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Effect of fungal fermentation on the nutrient digestibility of guar meal in *Penaeus vannamei*

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ABSTRACT

A 30-days indoor feeding trail was carried out to determine the nutrient digestibility of untreated and fermented guar meal (GRM) in *Penaeus vannamei*. The fermented guar meal was prepared by solid state fermentation using the fungus *Aspergillus niger.* A reference diet was formulated based on the dietary requirements of shrimp and test diets were prepared using the ingredient substitution method of reference diet and test ingredient at 7:3 ratio. Results revealed that the fungal fermentation significantly ($p<0.05$) increased dry matter digestibility of GRM from 48.86 to 54.27% and crude protein digestibility from 64.41 to 68.54%. Significant ($p<0.05$) improvement was observed in the digestibility of all the amino acids with fermented GRM compared to the untreated GRM. The range of essential amino acids digestibility was increased from 59.4-69.6% to 61.5-73.6% post-fermentation. Lysine in essential amino acids and serine in nonessential amino acids showed the highest digestibility in both untreated and fermented GRM whereas a better improvement was observed with arginine (9.79%) and glutamic acid (8.59%) due to fermentation. The present results of increased digestibility parameters in fermented GRM were attributed to the amelioration of anti-nutritional factors and reduction of fibre fractions.

Keywords: *Aspergillus niger,* Digestibility, Fungal fermentation, Guar meal*, Penaeus vannamei*

Introduction

Commercial shrimp feeds are generally formulated based on the nutritional composition of ingredients used for feed preparation and nutrient requirement of the cultured species. Adequate knowledge on feed ingredients helps to formulate nutritionally efficient feeds for aquatic species. In shrimp feed formulation, fishmeal is included in the range of 20 to 50% (Ali *et al*., 2004) due to higher palatability, digestibility and presence of all the essential nutrients to fulfill the dietary requirements of the cultured species. But in the last two decades, availability of fishmeal was drastically reduced due to various climatic events (FAO, 2015) which spurred nutritionists to reduce the usage of fishmeal quantity in feed formulation. Despite the oil seed meals have primarily been evaluated as a fishmeal alternate in the diet of shrimp (Lim and Dominy, 1990; Dayal *et al.,* 2011), there was also a concern about searching for various novel ingredients in order to increase affordability of feed ingredients. Cost of traditional plant based protein sources have increased in recent years as a result of high market demand from other feed industries (Kalanjiam *et al.,* 2014). Among the novel ingredients identified, guar meal (GRM) is having parallel nutritional composition like other traditional plant protein sources and it is also relatively inexpensive compared to soybean meal (Rajamohammed, 2012).

The GRM is a residual byproduct left after mechanical separation of galactomannan polysaccharide gum from guar seed. Global production of guar was about 2.34 million t in 2014 of which >80% was produced by India followed by Pakistan (15%) (Guar Outlook, 2015). GRM was used to certain extent in livestock feed (Lee *et al*., 2005) as well as in fish feed (Asad *et al.,* 2005; El-Saidy *et al.,* 2005; Kalanjium *et al.,* 2014) but its usage is very rare as an ingredient in shrimp feed. In our previous study, nutrient utilisation of GRM in the diet of *Penaeus vannamei* was evaluated and the results clearly indicated deleterious effects on the measures of growth, nutrient utilisation and feed efficiency due to the inclusion of GRM (Jannathulla *et al.,* 2016). Poor performance with GRM was attributed to undesirable chemical constituents especially, anti-nutritional factors (Asad *et al.,* 2005; Jannathulla *et al.,* 2016). The deleterious effects of anti-nutritional factors have been reported earlier in shrimp (Lim and Dominy, 1990; Chen *et al.,* 1996).

Microbial fermentation has been reported to be effective for enhancing the nutritional quality of plant based ingredients by partially or completely destroying the undesirable constituents (Shi *et al.,* 2015). Most of the earlier reports on fermentation have been restricted in evaluating the limited nutrients without paying attention

to digestibility parameters in candidate shrimp species (Hong *et al.,* 2004; Chen *et al.,* 2010; Jalil *et al.,* 2015; Shi *et al.,* 2015). Assessing digestibility is an important prerequisite to screen potential feed ingredients, which would be beneficial in developing nutritionally adequate diets as well as in reducing the waste produced by cultured species (Hajen *et al.,* 1993). Digestibility parameters of various traditional plant based ingredients have been investigated earlier in penaeid shrimps (Piedad *et al.,* 1990; Catacutan, 1991; Cruz-Suarez *et al.,* 2001) but to date, there are no available literatures on digestibility of untreated and fermented GRM. Hence in the present study, the digestibility parameters of GRM were evaluated after fermentation using the fungus, *Aspergillus niger* in *P. vannamei* to explore the suitability of the test ingredients, in particular fermented guar meal (FGRM) in the diet of shrimp.

Materials and methods

Fermentation methodology

GRM was purchased from the local market (n=6) and were ground to a particle size of <500 µm. The ground materials were subsequently sterilised by autoclaving at 121°C for 15 min and subsequently hydrated with water to bring the moisture content to a level of 60 to 65%. Cooled autoclaved ingredients were inoculated with *A. niger* (ATCC 6275; sourced from Himedia Laboratories, Mumbai, India) suspension (10^7 spores ml^{-1}) at the rate of 5% to the substrate. Fermentation was carried out at 35±1°C in an incubator for three days in 500 ml Erlenmeyer flask covered with non-absorbent cotton plugs to facilitate air transfer with three sets of replications (Shi *et al.,* 2015). At the end of fermentation, all the samples were dried at 35 to 40°C for 48 h to bring down the moisture content below 10%, ground to fine particles and then stored properly in a refrigerator at 4°C until further use. Chemical constituents of untreated (GRM) and fermented GRM (FGRM) are presented in Table 1.

Experimental diets

A reference diet (Table 2) was formulated based on the dietary requirement of *P. vannamei* using locally available ingredients with chromic oxide (0.5%) as an inert marker. Two test diets were prepared using 70% of reference diet and 30% of test ingredients *viz.,* GRM and fermented GRM according to Zhou *et al.* (2014). Diets were prepared by powdering the coarse ingredients listed in the formulae in a micropulveriser and passed through 250 µm mesh screen. All the ingredients including fish oil and lecithin were mixed in a domestic mixer for homogenisation. Water was added to the homogenised mash at the rate of 500 ml kg^{-1} and manually kneaded into dough. This dough was steamed for 5 min at atmospheric pressure and pelleted in a table top pelletiser having 2 mm dia die (Dayal *et al.,* 2003). The pellets were dried in a forced air oven at 60ºC for 12 h and stored in a refrigerator until being used. The proximate and amino acids composition of experimental diets are given in Table 3.

Table 1. Effect of fungal fermentation on chemical composition (% dry matter basis) of guar meal (n=6; mean±SD)

Treatments	Guar meal	
	GRM	FGRM
Proximate composition		
Crude protein	52.40^{b}±0.16	57.14^{a}±0.23
Ether extract	7.83^{a}±0.08	7.82^{a}±0.03
Crude fiber	7.94^{a}±0.07	6.06^{b}±0.08
Nitrogen free extract[1]	26.43^{a}±0.26	23.35^{b}±0.19
Total ash	5.40^{a}±0.12	5.63^{a}±0.77
Essential amino acids		
Arginine	6.19^{b}±0.19	6.70^{a}±0.18
Histidine	1.30^{b}±0.10	1.52^{a}±0.13
Isoleucine	2.15^{a}±0.10	2.18^{a}±0.09
Leucine	1.05^{b}±0.13	1.27^{a}±0.06
Lysine	2.06^{b}±0.16	3.33^{a}±0.17
Methionine	0.65^{b}±0.15	1.08^{a}±0.10
Phenylalanine	2.20^{a}±0.06	2.27^{a}±0.09
Threonine	2.47^{a}±0.09	2.49^{a}±0.08
Tryptophan	0.79^{b}±0.04	0.86^{a}±0.04
Valine	2.43^{a}±0.06	2.48^{a}±0.12
Nonessential amino acids		
Alanine	1.61^{b}±0.07	1.93^{a}±0.10
Aspartic acid	4.31^{a}±0.05	4.38^{a}±0.05
Cystine	0.71^{b}±0.15	1.18^{a}±0.15
Glutamic acid	6.54^{b}±0.07	6.81^{a}±0.05
Glycine	2.77^{b}±0.12	3.22^{a}±0.15
Proline	2.60^{a}±0.07	2.65^{a}±0.07
Serine	3.59^{b}±0.21	4.78^{a}±0.18
Tyrosine	1.91^{b}±0.09	2.18^{a}±0.07
Fiber fractions		
Neutral detergent fiber	19.71^{a}±0.30	11.59^{b}±0.09
Acid detergent fiber	9.01^{a}±0.17	7.66^{b}±0.11
Cellulose	7.90^{a}±0.25	5.72^{b}±0.23
Hemicellulose	10.70^{a}±0.53	3.93^{b}±0.05
Lignin	1.11^{a}±0.09	0.44^{b}±0.04
Anti-nutritional factors		
Phytic acid	2.57^{a}±0.16	1.19^{b}±0.03
Saponin	2.55^{a}±0.05	0.96^{b}±0.03
Tannin	0.39^{a}±0.01	0.33^{b}±0.02
Guar gum	10.99^{a}±0.53	10.17^{a}±0.58

Values with the same superscript letters in the same row are not significantly different ($p>0.05$)
[1]Calculated by difference

Table 2. Ingredient composition of reference diet used for *in vivo* digestibility trials (% as fed basis)

Ingredients	Inclusion (%)
Fishmeal[1]	25.0
Acetes	12.0
Prawn head	6.0
Squid meal	4.0
Corn gluten	2.5
Wheat gluten	1.5
Sesame cake	5.0
Rice bran	3.0
Broken rice	5.0
Maida	15.0
Wheat flour	14.5
Fish oil[1]	2.0
Lecithin	1.0
Vitamin mineral pre-mix [2]	2.0
Binder[3]	1.0
Chromium (III) oxide[4]	0.5

[1]Bismi Fisheries, Mayiladuthurai, Tamil Nadu, India

[2]Vitamins (kg^{-1}): Vitamin A (20 000 IU), B_1 (70 mg), B_2 (60 mg), B_6 (120 mg), B_{12} (60 mg), C (1000 mg), D_3 (300000 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 (1000 mg).

[2]Minerals (kg^{-1}): Iron (100 mg), Copper (5 mg), Zinc (50 mg), Manganese (40 mg), Selenium (20 mg), Cobalt (1 mg), Iodine (100 mg)

[3]Pegabind, Bentoli Agri Nutrition Asia Pvt Ltd, Singapore
[4]Sigma Aldrich (Cat. No: 393703)

Digestibility trails

A 30 days digestibility trial was conducted in *P. vannamei* (average weight: 14.23±1.47 g) procured from local farm and acclimatised to the indoor laboratory condition for 2 weeks with a control diet having 37% of crude protein. Post-acclimatisation, shrimps were randomly distributed into a 500 l (1.31 x 0.64 x 0.73 m) fiberglass reinforced plastics (FRP) tank. A total of thirty shrimps were used per treatment with each treatment having three replications (ten shrimps per replication) including control group fed a reference diet. The digestibility trial was conducted in static water system to prevent the leaching of faeces (Dayal *et al.,* 2011) however, 80% of water was exchanged daily, prior to first feeding. During the experimental period, shrimps were fed with respective diets thrice a day (at 07 00; 12 30 and 17 30 hrs) and the uneaten feed and other particles were removed after an hour of feeding. Faeces were gently siphoned off from the tanks on to a bolting silk cloth from second week onwards (Smith and Tabrett, 2004). The collected faeces were gently rinsed in distilled water, dried on filter paper and frozen immediately at -20°C for further analysis. In order to have a representative sample, dried faecal material of 30 days collections from each replication in a treatment were pooled to avoid possible variation and represented as one sample. From this, six replicates were taken for the analysis. The digestibility of dry matter, crude protein and amino acids were analysed according to Smith and Tabrett (2004). UV treated water was used in the present study and water quality parameters *viz.,* salinity (19 to 21 g l^{-1}), temperature (26.5 to 28.5°C), dissolved oxygen (5.8 to 7.8 mg l^{-1}), pH (8 to 8.5) and total ammonia-nitrogen (<0.1 mg l^{-1}) were monitored periodically following standard methods (APHA, 2012).

Biochemical analysis

Proximate composition of ingredients and experimental diets in terms of moisture, crude protein, ether extract, crude fiber and total ash were analysed as per standard methods (AOAC, 1997). Chromium content was analysed after Furukawa and Tsukahara (1966) by spectrophotometric method (350 nm) in both diets and faeces to calculate apparent digestibility coefficient of dry matter, crude protein and amino acids. Amino acid profiles were analysed using pre-column HPLC gradient system (Shimadzu Corp, LC-30AD) after hydrolysing the samples with 6 N hydrochloric acid in a sealed tube for 22 h at 110°C in an oven (Finlayson, 1964). Tryptophan, being labile to acid hydrolysis was measured after alkali hydrolysis by spectrophotometric method at 500 nm (Sastry and Tammuru, 1985). The partial oxidation of sulphur containing amino acids (cystine and methionine) during acid digestion was prevented using 0.1% phenol (Jajic *et al.,* 2013). Anti-nutritional factors such as saponin (AOAC, 1997), phytic acid (Davis and Reid, 1979), tannin (Price *et al.,* 1978) and guar gum (Das *et al.,* 1977) were analysed by standard methods. Fiber fractions namely, neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, hemicellulose and lignin of selected plant protein sources and experimental diets were estimated after Van Soest *et al.* (1991).

Statistical analysis

The data on digestibility were subjected to t-test to find significant difference if any due to fermentation. Prior to statistical evaluation, the data was checked for determining the homogeneity of variance after ascertaining the normal distribution. The entire data were analysed using SPSS version 16.0 and the statistical tests were evaluated at 5% significance ($p<0.05$).

Results and discussion

The nutritive value of ingredients varies based on the nutritional composition and hence, measuring the dry matter digestibility would be beneficial to ascertain

Table 3. Proximate and amino acid composition of reference and test diets (% fed basis)

Particulars	Reference diet	Test diets		R[1]
		GRM	FGRM	
Proximate composition				
Moisture	7.47	7.35	7.61	
Crude protein	37.42	39.63	41.13	
Ether extract	7.15	7.40	7.40	
Crude fiber	2.61	2.93	2.68	
Nitrogen free extract[2]	31.66	29.21	27.44	
Total ash	13.69	13.48	13.74	
Essential amino acids				
Arginine	2.02	3.27	3.43	2.32
Histidine	0.86	1.00	1.06	0.80
Isoleucine	1.42	1.64	1.66	1.01
Leucine	2.58	2.13	2.20	1.70
Lysine	1.98	2.00	2.38	1.64
Methionine	0.93	0.85	0.98	0.90
Phenylalanine	1.73	1.88	1.90	1.40
Threonine	1.42	1.74	1.74	1.51
Tryptophan	0.42	0.43	0.47	-
Valine	1.63	1.87	1.89	1.40
Nonessential amino acids				
Alanine	2.06	1.93	2.03	
Aspartic acid	2.99	3.39	3.41	
Cystine	0.49	0.56	0.70	
Glutamic acid	5.49	5.82	5.90	
Glycine	1.86	2.13	2.27	
Proline	1.79	2.04	2.05	
Serine	1.51	2.14	2.50	
Tyrosine	0.38	0.50	0.52	
Alanine	1.17	1.39	1.47	
Fiber fractions				
Neutral detergent fiber	29.20	25.82	23.70	
Acid detergent fiber	18.46	15.40	15.08	
Cellulose	2.53	2.63	2.38	
Hemicellulose	10.74	10.41	8.63	
Anti-nutritional factors				
Phytic acid	0.36	0.93	0.52	
Saponin	0.07	0.67	0.31	
Tannin	0.15	0.29	0.21	
Guar gum	nd[3]	2.78	2.74	

[1]Recommended levels of essential amino acids in *P. vannamei* (%) according to Macias-Sancho *et al.* (2014)
[2]Calculated by difference
[3]Not detected

the total quantity of nutrients that were digested and absorbed (Glencross *et al.,* 2007) since all the components of ingredients are not digested at equal proportion. In our study, dry matter digestibility of GRM (48.86%) was lower compared to crude protein digestibility (64.41%). Similar results have been reported for untreated GRM in *P. monodon* (Rajamohammed, 2012) and the findings of Akiyama *et al.* (1989) suggested that it can be attributed to better assimilation of crude protein. Fungal fermentation has significantly ($p<0.05$) increased dry matter digestibility

from 48.86 to 54.27% and crude protein digestibility from 64.41 to 68.54%. The challenging digestibility problem with untreated GRM was mainly attributed to the presence of anti-nutritional factors (El-Saidy *et al.,* 2005; Jannathulla *et al.,* 2016). Swick and Ivey (1992) reported that phytic acid has impaired protein digestibility in aquatic species by forming phytate-protein complexes. Tannin (Makkar and Becker, 1999) and phytic acid (Helsper *et al.,* 1993) interferes with protein digestibility by inhibiting protein hydrolytic ensymes in aquatic species. The feeding rate, growth and molting frequency of *Penaeus japonicus* has reduced due to the presence of saponin (Chen *et al.,* 1996). *A. niger* markedly reduced the quantity of phytic acid, saponin and tannin by 53.7, 62.4 and 15.4% respectively during fermentation (Table 1) though fermentation did not influence the level of galactomannan gum. The reduction of these anti-nutritional factors post-fermentation (Table 1 and 3) could have contributed to the increased dry matter and crude protein digestibility in our study (Fig. 1).

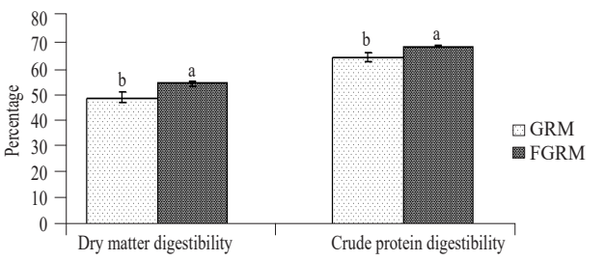

Fig. 1. Dry matter and crude protein digestibility of untreated and fermented guar meal in *P. vannamei.* Columns with same superscript letters between the categories are not significantly different ($p>0.05$)

Similarly the poor digestibility of untreated GRM was also related to the limited capability of *P. vannamei* to digest fibrous components. Typically, GRM had considerable quantity of fiber fractions (Table 1). Bureau *et al.* (1999) stated that the hydrolytic enzymes responsible for the degradation of fiber fractions are scarce in mono-gastric animals including shrimp. It could be the reason for lower digestibility observed with untreated GRM in the present investigation. The fiber fractions entrap the digestible nutrients and prevent digestive enzymatic degradation of these nutrients thereby decreasing the digestibility parameters. The fibre fractions also reduce the gut transit time (Brunson *et al.,* 1997). Lim *et al.* (1997) reported that *P. vannamei* had better growth and digestibility when fed with diet containing low fiber canola meal (14%) than those fed with high fiber canola meal (28%). Shi *et al.* (2015) documented that the fungus, *A. niger* is one of the beneficial microorganisms in reducing fibrous components by producing various fiber hydrolytic enzymes during fermentation. *A. niger* was found to produce 30 U g^{-1} of cellulase (Reddy *et al.,* 2015), 3099 U g^{-1} of xylanase (Maciel *et al.,* 2008) and 9 U g^{-1} of pectinase (Solis-Pereyra *et al.,* 1996) during solid state fermentation. The reduction of fiber fraction was in the range of 15 to 62% in fermented GRM used in the present study compared to those which are untreated (Table 1). Among fiber fractions, hemicellulose (by 62%) showed the highest reduction followed by lignin (by 59%) and cellulose (by 27%) which could be one of the reasons for enhancing digestibility parameters with fermented ingredient.

In comparing digestibility parameters with other traditional plant protein sources (Dayal *et al.,* 2011; Rajamohammed, 2012), still GRM showed poor digestibility even after fermentation. Researchers have suggested that it can be attributed to the presence of gum residues (Lee *et al.,* 2005; Kalanjiam *et al.,* 2014). The untreated GRM used in the present study had 10.99% of guar gum and its quantity did not significantly reduce even after fungal fermentation (10.17%). Daskiran *et al.* (2004) reported that the usage of fibrolytic enzymes alleviated the deleterious effect of guar gum by increasing intestinal viscosity of animals. *A. niger* would have produced these enzymes during fermentation as earlier reported by Solis-Pereyra *et al.* (1996), Maciel *et al.* (2008) and Reddy *et al.* (2015). However, no reduction in gum content was noticed in our study (Table 1). Similarly, Rajamohammed (2012) also observed that there was no change in the gum quantity of GRM after enzymatic treatment using customised enzyme mixture. It could be a reason for lowered digestibility with GRM compared to other traditional plant protein sources. However, enhanced digestibility parameters with fermentation compared to untreated GRM in our study indicates the suitability of ingredients is more after fermentation.

The utilisation of protein sources depends on the amino acid composition and also on the individual amino acid digestibility. Fungal fermentation showed a significant ($p<0.05$) increase in amino acid composition expected for phenylalanine, threonine, aspartic acid and proline (Table 1). Amino acid digestibility of untreated and fermented GRM is depicted in Table 4. The digestibility range was 59.4 to 69.6% for essential amino acids and 55.2 to 73.1% for nonessential amino acid in untreated GRM. Fermentation showed a significant ($p<0.05$) increase in the digestibility of all the amino acids in the present study and the range increased to 61.5-73.6% for essential amino acids and 57.8-76.5% for nonessential amino acids. Among amino acids, serine followed by lysine showed the highest digestibility in both untreated and fermented

Table 4. Amino acid digestibility (%) of untreated and fermented guar meal in *P. vannamei* (n=6; mean±6)

Particulars	Ingredients digestibility	
	GRM	FGRM
Essential amino acids		
Arginine	62.1^{b}±2.9	68.2^{a}±3.1
Histidine	66.5^{b}±1.8	70.0^{a}±2.0
Isoleucine	61.2^{b}±2.8	67.1^{a}±3.0
Leucine	63.5^{b}±1.2	65.7^{a}±1.3
Lysine	69.6^{b}±2.0	73.6^{a}±2.2
Methionine	63.9^{b}±1.3	66.5^{a}±1.5
Phenylalanine	59.4^{b}±1.1	61.5^{a}±1.3
Threonine	66.5^{b}±2.0	70.6^{a}±2.2
Tryptophan	68.7^{b}±2.4	73.6^{a}±2.6
Valine	64.6^{b}±1.2	66.7^{a}±1.4
Nonessential amino acids		
Alanine	55.2^{b}±1.3	57.8^{a}±1.5
Aspartic acid	65.9^{b}±1.7	69.2^{a}±1.9
Cystine	66.5^{b}±1.8	69.9^{a}±1.9
Glutamic acid	67.5^{b}±2.8	73.3^{a}±3.0
Glycine	57.9^{b}±1.8	61.5^{a}±1.9
Proline	59.7^{b}±1.5	62.6^{a}±1.7
Serine	73.1^{b}±1.8	76.5^{a}±2.0
Tyrosine	61.6^{b}±2.3	66.3^{a}±2.4

Values with the same superscript letters in the same row are not significantly different ($p>0.05$)

GRM whereas a better improvement was observed with arginine (9.79%) in essential amino acids and glutamic acid (8.59%) in nonessential amino acids due to fermentation. Though the quantity of phenylalanine, threonine, aspartic acid and proline did not differ significantly (Table 1), their digestibility coefficients have also increased significantly ($p<0.05$) due to fermentation. The findings of Ash (1985) suggested that it can be due to increased availability of the respective amino acids. Better growth and nutrient utilisation observed with fermented GRM compared to untreated GRM in *P. vannamei* (Jannathulla *et al.,* 2016) further confirms the present findings of increased amino acid digestibility due to fungal fermentation.

Even though there have been no reports on the *in vivo* amino acid digestibility regarding fermented ingredients, the results of our study are in agreement with the findings reported by Shi *et al.* (2015) relating to the increase in *in vitro* digestibility of amino acids for rapeseed meal fermented with *A. niger*. The increase of amino acids digestibility was attributed to the secretion of extra cellular proteolytic enzymes (proteases). Shi *et al.* (2015) reported that *A. niger* produced >800 U g^{-1} of protease during the fermentation of rapeseed meal. Among amino acids, both untreated and fermented ingredients have shown a poor digestibility for alanine and glycine (Table 4) and the findings of Akiyama *et al.* (1989) suggested that it can be due to the secretion of chitinous peritropic membrane which surrounds the faeces. The same was in agreement with the findings of Dayal *et al.* (2011) for sunflower oil cake (SFC) in the diet of *P. monodon*, who also reported that chitin had a higher content of alanine and glycine than other amino acids.

Results of the present study demonstrated that *P. vannamei* efficiently digests fermented GRM compared to the respective untreated ingredient as such. It indicates that the fermented plant protein source has a greater potential to be used as a protein source rather than as untreated material in the diet of shrimp. Hence the present study concludes that the fermented ingredients would help to formulate high quality feed rather fulfilling dietary quantitative requirements in the diet of shrimp.

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