Received: 24 November 2018 | Revised: 4 February 2019 | Accepted: 18 February 2019

DOI: 10.1111/are.14024

ORIGINAL ARTICLE

WILEY Aquaculture Research

Apparent digestibility coefficients of fungal fermented plant proteins in two different penaeid shrimps—A comparative study

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Funding information
Indian Council of Agricultural Research, Grant/Award Number: 2049/3001

Abstract

Using the 70:30 replacement method and chromium as an inert marker, the digestibility of four fermented oilseed meals/cakes (soybean meal (FSBM), groundnut oil cake (FGNC), rapeseed meal (FRSM), and sunflower oil cake (FSFC)) were determined in *Penaeus monodon* and *Penaeus indicus*. Apparent dry matter digestibility (ADMD) of fermented ingredients was ranked as FSBM > FGNC > FRSM > FSFC. The critical variations in apparent protein digestibility (APD) in *P. monodon* (0.816%) and *P. indicus* (0.608%) were lower than the ADMD. Appaprent amino acid digestibility (AAD) was >90% in FSBM for both the species and was lower for other ingredients. Protein had a higher digestibility than total amino acids and was in the range of 0.69%–2.71% in *P. monodon* and 0.32%–2.75% in *P. indicus*. A correlation between the ADC of total amino acids and protein was found to be $r = 0.8229$ in *P. indicus* and $r = 0.7447$ in *P. monodon*. Data were further subjected to two way analysis of variance for assessing the digestibility variations between the species. It was observed that *P. indicus* had higher values of ADMD than *P. monodon* in FSBM (2.97%) and FRSM (1.22%) and the reverse was true in FGNC and FSFC. The APD was high in *P. indicus* for FSBM, FGNC and FRSM but not for FSFC. However, significant variations could be noticed in AAD between the species.

KEYWORDS

Aspergillus niger, digestibility, fermentation, *Penaeus indicus*, *Penaeus vannamei*, shrimp feed

1 | INTRODUCTION

Aquaculture, in particular shrimp farming has become a fast growing food producing sector worldwide. India is one of the largest contributors to brackishwater aquaculture production which includes the culture of shrimp, mainly imported specific pathogen free *Penaeus vannamei* and the native species Black tiger shrimp, *Penaeus monodon* and Indian white shrimp, *Penaeus indicus* because of their excellent growth performance and tolerance to a wide range of salinity. In intensive shrimp culture, feed is an important component that accounts for 50%–70% of the total production cost. The growth of shrimp aquaculture has nowadays been related to an increase in shrimp feed production (Sookying, Davis, & Soller Dias Da Silva, 2013). However, the periodic shortage and high cost of fishmeal necessitated for searching suitable alternatives in relation to quality, economics and availability in last two decades. In this case, fermented plant proteins have been explored as potential protein sources in the diet of shrimp due to their improved nutritional quality compared to their respective untreated ones. In our earlier study (Jannathulla, Dayal, Vasanthakumar, Ambasankar, & Muralidhar,

2017), it was found that the microbial fermentation had significantly reduced fibre fractions and anti-nutrients, while the essential amino acids, especially methionine, lysine, and tryptophan were found to be increased. Though the fermented materials have been used with varying degree of success in the diet of shrimp, replacing fishmeal completely by using these ingredients is a quite challenging task for the researchers, which indicates the poor availability of nutrients from the ingredients even after fermentation. Consequently, the fungal fermented ingredients were tested for digestibility (Jannathulla, Dayal, Vasanthakumar, Ambasankar, & Muralidhar, 2018) and nutrient utilization (Jannathulla, Dayal, Ambasankar, & Muralidhar, 2018) along with the respective untreated ones in *P. vannamei* with varying degrees of success. In most of the earlier studies, the emphasis was restricted to analysing the nutritional value of the diets alone. Since the associated effects of each constituent in the formulation affected feed digestibility, assessing the digested value of the diet may not be an average of each ingredient present in the formulation (Akiyama, Coelho, Lawrence, & Roberson, 1989). Thus, assessing the nutritional value of each ingredient is vital to increase their utility economically and effectively. Hence, to explore the suitability of fermented ingredients, four different fungal (*Aspergillus niger*) fermented plant proteins were tested for apparent dry matter digestibility (ADMD), protein digestibility (APD) and essential amino acid digestibility (AAD) in two penaeid shrimps viz., *P. monodon* and *P. indicus*. Till date, there has been no report on such a study.

2 | MATERIALS AND METHODS

2.1 | Preparation of fermented plant proteins

Four commercial solvent extracted plant proteins viz., soybean meal (SBM), groundnut oil cake (GNC), rapeseed meal (RSM) and sunflower oil cake (SFC) were purchased from local markets in and around Chennai, India ($n = 6$). All the six replicates of each ingredient were pooled together to have a representative sample. From the pooled homogenate of an ingredient, six replications were taken for the fermentation process after grinding to a fine particle size using a hammer mill which passed through a <500 µm sieve. Prior to fermentation, the fungus, *A. niger* (ATCC-6275) listed under GRAS notifications (Generally Recognized As Safe) by FDA (GRAS Notice No. 35, 2010) acquired from Himedia Laboratories (Mumbai, India) was grown on potato dextrose agar (PDA) at 35 ± 1°C in an incubator. After five days, the fungal spores were collected using 0.1% Tween 80 and the microbial suspension was adjusted to 1×10^7 spores/ml using sterilized distilled water. This suspension was used as an inoculam for the fermentation with six replications for each test ingredients as described by Jannathulla et al. (2017). Briefly, the ground sample was weighed in a 500 ml Erlenmeyer flask to which deionized water was added to adjust the moisture content between 60% and 65%. The hydrated materials were sterilized by autoclaving at 121°C for 15 min and after cooling at room temperature, they were inoculated with the microbial suspension. The inoculated materials were hand-mixed and plugged using cotton for air transfer, then allowed to ferment at 35 ± 1°C in an incubator for 3 days. Post fermentation, the residual matters of all the replicates of an ingredient was pooled and dried at 40°C in an oven to reduce the moisture content to <10%. All the fermented samples were refrigerated at 4°C until use. The chemical composition of fermented test ingredients is given in Table 1.

2.2 | Preparation of experimental diets

A reference diet (Table 2) was formulated, to have ~375 g/kg crude protein and ~70 g/kg ether extract, using fishmeal, mantis shrimp meal, corn gluten meal and sesame cake as a major protein source. The experimental diets were prepared by the conventional

TABLE 1 Proximate and essential amino acid composition of test ingredients used in this study (g/kg dry matter basis)

Particulars	Test ingredients			
	FSBM[a]	FGNC[b]	FRSM[c]	FSFC[d]
Proximate composition				
Crude protein	598.5	520	467.5	375.4
Ether extract	7.8	19.5	22.8	15.6
Crude fibre	67.7	127.3	102.8	264.1
Nitrogen free extract[e]	246.0	252.9	337.5	264.3
Total ash	80.0	80.2	69.4	80.6
Essential amino acids				
Arginine	40.7	35.8	35.1	18.8
Histidine	19.5	10.9	16.6	5.6
Isoleucine	29	15.2	15.5	33.7
Leucine	40.1	11.1	23.9	18.7
Lysine	40.1	28.1	20.5	23.1
Methionine	9.9	10.3	13.1	17.8
Phenylalanine	25.2	31	12.6	17.2
Threonine	19.1	13.1	21.3	15.1
Tryptophan	7.7	5.3	4.9	4.5
Valine	17.6	27.9	25.4	14.9
Fibre fractions				
Neutral detergent fibre	114.7	209.5	253.0	403.6
Acid detergent fibre	73.9	138.1	185.1	257.2
Cellulose	55.8	68.5	76.3	156.0
Hemicellulose	40.1	71.5	68.1	146.4
Lignin	7.4	50.2	84.8	76.6
Anti-nutritional factors				
Trypsin inhibitor	0.1	–	–	–
Tannin	–	2.9	5.1	6.1
Glucosinolates	–	–	1.8	–

[a]Fermented soybean meal. [b]Fermented groundnut oil cake. [c]Fermented rapeseed meal. [d]Fermented sunflower oil cake. [e]Calculated by a difference.

methodology of mixing test ingredient with reference diet mix (Carvalho, Ota, Kadry, Tacon, & Lemos, 2016; Chen, Liu, Xie, Zhang, & Niu, 2016; Jannathulla, Dayal, Vasanthakumar et al., 2018; Zhou, Davis, & Buentello, 2014), where 30% of the reference diet was replaced (w/w) on an as fed basis by the respective test ingredient being analysed for digestibility. The diet preparation was carried out after Dayal, Rajaram, Ambasankar, and Ahamad Ali (2011). Briefly, all the listed dry ingredients (Table 2) were powdered in a hammer

TABLE 2 Ingredient, proximate and essential amino acid composition of reference and test diets used in this study

Particulars	Reference diet (Ref-D)	Test diets			
		FSBM-D	FGNC-D	FRSM-D	FSFC-D
Ingredient composition (g/kg as fed basis)					
Fishmeal	250	175	175	175	175
Mantis shrimp meal	120	84	84	84	84
Squid meal	40	28	28	28	28
FSBM	–	300	–	–	–
FGNC	–	–	300	–	–
FRSM	–	–	–	300	–
FSFC	–	–	–	–	300
Corn gluten	50	35	35	35	35
Sesame cake	60	42	42	42	42
Rice bran	40	28	28	28	28
Broken rice	50	35	35	35	35
Wheat	325	226	226	226	226
Fish oil	20	14	14	14	14
Soy-lecithin	10	7	7	7	7
Premix[a]	20	14	14	14	14
Binder[b]	10	7	7	7	7
Chromium oxide	5	5	5	5	5
Proximate composition (g/kg as fed basis)					
Moisture	74.7	53.5	49.7	52.6	73.4
Crude protein	374.2	410.8	381.5	377.3	369.8
Ether extract	71.6	56.3	65.3	64.4	55.2
Crude fibre	26.2	33.8	43.8	39.3	64.9
Nitrogen free extract	316.6	328.5	340.1	345.5	318.1
Total ash	136.7	117.1	119.6	120.9	118.6
Essential amino acids (g/kg of protein)					
Arginine	61.7	70.4	70.2	73.2	58.9
Histidine	23.4	30.7	24.9	30.4	21.2
Isoleucine	41.0	49.7	41.4	41.6	56.4
Leucine	70.5	75.2	57.4	69.3	65.1
Lysine	57.3	67.2	63.7	63.7	59.3
Methionine	22.4	21.9	23.1	26.7	30.2
Phenylalanine	46.3	48.4	56.4	45.1	46.7
Threonine	38.2	38.5	37.0	44.6	39.2
Tryptophan	11.1	13.6	11.8	11.9	11.5
Valine	45.7	42.4	53.5	53.4	44.5

[a]Premix (mg/kg): Vitamin A (20 000 IU), B_1 (70 mg), B_2 (60 mg), B_6 (120 mg), B_{12} (60 mg), C (1,000 mg), D_3 (300,000 IU), E (200 mg), K_3 (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), Iron (100 mg), Copper(5 mg), Zinc (50 mg), Manganese (40 mg) Selenium (20 mg), Cobalt (1 mg) and Iodine (100 mg)
[b]Pegabind, BentoliAgriNutrition Asia Pvt Ltd, Singapore

mill and passed through a 250 µm sevie. To this, the micro ingredients, including premix and binder were mixed followed by fish oil and soy-lecithin. Chromium oxide (5 g/kg) was included in all the formulations as an indigestible external marker and were homogenized for 10 min by hand. The homogenized mash was made into a dough by adding water at the rate of 500 ml/kg and was steam cooked at atmospheric pressure for 5 min. The resulting mixture was pelleted in a table-top pelletizer with 2 mm diameter die (5–6 mm long). The feed pellets were then dried at 60°C overnight in a hot air oven. The dry pellets were placed in a plastic container and refrigerated at 4°C until use.

2.3 | Animal husbandry and experimental condition

A 30-day digestibility trial was carried out at the Muttukadu Experimental Station of ICAR-Central Institute of Brackishwater Aquaculture, Chennai, India. Around 500 *P. monodon* shrimp were procured from a local farm near Gumudipoondi, Thiruvallur, India, whereas, *P. indicus* were collected from the sea near Kovalam, Chennai, India, with the help of local fishermen. Both the species were kept in a 2,000 L circular fibreglass reinforced plastic (FRP) tanks separately and were acclimatized to indoor laboratory condition for 4 weeks with a basal diet containing 370 g/kg crude protein and 70 g/kg ether extract without chromium oxide. The experimental tanks used in this study were oval-shaped, with the capacity of 500 L (1.31 × 0.64 × 0.73 m). All the tanks were fitted with a side standpipe for draining water and covered with fibremate to prevent escape of shrimp. Totally, 15 tanks were used for each species and were supplied with filtered water and continuous aeration through single air-stone. Ultraviolet treated water was used throughout the experimental periods and 80% of the water was exchanged daily prior to first feeding. The water quality parameters viz., salinity (19–21 g/L), temperature (26.5–28.5°C), dissolved oxygen (5.8–7.8 mg/L), pH (8–8.5) and total ammonia nitrogen (<0.1 mg/L) were measured periodically by standard methods (APHA, 2012) and all the parameters were maintained at the optimal level required for shrimp culture. A total of 150 each of *P. monodon* (15.04 ± 2.71 g) and *P. indicus* (14.59 ± 1.49 g) were randomly transferred from the acclimatized tanks to the experimental tanks (three replicates per diet and 10 shrimps per replicate). Shrimps were kept in a starving condition for 24 hr and allowed to feed on a respective diet thrice a day at 7.00 a.m., 12.30 p.m. and 5.30 p.m. Feed was given at the rate of 6% of the total biomass initially and was adjusted later based on the feed intake. Shrimps were allowed to feed for an hour each time they are fed. After an hour of feeding, the uneaten feed pellets and other particles, including faeces were siphoned out from all the experimental tanks and the bottom of the tanks was kept clean to prevent possible contamination. For the next one hour, the faeces were collected immediately after defecation with the help of a clean Falcon tube in a channel darting a silk fabric. They were rinsed with deionized water and transferred to a filter paper using forceps, dried and frozen immediately at −20°C (Carvalho et al., 2016; Jannathulla, Dayal, Vasanthakumar et al., 2018). The apparent digestibility of dry matter (ADMD), protein (APD) and essential amino acid (AAD) were analysed according to Smith and Tabrett (2004), Carvalho et al. (2016), and Jannathulla, Dayal, Vasanthakumar et al. (2018).

$$\text{Digestibility of feed } (\%) = 1 - (F/D \times \text{m}D/\text{m}F),$$

where D is the nutrient in the diet (%), F is the nutrient in the faeces (%), mD is the marker in the diet (%), and mF is the marker in the faeces.

$$\text{Digestibility of ingredients } (\%) = D_{TD} + [(D_{TD} - D_{RD}) \times (I_{RD} \times N_{RD}/I_I \times N_I)]$$

where D_{TD} is the digestibility of test diet, D_{RD} is the digestibility of reference diet, I_{RD} is the reference diet in the mash, N_{RD} is the nutrient in the reference diet mash, I_I is the test ingredient in the mash and N_I is nutrient in the test ingredient.

2.4 | Biochemical analysis

Proximate composition of ingredients and experimental diets were analysed according to the method of AOAC (1997). Chromium content in the diets and faeces was analysed by Furukawa (1966) method. Fibre fractions (Van Soest, Robertson, & Lewis, 1991), trypsin inhibitor (Kakade, Rackis, McGhee, & Puski, 1974), tannin (Price, Van Scoyoc, & Butler, 1978) and glucosinolate (McGhee, Kirk, & Mustakas, 1965) were analysed by the respective standard methods. Kjeltech (Model No: Kjeltech^{TM}-8100, Foss), Soxhlet (Model No: Scocs Plus-SCS:6, Pelican) and Fibertech (FOSS-2022, Foss) apparatus were used for proximate analysis. Chromic oxide and anti-nutrients were analysed by using UV–VIS spectrophotometer (UV-1800, Shimadzu).

The samples were hydrolysed using 6 N hydrochloric acid in a sealed tube at 110°C in an oven for 22 hr (Finlayson, 1964). After digestion, the concentrated acid was dried using a vacuum rotary evaporator (IKA, RE 10 C S84), and the residual matter was brought into a known volume of 0.1 N hydrochloric acid. Precolumn HPLC gradient system (Shimadzu Corp, LC- 30AD) was used to analyse the essential amino acids. The column used in this study was YMC- Triart C18, RRH (1.8 µm, 2.1 × 100 mm). The gradient elution was made using phosphate buffer (20 mmol) and a combination of acetonitrile: methanol: water (45:40:15) as a mobile phase A and B respectively. Mercaptopropionic acid, *O*- phthalaldehyde and fluorenylmethoxycarbonyl chloride were used as derivatizing agents. All the reagents, including samples were filtered through a 0.2-µm membrane syringe filter prior to injection. The gradient runs at the flow rate of 0.3 ml/min and 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. The eluted amino acids were quantified by a fluorescent detector (RF-20AXS) using the amino acid mixer as an external standard (Sigma Aldrich, Cat. No: AAS18) and norleucine as an internal standard. Tryptophan, being liable to acid hydrolysis, was measured after alkali hydrolysis by spectrophotometric method at 500 nm (Sastry &

Tammuru, 1985). To prevent the partial oxidization of sulphur containing amino acid, in particular methionine, due to acid digestion, 0.1% phenol was added along with digestive agent (Jajic, Krstovic, Glamocis, Jaksis, & Abramovic, 2013).

2.5 | Statistical analysis

The data were subjected to ANOVA (one way) to evaluate the suitability of fermented ingredients (FSBM, FGNC, FRSM and FSFC) in each species. Wherever significant differences were noticed between the groups, means were further compared with Tukey's test. The data were further subjected to ANOVA (two way) to assess the variation between the two species (*P. monodon* and *P. indicus*). Prior to statistical evaluation, data were checked for homogeneity of variance after ascertaining for normal distribution. Linear regressions between the ADC of total amino acids and protein was tested among the test ingredients in both the species. Data were analysed using SPSS version 16.0, and significance was tested at $p < 0.05$.

3 | RESULT

The ADMD, APD and AAD of various fermented plant proteins are given in Table 3 (*P. monodon*) and Table 4 (*P. indicus*). The ADMD was significantly affected by the ingredient in both the species ($p \leq 0.001$) and showed a trend as FSBM > FGNC > FRSM > FSFC, while no specific trend could be noticed for APD and AAD in relation to the ingredients. The ADMD was in the range of 49.75%–84.71% in *P. monodon* and 46.33%–87.68% in *P. indicus*. In both the species, FSBM showed the highest ADMD and a significantly ($p < 0.05$) lower value

TABLE 3 Apparent dry matter, protein and essential amino acid digestibilities (%) of fungal fermented plant proteins for *Penaeus monodon*

Particulars	Test ingredients				*SEM* (±)	*p*-value	CV (%)
	FSBM	FGNC	FRSM	FSFC			
Dry matter	84.71^{a}	61.79^{b}	55.94^{c}	49.75^{d}	0.847	<0.001	1.921
Protein	93.71^{a}	84.97^{c}	83.82^{c}	86.38^{b}	0.293	<0.001	0.816
Arginine	95.07^{a}	89.13^{b}	82.26^{c}	80.60^{c}	3.807	0.002	2.960
Histidine	95.06^{a}	87.24^{b}	86.74^{b}	91.34^{ab}	3.253	0.016	2.635
Isoleucine	93.56^{a}	87.24^{b}	83.11^{b}	93.57^{a}	4.058	0.007	2.967
Leucine	95.57^{a}	79.16^{c}	80.20^{c}	89.43^{b}	1.631	<0.001	1.952
Lysine	96.93^{a}	87.00^{b}	86.49^{b}	87.63^{b}	8.158	0.041	4.200
Methionine	96.90^{a}	86.06^{b}	85.62^{b}	87.21^{b}	8.008	0.029	4.187
Phenylalanine	93.51^{a}	82.13^{b}	81.71^{b}	79.15^{b}	9.259	0.018	4.760
Threonine	93.71^{a}	80.39^{b}	82.43^{b}	84.76^{b}	7.220	0.015	4.144
Tryptophan	90.70^{a}	82.67^{a}	82.30^{a}	81.37^{a}	12.859	0.154	5.601
Valine	90.70^{a}	86.54^{b}	87.64^{b}	88.89^{ab}	0.948	0.033	1.449

Note. Mean bearing same superscript in a row do not differ significantly ($p > 0.05$)

TABLE 4 Apparent dry matter, protein and essential amino acid digestibilities (%) of fungal fermented plant proteins for *Penaeus indicus*

Particulars	Test ingredients				*SEM* (±)	*p*-value	CV (%)
	FSBM	FGNC	FRSM	FSFC			
Dry matter	87.68^{a}	59.76^{b}	57.16^{c}	46.33^{d}	0.398	<0.001	1.324
Protein	95.12^{a}	89.23^{c}	91.37^{b}	86.54^{d}	0.175	<0.001	0.608
Arginine	97.62^{a}	91.11^{a}	91.27^{a}	83.48^{b}	7.785	0.019	4.041
Histidine	96.42^{a}	85.17^{b}	92.89^{a}	94.82^{a}	7.315	0.032	3.855
Isoleucine	95.25^{a}	91.74^{b}	88.12^{c}	87.91^{c}	1.743	0.006	1.915
Leucine	97.15^{a}	83.14^{b}	85.93^{b}	86.20^{b}	4.705	0.004	3.240
Lysine	93.43^{a}	87.69^{a}	92.73^{a}	85.09^{a}	7.032	0.071	3.889
Methionine	95.40^{a}	84.71^{b}	92.95^{a}	84.15^{b}	8.434	0.024	4.281
Phenylalanine	94.90^{a}	87.33^{bc}	91.35^{ab}	85.24^{c}	2.715	0.006	2.418
Threonine	94.85^{a}	85.02^{bc}	87.10^{b}	80.69^{c}	3.893	0.003	2.988
Tryptophan	95.37^{a}	88.15^{ab}	90.00^{ab}	82.74^{b}	8.190	0.034	4.229
Valine	94.92^{a}	89.54^{a}	92.64^{a}	90.84^{a}	7.775	0.038	3.990

Note. Mean bearing same superscript in a row do not differ significantly ($p > 0.05$)

was noticed with FSFC. The APD was more pronounced, in particular for FGNC, FRSM and FSFC, and also resulted in lower CV (0.816% in *P. monodon* and 0.608% *P. indicus*) than that of ADMD (1.324%–1.921%). As in dry matter, APD was also found to be the highest in FSBM for *P. monodon* (93.71%). The FSFC had the second highest digestible value of protein in *P. monodon*, while FGNC and FRSM did not differ significantly. The derived APD of test ingredients significantly ($p < 0.05$) differed from each other in *P. indicus* also and was found to be high in FSBM followed by FRSM, FGNC and FSCF. Of all the analysed ingredients, the AAD was significantly ($p < 0.05$) higher in FSBM and was >90% for both the species. Except tryptophan, all the essential amino acids significantly ($p < 0.05$) differed among the test ingredients in *P. monodon* (Table 3). However, lysine and valine were not affected due to the variation in ingredients in *P. indicus* (Table 4). The FSFC accounted for the least digestibility for most of the essential amino acids in *P. indicus*, but the result found to be higher in *P. monodon*. The mean AAD of FGNC was statistically similar to FRSM as in APD, in both the species with the exception of arginine in *P. monodon* and histidine and isoleucine in *P. indicus*. A higher correlation between the ADC of total amino acids and protein of fungal fermented plant proteins was found in *P. indicus* ($r = 0.8229$) (Figure 1) and was reduced to $r = 0.7447$ in *P. monodon* (Figure 2).

Two way analysis of digestibility parameters between the species (Table 5) revealed a significant ($p < 0.05$) difference in APD between the species but not in ADMD. The digestibility of arginine, leucine, phenyalanine, tryptophan and valine was significantly ($p < 0.05$) high in *P. indicus* compared *P. monodon* and the increase was in the range of 2.02%–5.58%, whereas the digestibility of other amino acids did not differ between the species. When the comparisoin was made among the ingredients irrespective of the species, the trend of ADMD was ranked as FSBM > FGNC > FRSM > FSFC as in one way ANOVA and the APD was significantly high in FSBM (94.41%) and low in FSFC (86.45%), while no difference was noticed between FGNC and FRSM. However, the mean values were significantly ($p < 0.05$) different between the species for all ingredients analysed. *P. indicus* dominated over *P. monodon* by 2.97% and 1.22% in FSBM and FRSM, respectively, whereas the reverse trend was true for FGNC and FSFC (Figure 3). As in APD, the digestibility of all the essential amino acids was significantly ($p < 0.05$) high in FSBM (93.03%–96.40%) compared to other ingredients tested (FGNC, FRSM and FSFC). However, the digestibility of valine did not vary among the ingredients selected (88.04%–92.81%). Though the protein digestibility was low, the FSFC had the higher ($p < 0.05$) digestibility for histidine, isoleucine and leucine than FGNC and FRSM.

4 | DISCUSSION

Nutrient availability and digestibility is a major factor affecting the utilization of ingredients in animals fed formulated feed. Having an adequate knowledge on the nutritional/digestible value of all the feed ingredients is vital to formulate a well balanced and economical diet. In our study, ADMD varied among the ingredients tested for both the species. It was in the range of 84.71%–87.68% in FSBM, 59.76%–61.79% in FGNC, 55.94%–57.16% in FRSM and 46.33%–49.75% in FSFC for both *P. monodon* and *P. indicus*. Our values are almost similar with the

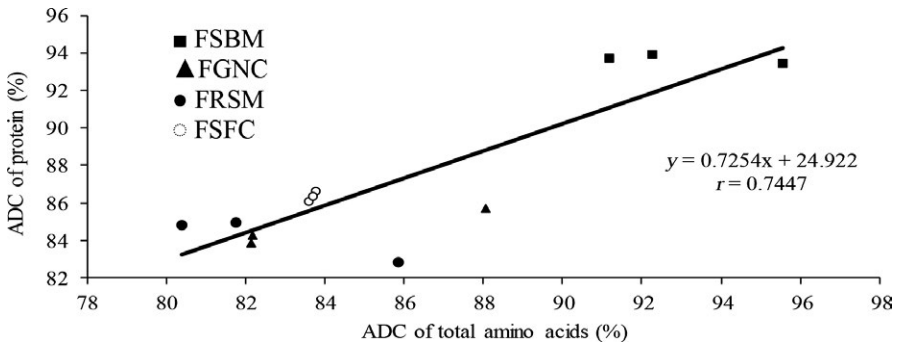

FIGURE 1 Relationship between ADC (Apparent digestibility coefficients) of total amino acids and protein of fermented plant proteins in *Penaeus monodon*

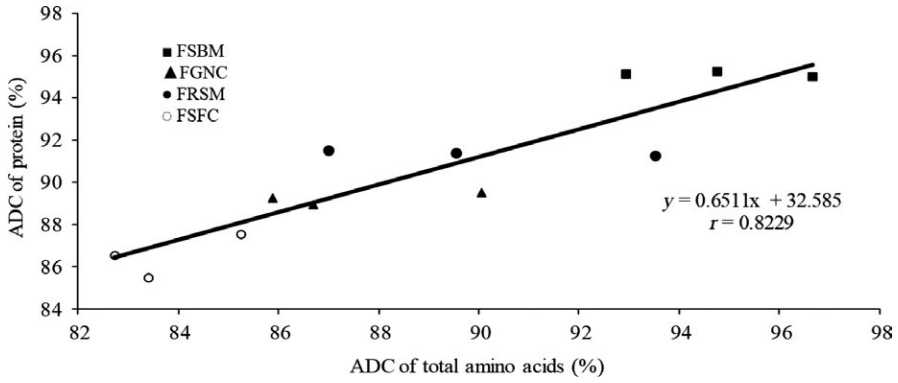

FIGURE 2 Relationship between ADC of total amino acids and protein of fermented plant proteins in *Penaeus indicus*

TABLE 5 Comparison of apparent dry matter, protein and essential amino acid digestibilities (%) of fungal fermented plant proteins in *Penaeus monodon* and *Penaeus indicus* after two way ANOVA

	Apparent digestibility coefficients											
Particulars	**Dry matter**	**Protein**	**Arginine**	**Histidine**	**Isoleucine**	**Leucine**	**Lysine**	**Methionine**	**Phenylalanine**	**Threonine**	**Tryptophan**	**Valine**
Peaneid shrimps (A)												
P. monodon	63.04^{a}	87.21^{b}	86.76^{b}	90.09^{a}	89.37^{a}	86.08^{b}	89.51^{a}	88.94^{a}	84.12^{b}	85.32^{a}	84.26^{b}	88.44^{b}
P. indicus	62.73^{a}	90.56^{a}	90.87^{a}	92.34^{a}	90.75^{a}	88.10^{a}	89.73^{a}	89.28^{a}	89.70^{a}	86.91^{a}	89.06^{a}	91.98^{a}
Fermented ingredients (B)												
FSBM	86.19^{a}	94.41^{a}	96.34^{a}	95.74^{a}	94.40^{a}	96.35^{a}	95.18^{a}	96.15^{a}	94.20^{a}	94.28^{a}	93.03^{a}	92.81^{a}
FGNC	60.77^{b}	87.09^{b}	90.12^{b}	86.20^{c}	89.49^{b}	81.15^{c}	87.34^{b}	85.38^{b}	84.73^{bc}	82.70^{b}	85.41^{b}	88.04^{a}
FRSM	56.54^{c}	87.59^{b}	86.76^{b}	89.84^{bc}	85.61^{c}	83.06^{c}	89.60^{b}	89.25^{b}	86.52^{b}	84.76^{b}	86.14^{b}	90.13^{a}
FSFC	48.04^{d}	86.45^{c}	82.04^{c}	93.08^{ab}	90.74^{b}	87.81^{b}	86.36^{b}	85.68^{b}	82.19^{c}	82.72^{b}	82.05^{b}	89.86^{a}
p-values												
A	0.436	<0.001	0.005	0.125	0.176	0.047	0.877	0.822	0.001	0.214	0.010	0.007
B	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	0.002	0.064
A×B	<0.001	<0.001	0.190	0.230	0.006	0.022	0.107	0.100	0.226	0.076	0.594	0.787
SEM (+)	0.535	0.201	5.151	6.546	3.251	2.970	6.849	7.547	6.079	5.172	9.132	4.393
CV (%)	1.530	0.663	3.363	3.691	2.635	2.604	3.843	4.057	3.733	3.476	4.589	3.058

Note. Mean bearing same superscript in a column within main effects and interactions between the categories do not differ significant ($p > 0.05$)

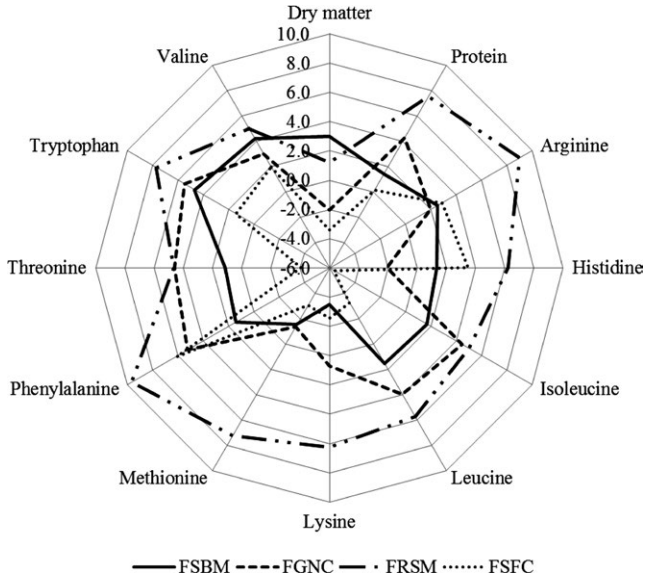

FIGURE 3 Per cent change of apparent digestibility of dry matter, protein and essential amino acids between *Penaeus indicus* and *Penaeus monodon*. FSBM: Fermented soybean meal; FGNC: Fermented groundnut oil cake; FRSM: Fermented rapeseed meal; FSFC: Fermented sunflower oil cake

values reported by Jannathulla, Dayal, Vasanthakumar et al. (2018) in *P. vannamei*. The ADMD of FGNC, FRSM and FSFC was significantly ($p < 0.05$) lower compared to FSBM in both the species. This could be explained by a lower content of fibre fractions in FSBM compared to other fermented ingredients (Table 1). Fibre fractions did not have any digestible energy for shrimp (Brunson, Romaire, & Reigh, 1997); however, carbohydrates other than fibre (NFE) can be digested with varying degrees of efficiency, based on the digestive capability of the cultured species. Carvalho et al. (2016) reported a higher digestibility for soy-based products due to the high protein and low level of fibre and anti-nutrients compared to other plant proteins. This indicates that dry matter digestibility could widely vary among plant products, though they have a similar chemical composition. Brunson et al. (1997) documented <70% of ADMD for various plant proteins in *P. setiferus* and who suggested that this is attributed to the insufficient utilization of carbohydrates. Chen et al. (2016) reported that the ADMD decreased with the increase in fibre content of feed ingredients. Dayal et al. (2011) reported lower digestibility and growth performance due to the inclusion of SFC in the diet of *P. monodon* and attributed this to the limited capability of monogastric animals, in particular shrimp to digest fibrous components. When shrimp were reared by feeding two varieties of canola meal, having a fibre level of 28% and 14%, the growth and digestibility were found to be depressed in the group fed a high fibre canola meal due to poor assimilation (Lim et al., 1997). The high fibre content increases gut transit time and reduces the availability of entangled essential nutrients (Bureau, Harris, & Cho, 1999). This phenomenon could be attributed to the lack of fibre digesting enzymes in shrimp. The fibre fractions of FSFC (Table 1) were comparatively higher than other ingredients tested in our study, which probably resulted in low ADMD of 49.75% in *P. monodon* and 46.33% in *P. indicus*. The ADMD of both FGNC and FSFC was higher in *P. monodon*, while *P. indicus* revealed higher ADMD for FSBM and FRSM. This disparity in the values between the species could partly be attributed to the variation in the nutritional requirement of the fed animals and nutritional composition of the test ingredients.

The APD of selected fermented plant proteins found to be in the range of 84.97% to 93.71% in *P. monodon* and 86.54% to 95.12% in *P. indicus*. The values found in our study were higher than those reported earlier for untreated plant protein sources in penaeid shrimps (Carvalho et al., 2016; Chen et al., 2016; Jannathulla, Dayal, Vasanthakumar et al., 2018). This indicates that the fermented plant proteins could effectively be used by the penaeid shrimps. However, APD significantly ($p < 0.05$) varied according to the ingredient type in our study. Of all the fermented plant proteins tested, FSBM had the highest digestibility for protein irrespective of the species (93.71%–95.12%). This could be due to a well balanced amino acid profile of FSBM compared to FGNC, FRSM and FSFC. The low APD of FGNC, FRSM and FSFC compared to FSBM in our study could possibly be due to known anti-nutrients, in particular tannin. Makkar and Becker (1999) stated that tannin makes protein become unavailable by forming an indigestible protein complex. Excluding FSBM, the other ingredients were ranked as FSFC > FGNC > FRSM in *P. monodon* and the trend was FRSM > FGNC> FSFC in *P. indicus* based on APD. According to the findings of Brunson et al. (1997), *P. setiferus* could digest 94.63% protein of SBM. Chen et al. (2016) reported that *P. vannamei* had an APD of SBM, GNC and RSM as 98.85%, 86.78% and 90.76% respectively. The APD was in the range of 71%–100% for various ingredients in *Palaemon serratus* and *Pandalus platyceros*. The difference in APD between the species can partly be attributed to the variation in the amino acid requirements. Lan and Pan (1993) reported lower APD when *P. monodon* were fed a diet with lower levels of lysine, arginine and phenylalanine than those fed a diet with the higher content of these amino acids. The APD of all the test ingredients was found to be high in *P. indicus* compared to *P. monodon* (Figure 3). The APD was significantly ($p < 0.05$) higher by 7.55% in FRSM, 4.26% in FGNC and 1.41% in FSBM for *P. indicus* compared to *P. monodon*, which indicates that the protein from fermented plant proteins could be effectively assimilated by *P. indicus*. However, there was no significant difference for FSFC between the species.

Besides the knowledge on ADMD and APD, the plant protein sources could effectively be used in feed formulation when their AAD values are known. The result of our earlier study (Jannathulla et al., 2017; Jannathulla, Dayal, Vasanthakumar et al., 2018) showed an enhanced digestibility of fungal fermented ingredients compared to their respective untreated ones in *P. vannamei*. In the present investigation, the average of total essential AAD was high in FSBM and was >90% for both the species, whereas the FRSM and FSFC had the lowest average in *P.monodon* and *P. indicus* respectively. The highest AAD for FSBM was recorded for lysine (96.93%) in *P. monodon* and arginine (97.62%) in *P. indicus*. However, in our previous study (Jannathulla, Dayal, Vasanthakumar et al., 2018) the highest digestibility to methionine (98.75%) was observed in *P. vannamei*. Nevertheless, the values reported earlier for essential amino acids varied slightly (Carvalho et

al., 2016; Chen et al., 2016; Jannathulla, Dayal, Vasanthakumar et al., 2018). In case of FGNC, FRSM, FSFC, the most digestible essential amino acids were arginine, valine and isoleucine for *P. monodon* and isoleucine, methionine and histidine for *P. indicus*.

The FSBM is the best source of all the essential amino acids according to our digestibility trial in *P. monodon* and *P. indicus* compared to other fermented ingredients (FGNC, GRSM and FSFC). The present results are corroborated with the earlier findings (Yang et al., 2009). However, the digestibility of tryptophan did not statistically differ among the ingredients tested in our study for *P. monodon* (Table 3). However, similar values were noticed between FSBM and FGNC (for arginine, lysine, tryptophan and valine), between FSBM and FRSM (for arginine, histidine, lysine, methionine, phenylalanine, tryptophan and valine) and between FSBM and FSFC (for histidine, lysine and valine). The result suggested that FGNC, FRSM and FSFC could also be used as a preferable ingredient as in FSBM in the diet of *P. indicus*. The available information is widely varied in the digestibility parameters for untreated plant proteins in various animals, including penaeid shrimp species and the details are very limited so far in relation to fermented ingredients. However, the present results are in agreement with the findings of Shi et al. (2015) who reported higher in-vitro digestibility of amino acids in FRSM with *A. niger*. The increase was mainly attributed to the secretion of extracellular proteolytic enzymes (proteases) produced by the microorganisms. The fungus, *A. niger* had produced >800 unit/g of protease during the fermentation of RSM. While comparing the penaeid shrimps, *P. indicus* had a significantly ($p < 0.05$) better digestibility for all the essential amino acids in FRSM compared to *P. monodon* and the improvement ranged from 4.67% to 9.01%. Arginine, trytophan and valine in FSBM, isoleucine, phenylalanine, threonine and tryptophan in FGNC, and phenylalanine in FSBM had a significantly ($p < 0.05$) lower digestibility in *P. mondon*, while the reverse was true for isoleucine. However, the digestibility of other essential amino acids was not significantly affected between the species.

The analysis of linear regression showed that the ADC between total amino acids and protein was highly correlated for fungal fermented plant proteins in *P. indicus* (r = 0.8229) compared to *P. monodon* ($r = 0.7447$). Similar results were reported in *P. vannamei* fed different wheat products (Nieto-López et al., 2011). In this study, protein had a higher digestibility than total amino acids in *P. monodon* (0.69%–2.71%) and the increase was in the range of 0.32%–2.75% in *P. indicus*. However, a higher correlation between the ADC of total amino acids and protein ($r = 0.99$) was observed in soy products (Cruz-Suárez et al., 2009). This confirmed that the total amino acids to protein varied for differently processed samples and corroborates the findings of Nieto-Lopez et al. (2011).

5 | CONCLUSION

It could be concluded that the fungal fermented plant proteins are highly suitable for the formulation of diets and could be considered as very good protein sources for both *P. monodon* and *P. indicus*. The results of the study clearly indicate that *P. indicus* shows a better digestibility than *P. monodon* for most of the nutrients analysed in the present experimental condition. These results give more precise information about the utilization of nutrients from different ingredients for two penaeid shrimp species and would help in formulating well balanced commercial feeds. Increasing the use of these fungal fermented plant proteins are not only to reduce the feed cost but also helps to reduce the dependence on fishmeal as the primary protein source in the aquaculture sector.

ACKNOWLEDGMENT

The authors are grateful to Dr. K.K. Vijayan, Director, ICAR-Central Institute of Brackishwater Aquaculture (CIBA), Chennai, India, for providing the necessary facilities to carry out the present work. This study was financially supported by the Indian Council of Agricultural Research (ICAR), New Delhi, India, through the project, National Innovations in Climate Resilient Agriculture (NICRA). The authors also acknowledge the help by Dr. G. Gopikrishna, Head, CIBA, Chennai (Rtd.), in preparing the manuscript .

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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How to cite this article: Jannathulla R, Dayal JS, Vasanthakumar D, Ambasankar K, Panigrahi A, Muralidhar M. Apparent digestibility coefficients of fungal fermented plant proteins in two different penaeid shrimps—A comparative study. *Aquac Res*. 2019;00:1–10. https://doi.org/10.1111/are.14024