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Effect of fungal fermentation on apparent digestibility coefficient for dry matter, crude protein and amino acids of various plant protein sources in *Penaeus vannamei*

R. Jannathulla | J.S. Dayal | D. Vasanthakumar | K. Ambasankar | M. Muralidhar

ICAR-Central Institute of Brackishwater Aquaculture, Chennai, India

Correspondence
Jagabathula Syama Dayal, ICAR-Central Institute of Brackishwater Aquaculture, Chennai, India.
Email: symadayal@rediffmail.com

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Abstract

Four commercial solvent-extracted oilseed meals/cakes, viz. soybean meal (SBM), groundnut oil cake (GNC), rapeseed meal (RSM) and sunflower oil cake (SFC), were fermented with fungus *Aspergillus niger*, and its effect on apparent digestibility coefficient (ADC) was studied in *Penaeus vannamei*. Reference diet and eight experimental diets containing 700 g/kg reference diet and 300 g/kg test ingredient with 5 g/kg chromic oxide were formulated. Each diet was randomly allotted to three tanks containing ten shrimps. Shrimps were adapted to the experimental diets for a week, and faeces were collected using Falcon tube from second week onwards. The ADC of all the ingredients significantly ($p < .05$) increased with fermentation and the increase being higher in SBM (78.46%–91.71%) for dry matter and in SFC (71.51%–87.02%) for protein. Analysis of variance showed that the ADC of both dry matter and protein significantly ($p < .05$) differed in treatments ($p = <.001$) and ingredients ($p = <.001$). The average ADC of ingredients was ranked as SBM > GNC > RSM > SFC. The most digestible essential amino acid (EAA) in fermented ingredients was methionine in SBM, arginine in GNC, valine in RSM and histidine in SFC. A better improvement in amino acid digestibility was observed in fermented SFC. Results indicated that *P. vannamei* efficiently digests fermented ingredients compared to unfermented ones.

KEYWORDS

apparent digestibility coefficients, *Aspergillus niger*, *Penaeus vannamei*, plant protein sources, shrimp feed, solid-state fermentation

1 | INTRODUCTION

A formulated diet is an excellent source of nutrients due to its nutritional composition, but this will be wasted and detrimental to the environment, if not efficiently digested or absorbed. It is essential to have a proper understanding of the nutritional requirements of cultured species and nutrient availability of the ingredients required for formulating nutritionally efficient feeds. Among the various protein sources available commercially, fishmeal is used mainly due to well-balanced nutrient profile with a higher palatability and digestibility. However, the decrease in production of fishmeal coupled with its increasing cost during the past two decades led to substituting fishmeal with possible cheaper ingredients. Oilseed cakes/meals are predominantly used as potential substitutes for fishmeal due to their easy availability, desirable nutrient content and reasonable price. Among them, soybean meal (SBM) is the most reliable ingredient (Sookying, Davis, & Soller Dias Da Silva, 2013) and often used in the range of 200-350 g/kg in commercial shrimp feed formulations. The use of SBM has been studied in numerous aquatic species (Amaya, Allen Davis, & Rouse, 2007; Refstie, Storebakken, & Roem, 1998); however, information on SBM as a major or sole protein source in the diet of shrimp is scanty. This could partly be attributed to inadequacy of sulphur-containing

amino acids, especially methionine, and the presence of antinutritional factors, particularly trypsin inhibitor (Shiu, Wong, Guei, Shin, & Liu, 2015). Similarly, as with SBM, a few reports have focused on the usage of other oilseed meals/cakes in various aquatic species for increasing the affordability of feed ingredients (Davis, Arnold, & McCallum, 2002; Dayal, Rajaram, Ambasankar, & Ali, 2011; Rajaram, 2010; Thiessen, Maenz, Newkirk, Classen, & Drew, 2004). These authors suggested that the acceptability of oilseed meals/cakes was much lower (20-50 g/kg) than that of SBM in the diet of shrimp due to the high content of fibre fractions and antinutritional factors.

Microbial fermentation, using bacteria (Imelda, Raj, & Bhatnagar, 2008), fungus (Shi et al., 2015; Jannathulla, Syama Dayal, Ambaskar, & Muralidhar, 2017; Jannathulla, Syama Dayal, Vasanthakumar, Ambasankar, & Muralidhar, 2017) and yeast (Sharawy, Goda, & Hassaan, 2016) species, is being employed for enhancing the nutritional quality of plant protein sources by detoxifying or reducing the associated constraints. Due to this positive effect, the substitution of fishmeal increased from 374.2 to 616.7 g/kg using fermented SBM in *Penaeus vannamei* (Shiu et al., 2015) and was more than 500 g/kg in *Penaeus monodon* (Imelda et al., 2008) and *Penaeus indicus* (Sharawy et al., 2016). However, earlier reports on fermentation were restricted to evaluating growth parameters in candidate shrimp species without considering the bioavailability and digestibility (Imelda et al., 2008; Jannathulla, Syama Dayal, Vasanthakumar, et al., 2017; Sharawy et al., 2016; Shiu et al., 2015). Determination of apparent digestibility coefficients (ADCs) of any ingredients, prior to use in the formulation, is an important prerequisite for formulating cost-effective feeds. This would not only be beneficial in developing nutritionally adequate diets but also reduced the waste produced by the cultures species (Hajen, Beames, Higgs, & Dosanjh, 1993). The ADC of various traditional plant-based ingredients as such had been investigated earlier in penaeid shrimps (Catacutan, 1991; Cruz-Suarez, Ricque-Marie, Tapia-Salazar, McCallum, & Hickling, 2001; Piedad-Pascual, Cruz, & Sumalangcay, 1990), but there is paucity of information with reference to fermented ingredients. Moreover, the complete replacement of fishmeal in shrimp feed has not yet been achieved even using fermented plant-based ingredients. It is important to understand the availability of nutrients, in particular amino acids in fermented ingredients as well. Hence, in the present study, the ADCs for dry matter, crude protein and amino acids of four commercially available plant protein sources were evaluated as such and also postfermentation using the fungus, *Aspergillus niger* in *P. vannamei* to explore their suitability in shrimp feed.

2 | MATERIAL AND METHODS

2.1 | Methodology of fermentation

Fermentation was conducted at the Nutrition Laboratory of ICAR-Central Institute of Brackishwater Aquaculture, Chennai, India, using the method described by Shi et al. (2015). Four commercial solvent-extracted oilseed meals/cakes including soybean meal (SBM), groundnut oil cake (GNC), rapeseed meal (RSM) and sunflower oil cake (SFC) were collected from six different places (n = 6) in and around Chennai, India. All six replicates of each ingredient were pooled together to have a representative sample. From this pooled homogenate of an ingredient, six replications were taken for fermentation after grinding to a particle size of <500 μm. The ground materials were hydrated with water to adjust the moisture content between 600 and 650 g/kg, and thereafter sterilized by autoclaving at 121°C for 15 min. The cooled sterilized samples were inoculated with *A. niger* (ATCC 6275) suspension (10^{-7} spores/ml), obtained from HiMedia Laboratories (Mumbai, India) at the rate of 50 g/kg. Fermentation was carried out in a 500-ml Erlenmeyer flask plugged with cotton to facilitate air transfer at 35 ± 1°C in an incubator for 3 days. Postfermentation, all the samples were dried at 40°C for 48 hr to bring down the moisture content below 100 g/kg and then stored at 4°C until use. The nutrient composition of test ingredients as such and postfermentation is given in Table 1.

2.2 | Experimental diets

Reference diet (Table 2) was formulated based on the nutritional requirements of *P. vannamei* using locally available ingredients. In addition, eight experimental diets containing 70% reference diet and 30% of each one of the ingredients (as such and fermented) were prepared by mixing as suggested by Brunson, Romaire, and Reigh (1997), Davis et al. (2002), Yang et al. (2009), Zhou, Davis, and Buentello (2014), and Chen, Liu, Xie, Zhang, and Niu (2016). While preparing experimental diets, all the dry solid materials were weighed as per formulae and ground to pass through a 250-μm sieve. Premix, including vitamin–mineral mix, binder and oil sources were added to the ground materials and mixed in an electric blender for 20 min for homogenization. The homogenized mash was manually kneaded into a dough by adding water at the rate of 500 ml/kg. The dough was steamed at atmospheric pressure for 5 min and pelletized in a tabletop pelletizer having a 2-mm-diameter die. The pellets were dried at 60°C overnight in a hot air oven and stored in a plastic container at 4°C until further use. Chromium (III) oxide (Sigma Aldrich, Cat. No: 393703) was added at the rate of 5 g/kg as an external marker to determine the ADC of test ingredients. The proximate and amino acid composition of reference and test diets are depicted in Table 3.

2.3 | Experimental condition

A 30-day digestibility trial was conducted at the Muttukadu Experimental Station of ICAR-Central Institute of Brackishwater Aquaculture, Chennai, India, in *P. vannamei*. Approximately, 500 shrimp were procured from a local farm near Chennai, India, and were acclimatized to indoor laboratory and experimental condition for 2 weeks with a basal diet containing 374.2 g/kg of crude protein. Postacclimatization, a total of 270 healthy shrimp (15.26 g) were randomly transferred to twenty-seven 500-L oval-shaped fibreglass experimental tanks (1.31 × 0.64 × 0.73 m) at the rate of ten shrimp per tank, with three replicates in each treatment. All three replicates in a treatment were fed with the respective diet thrice daily (7.00 a.m., 12.30 p.m. and 5.30 p.m.) and allowed to feed for an hour after which, all the particles, including faeces, were siphoned out and the bottom

TABLE 1 Proximate, amino acid, fibre fractions and antinutritional composition of test ingredients used in the present study (g/kg dry matter basis)

Particulars	Test ingredients							
	SBM[a]	FSBM[b]	GNC[c]	FGNC[d]	RSM[e]	FRSM[f]	SFC[g]	FSFC[h]
Proximate composition								
Crude protein	524.0	598.5	429.3	520.0	417.3	467.5	356.1	375.4
Ether extract	10.9	7.8	21.5	19.5	26.4	22.8	17.3	15.6
Crude fibre	69.6	67.7	128.7	127.3	106.1	102.8	288.5	264.1
Nitrogen-free extract[i]	320.1	246.0	343.2	252.9	382.6	337.5	259.8	264.3
Total ash	75.4	80.0	77.3	80.3	67.6	69.4	78.3	80.6
Gross energy (KJ/g)[j]	19.3	19.7	18.9	19.4	19.1	19.3	18.2	18.3
Essential amino acids								
Arginine	30.0	40.7	30.4	35.8	36.2	38.2	16.2	18.8
Histidine	17.5	19.5	9.1	10.9	17.3	17.8	4.7	5.6
Isoleucine	27.3	29.0	13.6	15.2	15.6	16.6	33.7	33.7
Leucine	39.3	40.1	10.9	11.1	20.4	25.6	14.6	18.7
Lysine	12.5	40.1	14.2	35.1	11.0	30.1	11.8	23.1
Methionine	7.4	9.9	5.6	10.3	8.8	14.1	17.0	17.7
Phenylalanine	20.2	25.2	30.0	31.0	9.9	16.3	16.0	17.2
Threonine	17.1	19.1	9.5	13.1	21.5	22.8	10.2	15.0
Tryptophan	6.7	7.7	4.3	5.2	4.7	5.3	4.2	4.4
Valine	16.3	17.6	27.1	27.9	23.6	27.2	14.8	14.9
Nonessential amino acids								
Alanine	33.2	34.2	20.2	21.4	26.9	28.4	10.9	12.4
Aspartic acid	50.3	52.5	32.4	41.9	22.4	25.4	16.3	18.9
Cystine	8.0	10.7	6.1	11.1	11.2	17.9	12.3	12.9
Glutamic acid	79.5	80.2	62.7	70.9	51.0	51.1	69.5	69.8
Glycine	10.7	11.9	20.3	24.4	32.1	33.1	10.9	13.2
Proline	27.4	26.7	16.8	22.0	23.2	25.4	13.3	15.7
Serine	25.1	31.3	20.4	24.8	24.9	26.7	14.0	15.1
Tyrosine	23.4	24.3	35.4	35.6	17.2	18.2	16.4	17.1
Fibre fraction								
Neutral detergent fibre	119.2	114.7	215.4	209.5	265.8	253.0	438.6	403.6
Acid detergent fibre	78.26	73.91	139.0	138.1	193.4	185.1	275.9	257.2
Cellulose	69.6	55.8	87.53	68.5	86.2	76.3	197.7	156.0
Hemicellulose	41.5	40.1	76.4	71.5	72.5	68.1	162.7	146.4
Lignin	8.6	7.38	51.5	50.2	107.2	84.8	78.2	76.6
Antinutritional factors								
Trypsin inhibitor	2.41	0.14	–	–	–	–	–	–
Phytic acid	13.36	6.53	10.3	6.4	27.4	8.9	–	–
Tannin	–	–	17.56	2.88	8.8	5.1	8.79	6.10
Saponin	10.03	2.10	7.36	3.75	–	–	6.42	2.17
Glucosinolates	–	–	–	–	3.1	1.8	–	–

All the plant protein sources are solvent-extracted and sourced (n = 6) from the local markets in and around Chennai, India.
[a]Untreated soybean meal.
[b]Fermented soybean meal.
[c]Untreated groundnut oil cake.
[d]Fermented groundnut oil cake.
[e]Untreated rapeseed meal.
[f]Fermented rapeseed meal.
[g]Untreated sunflower oil cake.
[h]Fermented sunflower oil cake.
[i]Calculated by difference.
[j]Estimated according to Jobling (1983) using the factor 5.65, 9.45 and 4.00 for crude protein, ether extract and carbohydrate, respectively.

TABLE 2 Ingredient composition of reference diet used in the present study (g/kg as fed basis)

Ingredients	Inclusion (g/kg)
Fishmeal[a]	250
Mantis shrimp meal[b]	120
Squid meal[c]	40
Corn gluten[d]	50
Sesame cake[d]	60
Rice bran[d]	40
Broken rice[d]	50
Wheat (Whole)[d]	325
Fish oil[a]	20
Lecithin[d]	10
Vitamin mineral mix[e]	20
Chromic oxide[f]	5
Binder[g]	10

[a]Bismi Fisheries, Mayiladuthurai, Tamil Nadu, India.
[b]Khaja Mohammed Store, Chennai, Tamil Nadu, India
[c]Kings Fish Products Pvt. Ltd., Veraval, Gujarat, India.
[d]Local markets, Chennai, Tamil Nadu, India.
[e]Vitamin mineral mix (mg/kg): Retinol (20 000 IU), thiamine (70 mg), riboflavin (60 mg), pyridoxine (120 mg), cyanocobalamin (60 mg), ascorbic acid (1,000 mg), cholecalciferol (300,000 IU), tocopherol (200 mg), menadione (7 mg), niacin (500 mg), folic acid (500 mg), D-calcium pantothenate (140 mg), biotin (0.50 mg), choline chloride (800 mg), inositol (1,000 mg), ferrous citrate (100 mg), copper sulphate (5 mg), zinc sulphate (50 mg), manganese sulphate (40 mg), sodiun selenite (20 mg), cobalt chloride (1 mg) and potassium iodide (100 mg).
[f]Sigma-Aldrich (Cat. No: 393703).
[g]Pegabind, Bentoli AgriNutrition Asia Pvt Ltd, Singapore.

of the experimental tanks kept clean to avoid contamination. The uncontaminated faeces were gently siphoned off using a clean Falcon tube (Carvalho, Ota, Kadry, Tacon, & Lemos, 2016) from each experimental tank into a channel darting a silk fabric after 2 hr of each feeding from second week of the experiment onwards. This 2 hr includes feeding time (1 hr) and defecation (1 hr). During the experimental period, water at the rate of 80% was exchanged daily prior to the first feeding. Shrimp were maintained at a natural photoperiod of 12-hr light and 12-hr darkness throughout the experiment. The collected faeces were then gently rinsed in distilled water, dried on a filter paper and frozen immediately at −20°C for further analysis. To have a representative sample, a 30-day collection of dried faeces of each replicate in a treatment was pooled to avoid possible variation. The ADCs for dry matter, crude protein and amino acids were analysed according to Smith and Tabrett (2004) and Carvalho et al. (2016).

$$ADC_D = 1 - (F/D \times mD/mF),$$

where ADC_D is apparent digestibility coefficient (dry matter/crude protein/amino acids) of diet, D is nutrient of the diet (%), F is nutrient of the faeces (%), mD is marker in the diet (%) and mF is marker in the faeces (%).

$$ADC_I = ADC_{TD} + [(ADC_{TD} - ADC_{RD}) \times (I_{RD} \times N_{RD}/I_I \times N_I)],$$

where ADC_I is apparent digestibility coefficient (dry matter/crude protein/amino acids) of ingredients, ADC_{TD} is apparent digestibility coefficient of test diet, ADC_{RD} is apparent digestibility coefficient of reference diet, I_{RD} is reference diet in the mash (%), N_{RD} is nutrient in the reference diet mash (%), I_I is test ingredient in the mash (%) and N_I is nutrient in the test ingredient (%).

2.4 | Water quality parameters

Ultraviolet-treated water was used after filtering the same using a 5-μm cartridge filter. Water quality parameters, viz. salinity, temperature, dissolved oxygen and pH, were recorded daily. Total ammonia nitrogen, nitrite nitrogen and nitrate nitrogen were determined weekly using standard methods (APHA 2012). The analysed values are depicted in Table 4.

2.5 | Biochemical analysis

Proximate composition of ingredients and experimental diets were analysed according to the method of AOAC (1997). Briefly, the moisture content was determined by drying the samples at 105°C in a hot air oven overnight. Nitrogen content was analysed by the micro-Kjeldahl method (Kjeltec™-8100, Tecator™ Line), and the analysed nitrogen was converted into crude protein by multiplying with common empirical factor of 6.25. The ether extract was estimated using petroleum ether (60–80°C) in Soxhlet extraction unit (Scocs Plus-SCS 6). The samples were digested using 1.25% sulphuric acid (30 min) followed by 1.25% sodium hydroxide (30 min) using a Fibre cap method (FOSS-2022, Tecator™) for determining the crude fibre level. Total ash content was measured by incinerating the samples at 540°C in a muffle furnace for 6 hr. The nitrogen-free extract was calculated by a difference (1,000-sum of all other nutrients in g/kg). The gross energy of ingredients and experimental feeds was estimated according to Jobling (1983) using the factor 5.65, 9.45 and 4.00 for crude protein, ether extract and carbohydrate, respectively.

Amino acid profiles were analysed using precolumn HPLC gradient system (Shimadzu Corp, LC-30AD) after hydrolysing the samples with 6 N hydrochloric acid in a sealed tube at 110°C in an oven for 22 hr (Finlayson, 1964). The acid was dried using a vacuum rotary evaporator (IKA, RE 10 C S84), and the residue was brought into a diluent (0.1 N hydrochloric acid) and thereafter filtered using a 0.2-μm membrane syringe filter. The YMC-Triart C18, RRH (1.8 μm, 2.1 × 100 mm) column was used to separate the amino acids after derivatization with mercaptopropionic acid, O-phthalaldehyde and fluorenylmethoxycarbonyl chloride under gradient elution using phosphate buffer (20 mmol as mobile phase A) and combination of acetonitrile: methanol: water (45:40:15 as mobile phase B) at the flow rate of 0.3 ml/min. The gradient was changed by increasing mobile phase B concentration at the rate of 11%–13% at 3 min, 31% at 5 min, 37% at 15 min, 70% at 20 min, 100% at 25 min. Amino acids were quantified by a fluorescent detector (RF-20AXS) using amino acid mixer as an external standard (Sigma Aldrich, Cat. No: AAS18) and norleucine as an internal standard. Tryptophan, being labile to acid hydrolysis, was measured after

TABLE 3 Proximate and amino acid composition of reference and test diets used in the present study

Particulars	Reference diet	Test diets							
		SBM	FSBM	GNC	FGNC	RSM	FRSM	SFC	FSFC
Proximate composition (g/kg dry matter basis)									
Moisture	74.7	55.3	53.5	49.9	49.7	54.9	52.6	65.5	73.4
Crude protein	374.2	399.9	410.8	375.2	381.5	368.5	377.3	355.2	369.8
Ether extract	71.6	56.1	56.3	65.6	65.3	66.5	64.4	55.9	55.2
Crude fibre	26.2	36.4	33.8	47.3	43.8	39.2	39.3	65.5	64.9
NFE[a]	316.6	335.6	328.5	343.0	340.1	356.5	345.5	340.5	318.1
Total ash	136.7	116.7	117.1	119.0	119.6	114.4	120.9	117.4	118.6
Gross energy (KJ/g)[b]	17.4	17.9	18.0	18.0	18.0	17.9	17.9	17.4	17.3
Essential amino acids (g/kg protein basis)									
Arginine	61.7	64.3	70.4	66.9	70.2	73.0	73.2	59.1	58.9
Histidine	23.4	27.8	30.7	22.7	24.9	30.7	30.4	21.2	21.2
Isoleucine	41.0	46.0	49.7	38.9	41.4	41.8	41.6	58.6	56.4
Leucine	70.5	77.5	75.2	57.6	57.4	66.8	69.3	64.3	65.1
Lysine	57.3	47.8	67.2	50.6	63.7	49.6	63.7	52.2	59.3
Methionine	22.4	20.8	21.9	19.7	23.1	23.1	26.7	30.8	30.2
Phenylalanine	46.3	46.0	48.4	56.0	56.4	40.9	45.1	47.7	46.7
Threonine	38.2	38.5	38.5	33.0	37.0	44.6	44.6	36.8	39.2
Tryptophan	11.1	12.8	13.6	10.7	11.8	11.7	11.9	11.8	11.5
Valine	45.7	39.8	42.4	53.6	53.5	51.7	53.4	46.2	44.5
Nonessential amino acids (g/kg protein basis)									
Alanine	51.9	59.5	59.2	52.2	52.9	58.9	58.6	47.5	46.9
Aspartic acid	88.5	94.8	95.7	86.4	91.5	81.2	81.7	79.0	78.1
Cystine	12.9	14.0	16.6	14.7	17.3	18.3	23.2	19.9	19.6
Glutamic acid	155.6	160.8	158.5	158.8	163.3	152.2	148.7	173.5	166.9
Glycine	48.8	39.5	40.9	49.0	53.2	60.8	60.2	45.2	45.2
Proline	47.4	50.8	50.1	45.6	50.1	52.6	53.1	46.2	46.3
Serine	43.0	46.0	50.6	46.9	49.5	50.8	51.1	43.5	42.7
Tyrosine	31.7	37.8	38.7	49.3	50.9	36.6	36.5	37.2	36.3

[a]Nitrogen-free extract (calculated by difference).
[b]Estimated according to Jobling (1983) using the factor 5.65, 9.45 and 4.00 for crude protein, ether extract and carbohydrate, respectively.

alkali hydrolysis by spectrophotometric method at 500 nm (Sastry & Tammuru, 1985). The partial oxidation of sulphur-containing amino acids like cystine and methionine during acid digestion was prevented by adding 0.1% phenol (Jajic, Krstovic, Glamocis, Jaksis, & Abramovic, 2013).

Antinutritional factors, such as trypsin inhibitor (Kakade, Rackis, McGhee, & Puski, 1974), saponin (AOAC 1997), phytic acid (Davies & Reid, 1979), tannin (Price, Van Scoyoc, & Butler, 1978) and glucosinolates (McGhee, Kirk, & Mustakas, 1965), were analysed by standard methods. Fibre fractions, namely neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose, hemicellulose and lignin of selected plant protein sources, were estimated following the method of Van Soest, Robertson, and Lewis (1991). Chromium content was analysed in both diets and faeces using Furukawa (1966) by spectrophotometric method (350 nm) to calculate the ADC for dry matter, crude protein and amino acids of test ingredients.

2.6 | Statistical analysis

Experimental data were subjected to *t* test to find the specific effect of fermentation on each ingredient and were further subjected to 2 × 4 factorial ANOVA using two factors, viz. treatments (untreated and fermented) and ingredients (SBM, GNC, RSM and SFC) to assess the effect of treatment and ingredient on the digestibility parameters. Prior to statistical evaluation, data were checked for homogeneity of variance after ascertaining the normal distribution. The entire data were analysed using SPSS version 16.0, and statistical tests were performed at $p < .05$.

TABLE 4 Minimum and maximum values of water quality parameters observed during the experimental period of 30 days

Particulars	Water quality parameters	
	Average	(Minimum and maximum)
Salinity (g/L)	22.37 ± 1.25	(21.0–24.0)
Dissolved oxygen (mg/L)	6.35 ± 0.72	(5.2–7.6)
Temperature (°C)	28.45 ± 0.67	(27.1–29.6)
pH	8.15 ± 0.21	(7.9–8.5)
Total ammonia nitrogen (mg/L)	0.12 ± 0.03	(0.09–0.16)
Nitrite nitrogen (mg/L)	0.43 ± 0.09	(0.31–0.52)
Nitrate nitrogen (mg/L)	2.54 ± 0.10	(2.43–2.67)

3 | RESULTS

The chemical composition of untreated and fermented ingredients used in the present study is presented in Table 1. The nutritional composition of fermented ingredients was comparatively better than the respective untreated materials. Fungal fermentation increased protein and limiting amino acids like methionine and lysine. A marginal reduction was observed in crude fibre and its fractions, but all the analysed antinutritional factors found to be much lower in fermented ingredients than that in untreated ingredient.

The ADC for dry matter, crude protein and amino acids is presented in Table 5 in an effort to assess the specific effect of fermentation on each ingredient (*t* test). Of all the untreated ingredients tested in the present study, SBM had the highest ADC for dry matter (78.46%) and crude protein (89.46%) and the lowest values were noticed with SFC (44.12% and 71.51%) while both GNC and RSM were in comparable. The ADC values of all the test ingredients significantly ($p < .05$) increased with fungal fermentation, and the increase was high in SBM (78.46%–91.71%) for dry matter and high in SFC (71.51%–87.02%) for crude protein. In amino acids, methionine (97.16%) in SBM had the highest ADC and alanine (63.47%) in SFC had the lowest ADC. Effect of fermentation on the ADC for amino acids had a similar tendency as in protein. Fungal fermentation significantly ($p < .05$) increased amino acid digestibility except arginine and proline in SBM, glutamic acid in GNC and histidine, alanine, glutamic acid, glycine and serine in RSM, while the digestibility of all the amino acids was significantly ($p < .05$) high in fermented SFC. The most digestible essential amino acid (EAA) in fermented ingredients was methionine in SBM, arginine in GNC, valine in RSM and histidine in SFC. Likewise, the most digestible nonessential amino acids (NAA) were serine, proline, tyrosine and cystine in SBM, GNC, RSM and SFC, respectively. A better improvement in amino acids digestibility was observed in fermented SFC compared to other ingredients as in crude protein.

To assess the effect of treatment and ingredients on digestibility parameters, the data were further subjected to ANOVA using two factors, viz. treatments (untreated and fermented) and ingredients (SBM, GNC, RSM and SFC). The results revealed that the ADCs of both dry matter and crude protein were significantly ($p < .05$) different in both treatments ($p = <.001$) and ingredients ($p = <.001$). The ADC significantly ($p < .05$) increased with fermentation from 58.74% to 67.15% in dry matter ($p = <.001$) and from 79.48% to 88.74% in crude protein ($p = <.001$). The mean ADC of test ingredients was ranked as SBM > GNC > RSM > SFC for both dry matter as well as crude protein. Histidine was the most digestible amino acid among the EAA prior to and postfermentation (Table 6). However, glutamic acid had the highest digestibility in untreated ingredients among the NAA, while proline showed a better digestibility after fermentation (Table 7). The average ADC for EAA of SBM was the highest (93.58%), and SFC had the lowest average (78.95%). The GNC and RSM had comparable averages of 82.02% and 82.32%, respectively; and there were significant differences in individual amino acids between them. For example, digestibility of arginine was high in GNC (85.23%) and low in RSM (81.49%), whereas on the contrary, valine showed a higher value in RSM (86.45%) compared to GNC (83.14%). The average digestibility pattern was almost similar in NAA for all the ingredients (Table 7) as in EAA.

4 | DISCUSSION

Determining the digestibility coefficient is an important prerequisite to screen potential feed ingredients for formulating nutritious feed for a species (Hajen et al., 1993). Measuring the dry matter digestibility would be advantageous to ascertain the total quantity of nutrients that are digested and absorbed (Glencross, Booth, & Allan, 2007), as all the components of the ingredients are not digested at equal proportion. The present results indicated that there were variations in the ADC values among the ingredients prior to and postfermentation. Mu, Lam, Guo, and Shim (2000) reported that SBM had the highest digestibility among the plant protein sources in various crustacean species and our results corroborate their findings. The ADCs for dry matter of untreated SFC (44.12%), GNC (57.52%) and RSM (54.88%) were lower than that of SBM (78.46%). This disparity in the values between the ingredients could partly be attributed to the variations in the nutritional composition. The protein is one of the major factors that primarily influences shrimp growth. The nitrogen fraction of faecal matter, leftover feed, moults and dead shrimp contributes to the accumulation nitrogen in soil and water, which in turn hinders shrimp growth by causing various diseases, in addition to the pollution and eutrophication of water (Lin, Li, Chen, Zheng, & Yang, 2006). Various research efforts like fermentation (Mukhopadhyay & Ray, 1999), exogenous enzymes (Dalsgaard et al., 2012) have been attempted to reduce the faecal nitrogen excretion by improving the digestibility and thereby increasing the availability of nutrients, in particular amino acids to aquatic species. The pattern of protein digestibility for untreated ingredients was similar as in dry matter digestibility (Table 5), but the values were comparatively higher in protein digestibility than dry matter digestibility regardless of ingredients tested. The results were comparable with

TABLE 5 Effect of fungal fermentation on apparent digestibility coefficient (%) for dry matter, crude protein and amino acids of test ingredients in *Penaeus vannamei* using *t* test (means ± *SD*)

	Soybean meal		Groundnut oil cake		Rapeseed meal		Sunflower oil cake	
Particulars	**SBM**	**FSBM**	**GNC**	**FGNC**	**RSM**	**FRSM**	**SFC**	**FSFC**
Dry matter	$78.46^{b} \pm 0.54$	$91.71^{a} \pm 1.48$	$57.52^{b} \pm 1.01$	$62.96^{a} \pm 2.33$	$54.88^{b} \pm 0.53$	$64.63^{a} \pm 4.41$	$44.12^{b} \pm 1.41$	$49.34^{a} \pm 1.05$
Crude protein	$89.46^{b} \pm 0.30$	$95.49^{a} \pm 0.63$	$78.84^{b} \pm 0.49$	$87.09^{a} \pm 1.00$	$78.12^{b} \pm 0.63$	$85.39^{a} \pm 2.71$	$71.51^{b} \pm 0.99$	$87.02^{a} \pm 0.85$
Essential amino acids								
Arginine	$92.92^{a} \pm 2.30$	$95.51^{a} \pm 2.93$	$79.60^{b} \pm 2.76$	$90.87^{a} \pm 3.12$	$79.12^{b} \pm 2.17$	$83.86^{a} \pm 1.45$	$70.05^{b} \pm 1.30$	$83.62^{a} \pm 4.99$
Histidine	$91.74^{b} \pm 1.49$	$96.86^{a} \pm 2.49$	$80.14^{b} \pm 2.06$	$89.06^{a} \pm 2.33$	$83.86^{a} \pm 2.32$	$85.99^{a} \pm 3.43$	$76.95^{b} \pm 1.64$	$92.98^{a} \pm 5.41$
Isoleucine	$90.74^{b} \pm 1.49$	$95.57^{a} \pm 1.43$	$78.29^{b} \pm 1.53$	$89.14^{a} \pm 1.73$	$81.37^{b} \pm 0.97$	$83.60^{a} \pm 2.07$	$72.37^{b} \pm 1.97$	$90.88^{a} \pm 5.55$
Leucine	$92.24^{b} \pm 1.91$	$97.38^{a} \pm 2.34$	$73.20^{b} \pm 2.76$	$80.66^{a} \pm 3.12$	$73.49^{b} \pm 2.96$	$81.64^{a} \pm 0.71$	$67.87^{b} \pm 4.13$	$86.14^{a} \pm 6.36$
Lysine	$91.74^{b} \pm 2.99$	$97.99^{a} \pm 1.12$	$77.08^{b} \pm 1.77$	$88.86^{a} \pm 2.00$	$79.37^{b} \pm 2.45$	$88.05^{a} \pm 0.56$	$70.45^{b} \pm 3.35$	$86.74^{a} \pm 3.65$
Methionine	$97.16^{b} \pm 1.12$	$98.75^{a} \pm 0.32$	$76.79^{b} \pm 2.85$	$87.71^{a} \pm 3.23$	$78.10^{b} \pm 0.86$	$87.16^{a} \pm 1.36$	$68.87^{b} \pm 1.90$	$88.41^{a} \pm 4.77$
Phenylalanine	$90.24^{b} \pm 2.23$	$95.49^{a} \pm 3.18$	$78.63^{b} \pm 2.16$	$83.80^{a} \pm 2.44$	$79.31^{b} \pm 1.47$	$83.18^{a} \pm 0.86$	$70.42^{b} \pm 2.68$	$82.49^{a} \pm 4.10$
Threonine	$90.44^{b} \pm 4.16$	$93.15^{a} \pm 2.03$	$76.85^{b} \pm 1.87$	$82.07^{a} \pm 2.12$	$77.68^{b} \pm 1.95$	$83.92^{a} \pm 0.73$	$68.18^{b} \pm 2.71$	$85.66^{a} \pm 4.50$
Tryptophan	$88.64^{b} \pm 3.14$	$93.12^{a} \pm 2.02$	$77.14^{b} \pm 2.78$	$84.25^{a} \pm 3.14$	$80.99^{b} \pm 1.52$	$83.50^{a} \pm 0.57$	$70.86^{b} \pm 1.82$	$81.94^{a} \pm 4.84$
Valine	$87.54^{b} \pm 2.23$	$89.37^{a} \pm 1.47$	$77.78^{b} \pm 1.16$	$88.49^{a} \pm 1.31$	$83.70^{b} \pm 2.96$	$89.22^{a} \pm 1.16$	$74.39^{b} \pm 1.28$	$89.83^{a} \pm 1.17$
Nonessential amino acids								
Alanine	$85.23^{b} \pm 1.11$	$89.31^{a} \pm 2.20$	$76.14^{b} \pm 1.82$	$83.87^{a} \pm 2.06$	$74.85^{a} \pm 2.03$	$76.23^{a} \pm 0.83$	$63.47^{b} \pm 2.68$	$79.46^{a} \pm 1.14$
Aspartic acid	$85.23^{a} \pm 3.28$	$94.75^{a} \pm 3.25$	$79.67^{b} \pm 1.97$	$87.08^{a} \pm 2.23$	$78.93^{b} \pm 2.63$	$84.32^{a} \pm 1.22$	$69.17^{b} \pm 2.71$	$83.52^{a} \pm 1.22$
Cystine	$89.54^{b} \pm 1.81$	$96.41^{a} \pm 2.97$	$76.15^{b} \pm 2.30$	$81.03^{a} \pm 2.61$	$82.96^{b} \pm 1.39$	$85.62^{a} \pm 1.55$	$77.12^{b} \pm 1.55$	$85.66^{a} \pm 2.62$
Glutamic acid	$93.45^{b} \pm 3.50$	$92.08^{a} \pm 5.62$	$81.15^{a} \pm 4.62$	$87.43^{a} \pm 5.23$	$82.85^{a} \pm 3.95$	$83.18^{a} \pm 1.01$	$74.66^{b} \pm 2.04$	$80.09^{a} \pm 1.48$
Glycine	$83.94^{b} \pm 3.15$	$85.49^{a} \pm 5.81$	$75.71^{b} \pm 1.09$	$80.98^{a} \pm 1.24$	$76.06^{a} \pm 2.27$	$77.74^{a} \pm 0.55$	$65.66^{b} \pm 3.86$	$71.95^{a} \pm 4.05$
Proline	$91.14^{a} \pm 2.20$	$95.45^{a} \pm 3.37$	$78.67^{b} \pm 1.48$	$89.56^{a} \pm 1.67$	$79.61^{b} \pm 2.03$	$85.59^{a} \pm 3.47$	$70.65^{b} \pm 1.42$	$85.32^{a} \pm 1.44$
Serine	$91.34^{b} \pm 3.32$	$97.71^{a} \pm 3.97$	$73.86^{b} \pm 3.58$	$84.54^{a} \pm 4.06$	$81.66^{a} \pm 4.54$	$83.75^{a} \pm 1.15$	$76.92^{b} \pm 1.73$	$82.91^{a} \pm 1.58$
Tyrosine	$93.95^{b} \pm 2.51$	$98.52^{a} \pm 2.73$	$76.25^{b} \pm 3.41$	$83.15^{a} \pm 3.86$	$82.02^{b} \pm 2.64$	$86.60^{a} \pm 1.02$	$74.02^{b} \pm 2.20$	$82.77^{a} \pm 5.51$

Values are means ± *SD* of six replication.

Mean bearing same superscript letters in a row between untreated and respective fermented samples does not differ significantly ($p > .05$).

TABLE 6 Effect of fungal fermentation on apparent digestibility coefficient (%) for essential amino acids of test ingredients in *Penaeus vannamei* using factorial ANOVA (n = 6)

	Apparent digestibility coefficient of essential amino acids									
Main effects	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Try	Val
Treatment (A)										
Untreated	80.42^{b}	83.17^{b}	80.69^{b}	76.70^{b}	79.66^{b}	80.22^{b}	79.31^{b}	78.28^{b}	79.40^{b}	80.85^{b}
Fermented	88.46^{a}	91.22^{a}	89.79^{a}	86.45^{a}	90.40^{a}	90.50^{a}	86.32^{a}	86.78^{a}	85.56^{a}	90.16^{a}
SEM (±)	3.13	3.33	1.72	4.25	1.98	2.52	2.59	2.66	3.45	0.84
Ingredient (B)										
SBM	94.21^{a}	94.30^{a}	93.15^{a}	94.80^{a}	94.86^{a}	97.95^{a}	92.36^{a}	92.96^{a}	90.89^{a}	90.32^{a}
GNC	85.23^{b}	84.59^{b}	83.72^{b}	76.92^{b}	82.96^{b}	82.25^{b}	81.21^{b}	79.45^{b}	80.69^{b}	83.14^{c}
RSM	81.49^{c}	84.92^{b}	82.48^{bc}	77.56^{b}	83.71^{b}	82.63^{b}	81.24^{b}	80.80^{b}	81.94^{b}	86.45^{b}
SFC	76.83^{d}	84.96^{b}	81.62^{c}	77.00^{b}	78.59^{c}	78.63^{c}	76.45^{c}	76.91^{c}	76.39^{c}	82.11^{c}
SEM (±)	3.13	3.33	1.72	4.25	1.98	2.52	2.59	2.66	3.45	0.84
p-Value										
A	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
B	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
A × B	<.001	<.001	<.001	<.001	<.001	<.001	.002	<.001	.004	<.001
CV (%)	3.278	3.275	2.411	3.954	2.591	2.912	3.043	3.092	3.524	1.673

Mean bearing same superscript letters in a column within main effects and interactions between the categories does not differ significant (p > .05).

TABLE 7 Effect of fungal fermentation on apparent digestibility coefficient (%) for non-essential amino acids of test ingredients in *Penaeus vannamei* using factorial ANOVA (n = 6)

	Apparent digestibility coefficient of nonessential amino acids							
Main effects	Ala	Asp	Cyt	Glu	Gly	Pro	Ser	Tyr
Treatment (A)								
Untreated	74.92^{b}	78.25^{b}	81.44^{b}	83.03^{b}	75.34^{b}	80.01^{b}	80.94^{b}	81.56^{b}
Fermented	82.23^{a}	86.06^{a}	86.76^{a}	86.77^{a}	79.04^{a}	89.03^{a}	86.66^{a}	87.56^{a}
SEM (±)	1.05	1.56	2.06	6.02	3.00	1.49	3.44	3.87
Ingredient (B)								
SBM	87.29^{a}	87.27^{a}	92.14^{a}	94.93^{a}	84.71^{a}	93.40^{a}	93.39^{a}	95.83^{a}
GNC	80.00^{b}	83.37^{b}	78.59^{d}	84.28^{b}	78.35^{b}	84.10^{b}	79.20^{c}	79.70^{c}
RSM	75.53^{c}	81.62^{c}	84.29^{b}	83.01^{b}	76.90^{b}	82.60^{b}	82.70^{b}	84.31^{b}
SFC	71.46^{d}	76.34^{d}	81.39^{c}	77.37^{c}	68.80^{c}	77.98^{c}	79.91^{c}	78.39^{c}
SEM (±)	1.05	1.56	2.06	6.02	3.00	1.49	3.44	3.87
p-Value								
A	<.001	<.001	<.001	.002	<.001	<.001	<.001	<.001
B	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
A × B	<.001	<.001	.026	.243	.081	<.001	.007	.202
CV (%)	2.036	2.376	2.670	4.521	3.512	2.258	3.465	3.643

Mean bearing same superscript letters in a column within main effects and interactions between the categories does not differ significant (p > .05).

earlier reports of Akiyama, Coelho, Lawrence, and Roberson (1989) in *P. vannamei* who suggested that it can be attributed to the better assimilation of crude protein. The variation observed in the ADC of crude protein between the ingredients could be ascribed to the differences in protein solubility and dispersibility indices (Zhou et al., 2014). In our earlier study (Jannathulla, Dayal, et al., 2017), it was found that SBM (84.11%) had higher KOH-protein solubility than GNC (81.75%), RSM (77.85%) and SFC (75.31%). However, fungal fermentation significantly (p < .05) increased the ADC of both dry matter and crude protein (Tables 5 and 8).

Untreated SBM had 13.36 and 10.03 g/kg of phytic acid and saponin, respectively. Swick and Ivey (1992) reported that the presence of phytic acid impaired growth rate in aquatic species by affecting protein digestibility due to the formation of phytic acid–protein complexes. Similarly, decreased feeding rate, growth and digestibility have been reported in *P. japonicus* when exposed to >0.1 mg/L of saponin (Chen, Chen, & Chen, 1996). The fungus *A. niger* has reduced trypsin inhibitor, phytic acid and saponin by 94.2%, 51.1% and 79.1%, respectively, during fermentation of SBM (Table 1). Reddy and Pierson (1994) reported that the reduction in phytic acid was attributed to its degradation into inositol and orthophosphate by the endogenous enzyme phytase produced during fermentation. *Aspergillus niger* had produced 58 U/g of phytase during solid-state fermentation as reported by Gull, Hameed, Aslam, and Athar (2013). Makkar and Becker (1999) reported the production of glycosidase enzymes, by microorganisms during fermentation, could break the saponin content into sapogenin and sugar moieties. The major reason for the poor digestibility of untreated GNC compared to fermented GNC was attributed to the high content of tannin (Table 1). Tannin hindered the accessibility of essential nutrients, particularly protein to the cultured species by forming indigestible protein complex (Makkar & Becker, 1999). Furthermore, Krogdahl (1989) documented the negative influence on the feed palatability due to bitter taste of tannin. Fungal fermentation reduced the tannin content from 17.56 to 2.88 g/kg in GNC. Emambux and Taylor (2003) suggested that it could partly be attributed to the production of enzyme tannase or microbial phenyl oxidase action. It was reported that *A. niger* has produced 12.26 U/g of tannase during solid-state fermentation (Liu et al., 2016). Glucosinolates is a major factor restricting the digestibility and utilization of RSM (Shi et al., 2015). Untreated RSM had 3.1 g/kg of glucosinolates, while it is not present in other ingredients tested in the present study. The pernicious impact of glucosinolates has been reported earlier in various aquatic species fed with a semipurified practical diet containing a higher level of RSM and mustard seed meal (Davies, McConnell, & Bateson, 1990; Gomes, Corraze, & Kaushik, 1993). The level of glucosinolates was reduced by 72% postfermentation in the present investigation. This is in agreement with Shi et al. (2015) who stated that the reduced glucosinolates in the fermented ingredients observed due to the utilization of glucose and sulphur moieties by the microorganisms during fermentation.

TABLE 8 Effect of fungal fermentation on apparent digestibility coefficient (%) for dry matter and crude protein of test ingredients in *Penaeus vannamei* using factorial ANOVA (n = 6)

	Apparent digestibility coefficients	
Main effects	**Dry matter**	**Crude protein**
Treatment (A)		
Untreated	58.74[b]	79.48[b]
Fermented	67.15[a]	88.74[a]
SEM (±)	1.16	0.44
Ingredients (B)		
SBM	85.08[a]	92.47[a]
GNC	60.23[b]	82.96[b]
RSM	59.75[b]	81.75[c]
SFC	46.72[c]	79.26[d]
SEM (±)	1.16	0.44
p-Value		
A	<.001	<.001
B	<.001	<.001
A × B	<.001	<.001
CV (%)	2.675	1.237

Mean bearing same superscript letters in a column within main effects and interactions between the categories does not differ significant (p > .05).

The challenging problem of digestibility with untreated SFC was mainly related to the limited capability of *P. vannamei* to digest fibrous components. This is in agreement with the findings of Dayal et al. (2011), who reported that the digestibility and growth performance together significantly (p < .05) reduced with the inclusion of SFC in the diet of *P. monodon* due to the higher content of fibre. Typically, untreated SFC used in the present study had the highest content of fibre (288.5 g/kg) than other ingredients (69.6–128.7 g/kg). Lim et al. (1997) stated that *P. vannamei* fed a diet containing canola meal with high fibre (28%) exhibited depressed growth rate than those fed with low-fibre canola meal (14%). Bureau, Harris, and Cho (1999) suggested that the lack of fibre hydrolytic enzymes in monogastric animals, including shrimp, reduced the entangled essential nutrients by increasing gut transit time. The fungus, *A. niger*, is one of the beneficial microorganisms in reducing fibrous components by producing various fibre hydrolytic enzymes during fermentation (Shi et al., 2015). *Aspergillus niger* had produced 30 U/g of cellulose, 3,099 U/g of xylanase and 9 U/g of pectinase as reported by Reddy et al. (2015), Maciel et al. (2008) and Solis-Pereyra et al. (1996), respectively. In addition, fermented SFC also had a low level of antinutritional factors compared to the untreated one.

The database on amino acid digestibility would be more helpful and also provides flexible information on the usage and constraint about the protein sources in feed formulation. The plant-based protein sources in general are low in essential amino acids like methionine and lysine. Thus, Akiyama et al. (1989) recommended supplementing these amino acids to provide a good growth response. However, Williams, Barlow, and Rodgers (2001) have reported that supplementing crystalline amino acids into the diet led to higher leaching in water, faster degradation by enterocytes and microflora in the gastrointestinal tract and expanded the rate of absorption than protein-bound amino acids. These drawbacks have been overcome by fermentation using bacterial, fungal and yeast species (Ravindra, 2000). In the present study, the increase in limiting amino acids, viz. methionine, lysine and tryptophan, was noticed with fungal fermentation (Table 1). The digestibility of individual amino acids was highly variable between the ingredients tested in our study. In case of SBM, methionine showed the highest digestibility in the EAA, while arginine, valine and histidine showed a better digestibility in GNC, RSM and SFC, respectively, in both prior to as well as postfermentation (Tables 5 and 6). Fungal fermentation significantly (p < .05) increased the digestibility of most of the amino acids regardless of ingredients tested and the increase was superior

with fermented SFC compared to others as in crude protein digestibility. Although there are no reports on the in vivo amino acid digestibility with regard to fermented ingredients, the results of our study are in agreement with the findings of Shi et al. (2015) relating to the increase in in vitro digestibility of amino acids in fermented RSM with *A. niger*. The increase in amino acid digestibility due to fermentation could be attributed to the secretion of extracellular proteolytic enzymes (proteases). Shi et al. (2015) reported that *A. niger* had produced >800 unit/g of protease during the fermentation of RSM. Of all amino acids, both untreated and fermented ingredients have shown a poor digestibility for alanine and glycine in SBM, RSM and SFC (Table 5). Akiyama et al. (1989) suggested that it can be due to the secretion of chitinous peritrophic membrane, which surrounds the faeces. This is similar to the findings of Dayal et al. (2011) for SFC in the diet of *P. monodon*, who reported that chitin had a higher content of alanine and glycine than other amino acids. However, in contrast, leucine and serine had lower digestibility in fermented GNC.

5 | CONCLUSION

The results of the present study demonstrated that ADC was significantly affected by both treatments (untreated and fermented) and ingredients (SBM, GNC, RSM and SFC). *P. vannamei* efficiently digests fermented ingredients compared to the untreated ones in the present experimental condition.

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ORCID

J.S. Dayal http://orcid.org/0000-0002-8601-1186

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