively, the first five PCs explained 83% of the total sample variance. According to the loading values, PC1 was represented by sucrose (−0.31) and iron (0.33) and PC2 was represented by sodium (0.42), sulphur (0.38), non-phytate phosphorus (0.37), zinc (0.31), and phosphorus (0.29). Phosphorus

TABLE 5 Composition of minerals[a] in the different Soybean meal used in diets of Pacific white shrimp, *Litopenaeus vannamei*

	Minerals																
Soybean meal sample key	Ca, %	P, %	P in PA, %	Total PA, %	Non-phytate P, %	Cr, ppm	Cobalt, ppm	Cu, ppm	Fe, ppm	Mg, %	Mn, ppm	Molybdenum, ppm	K, %	Se, ppm	Na, ppm	S, %	Zn, ppm
AU Soy	0.32	0.64						9.7		0.24							46.8
45531	0.20	0.66	0.52	1.85	0.14	19.8	<0.2	7.74	120	0.25	31.1	2.72	2.08	<4	9.45	0.42	44.6
45532	0.18	0.70	0.54	1.9	0.17	<0.1	<0.2	7.96	114	0.25	33.2	3.24	2.07	<4	7.80	0.43	45.3
45533	0.18	0.68	0.55	1.96	0.13	<0.1	<0.2	7.41	105	0.25	31.2	2.23	2.13	<4	5.32	0.42	44.2
45534	0.18	0.70	0.55	1.96	0.15	<0.1	<0.2	7.65	111	0.25	31.3	2.38	2.08	<4	5.32	0.43	44.5
45535	0.19	0.69	0.54	1.9	0.15	<0.1	<0.2	7.38	106	0.25	30.9	2.54	2.07	<4	<0.2	0.42	43.5
45536	0.25	0.68	0.53	1.87	0.15	2.41	<0.2	11.3	90.3	0.28	44.9	9.93	2.30	<4	4.64	0.42	41.3
45537	0.24	0.67	0.52	1.86	0.15	<0.1	<0.2	11.3	78.5	0.28	41.3	8.24	2.28	<4	2.27	0.41	40.7
45538	0.26	0.69	0.50	1.77	0.19	<0.1	<0.2	11.4	130	0.30	42.5	7.90	2.30	<4	117	0.41	43.0
45539	0.24	0.70	0.53	1.89	0.17	<0.1	<0.2	11.0	68.1	0.29	39.8	8.42	2.31	<4	6.38	0.42	39.6
45540	0.29	0.63	0.45	1.58	0.18	<0.1	<0.2	11.7	105	0.29	38.1	6.64	2.25	<4	11.6	0.40	45.4
45541	0.28	0.61	0.43	1.53	0.18	<0.1	<0.2	8.22	172	0.32	26.9	4.14	2.11	<4	7.67	0.39	50.3
45542	0.32	0.59	0.40	1.42	0.19	<0.1	<0.2	10.5	256	0.30	34.8	2.42	2.08	<4	43.8	0.42	50.9
45543	0.28	0.62	0.46	1.62	0.16	<0.1	<0.2	7.42	141	0.32	30.5	5.34	2.27	<4	<0.2	0.43	49.3
45544	0.30	0.64	0.46	1.64	0.17	<0.1	<0.2	9.49	79.5	0.31	27.8	4.15	2.20	<4	19.5	0.42	49.0
45545	0.33	0.65	0.47	1.67	0.18	<0.1	<0.2	9.74	110	0.33	29.0	3.73	2.17	<4	2.97	0.43	53.9
45546	0.32	0.64	0.47	1.67	0.17	<0.1	<0.2	10.6	82.8	0.27	31.5	2.76	2.20	<4	3.55	0.43	41.0
45547	0.24	0.63	0.47	1.65	0.16	<0.1	<0.2	12.5	101	0.28	39.4	3.49	2.12	<4	53.6	0.43	47.1
45548	0.26	0.61	0.43	1.54	0.17	<0.1	<0.2	11.6	109	0.26	26.7	11.5	2.15	<4	8.66	0.44	48.1
45549	0.57	0.64	0.45	1.58	0.20	<0.1	<0.2	44.1	167	0.28	61.3	4.13	2.14	<4	371	0.42	153
45550	0.48	0.81	0.51	1.80	0.30	<0.1	<0.2	14.8	331	0.42	71.2	2.96	2.17	<4	1470	0.52	97.1
45551	0.53	0.61	0.44	1.57	0.17	<0.1	<0.2	14.1	1590	0.35	78.0	0.187	2.01	<4	22.6	0.41	54.7
45552	0.43	0.59	0.43	1.54	0.16	<0.1	<0.2	15.0	713	0.34	48.2	2.03	2.00	<4	12.2	0.40	56.3
45553	0.34	0.57	0.41	1.45	0.16	<0.1	<0.2	16.2	395	0.32	46.3	3.29	2.03	<4	9.59	0.43	59.2
45554	0.35	0.60	0.43	1.52	0.18	<0.1	<0.2	17.0	695	0.34	53.7	1.88	2.07	<4	11.1	0.43	58.9

[a]Monogastric Nutrition Laboratory, Division of Nutritional Sciences, University of Illinois at Urban-Champaign. Results are expressed on an "as is" basis unless otherwise indicated (Lagos & Stein, 2017).

TABLE 6 Codes for different soybean meal used in the experiment

Diet number	Ingredient code	Diet number	Ingredient code
1	AU Soy	14	45543
2	45531	15	45544
3	45532	16	45545
4	45533	17	45546
5	45534	18	45547
6	45535	19	45548
7	45536	20	45549
8	45537	21	45550
9	45538	22	45551
10	45539	23	45552
11	45540	24	45553
12	45541	25	45554
13	45542		

TABLE 7 Composition (% as is) of the basal diets used in the growth trials

Ingredient (As basis g/kg feed)	Basal diet for growth trial
Fishmeal[b]	6.00
Soybean meal[c]	51.70[a]
Corn protein concentrate[d]	7.00
Menhaden fish oil[b]	5.76[a]
Lecithin[e]	1.00
Cholesterol[f]	0.05
Whole wheat[g]	23.0
Corn Starch[f]	0.39[a]
Mineral premix[h]	0.50
Vitamin premix[i]	1.80
Choline chloride[j]	0.20
Stay C 35% active[k]	0.10
CaP-dibasic[j]	2.50

[a]See Table 8 for adjustments for test diets. [b]Omega Protein, Houston, TX, USA. [c]De-hulled Solvent Extracted Soybean Meal, Bunge Limited, Decatur, AL, USA. [d]Empyreal® 75, Cargill Corn Milling, Cargill, Blair, NE, USA. [e]The Solae Company, St. Louis, MO, USA. [f]MP Biomedicals, Solon, OH, USA. [g]Bob's red mill, Milwaukie, OR, USA. [h]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. [i]Vitamin premix (g/kg premix): Thiamin 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. [j]VWR Amresco, Suwanee, GA, USA. [k]Stay-C® (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins, Parsippany, NJ, USA.

in phytic acid (0.35), total phytic acid (0.35), ADF (0.29), NDF (0.31), fructose (0.31), phosphorus (0.30) and raffinose (-0.30) were the components in PC3.

3.4 | Multiple linear regression

The results of multiple linear regression of TGC on the first five PCs are presented in Table 17. The *p*-value for the entire model was less than 0.05, but only PC2 and PC3 had positive ($p < 0.05$) impacts on TGC. Combining the results of PCA and multiple linear repression, it was concluded that the phosphorus, non-phytate phosphorus, sodium, sulphur, zinc, phosphorus in phytic acid, total phytic acid, fructose, ADF and NDF has a positive attribute for TGC, whereas raffinose has a negative impact on TGC.

3.5 | Pearson correlation coefficients

Pearson correlation coefficients of TGC with raffinose, ADF, NDF, phosphorus, phosphorus in phytic acid, total phytic acid, non-phytate phosphorus, sodium, sulphur and zinc are presented in Table 18. Only phosphorus, phosphorus in phytic acid and total phytic acid levels were positively correlated with TGC, whereas raffinose ($p = 0.086$) appeared as the only negative correlation with TGC of the selected variables representing PC2 and PC3.

Except for the variables from PCA, Pearson correlation coefficients were calculated for the protein level of SBM, pepsin digestibility and trypsin activity of diets against the TGC of shrimps. A negative correlation was detected with protein in SBM ($p = 0.001$, $R^2 = 0.37$) and a positive correlation was observed with trypsin inhibitor level in diets ($p = 0.042$, $R^2 = 0.18$). There tended to be a negative correlation with pepsin digestibility of diets against TGC ($p = 0.152$, $R^2 = 0.09$), and a positive correlation was observed with SBM inclusion level in the diet ($p = 0.001$, $R^2 = 0.40$).

4 | DISCUSSION

Historically, fishmeal has been the primary protein source used in shrimp feed formulations. However, as the aquaculture industry expands so does demand resulting in increases in the price of fish meal, which then results in reduced concentrations of protein in the diets and use of alternative protein sources (Davis, Roy & Sookying, 2008). Hardy (2010) argued that the fish meal demands for the production of feed may eventually exceed the world production of fish meal based on the expected growth rates of aquaculture and rates of fish meal utilization. As an alternative to the use of fish meal in fish feed formulations, a variety of plant-based dietary ingredients have been tested (NRC 2011). Soybean meal attracted most of the attention due to its comparable amino acid profile, worldwide availability, low price and consistent composition (Amaya et al., 2007b; Davis & Arnold, 2000; Dersjant-Li, 2002).

TABLE 8 Basal diet ingredient modification (g/100g as is) to create the test diets. All other ingredients are the same as that of the basal diet (Table 1)

Diet #	Soybean meal	Corn starch	Fish oil	Diet #	Soybean meal	Corn starch	Fish oil
2	49.30	2.87	5.68	14	44.30	7.62	5.93
3	48.70	3.59	5.56	15	45.00	7.69	5.16
4	49.80	2.44	5.61	16	44.30	7.94	5.61
5	49.40	2.69	5.76	17	47.40	4.88	5.57
6	49.30	2.78	5.77	18	47.30	5.14	5.41
7	47.60	4.36	5.89	19	45.30	7.15	5.40
8	48.50	3.73	5.62	20	48.10	4.14	5.61
9	47.30	4.9	5.65	21	49.80	2.5	5.55
10	47.90	4.31	5.64	22	47.10	5.14	5.61
11	47.70	5.5	4.65	23	46.10	6.29	5.46
12	47.80	4.44	5.61	24	44.40	7.54	5.91
13	47.10	5.45	5.30	25	44.40	7.44	6.01

TABLE 9 Chemical analysis[a] (proximate composition, pepsin digestibility and trypsin inhibitors) of different diets fed to the Pacific white shrimp, *Litopenaeus vannamei*

Diet	Crude protein	Moisture	Crude fat	Crude fiber	Ash	Pepsin digestibility	Trypsin inhibitor/TIU/g
1	36.41	10.41	8.27	4.29	6.63	93.65	1924
2	34.10	11.88	7.83	4.62	6.16	93.52	1034
3	34.34	9.09	7.94	5.20	6.41	90.29	1036
4	35.40	8.10	7.25	4.77	6.43	93.31	849
5	35.93	7.23	7.06	4.77	6.80	93.27	1085
6	35.85	7.05	11.11	4.97	6.63	90.60	1087
7	35.07	8.85	13.17	3.98	6.57	92.54	1129
8	35.21	9.23	10.58	3.85	6.56	93.40	1167
9	36.45	6.90	8.21	3.85	6.76	94.17	1041
10	36.53	6.20	8.81	3.30	6.70	93.83	535
11	36.35	6.43	7.89	3.80	6.64	94.25	524
12	36.66	6.10	8.05	5.09	6.66	94.57	738
13	36.45	6.24	10.46	4.25	6.51	94.13	842
14	36.56	6.30	11.75	3.61	6.55	93.97	819
15	36.46	6.02	16.41	3.25	6.45	95.25	861
16	36.95	6.30	13.37	3.49	6.46	92.80	303
17	36.37	6.78	6.60	3.71	6.53	94.97	284
18	36.01	7.92	8.43	3.93	6.36	95.86	435
19	36.20	6.99	8.62	3.22	6.28	94.57	625
20	36.35	6.83	8.67	3.89	6.77	95.78	767
21	36.43	6.65	8.74	4.27	7.40	93.97	821
22	36.41	6.51	7.29	6.47	7.68	91.96	1455
23	36.23	7.06	9.75	4.63	6.74	94.30	1059
24	36.32	7.07	13.37	4.07	6.36	94.03	887
25	36.58	5.62	9.22	4.19	6.56	94.51	867

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

TABLE 10 Indispensable Amino acid profile[a] (as is basis) of the different Soybean meal used in diets of Pacific white shrimp, *Litopenaeus vannamei*

Diet	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophan	Valine	Total
1	2.24	0.98	1.60	3.18	2.00	0.60	1.92	1.33	0.47	1.71	16.03
2	2.09	0.91	1.45	2.87	1.87	0.56	1.76	1.18	0.43	1.54	14.66
3	2.12	0.93	1.51	2.94	1.92	0.57	1.78	1.19	0.46	1.62	15.04
4	2.18	0.88	1.55	3.01	1.93	0.59	1.78	1.24	0.46	1.68	15.30
5	2.17	0.92	1.56	3.09	1.96	0.60	1.82	1.28	0.46	1.67	15.53
6	2.22	0.98	1.57	3.05	2.01	0.60	1.85	1.29	0.50	1.69	15.76
7	2.10	0.88	1.58	3.06	1.92	0.61	1.80	1.32	0.49	1.71	15.47
8	2.09	0.96	1.57	3.06	1.96	0.60	1.86	1.31	0.47	1.68	15.56
9	2.21	0.99	1.60	3.16	1.99	0.60	1.92	1.30	0.49	1.73	15.99
10	2.19	0.99	1.62	3.14	2.06	0.63	1.92	1.37	0.51	1.74	16.17
11	2.14	0.90	1.60	3.16	1.95	0.60	1.86	1.36	0.49	1.72	15.78
12	2.14	0.92	1.61	3.12	1.96	0.60	1.89	1.34	0.50	1.70	15.78
13	2.19	0.98	1.66	3.17	2.01	0.60	1.93	1.28	0.49	1.76	16.07
14	2.24	0.99	1.68	3.21	2.03	0.59	1.97	1.30	0.49	1.79	16.29
15	2.19	0.94	1.68	3.25	1.97	0.61	1.94	1.31	0.48	1.78	16.15
16	2.22	0.99	1.70	3.32	2.00	0.58	2.01	1.31	0.46	1.78	16.37
17	2.17	0.98	1.63	3.15	2.03	0.62	1.91	1.35	0.51	1.73	16.08
18	2.18	0.98	1.59	3.11	2.00	0.60	1.88	1.32	0.47	1.69	15.82
19	2.18	0.95	1.57	3.16	1.99	0.61	1.87	1.35	0.50	1.68	15.86
20	2.18	0.90	1.60	3.18	1.95	0.59	1.86	1.32	0.48	1.71	15.77
21	2.18	0.98	1.58	3.14	2.03	0.59	1.90	1.31	0.51	1.69	15.91
22	2.13	0.94	1.64	3.20	1.93	0.61	1.85	1.32	0.46	1.76	15.84
23	2.16	0.97	1.62	3.12	1.99	0.60	1.88	1.29	0.49	1.73	15.85
24	2.22	0.98	1.65	3.23	2.00	0.60	1.93	1.29	0.49	1.77	16.16
25	2.25	1.00	1.64	3.19	2.02	0.60	1.93	1.33	0.49	1.75	16.20

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

TABLE 11 Dispensable Amino acid profile[a] (as is basis) of the different soybean meal used in diets of Pacific white shrimp, *Litopenaeus vannamei*

Diet	Alanine	Aspartic acid	Cysteine	Glutamic acid	Glycine	Proline	Serine	Taurine	Hydroxy-proline	Tyrosine	Hydroxy-lysine	Total
1	1.81	3.45	0.52	6.71	1.57	2.20	1.52	0.16	0.07	1.41	0.20	19.62
2	1.64	3.03	0.49	6.04	1.45	1.73	1.36	0.15	0.09	1.27	0.21	17.46
3	1.71	3.13	0.51	6.27	1.52	1.83	1.37	0.17	0.12	1.26	0.19	18.08
4	1.77	3.27	0.53	6.49	1.59	1.89	1.43	0.17	0.12	1.27	0.10	18.63
5	1.82	3.31	0.54	6.77	1.57	1.96	1.59	0.17	0.14	1.29	0.13	19.35
6	1.77	3.35	0.54	6.56	1.57	2.05	1.47	0.18	0.09	1.32	0.19	19.09
7	1.78	3.33	0.53	6.53	1.55	1.92	1.48	0.17	0.07	1.31	0.11	18.78
8	1.78	3.30	0.51	6.48	1.56	2.09	1.48	0.16	0.08	1.30	0.20	19.00
9	1.83	3.34	0.51	6.69	1.60	1.98	1.51	0.18	0.11	1.38	0.20	19.40
10	1.83	3.44	0.53	6.72	1.57	2.15	1.57	0.17	0.09	1.35	0.20	19.68
11	1.83	3.40	0.49	6.72	1.55	2.11	1.55	0.18	0.07	1.34	0.12	19.36
12	1.80	3.43	0.52	6.65	1.61	1.97	1.56	0.17	0.11	1.34	0.17	19.33
13	1.83	3.37	0.52	6.68	1.61	2.17	1.46	0.17	0.09	1.36	0.21	19.47
14	1.86	3.44	0.52	6.81	1.62	2.04	1.49	0.18	0.09	1.39	0.20	19.64
15	1.80	3.43	0.51	6.85	1.49	2.08	1.51	0.17	0.09	1.38	0.15	19.46
16	1.88	3.48	0.50	6.90	1.58	2.09	1.54	0.18	0.07	1.44	0.20	19.86
17	1.82	3.49	0.53	6.71	1.60	2.41	1.52	0.18	0.09	1.36	0.20	19.98
18	1.79	3.36	0.52	6.61	1.57	1.97	1.52	0.18	0.08	1.35	0.19	19.14
19	1.85	3.46	0.54	6.91	1.60	2.01	1.64	0.18	0.10	1.35	0.13	19.81
20	1.82	3.36	0.48	6.74	1.59	1.97	1.53	0.17	0.09	1.36	0.12	19.23
21	1.81	3.32	0.52	6.64	1.58	2.01	1.54	0.18	0.09	1.36	0.20	19.25
22	1.83	3.41	0.52	6.73	1.62	1.98	1.48	0.20	0.11	1.34	0.08	19.33
23	1.78	3.41	0.50	6.68	1.57	2.00	1.46	0.19	0.08	1.34	0.14	19.15
24	1.84	3.41	0.51	6.85	1.60	2.09	1.47	0.18	0.09	1.39	0.17	19.60
25	1.82	3.44	0.52	6.82	1.59	2.03	1.50	0.19	0.08	1.39	0.19	19.57

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

TABLE 12 Water quality data (mean ± *SD*[a]) of the growth trials, 1 and 2

	Trial 1	Trial 2
Dissolved oxygen (mg/L)	6.02 ± 0.89	6.78 ± 0.30
Salinity (ppt)	7.51 ± 0.37	7.20 ± 0.49
Temperature (°C)	28.19 ± 2.04	29.53 ± 0.60
pH	7.48 ± 0.48	7.45 ± 0.52
TAN[b] (mg/L)	0.11 ± 0.05	0.12 ± 0.08
Nitrite (mg/L)	0.07 ± 0.02	0.13 ± 0.02

[a]*SD* = Standard Deviation. [b]TAN = Total Ammonia Nitrogen.

SBM is available worldwide and is used as a primary protein source in shrimp and fish diet formulations, but information about the complete nutritional profile of SBM sourced from different locations is scarce and there is no information on how differences among sources of SBM may affect the production performance of shrimps or fish. Palmer et al. (1996), Verma and Shoemaker (1996) and Van Kempen et al. (2002) clearly stated that the location of production may affect the growth characteristics, yield and nutritional value. Maestri et al. (1998) observed negative correlations between protein and oil contents in soybeans and total precipitation during the growing season in Argentina, whereas neither protein content nor fatty acid composition were affected by temperatures during seed maturation at production locations. The protein content of soybeans is inversely correlated with latitude, and a positive correlation between protein and oil contents in soybeans and growing altitude was observed (Maestri et al., 1998). A study conducted by Van Kempen et al. (2002) revealed that SBM collected from four regions within the United States varied a little in nutrient quality compared with SBM sampled from the Netherlands, which had reduced amino acid content causing negative effects on digestibility of amino acids by pigs. Therefore, there is evidence indicating that variations in nutrient quality of soybeans grown in different environmental conditions in different geographical locations (Natarajan et al., 2016) may also result in differences in the production performances of shrimps or fish.

Protein content of the SBM sources used in the present study was in the range of 44% to 51% and the 24 sources of SBM were separated into five major groups based on the complete chemical profile through the cluster analysis, which was also indicated from the PCA. The limited groupings are in part due to the narrow variations and homogeneous chemical characteristics of the ingredients as well as specifications used in sourcing the materials. The three individual points that were observed for the SBM used in diet 20, diet 21 and diet 22 is likely due to the elevated levels of Cu, Na and Fe in these meals compared with the other sources of SBM.

Differences in growth performances were not clearly overlaid through the SBM cluster analysis. However, the SBM used in diet 21, which was different from the other sources of SBM, resulted in the best growth of shrimp. No biological responses were observed in

TABLE 13 Response of juvenile shrimp (0.23 ± 0.02 g) fed with diets contained different sources of soybean meal over a 6-weeks experimental period (Trial 1). Values represented the mean of eight replicates for the basal diets and four replicates for the rest

Trt.	Final mean weight (g)	Weight gain (g)	Weight gain (%)	FCR	Survival (%)	TGC
1	5.69	5.46	2302	1.73^{ab}	85.0	0.098
2	5.78	5.54	2283	1.70^{ab}	90.0	0.099
3	5.54	5.31	2269	1.73^{ab}	90.0	0.097
4	5.94	5.71	2458	1.60^{b}	87.5	0.101
5	5.71	5.50	2602	1.64^{b}	85.0	0.101
6	5.61	5.38	2365	1.68^{ab}	85.0	0.098
7	5.58	5.36	2466	1.69^{ab}	95.0	0.099
8	5.06	4.84	2210	1.97^{a}	80.0	0.094
9	5.28	5.05	2231	1.78^{ab}	82.5	0.095
10	5.34	5.10	2152	1.73^{ab}	92.5	0.095
11	5.62	5.39	2371	1.71^{ab}	80.0	0.099
12	5.18	4.96	2259	1.75^{ab}	97.5	0.095
13	5.42	5.19	2290	1.70^{ab}	90.0	0.097
14	5.23	4.99	2165	1.80^{ab}	85.0	0.095
PSE	0.39	0.38	217.65	0.13	7.94	0.003
p-value	0.07	0.07	0.23	0.06	0.07	0.067

Values with different superscripts within the same column are significantly different based on Tukey Pairwise Comparisons.
Feed conversion ratio (FCR): feed offered/(final weight-initial weight).
Weight gain: (final weight-initial weight)/initial weight × 100%.
Thermal Growth Coefficient (TGC): (Final weight$^{1/3}$ − Intial weight$^{1/3}$)/Σ(Temp × days) × 1,000.
PSE: Pooled standard error.

TABLE 14 Response of juvenile shrimp (0.67 ± 0.02 g) fed with diets contained different sources of soybean meal over a 5-weeks experimental period (Trial 2). Values represented the mean of five replicates

Trt.	Final mean weight (g)	Weight gain (g)	Weight gain (%)	FCR	Survival (%)	TGC
1	6.07^{ab}	5.40^{ab}	811^{ab}	1.86^{ab}	86^{ab}	0.092^{ab}
15	5.53^{b}	4.86^{b}	731^{ab}	1.93^{ab}	92^{ab}	0.087^{ab}
16	5.36^{b}	4.70^{b}	712.2^{b}	2.02^{a}	96^{a}	0.085^{b}
17	5.44^{b}	4.76^{b}	697^{b}	2.04^{a}	90^{ab}	0.085^{b}
18	5.52^{b}	4.85^{b}	717^{b}	1.97^{ab}	96^{a}	0.086^{b}
19	6.02^{ab}	5.36^{ab}	807^{ab}	1.81^{ab}	88^{ab}	0.092^{ab}
20	5.97^{ab}	5.31^{ab}	807^{ab}	1.79^{ab}	96^{a}	0.091^{ab}
21	6.33^{a}	5.66^{a}	851^{a}	1.67^{b}	92^{ab}	0.095^{a}
22	5.89^{ab}	5.20^{ab}	749^{ab}	1.84^{ab}	90^{ab}	0.089^{ab}
23	6.08^{ab}	5.39^{ab}	791^{ab}	1.77^{ab}	92^{ab}	0.091^{ab}
24	5.85^{ab}	5.17^{ab}	764^{ab}	1.84^{ab}	92^{ab}	0.089^{ab}
25	5.55^{ab}	4.86^{b}	707^{b}	1.99^{a}	80^{b}	0.086^{b}
PSE	0.37	0.37	60.08	0.14	7.19	0.004
p-value	0.001	0.001	0.001	0.002	0.041	0.001

Values with different superscripts within the same column are significantly different based on Tukey Pairwise Comparisons. PSE: Pooled standard error.

TABLE 15 Total growth coefficients (TGC) of juvenile shrimp (as a percentage from TGC of basal diet) fed with diets contained different sources of soybean meal (Trial 1 & 2 combined data). PSE = 3.87 and *p*-value < 0.001

Trt.	TGC	Trt.	TGC
2	100.42^{abcd}	14	96.08^{abcd}
3	98.885^{abcd}	15	94.08^{abcd}
4	102.57^{ab}	16	92.45^{cd}
5	102.16^{abc}	17	92.34^{d}
6	99.94^{abcd}	18	93.67^{abcd}
7	100.43^{abcd}	19	99.62^{abcd}
8	95.39^{abcd}	20	99.27^{abcd}
9	96.97^{abcd}	21	102.74^{a}
10	96.57^{abcd}	22	96.9^{abcd}
11	100.11^{abcd}	23	99.36^{abcd}
12	96.49^{abcd}	24	97.14^{abcd}
13	98.3^{abcd}	25	93.4^{bcd}

Values with different superscripts are significantly different based on Tukey Pairwise Comparisons.

shrimp for trypsin inhibitor level and pepsin digestibility of the diets or protein content of SBM (along with dispensable and indispensable amino acids of SBM), emphasizing the importance of considering the complete nutritional profile of an ingredient rather than individual variables (Francis, Makkar & Becker, 2001). Biological responses to various meals are likely due to their combined interactions of nutrient level, digestion and absorption. The observed positive correlation of inclusion level on growth performances infer the augmented positive impact of another variable (or combination of several) in SBM which was natural occurrence during the diet formulation while balancing the protein content of the diet through inserting different SBM sources at variable levels to standardize protein. Simply put, SBM sources added to the diet in greater quantities due to their lower protein value in general performed better over SBM with higher protein value, possibly overturning the individual biological effects of protein and trypsin activity on the growth performances of pacific white shrimps. This may also point to the fact that to maintain a higher protein level the meal may go through harsher processing resulting in poorer performance.

Phosphorous is considered a critical element within the minerals required by penaeid shrimps due to its direct involvement in all energy-yielding reactions and the role as a structural material of nucleic acids, phospholipids, phosphoproteins, ATP and several key enzymes (Lovell, 1989).

According to the NRC (2011), different dietary requirements for phosphorous were mentioned for *Marsupenaeus Japonicas (Kanazawa, Teshima & Sasaki, 1984)*, *Penaeus monodon (Peñaflorida, 1999)* and *Littopenaeus vannamei (Davis, Lawrence & Gatlin, 1993b)* while most of the researchers emphasized the interaction between calcium and phosphorous due to the elevated phosphorous requirements at the presence of higher calcium levels. Therefore, an optimal Ca:P ratios were suggested for different species, such as 1:1.7 for *F. chinensis* (Li, Huang, Lou & Xu, 1986) and 1:1 for *M. japonicas (Kanazawa* et al., *1984)*.

According to Davis et al. (1993b), dietary levels of 0.5–1% and 1–2% phosphorus is required to maintain normal growth of the shrimp in the presence of 1% and 2% supplemental calcium respectively and revealed a poor growth performances at higher calcium levels. Phosphorous levels of the SBM used during the present study varied from 0.57% to 0.81% showing a positive correlation with TGC while calcium levels ranged from 0.18%

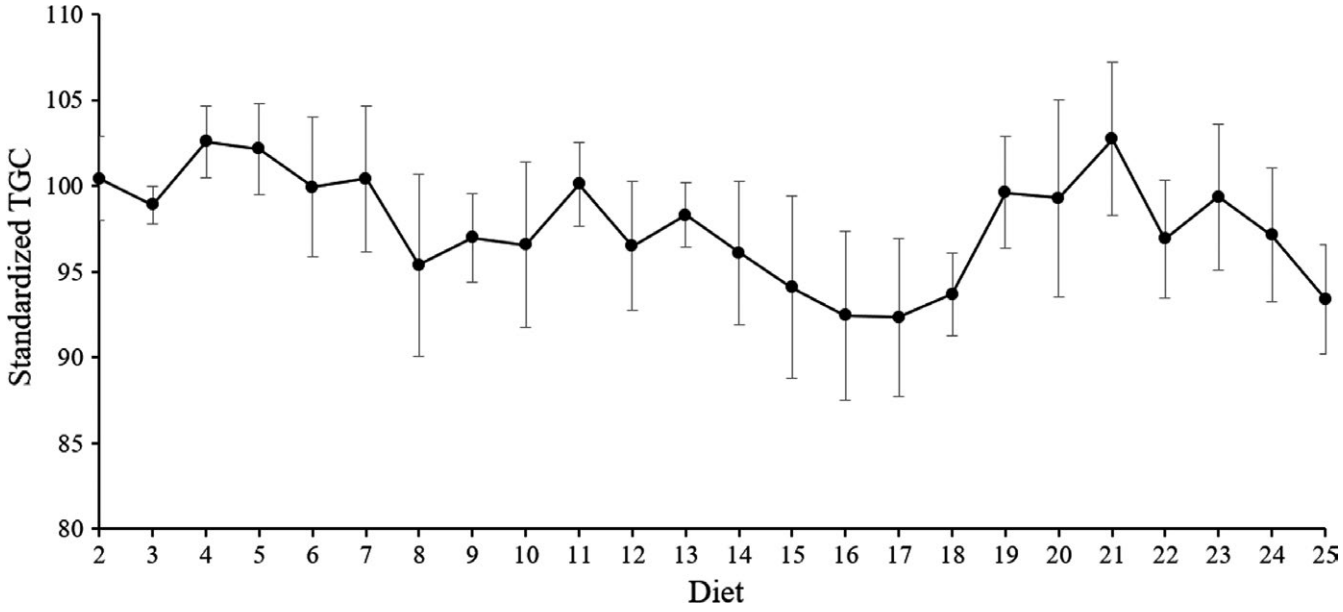

FIGURE 1 Interval plot of standardized total growth coefficients (TGC) of juvenile shrimp (as a percentage from TGC of basal diet) fed with diets contained different sources of soybean meal (Trials 1 & 2 combined data)

TABLE 16 Principle component analysis of chemical characteristics of SBM sources

Variable	PC1	PC2	PC3	PC4	PC5
Trypsin Inhibitor	0.2138	−0.0050	0.0739	−0.3508	−0.1167
Fructose	0.1786	−0.0550	0.3096	0.3782	0.1568
Glucose	0.2267	−0.0594	0.2669	0.3216	0.1670
Sucrose	−0.3112	0.1642	0.0682	−0.1364	0.2045
Raffinose	0.1874	0.0245	−0.3020	−0.1670	−0.1315
Stachyose	−0.1868	0.2506	−0.1062	−0.2097	0.2323
ADF	0.2267	−0.1123	0.2917	−0.1822	−0.0175
NDF	0.1904	−0.0955	0.3059	−0.1194	−0.0022
Lignin	0.0105	−0.1362	−0.1243	0.2744	−0.1507
Ca	0.2973	0.2157	−0.1229	0.1022	0.1329
P	−0.1727	0.2926	0.2966	0.0502	−0.1050
P in PA	−0.2487	0.0980	0.3494	−0.0185	0.0911
Total PA	−0.2468	0.0900	0.3534	−0.0176	0.0833
Non-phytate P	0.1013	0.3683	−0.0315	0.0939	−0.2835
Cu	0.1345	0.2130	−0.2365	0.0576	0.5020
Fe	0.3297	−0.0279	0.1597	0.0647	0.0656
Mg	0.2447	0.2164	−0.0286	0.0541	−0.4240
Mn	0.2428	0.2542	0.0932	0.1746	0.1493
Mo	−0.2113	0.0006	−0.1997	0.4188	−0.0452
K	−0.2281	0.0951	−0.0520	0.4057	−0.1609
Na	0.0538	0.4160	0.0612	0.0040	−0.1989
S	−0.0456	0.3789	0.1278	−0.1142	−0.1657
Zn	0.1336	0.3068	−0.1470	−0.0292	0.3467
Eigen value	7.0844	5.0033	3.2787	2.0938	1.5463
% variance	30.8	21.8	14.3	9.1	6.7
Cumulative %	30.8	52.6	66.8	75.9	82.6

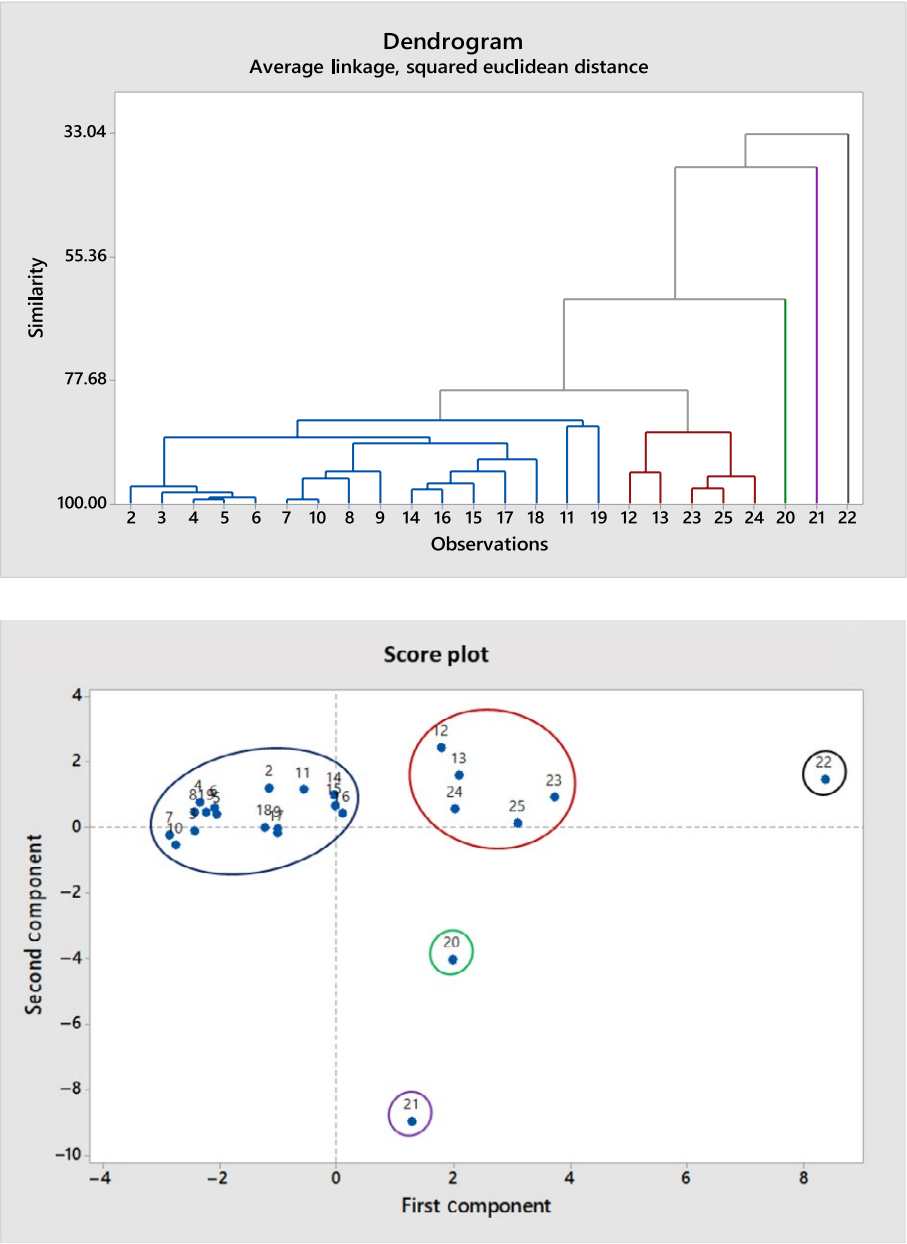

FIGURE 2 Dendrogram of Cluster analysis (grouping of SBM diets base on their chemical characteristics) and score plot of PCA (grouping of SBM diets base on their chemical characteristics over the component 1 (31% of variation) and component 2 (22% of variation) of PCA) [Colour figure can be viewed at wileyonlinelibrary.com]

to 0.57% revealing a non-significant negative trend with TGC. Ca:P ratio of the SBM used during the study ranged from 1:1.1 to 3.9 which showed a positive trend ($p = 0.097$) with TGC of shrimps.

Alhough dietary phosphorous requirement is vital in shrimp nutrition, approximately two-thirds of total phosphorus in various grains is present as phytate or inositol hexaphosphate (1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate) (Raboy, 1997) which is less digestible to monogastric animals such as fish and shrimps. In addition, phytic acid has a potential to produce indigestible complexes with minerals such as Zn^{+2}, Fe^{+2}, Fe^{+3}, Ca^{+2}, Mg^{+2}, Mn^{+2}, Cu^{+2} and protein, restricting their availability as well (Adeola & Sands, 2003; Cheryan & Rackis, 1980, Chowdhury, Martie & Bureau, 2015; Cosgrove & Irving, 1980; D'Mello, Duffus & Duffus, 1991; Denstadli, Skrede, Krogdahl, Sahlstrøm & Storebakken, 2006; Laining et al., 2010; Liener, 1989; NRC 2011). According to Francis et al. (2001) commercial SBM contains 1.0–1.5% phytate while Gatlin et al. (2007) stated the phytate fraction in SBM as 4%. Phosphorus bio-availability in SBM range from virtually nil (Riche & Brown, 1996) to 22% in the rainbow trout (Sugiura, Dong, Rathbone & Hardy, 1998). Reduced growth performance in cultured fish species such as carp, tilapia, trout and salmon due to phytate containing ingredients in the diets were well documented, attributed to various factors such as reduced mineral bioavailability, impaired protein digestibility and depressed absorption of nutrients (Francis et al., 2001; NRC 2011; Spinelli, Houle &

TABLE 17 Multiple linear regression of Thermal growth coefficient (TGC) with principle components (PC1, PC2, PC3, PC4, PC5)

Model *p*-value = 0.016 R^2 = 0.127	Parameter estimates	*p*-value for each variable
PC1	−0.1643	0.3108
PC2	0.4516	0.0195
PC3	0.5929	0.0142
PC4	−0.1286	0.6726
PC5	0.4413	0.2052

Wekell, 1983). Davis, Lawrence and Gatlin (1993a) and Qiu and Davis (2017), reported low bioavailability of phytate phosphorus to shrimp (*P. vannamei*) and emphasized the reductions in zinc bioavailability due to the effect of Phytic acid. In addition to negative effects on the growth performances of fish and shrimp, Kies, Van Hemert and Sauer (2001) and Baruah, Sahu, Pal and Debnath (2004) emphasized the potential environmental pollution due to high phosphorous concentration in the manure from animals fed with phytate-containing diets which is one of the major concerns as well. During the current study, significantly positive correlations were observed for TGC with phytic 10 acid and phytate phosphorous levels of the diets. Given the well-documented negative effects of phytate, the positive response is likely due to a correlated effect from some other variable.

According to Snyder and Kwon (1987) and Refstie, Svihus, Shearer and Storebakken (1999) raw soybeans contain approximately 100 g kg^{-1} di- and oligosaccharides including sucrose, raffinose and stachyose which some of them are indigestible due to a lack of α-galactosidases in fish and shrimps (Gatlin et al., 2007).

In fish, their negative effects may be either due to binding to bile acids, interfere with the uptake of nutrients through increasing the viscosity of the chime in the digestive tract (Refstie, Storebakken & Roem, 1998; Storebakken, Shearer & Roem, 1998). According to the present study, SBM raffinose levels were ranged from 1.04% to 2.23%, which showed a negative correlation (p = 0.086) with TGC of shrimps. Thus confirming the negative effects of raffinose as we also observed by Zhou, Davis and Buentello (2015).

Most of the studies relevant to the ANFs have been conducted using an ingredient rich in one particular factor and the observed effects have been attributed to the particular factor without considering the other anti-nutrients present in the ingredient, or interactions between them (Francis et al., 2001). For this research, holistic changes in antinutrients and nutrients occurred making it difficult to make firm conclusion about a specific culprit for the resulted growth performances of pacific white shrimp and their threshold levels in shrimp diet might be due to their interactive effects. However, based on the statistical outcomes from the present study, phosphorous, phosphorous in phytic acid and total phytic acid and Raffinose were screened with significant correlations, which could cause major effects on the growth performances of pacific white shrimp.

5 | CONCLUSION

It is difficult to make a firm conclusion about a specific culprit for the resulted fluctuations in the growth performances of pacific white shrimp and their threshold levels might due to their interactive positive and negative effects. However, there is clear evidence that phosphorous, phosphorous in phytic acid and total phytic acid and Raffinose were selected as vital chemical variables in SBM, which could cause significant effects on the growth performances of pacific white shrimp. The results of this study demonstrate that there are differences even in reasonably similar sources of soybean meal.

Hence, if we are to understand and predict the biological performance on animals we need systematic research to look at various processing and nutritional changes and how they influence the performance of the animals.

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TABLE 18 Pearson correlation coefficients of TGC with raffinose, ADF, NDF, phosphorus, phosphorus in phytic acid, total phytic acid, non-phytate phosphorus, sodium, sulphur and zinc

Variable	*r*-value	*p*-value	Variable	*r*-value	*p*-value
Raffinose	-0.358	0.086	Total phytic acid	0.426	0.038
ADF	0.256	0.228	Non-phytate phosphorus	0.140	0.514
NDF	0.298	0.157	Sodium	0.353	0.091
Phosphorus	0.469	0.021	Sulphur	0.327	0.119
Phosphorus in phytic acid	0.429	0.037	Zinc	0.199	0.351

does not imply its approval to the exclusion of other products that may also be suitable.

ORCID

Harsha Sameera Chathuranga Galkanda Arachchige https://orcid.org/0000-0002-5464-1458

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ORIGINAL ARTICLE

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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

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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.

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 ($ADMD_I$), protein (APD_I) and energy (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_{ref} = % nutrient (or KJ/g gross energy) of basal diet (dry weight)

D_{ingr} = % 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 ($ADMD_I$, AED_I and 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 ($ADMD_I$) in SBM ranged from 45% to 90%, while apparent energy digestibility (AED_I) and protein digestibility (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_I), energy (AED_I) and dry matter ($ADMD_I$) digestibility coefficients of test ingredients and principle component scores

	APD_I		AED_I		$ADMD_I$	
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

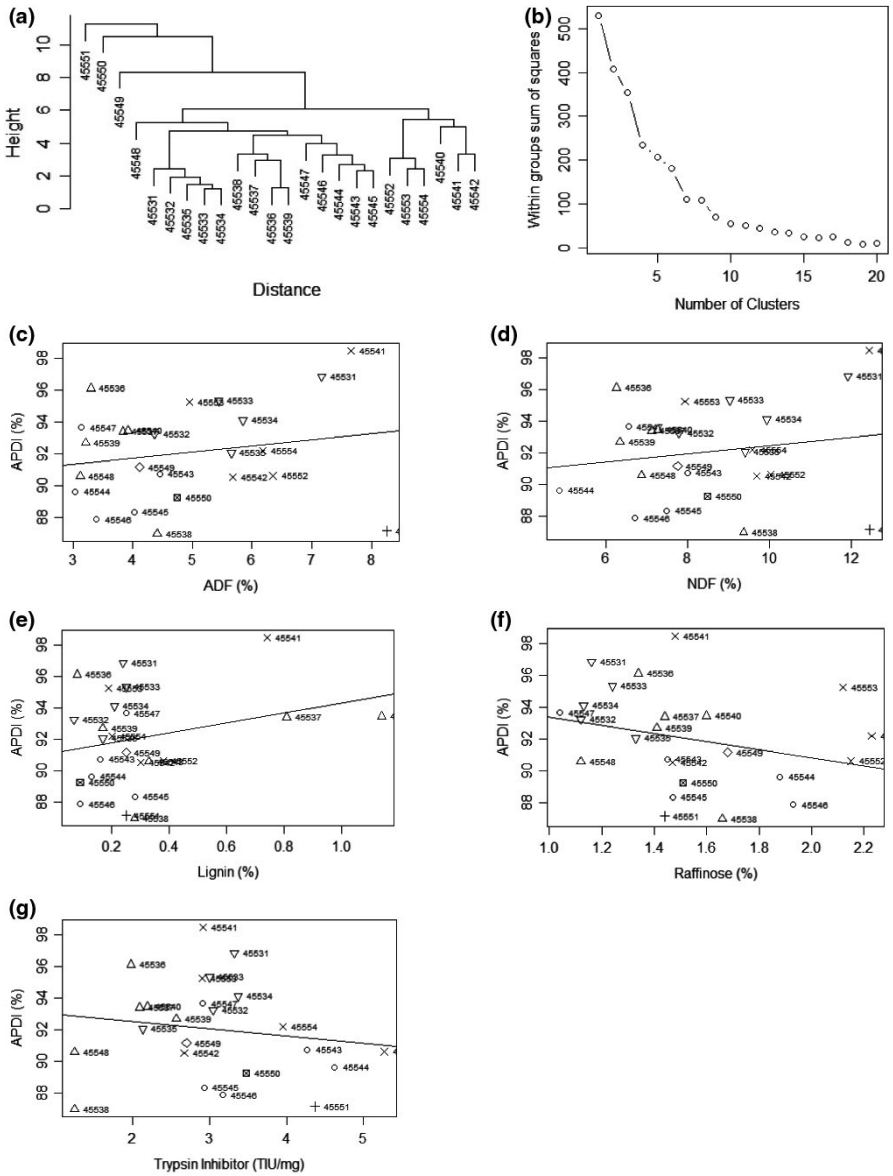

FIGURE 1 Dendrogram of cluster analysis (grouping of soybean meal (SBM) based on chemical characteristics) (a), scree plot (b) and patterns of association between PCA selected chemical parameters of SBM (acid detergent fibre/ADF, neutral detergent fibre/NDF, lignin, raffinose and trypsin inhibitor) and 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).

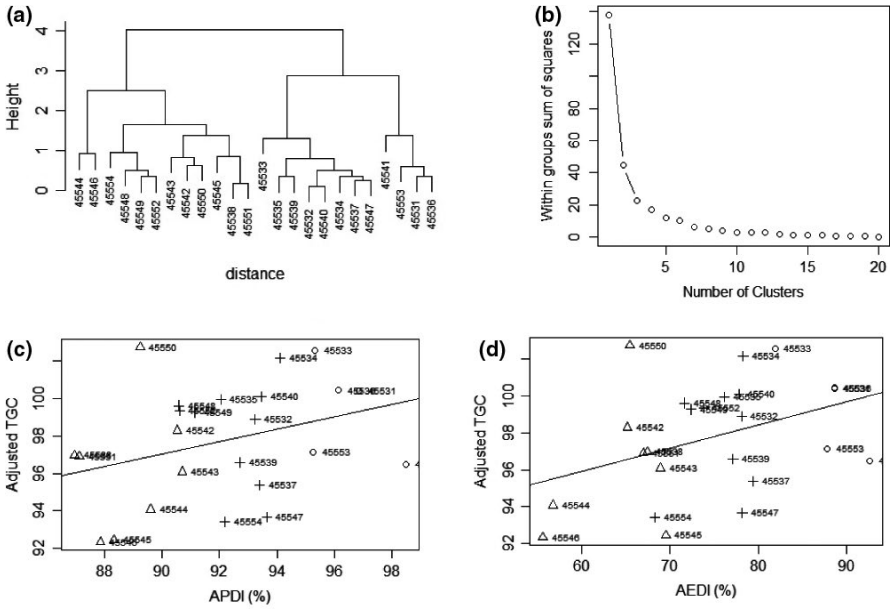

FIGURE 2 Dendrogram of cluster analysis (grouping of soybean meal based on digestibility characteristics) (a) scree plot (b) and patterns of association between ingredient (I) digestibility parameters (apparent digestibility coefficients for protein/APD and energy/AED) and standardized thermal growth 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.

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