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Evaluation of Fungal Fermented Rapeseed Meal as a Fishmeal Substitute in the Diet of *Penaeus vannamei*

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ABSTRACT

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Rapeseed meal (RSM) was fermented using the fungus, *Aspergillus niger* and was evaluated as a fishmeal substitute in the diet of *Penaeus vannamei*. A 45-days' growth trial was carried out using nine iso-nitrogenous and iso-lipidic diets. Raw/fermented RSM was included in experimental diets at the rate of 0 (control), 25, 50, 75 and 100 g/kg by replacing fishmeal (w/w). Results revealed that shrimp fed a control diet had a weight gain of 211.65% and was comparable up to the treatments fed diets containing 25, 50 and 75 g/kg fermented RSM (221.56, 211.77 and 202.85%, respectively). However, the inclusion of >25 g/kg raw RSM tended to decrease ($P<0.05$) the weight gain. The broken-line model indicated that the maximum inclusion was 64.4 g/kg for fermented RSM. Though feed conversion ratio (1.71-1.94) and apparent protein utilization (21.42-24.93) were not affected due to dietary modifications, numerically better results were observed with fermented RSM compared to the respective level of raw RSM. No significant difference was observed in survival and was in the range of 86.67 to 96.67%. Carcass lipid level was high in shrimp fed with the diets having test ingredients (9.50-10.23 g/kg wet weight) than those fed a control diet (8.83 g/kg). Haemolymph indices have shown significant differences in total protein, glucose, cholesterol and triglycerides between the control and test diets. The present investigation concluded that fermented RSM could replace higher level (64.4 g/kg) of fishmeal in the diet of *P. vannamei* when compared to their raw counterpart (25 g/kg).

ADDITIONAL INDEX WORDS: Aspergillus niger, *hemolymph indices, nutrient utilization,* Penaeus vannamei, *rapeseed meal, shrimp feed.*

INTRODUCTION

Aquaculture is the fastest growing food-producing sector, in which shrimp farming is an important component. Shrimp feeds generally contain >20% of fishmeal in commercial formulations due to its balanced nutrient profiles, in particular, essential amino acids, higher palatability and digestibility. The global production of fishmeal declined from 6.2 million metric tons (Mt) in 2000 to 4.3 Mt in 2015, which led to the escalation of fishmeal cost from 452 to 2169 USD/ton during this period (World Bank Commodity Price Data, 2016). Therefore, identifying potential alternatives to fishmeal has become an important task for the researchers. The wide availability, reasonable price and desirable protein content have made interest on various plant protein sources, mainly oilseed cakes/meals and one among them is rapeseed meal (RSM). The global production of RSM was 39.9 Mt in 2015 and India accounts about 9% of the total production (World Bank Commodity Price Data, 2016). In an earlier study (Rajaram, 2010), the inclusion of RSM beyond 2.5% significantly ($p<0.05$) reduced the growth performance and digestibility of *Penaeus monodon* due to the presence of anti-nutritional factors, particularly glucosinolates. The lower content of essential amino acids like methionine and lysine may also be a reason for limiting the usage of RSM in shrimp feed (Jannathulla *et al*., 2017b).

Over the past few years, various methodologies were used to deactivate the associated constraints from plant protein sources, yet most of the techniques have not shown a positive response because of the various bottlenecks such as nutrient loss, commercial infeasibility, environmental pollution etc., (Shi *et al.,* 2015). Notwithstanding minor drawbacks, fermentation improves the nutritional quality of plant protein sources by producing various microbial syntheses (Wee, 1991). In an earlier study, solid-state fermentation with the fungus, *Aspergillus niger* has significantly ($p<0.05$) reduced anti-nutritional factors and fibre fractions with simultaneous improvement in major essential nutrients, particularly limiting amino acids (methionine and lysine) in various plant protein sources (Jannathulla *et al.*, 2017a and b). Though, the effect of fermented soybean meal (Jannathulla *et al*., 2018a); Sharawy *et*

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al., 2016), sunflower oil cake (Jannathulla *et al*., 2018a), groundnut oil cake (Jannathulla *et al*., 2018b) cottonseed meal (Sun *et al*., 2016), plant protein mix (Imelda *et al.*, 2008) as a fishmeal alternative has been documented in shrimp, but the information is minimal on the utilization of fermented RSM.

Hence, the present study investigated the effect of *A. niger* fermented RSM as a fishmeal alternative in the diet of *P. vannamei.*

METHODS

Fermentation Methodology

The fungus, *A. niger* listed under GRAS notifications (Generally Recognized as Safe) by FDA (GRAS Notice No. 35, 2010) was used for fermentation. The ATCC (6275) culture of *A. niger* acquired from Himedia Laboratories (Mumbai, India) was grown on potato dextrose agar (PDA) for five days at 35±1°C in an incubator. Tween 80 (0.1%) was used to harvest the fungal spores, and the suspension was approximately adjusted to 10^7 spores/ml.

Table 1. *Chemical composition of fishmeal, raw and fermented rapeseed meal used in the present study (g/kg dry matter basis).*

Particulars	Fishmeal	Rapeseed meal (RSM)	
		Raw	Fermented
Proximate composition			
Crude protein	631.67	417.33	467.53
Ether extract	105.31	26.44	22.81
Crude fibre	5.39	106.11	102.84
Nitrogen free extract[1]	68.06	382.49	337.45
Total ash	189.57	67.63	69.37
Essential amino acids			
Arginine	43.77	33.54	35.60
Histidine	16.94	15.99	16.60
Isoleucine	29.65	14.40	15.47
Leucine	50.83	18.92	23.85
Lysine	52.95	10.17	20.52
Methionine	19.06	8.16	13.12
Phenylalanine	27.53	9.17	12.59
Threonine	28.95	19.93	21.25
Tryptophan	7.06	4.35	4.94
Valine	34.59	21.86	25.35
Fibre fractions			
Neutral detergent fibre	10.04	265.87	253.01
Acid detergent fibre	8.16	193.43	185.17
Cellulose	-	86.21	76.38
Hemicellulose	-	72.51	68.02
Lignin	-	107.22	84.82
Anti-nutritional factors			
Glucosinolates	-	3.13	1.28
Tannin	-	8.89	5.09
Phytic acid	-	27.45	8.97

[1]*Calculated by a difference (g/kg): 1000 - (moisture + crude protein + ether extract + crude fibre + total ash).*

Meanwhile, the commercial defatted RSM was purchased (n=6) from the markets in and around Chennai, India was ground to a particle size of <500 µm. Fermentation was carried out by the method described by Jannathulla *et al.* (2017b). Briefly, the ground RSM was hydrated to obtain the moisture content of 60 to 65% and sterilized by autoclaving at 121°C for 15 min. The cooled autoclaved samples were inoculated with 5% of *A. niger* suspension and allowed to ferment at 35±1°C in an incubator for three days. Fermentation was carried out in a 500 ml Erlenmeyer flask plugged with cotton to facilitate air transfer with three sets of replications. Post fermentation, all the replicates were oven dried at 40 to 45°C to bring down the moisture content below 10%. To have a representative sample, all the replicates were pooled to avoid a possible variation and were stored at 4°C until further use after grinding to fine particles of <250 µm. The chemical composition of test ingredients (raw and fermented RSM) and fishmeal are presented in Table 1 (n=6).

Experimental Diets

The experimental diets were formulated based on the nutrient requirements for *P. vannamei* using locally available ingredients, according to Dayal *et al.*, 2003). In preparing the experimental diets, all dry ingredients (Table 2) were powdered in a micro-pulverizer and passed through <250 µm mesh screen. The test ingredients such as raw and fermented RSM was serially included at the rate of 0 (control), 25, 50, 75 and 100 g/kg by replacing fishmeal (w/w).

Raw and fermented RSM used in the present study had different protein contents (Table 1); hence fishmeal was replaced with test ingredients on a w/w basis for testing the specific effect of fermentation on nutrient utilization in shrimp. However, the diets were formulated to iso-nitrogenous by adjusting with corn gluten meal. Proximate composition of experimental diets is presented in Table 2 (n=3).

Growth Trial

A 45-days' growth trial was conducted with juveniles of *P. vannamei* (3.08±0.14 g) in 500 l (1.31x0.64x0.73 m) oval shape fibreglass reinforced plastic (FRP) tanks equipped with flow-through system (1.5 ml/min) in an indoor wet laboratory at the Muttukadu Experimental Station of ICAR-Central Institute of Brackishwater Aquaculture, Chennai, India. The juveniles were acclimatized to the experimental conditions for 15 days and fed with control diet having 37% of crude protein before the experiment began. Thirty shrimps were randomly assigned to each treatment having three replicate tanks. Shrimps were fed respective experimental diets thrice a day (7.00 AM, 12.30 PM and 5.30 PM) and the amount of diet provided was adjusted according to survival, body weight and intake. The uneaten feed particles (if any) were removed from the tanks using a clean Falcon tube after an hour of feeding and were dried overnight at 60°C in a hot air oven to measure the feed intake on a daily basis. Shrimp were maintained under the natural photoperiodicity of 12 h L: 12 h D during the experimental period. At the end of the growth trial, weight gain (WG %), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency measures like protein efficiency ratio (PER), and apparent protein utilization (APU) were determined along with survival for each dietary treatment.

$$SGR = [\ln(\text{Final body weight}) - \ln(\text{Initial body weight})] / \text{Days of experiment} \times 100$$

$$FCR = \text{Feed intake (g)} / \text{Weight gain (g)}$$

Table 2. *Ingredient, proximate and essential amino acid composition of experimental diets containing graded levels of raw and fermented rapeseed meal by replacing fishmeal (g/kg as is basis).*

Particulars	Experimental diets								
	RSM 0	RSM 25	RSM 50	RSM 75	RSM 100	FRSM 25	FRSM 50	FRSM 75	FRSM 100
Ingredient composition									
Fishmeal[1]	250	225	200	175	150	225	200	175	150
RSM	-	25	50	75	100	-	-	-	-
FRSM	-	-	-	-	-	25	50	75	100
Acetes[2]	80	80	80	80	80	80	80	80	80
Squid meal	15	15	15	15	15	15	15	15	15
Soybean meal	200	200	200	200	200	200	200	200	200
Corn gluten	20	29	38	47	56	26	34	42	50
Sesame cake	50	50	50	50	50	50	50	50	50
Broken rice	80	80	80	80	80	80	80	80	80
Wheat	244	233	222	211	200	236	226	216	206
Fish oil[1]	20	20	20	20	20	20	20	20	20
Palm oil	-	2	4	6	8	2	4	6	8
Lecithin	10	10	10	10	10	10	10	10	10
Pre-mix[3]	20	20	20	20	20	20	20	20	20
Binder[4]	10	10	10	10	10	10	10	10	10
BHT[5]	1	1	1	1	1	1	1	1	1
Proximate composition									
Moisture	87.64	87.72	84.37	86.74	85.01	87.81	87.08	86.74	85.03
Crude protein	374.37	368.56	375.44	377.58	380.19	375.81	380.60	375.78	377.18
Ether extract	67.63	68.31	66.94	68.63	66.87	68.41	68.33	67.41	68.14
Crude fibre	29.81	31.87	33.67	35.77	37.43	30.57	33.11	34.90	36.62
Neutral detergent fibre	291.6	310.57	336.29	360.49	390.14	299.03	323.81	342.19	357.98
Acid detergent fibre	124.4	134.31	147.44	152.76	163.48	130.11	137.16	148.27	156.14s
Nitrogen free extract[6]	297.08	302.82	301.03	296.17	300.34	295.62	292.11	299.33	301.89
Total ash	143.47	140.72	138.55	135.11	130.16	141.78	138.77	135.84	131.14

[1] *Bismi Fisheries, Mayiladuthurai, Tamil Nadu, India*.
[2] *Mantis shrimp species having approximately 60% of crude protein and 2-4% of ether extract mainly used as a protein source*.
[3] *Premix (mg 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), Iron (100 mg), Copper(5 mg), Zinc (50 mg), Manganese (40 mg) Selenium (20 mg), Cobalt (1 mg) and Iodine (100 mg).*
[4] *Pegabind, Bentoli AgriNutrition Asia Pvt Ltd, Singapore*.
[5] *Butylated hydroxytoluene: Sigma Aldrich (Cat. No: PHR1117).*
[6] *Calculated by a difference (g/kg): 1000 - (moisture + crude protein + ether extract + crude fibre + total ash).*

PER = Weight gain (g) / Protein intake (g)

APU= Protein gain (g) / Protein intake (g)

Survival (%) = Final number of animals / Initial number of animals X 100

Twelve shrimps were randomly selected from each treatment, and about 0.5 ml of hemolymph was withdrawn from the ventral sinus in the first abdominal segment using a 26-gauge hypodermic needle on 1 ml syringe with 2 mm thickness containing 0.3 ml of anti-coagulant solution. Other whole shrimp, not used for hemolymph collection, were used for the analysis of carcass composition.

Water Quality Parameters

Ultraviolet treated water was used throughout the experimental period. Water quality parameters like salinity (20 g/L), temperature (26.5 to 28.5°C), dissolved oxygen (6.4 to 7.9 mg/L) and pH (8 to 8.3) were measured daily. Total ammonia-nitrogen (<0.1 mg/L), nitrite-nitrogen (0.34 to 0.49 mg/L) and nitrate-nitrogen (2.36 to 2.61 mg/L) were measured once in a week by standard methods (APHA, 2012).

Biochemical Analysis

Proximate composition of ingredients, experimental diets and shrimp carcass were analyzed as per the method of AOAC (1997). Briefly, the moisture content was determined by drying the samples at 105°C in a hot air oven for overnight. Nitrogen content was analyzed by the micro Kjeldahl method (KjeltecTM-8100, TecatorTM Line), and the analyzed nitrogen was converted into crude protein by multiplying with a common empirical factor of 6.25. The ether extract was estimated using petroleum ether (60 to 80°C) in Soxhlet extraction unit (Scocs Plus-SCS 6). The samples were digested in 1.25% sulphuric acid (30 min) followed by 1.25% sodium hydroxide (30 min) using a Fiber cap method (FOSS-2022, TecatorTM) for determining the crude fiber level. Total ash content was measured by incinerating samples at 540°C in a muffle furnace for 6 h. The

nitrogen-free extract was calculated by difference.

Amino acid profiles were analyzed using pre-column HPLC gradient system (Shimadzu Corp, LC-30AD) after hydrolyzing the samples with 6 N hydrochloric acid in a sealed tube for 22 h at 110°C in an oven (Finlayson, 1964). The YMC-Triart C18, RRH (1.8 μm, 2.1x100 mm) column was used to separate the amino acids after derivatization with mercaptopropionic acid, O-pthalaldehyde 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). Amino acids were quantified by a fluorescent detector (RF-20AXS) using the amino acid mixer (Sigma Aldrich) as an external standard and norleucine as an internal standard. Tryptophan, being liable to acid hydrolysis, was measured after alkali hydrolysis by the spectrophotometric method at 500 nm (Sastry and Tammuru, 1985). The partial oxidation of methionine during acid digestion was prevented by adding 0.1% of phenol (Jajic *et al.*, 2013).

Anti-nutritional factors, such as glucosinolates (McGhee et al., 1965), phytic acid (Davis and Reid, 1979) and tannin (Price et al., 1978) were analyzed by the standard methods. Fibre fractions namely, neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose, hemicellulose and lignin contents were estimated as per the method of Van Soest (1963). The haemolymph indices such as total protein, glucose, cholesterol and triglycerides were estimated using the respective commercial kits obtained from Sigma-Aldrich (Code No: TP0100, GAHK20, MAK043 and TR0100, respectively) in a UV-spectrophotometer (Shimadzu, UV-1800) at 595, 340, 570 and 540 nm, respectively, according to the accredited methodologies developed by Sigma-Aldrich (http://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs /Sigma/Bulletin/(Code No)bul.pdf).

Statistical Analysis

The experimental data were subjected to two-way analysis of variance (ANOVA) to assess the effect of processing (raw and fermented) and inclusion level (0, 25, 50, 75 and 100 g/kg) on shrimp. Multiple comparisons were done using Tukey's to find significant differences among the treatments. Prior to the statistical evaluation, data were checked for determining the homogeneity of variance after ascertaining the normal distribution. The entire data were analyzed using SPSS version 16.0, and statistical tests were evaluated at 5% significance ($P < 0.05$). Broken-line regression was performed to determine the optimum inclusion level of fermented RSM by replacing fishmeal in the diet of *P. vannamei*.

RESULTS

The growth performance, survival, feed and protein efficiency measures of *P. vannamei* fed experimental diets containing graded levels of raw and fermented RSM are depicted in Table 3. The average body weight of shrimp was significantly ($p < 0.05$) affected by both processing (raw and fermented) and inclusion level (0, 25, 50, 75 and 100 g/kg). SGR was found to be higher ($p < 0.05$) in shrimp fed fermented RSM (9.39) compared to those fed raw RSM (8.88) irrespective of the inclusion level. However, the result of interaction between the processing and inclusion level showed that the growth performance in terms of SGR was significantly ($P<0.05$) lowered in shrimp fed with diets having above 25 g/kg of raw RSM and 75 g/kg of fermented RSM compared to the control group. FCR remained unchanged significantly regardless of the processing and inclusion level of both raw and fermented RSM by replacing fishmeal, however; lower FCR was noticed in the group fed fermented RSM than those fed with the respective level of raw RSM. Of all the treatments, nutrient utilization of protein measures in terms of PER and APU were better in RSM 25 diet followed by FRSM 25 diet. No significant difference was observed in survival between the dietary treatments and was in the range of 86.67 to 96.67%. The correlation between WG and inclusion level of fermented RSM by replacing fishmeal was described using the broken-line regression analysis, which indicated that the maximum inclusion level was 64.4 g/kg for fermented RSM (Figure 1).

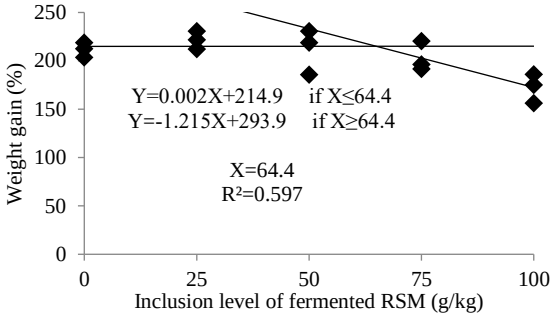

Figure 1. Estimation of optimal inclusion level of fermented RSM by replacing fishmeal in the diet of *Penaeus vannamei* using broken line analysis.

Carcass composition was not affected due to processing (raw and fermented) but a significant ($p < 0.05$) variation was observed for crude protein and ether extract due to the variation in inclusion level (Table 4). Interaction results revealed that ether extract found to be significantly ($p < 0.05$) high in shrimp fed with diets having raw or fermented RSM than those fed a control diet, whereas other parameters were not significantly affected by the treatments (Table 4).

Hemolymph indices of *P. vannamei* fed experimental diets having graded levels of raw and fermented RSM is presented in Table 5. Shrimp fed diets with fermented RSM showed significantly ($p < 0.05$) higher hemolymph total protein (8.09 g/dl) and was 7.78 g/dl in the groups fed raw RSM. However, glucose, cholesterol and triglycerides were not affected due to the processing. Hemolymph glucose was significantly ($p < 0.05$) decreased with increasing the inclusion level of test ingredients (raw and fermented RSM), while the reverse trend was true for other hemolymph indices (total protein, cholesterol and triglycerides).

DISCUSSION

Finding an alternative to fishmeal, due to its demand and high cost, is important nowadays to increase or sustain the present growth rate of aquaculture. In the meantime, replacing fishmeal using plant protein sources remains a challenging task

Table 3. *Growth performances of* Penaeus vannamei *juveniles fed experimental diets having graded levels of raw and fermented rapeseed meal by replacing fishmeal (n=3).*

Particulars	Growth performance						
	Initial wt. (g)	Final wt. (g)	SGR[1]	FCR[2]	PER[3]	APU[4]	Survival (%)
Processing							
Raw	3.08[a]	8.88[b]	2.34[b]	1.87[a]	1.44[a]	23.14[a]	94.67[a]
Fermented	3.09[a]	9.39[a]	2.46[a]	1.80[a]	1.48[a]	23.93[a]	92.00[a]
Inclusion level (g/kg)							
0	3.06[a]	9.52[ab]	2.52[ab]	1.86[a]	1.44[a]	23.79[a]	93.33[a]
25	3.11[a]	9.87[a]	2.56[a]	1.72[a]	1.57[a]	25.25[a]	93.33[a]
50	3.11[a]	9.28[bc]	2.42[bc]	1.82[a]	1.46[a]	23.61[a]	93.33[a]
75	3.08[a]	8.81[c]	2.33[c]	1.86[a]	1.44[a]	23.04[a]	95.00[a]
100	3.09[a]	8.20[d]	2.16[d]	1.92[a]	1.38[a]	21.99[a]	91.67[a]
Processing (A)	0.816	0.010	0.008	0.221	0.309	0.309	0.491
Inclusion level (B)	0.623	<0.001	<0.001	0.174	0.105	0.133	0.988
A x B	0.693	0.446	0.366	0.855	0.798	0.777	0.898
Pooled SEM (±)	0.002	0.132	0.007	0.011	0.007	2.408	62.226
CV (%)	2.009	5.224	4.690	7.375	7.771	8.677	11.123

Mean bearing the same superscript in a column within the category do not differ significant (p >0.05).
[1]*Specific growth rate;* [2]*Feed conversion ratio;* [3]*Protein efficiency ratio;* [4]*Apparent protein utilization.*

Table 4. *Carcass composition (g/kg wet basis) of* Penaeus vannamei *juveniles fed experimental diets having graded levels of raw and fermented rapeseed meal by replacing fishmeal (n=3).*

Particulars	Carcass composition			
	Moisture	Crude protein	Ether extract	Total ash
Processing				
Raw	784.59[a]	160.56[a]	9.62[a]	28.71[a]
Fermented	783.19[a]	161.02[a]	9.68[a]	29.17[a]
Inclusion level (g/kg)				
0	780.97[a]	163.39[a]	8.83[b]	29.09[a]
25	784.90[a]	160.26[b]	9.86[a]	28.98[a]
50	783.83[a]	161.00[ab]	9.80[a]	28.87[a]
75	784.03[a]	160.08[b]	9.86[a]	28.77[a]
100	785.73[a]	159.18[b]	9.86[a]	28.97[a]
P-values				
Processing (A)	0.167	0.586	0.624	0.152
Inclusion level (B)	0.060	0.048	<0.001	0.972
A x B	0.761	0.874	0.036	0.040
Pooled SEM (±)	4.086	2.971	0.061	0.417
CV (%)	0.339	1.411	3.378	2.938

Mean bearing the same superscript in a column within the category do not differ significant (p >0.05).

due to the associated imperatives. The present study revealed that the inclusion of raw RSM could be limited to 25 g / kg beyond thatlevel the growth and nutrient utilization was significantly (P<0.05) reduced in *P. vannamei.* Similar results were corroborated with the finding of Rajaram (2010) in *P. monodon* and who inferred that the reduced performance of shrimp with a higher inclusion of RSM was mainly attributed to the anti-nutritional factors, particularly glucosinolates. Hossain and Jauncey (1988) have documented the toxicity of purified glucosinolates (ally1 isothiocyanate) in common carp, *Cyprinus carpio.* A similar negative effect was reported in other aquatic species fed with a semi-purified practical diet containing a higher level of RSM and mustard seed meal (Davies *et al*., 1990; Gomes *et al*., 1993).

Tannin forms indigestible protein complexes with protein by inhibiting protease enzymes and limits protein accessibility and digestibility (Helsper *et al.*, 1993). Hossain and Jauncey (1988) reported that the diet with a high content of tannin (1.14%) caused adverse effects in *C. carpio* than those fed 0.57% of tannin. Besides, the bitter taste of tannin reduced feed palatability and thereby hindering feed intake and growth (Krogdahl, 1989). In general, phytate-bound phosphorus is not promptly accessible to monogastric animals, including shrimp, because of lack of the enzyme phytase (Wu *et al*., 2009). Liener (1994) reported that undigested phytate reduced the accessibility of essential elements, including calcium, magnesium and potassium, notwithstanding phosphorous. Phytate likewise reduced protein accessibility to the cultured species by inhibiting the activity of the enzyme trypsin (Helsper *et al*., 1993). In the present study, fermentation has reduced glucosinolates by 59% (Table 1).

A similar reduction was reported by Shi *et al.* (2015) in *A. niger* treated RSM who stated that the reduced glucosinolates during fermentation could mainly be attributed to the utilization

of glucose and sulphur moieties by the microorganisms. In addition, fermented RSM had lowered content of tannin and phytic acid compared to raw RSM (Table 1), and the reduction could be attributed to the production of enzyme tannase and phytase, respectively. *Aspergillus niger* had produced 12.26 U/g of tannase and 58 U/g of phytase as earlier reported by Liu *et al.* (2016) and Gull *et al.* (2013), respectively.

Table 5. *Haemolymph indices of* Penaeus vannamei *juveniles fed experimental diets having graded levels of raw and fermented rapeseed meal by replacing fishmeal (n=3).*

Particulars	Haemolymph indices			
	Total protein (g/dl)	Glucose (g/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Processing				
Raw	7.78[b]	1.59[a]	19.79[a]	52.95[a]
Fermented	8.09[a]	1.58[a]	19.92[a]	52.39[a]
Inclusion level (g/kg)				
0	9.28[a]	1.45[d]	23.97[a]	65.52[a]
25	8.33[b]	1.48[d]	21.96[b]	62.45[b]
50	8.03[c]	1.58[c]	19.78[c]	53.99[c]
75	7.67[d]	1.63[b]	17.66[d]	45.73[d]
100	6.38[e]	1.76[a]	15.88[e]	35.64[e]
P-values				
Processing (A)	0.001	0.532	0.585	0.487
Inclusion level (B)	<0.001	<0.001	<0.001	<0.001
A x B	0.003	0.903	0.486	0.261
Pooled SEM (±)	0.027	0.001	0.249	2.675
CV (%)	2.729	1.989	3.304	4.086

Mean bearing the same superscript in a column within the category do not differ significant (p >0.05).

The poor utilization of raw RSM might also relate to the limited capability of *P. vannamei* in digesting fibrous components. Raw RSM had a considerable quantity of fibre fractions (Table 1), which entrap the digestible nutrients by preventing enzymatic degradation and 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 a diet containing low fibre canola meal (14%) than those fed with high fibre 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 fibre hydrolytic enzymes during fermentation. *Aspergillus niger* had produced 30 U/g of cellulase (Reddy *et al*., 2015), 3099 U/g of xylanase (Maciel *et al*., 2008) and 9 U/g of pectinase (Solis-Pereyra *et al*., 1996) during solid state fermentation. Furthermore, plant protein sources are deficient in one or more essential amino acids, particularly methionine and lysine. Therefore, there is need to supplement the respective deficient amino acid during feed formulation. However, Williams *et al.* (2001) had reported that the supplementation crystalline amino acids had higher leaching and faster rate of absorption than protein-bound amino acids. Due to these drawbacks, there is a need to improve the protein quality of plant-based ingredients with enhanced amino acid content. This could be achieved through the methodology of fermentation using microbial species, including fungus, bacteria and yeast (Ravindra, 2000).

In the present study, fungal fermentation has increased methionine from 8.16 to 13.12 g/kg and lysine from 10.17 to 20.52 g/kg. Though FCR remained unchanged significantly between the experimental groups, lower FCR was observed in shrimp fed fermented RSM than those fed the respective level of raw RSM. This indicates that the utilization of fermented RSM was superior to the raw material. The protein efficiency measures viz., PER and APU followed a similar pattern as that observed for growth in the respective diets. No significant difference observed in survival between the dietary treatments indicates the acceptability of RSM by the shrimp is more even at a higher level of inclusion.

The carcass composition of *P. vannamei* did not differ significantly among the dietary treatments except ether extract (Table 5). Similar observations were reported by Yue *et al.* (2012) and Sun *et al*. (2016) while replacing fishmeal using fermented cottonseed meal and a blend of soybean meal and groundnut oil cake, respectively in the diet of *P. vannamei*. Kaushik *et al.* (2004) reported that increasing dietary plant protein source was related to enhanced activity of hepatic lipogenic enzymes that induced fat retention in the body, which could be a reason behind getting higher ether extract values in shrimp fed diet with higher plant proteins in the present investigation. Moreover, the inclusion of vegetable oils by replacing dietary fish oil increased ether extract level in Gilthead seabream (Menoyo *et al*., 2004). In the present study, palm oil was included in the test diets to compensate the lipid loss which occurred due to fishmeal replacement. It could also be a possible reason for observing a higher content of ether extract in shrimp fed test diets than those fed a control diet. Shrimp fed diets with raw and fermented RSM showed lower total protein content in hemolymph than those fed with the control diet (RSM 0).

The result is corroborated with the findings of Yun *et al*. (2017) in *P. vannamei* and who reported that fishmeal containing diet had higher haemolymph protein than those diets were substituted using soybean meal. Yue *et al*. (2012) have also observed the hypolipidemic effect while replacing fishmeal using plant protein sources in the diet of *P. vannamei*. This could be a reason for lower triglyceride and cholesterol level in the hemolymph of shrimp fed fishmeal replaced diets in the present study. The inclusion of RSM has increased the hemolymph glucose level in the present investigation, and the authors suggested that this would be partly attributed to an increment of carbohydrates.

CONCLUSIONS

It can be concluded that fermented RSM could replace more fishmeal compared to raw RSM in the diet of *P. vannamei*. Fermented RSM could be included to the extent of 64.4 g/kg of shrimp feed in the given combination of ingredients, without having any negative impact on shrimp growth and survival, whereas raw RSM could replace fishmeal to the extent of 25 g/kg. Solid state fermentation appears to be an ideal processing method to enhance the nutritional value of plant ingredients in aquafeeds by suppressing various anti-nutritional factors.

However, the present study is suggesting for further investigations for up-scaling fermentation technique before advocating into commercial applications.

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