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Carcass amino acid, fatty acid and mineral composition of *Penaeus vannamei* Boone, 1931 fed diets with fermented oilseed meals/cakes

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Abstract
Commercial de-fatted groundnut oil cake (GNC) and rapeseed meal (RSM) were fermented using the fungus, *Aspergillus niger* to evaluate for carcass amino acid, fatty acid and minerals in *Penaeus vannamei* after 45-days. Fermented GNC and RSM was included at 100 and 75 g/kg by replacing fishmeal in test diets. A total of 180 shrimps (3.12±0.09 g) were distributed randomly into nine 500-L oval-shaped fiberglass tanks (1.31x0.64x0.73m) (20 shrimps per tank) with three replication for each dietary treatment. The results revealed that shrimp fed fermented ingredients had no effect on carcass amino acids and poly unsaturated fatty acids in particular, eicosapentaenoic and docosahexaenoic acids. Calcium was significantly (p<0.05) increased in test groups than those fed a control. The present results concluded that the fermented ingredients could be used as a potential substitute for fishmeal in the diet of *P. vannamei*, hence they would not have any negative effect on carcass micronutrients.

Keywords: Amino acids, *Aspergillus niger*, fatty acids, minerals, oilseed meals/cakes, *Penaeus vannamei*

1. Introduction
The nutritional composition of feeds mainly influences the body composition of aquatic species in particular, shrimp. Before two decades, fishmeal was used as a primary protein source in commercial shrimp feed formulation with the inclusion of 25-50% due to its richness of essential amino acids (methionine, lysine and tryptophan), essential fatty acids (eicosapentaenoic acid (EPA) and docosahexenoic acid (DHA)) and higher content of available phosphorus with good palatability and digestibility compared to other ingredients. Over the period of 1986 to 2000, the average global production of fishmeal was 6.2 million metric tonnes (mmt) and was significantly reduced to 4.2 mmt in 2015 [1]. This drasttical reduction increases the cost of fishmeal from 452 to 2169 USD tonne^{-1}, which consequently increases the feed cost. As feed cost accounts more than 70% of total production cost, the researchers reduced considerable quantity of fishmeal by using various alternatives with varying degree of success. One among them is fermented plant protein sources [2, 3, 4, 5]. In our earlier studies, fishmeal could be replaced up to 60 and 20% using fermented soybean meal (SBM) and sunflower oil cake (SFC), respectively [5]. Though microbial fermentation enhanced the nutrient utilization of plant-based ingredients, carcass composition of cultured species would be varied due to fishmeal substitution. Jannathulla *et al.* [5] found a higher deposition carcass lipid in *Penaeus vannamei* fed diets with fermented SBM and SFC compared those fed a control feed which did not have fermented ingredients. The similar result was observed while replacing fishmeal using fermented guar meal in the diet of *P. vannamei* [6]. The impact of fermented ingredients on the carcass composition has also been reported in *Penaeus monodon* [7], *Epinephelus coioides* [2] and *Feneropenaeus indicus* [3].
As shrimps have been recognized a healthy choice of food due to its wholesome attributes like rich source of protein, vitamin B_{12}, minerals, ω-3 highly unsaturated fatty acids (HUFA), astaxanthin and a potent natural antioxidant [8], the increased awareness of the consumers for purchasing good quality food to maintain a healthy food regime. The Act of Nutrition Labelling and Education (NLEA) of 1990 in the USA and the Association of Official Analytical Chemists (AOAC) have already been invented to label the processed food materials

which had information on the nutrient contents of the respective food materials. Hence, producing good quality shrimp is obligatory nowadays. The majority of the earlier works related to fermentation have been restricted in assessing only macro nutrients (proximate composition), but till to date, there is scarce of information regarding fermented ingredients on micronutrients (amino acids, fatty acids and minerals). Hence, in the present study, it aimed to evaluate the effect of inclusion of two fermented oilseed cakes/meals viz., groundnut oil cake (GNC) and rapeseed meal (RSM) with the fungus, *Aspergillus niger* on carcass composition of amino acids, fatty acids and minerals in *P. vannamei*. The output from this study would help to explore the usage and limitation of fermented ingredients in producing good quality shrimp.

2. Materials and method

2.1. Fermentation methodology

The fungus, *A. niger* listed under GRAS notifications (Generally Recognized As Safe) by FDA (GRAS Notice No. 35, 2010) was used to ferment the selected ingredients. Prior to fermentation, the parent culture of *A. niger* (ATCC 6275) purchased from Himedia Laboratories (Mumbai, India) and was grown on potato dextrose agar (PDA) for five days at 35±1 °C in an incubator. The microbial suspension was prepared at the rate of 10^7 spores ml^{-1} according to Jannathulla *et al.* (2017c). Meanwhile, two commercial solvent extracted oilseed meals/cakes viz., groundnut oil cake (GNC) and rapeseed meal (RSM) were purchased from the local markets (n=6) and were ground to a particle (<500 µm). The ground materials were sterilized by autoclaving at 121 °C (105 kPa) for 15 min after hydrated with water and then inoculated with 5% *A. niger* suspension (10^7 spores ml^{-1}). Fermentation was carried out in 500 ml Erlenmeyer flasks plugged with cotton at 35±1 °C in an incubator for three days with three sets of replications for each ingredient [6]. All the fermented samples were dried at 50 °C for 48 h to bring down the moisture content below 10%. In order to have a representative sample, all the replicates of an ingredient was pooled to avoid a possible variation. The fermented materials were ground to fine particles (<250 µm) and stored at 4 °C until further use. The nutritional composition of test ingredients is presented in Table 1.

Table 1: Proximate, amino acid, fatty acid and mineral composition of fishmeal and test ingredients used in the present study (g kg^{-1} dry matter basis)

Particulars	Fishmeal	Test ingredients	
		FGNC[1]	FRSM[2]
Proximate composition			
Crude protein	631.67	520.03	467.53
Ether extract	105.31	19.51	22.81
Crude fiber	5.39	127.31	102.84
Nitrogen free extract[5]	68.06	252.91	337.45
Total ash	189.57	80.24	69.37
Essential amino acids			
Arg	43.77	35.84	35.64
His	16.94	10.94	16.66
Ile	29.65	15.2	15.47
Leu	50.83	11.13	23.85
Lys	52.95	28.12	20.52
Met	19.06	10.27	13.12
Phe	27.53	31.04	12.59
Thr	28.95	13.1	21.25
Try	7.06	5.23	4.94
Val	34.59	27.9	25.35
Major fatty acids			
C14:0	11.99	0.01	0.03
C16:0	24.45	1.85	0.94
C16:1	11.33	0.02	0.11
C18:0	5.28	0.53	0.28
C18:1	5.00	8.79	2.26
C22:1	-	0.02	6.77
C18:2	0.97	3.36	3.84
C18:3	0.57	0.17	3.01
C20:4	1.49	0.02	0.04
C20:5	4.73	-	-
C22:6	2.68	-	-
Macro minerals			
Ca	47.64	3.55	5.31
Mg	2.91	4.27	4.14
P	28.46	5.28	11.55
K	9.64	14.68	12.03
Na	12.30	0.28	0.18

[1]Fermented groundnut oil cake
[2]Fermented rapeseed meal
[3]Calculated by a difference

2.2. Experimental diets

A control diet contained 250 g kg^{-1} fishmeal and was partially replaced (w/w) with 100 and 75 g kg^{-1} of fermented GNC and RSM, respectively in the test diets based on our previous work (Communicated data), since the growth performance of these test diets was on par with the control diet. Prior to preparing the experimental diets, all the listed ingredients (Table 2) were pulverized and sieved through 250 µm mesh. To the sieved mesh of each diet, pre-mix (vitamin-mineral mixture, binder and butylated hydroxytoluene) were added. After 2 to 3 min of the manual mixing, oil sources (fish oil, palm oil and soy-lecithin) were dispersed and blended in an electric blender for 20 min. The resulting homogenised mixture was steamed at atmospheric pressure for 5 min after making dough using water (500 ml kg^{-1}) and then pelleted in a table top pelletizer having a 2 mm diameter die [9]. The diets were dried in an oven at 60 °C overnight and were placed in a plastic container and then stored -20 °C until used. The nutritional composition of experimental diets is given in Table 2.

Table 2: Ingredients and nutritional composition of experimental diets having fermented ingredients by replacing fishmeal (g kg^{-1} as fed basis)

Particulars	Experimental diets		
	Control	**FGNC 100**	**FRSM 75**
Ingredient composition			
Fishmeal[1]	250	150	175
FSBM	-	-	-
FGNC	-	100	-
FRSM	-	-	75
FSFC	-	-	-
Acetes[2]	80	80	80
Squid meal	15	15	15
Soybean meal	200	200	200
Corn gluten	20	40	42
Sesame cake	50	50	50
Wheat flour	324	296	296
Fish oil[1]	20	20	20
Palm oil	-	8	6
Lecithin	10	10	10
Pre-mix[3]	20	20	20
Binder[4]	10	10	10
BHT[5]	1	1	1
Proximate composition			
Moisture	87.65	81.56	86.76
Crude protein	374.46	369.94	375.84
Ether extract	67.64	71.01	67.47
Crude fiber	29.85	39.46	34.92
Nitrogen free extract	296.83	315.24	299.12
Total ash	143.57	122.79	135.89
Essential amino acids			
Arg	23.10	23.53	23.74
His	8.84	9.12	10.11
Ile	15.36	14.95	15.36
Leu	26.47	25.44	25.67
Lys	21.44	21.78	20.86
Met	8.45	8.04	9.12
Phe	17.39	18.95	16.84
Thr	14.37	13.61	14.28
Try	7.21	6.90	6.74
Val	17.12	17.56	17.64
Major fatty acids			
C14:0	4.93	3.94	3.96
C15:0	0.37	0.25	0.22
C16:0	14.79	14.74	14.04
C17:0	0.47	0.31	0.31
C18:0	3.19	2.64	2.75
C20:0	0.35	0.26	0.47
C24:0	0.21	0.18	0.16
C16:1	4.89	3.61	3.84
C18:1	9.09	14.63	10.98
C20:1	0.25	0.21	0.24
C18:2	11.22	11.67	11.25
C18:3	1.24	1.05	1.35
C20:4	0.74	0.54	0.53

C20:5	3.56	2.99	3.27
C22:6	2.01	1.71	1.85
Macro minerals			
Ca	29.71	23.73	25.34
P	14.81	11.86	12.76
Na	2.02	1.66	1.75
K	5.60	6.88	5.93
Mg	3.31	2.92	3.27

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

[2]Mantis shrimp having approximately 60% crude protein and 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)

2.3. Experimental conditions

Juveniles of *P. vannamei* were obtained from a semi-intensive culture pond near Chennai, India. Shrimp were acclimatized to the experimental conditions and fed a control diet for two weeks before the experiment started. A total of 180 shrimp with an initial body weight of 3.12±0.09 g were distributed randomly into nine 500 L oval shaped fiberglass tanks (1.31x0.64x0.73 m) at the rate of twenty shrimps per tank with three replication for each dietary treatment. All the tanks were equipped with aquaculture flow-through system (1.5 ml min^{-1}) and covered with a fiber mat to prevent the light intensity and shrimp from jumping out. The shrimp were cultured indoors and subjected to natural photoperiods (12 h light: 12 h dark). During the experimental periods, ultraviolet treated water was used after filtering through a 5 µm cartridge filter. The 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 recorded and found to be within the normal range. All shrimp in each tank were initially fed 6% of total biomass daily at a frequency of three times per day (7.00 AM, 12.00 PM and 5.30 PM) and lasted for 45 days. During the experimental period, the amount of diet given was progressively adjusted according to body weight, survival and consumption and all residual/uneaten feed (if any) were siphoned out from the tanks. After 45 days of the trial, a total of 45 shrimps from each treatment (15 shrimps per replication) were collected and washed with de-ionized water to remove the adhering contaminations and transferred to the Nutrition Laboratory for the analysis of nutrient contents.

2.4. Biochemical analysis

Proximate composition of ingredients, experimental diets was analyzed as per the method of AOAC [10]. Amino acid profiles were analyzed using a pre-column derivatization HPLC gradient system (Shimadzu Corp, LC-30AD) after hydrolyzing the samples with 6 N hydrochloric acid in a sealed tube filled with nitrogen for 22 h at 110 °C in an oven [11]. Tryptophan, being liable to acid hydrolysis, was measured after alkali hydrolysis by the spectrophotometric method at 500 nm [12]. The partial oxidation of sulphur containing amino acids like cystine and methionine during acid digestion was prevented by adding 0.1% of phenol [13]. Lipid was extracted by using chloroform and methanol (2:1) by Folch *et al.* [14] method and the respective fatty acid methyl esters (FAMEs) were prepared by Metcalfe *et al.* [15] method and finally, FAMEs were extracted into petroleum ether. Routine analysis of methyl esters was performed by a gas chromatograph (GC-2014 Shimadzu) on an RTX wax capillary column (100 m length X 0.25 mm I.D X 0.2 µm film thickness). Nitrogen was used as a carrier gas at a linear velocity of 20.9 cm sec^{-1} with 3 ml min^{-1} of purge flow. The quantity of fatty acids (mg kg^{-1}) was calculated according to Aziz *et al.* [16]. The sample was digested using microwave digestion method (Anton Par microwave system) for mineral analysis with a combination of nitric acid and hydrogen peroxide in inert polymeric microwave vessels. The minerals were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES; Agilent 5100 SUV) using the 5.2 software [17].

2.5. Statistical analysis

All data are presented as means±SEM and subjected to one-way analysis of variance (ANOVA) to test the effects of experimental diets on carcass composition of *P. vannamei* using the software of the SPSS for windows (16.0 ver.). Tukey's test was used to resolve the differences among treatment means. Statistical significance was examined at $P<0.05$. Prior to statistical evaluation, data were checked for ascertaining a normal distribution and then determining the homogeneity of variance.

3. Results and Discussion

Carcass amino acid composition of *P. vannamei* fed different experimental diets is presented in Table 3. In essential amino acids (EAA), histidine followed by leucine, arginine and lysine had >10 g kg^{-1} and glutamic acid was dominated in nonessential amino acids (NAA) irrespective of the treatments. However, the dietary substitution of fishmeal with fermented GNC and RSM did not influence the body amino acid composition. The result is corroborated with the findings of Rajaram [18] and Dayal *et al.* [9] in *P. monodon* while using GNC, RSM and SFC as a substitute of fishmeal. But, the contrast result was reported by Jeyasantha and Patterson [19] in *P. monodon,* which had a higher EAA in the body muscle while fed a diet with high content of trash fishes (50%) as a major ingredient compared to a commercial feed. Though plant origins could not meet the nutritional requirement of cultured species like fishmeal, partial replacement of fishmeal with fermented ingredients did not have any negative impact on carcass amino acids in our study. This would be attributed to the microbial contribution.

Table 3: Amino acid composition of *Penaeus vannamei* fed experimental diets having fermented ingredients by replacing fishmeal (g kg^{-1} wet basis)

Particulars[1]	Experimental diets			SEM	p-value	CV (%)
	Control	FGNC 100	FRSM 75			
Essential amino acids						
Arg	11.07	10.79	10.56	0.065	0.288	3.114
His	14.02	14.07	13.78	0.887	0.433	9.101
Ile	6.51	6.71	6.30	0.074	0.449	5.502
Leu	11.47	11.88	11.12	0.173	0.339	4.768
Lys	10.41	11.55	10.55	0.295	0.220	6.600
Met	3.84	3.95	3.47	0.095	0.393	10.824
Phe	5.96	6.31	5.76	0.061	0.234	5.421
Thr	4.25	4.46	4.08	0.063	0.454	7.742
Trp	1.15	1.12	1.21	0.005	0.538	8.077
Val	6.66	6.89	6.57	0.075	0.581	5.389
Nonessential amino acids						
Ala	8.49	8.66	8.19	0.022	0.094	2.310
Asp	12.75	13.04	12.11	0.206	0.260	4.730
Cyt	1.04	0.87	0.86	0.008	0.219	12.449
Glu	21.60	22.44	21.64	0.154	0.197	2.359
Gly	12.00	12.23	12.40	0.062	0.415	2.689
Pro	8.39	8.92	8.34	1.137	0.860	16.414
Ser	4.84	4.76	4.50	0.053	0.439	6.465
Tyr	4.72	4.79	4.52	0.007	0.079	2.353

All the values are means of three replications
[1]No significant difference

Jannathulla *et al.* [20] found the increase of methionine and lysine in double in various oilseed cakes/meals fermented with *A. niger* than those were untreated. Ravindra [21] suggested that it could be due to the higher content of these amino acids in *A. niger* than other microbial species. The quality of protein according to the amino acid requirements of human is assessed nowadays based on Protein Digestibility Corrected Amino Acid Score (PDCAAS), which was 1 for shrimp indicating that shrimp has a superior quality of protein. The ratio of EAA/NAA was in the range of 1.01 to 1.03 the present study. A similar result (1.05) was observed in *Macrobrachium vollenhovenii* [22] while the lower values were reported in fish (0.70) and in crab and squid (0.56) by Iwasaki and Harada [23]. Presently, a new concept has been arrived with functional amino acids (FAA) which includes arginine, cystine, leucine, methionine, tryptophan, tyrosine, aspartic acid, glutamic acid, glycine and proline for human nutrition. These amino acids mainly helped to regulate the key metabolic pathways to improve the health, growth, lactation and reproduction in addition to preventing various diseases and disorders [24]. All these amino acids were in considerable quantity in *P. vannamei* reared in the present study.

The fatty acid composition of feed mainly influences the carcass fatty acids [25]. Certain fatty acids in particular, C16:0, C18:0, C16:1, C18:1, C18:2, C20:5 and C22:6 were high in experimental feeds (Table 2) and to a certain extent; the same was reflected in shrimp carcass compositions in our study. The similar results were corroborated in earlier findings in *P. monodon* [25] and *P. indicus* [26]. In SFAs, C16:0 and C18:0 were dominated and were significantly ($p<0.05$) reduced due to fishmeal substitution (Table 4). Among the MUFAs, C18:1 was predominant in both diets and shrimp but its level was low in control diet than test diets and reverse trend was noticed in shrimp carcass. Gonzalez-Félix *et al.* [27] suggested that this effect would probably be due to the preferential utilization of fatty acids by the shrimps. Sparing and retention of fatty acids had already been demonstrated in *F. chinensis* [28], *P. monodon* [25] and *P. vannamei* [27].

A plant-based diet, in which 90% of fish oil was replaced by soy oil, had no adverse effects on shrimp growth performance, but dramatically reduced body PUFAs content in particular EPA and DHA [27]. This result is in agreement with the findings of Montero *et al.* [29] in *Dicentrarchus labrax* fed fish oil replaced diet with various plant oils viz., rapeseed, linseed and palm oils. The authors suggested to switch over the aquatic species fed plant oil to fish oil containing diet (finisher diet) prior to harvesting for an appropriate time to restore both EPA and DHA as they are most important fatty acids in human nutrition. A study with juveniles of *P. monodon* [30] shown that a period of 30-days was required to restore both EPA and DHA by using a finisher diet (control diet with no fishmeal replacement) in both yard and laboratory condition, whereas finishing diet phase was 16 to 20 weeks in Salmon fed a blend of rapeseed, linseed and fish oil [31]. The variation in time phase between the studies could be due to the species difference, weight of the species, rearing and environmental conditions. However, fishmeal substitution did not affect these fatty acids in the present study, which would be due to maintaining similar fish oil level in both the test diets as like control diet.

Intake of 500 mg day^{-1} or 3.50 g $week^{-1}$ of EPA and DHA together was recommended to promote good cardiac health in human adults according to International Society for the Study of Fatty Acids and Lipids [32]. However, the level was revised to 250 mg day^{-1} or 1.75 g $week^{-1}$ in the later period of 2010 by the US dietary guidelines [33]. The US dietary guidelines of EPA and DHA can be met by consuming approximately 184 to 192 g of shrimp fed on control and other experimental diets. However, the impact of further processing or cooking on the fatty acid fluctuations needs to be considered. In total PUFA, the highest proportion was contributed by EPA and DHA. The result of the present study indicates that EPA and DHA together was 46.8-56.5% total PUFAs in the experimental shrimps. Having more EPA than DHA is a

distinguished feature of crustaceans and the same was agreed in the present study. Health Education Authority [34] stated that the ratio of PUFA/SFA in a healthy food should be above 0.54 and the shrimp assessed in the present study had a PUFA/SFA ratio in the range of 1.06, 1.12 and 1.18 in control, FGNC 100 and FRSM 75 diet, respectively. This indicates that the dietary substitution of fishmeal using fermented ingredients did not have any negative impact on shrimp fatty acids, particularly on PUFAs.

Table 4: Fatty acid composition of *Penaeus vannamei* fed experimental diets having fermented ingredients by replacing fishmeal (mg kg^{-1} wet basis)

Particulars	Experimental diets			SEM	p-value	CV (%)
	Control	FGNC 100	FRSM 75			
Saturated fatty acids (SFAs)						
C14:0	52.32^{a}	35.03^{b}	30.02^{c}	0.371	0.000	2.050
C15:0	45.03^{a}	48.37^{a}	28.91^{b}	1.347	0.000	3.747
C16:0	1490.42^{a}	1183.66^{b}	1034.68^{c}	54.317	0.000	0.785
C17:0	55.99^{b}	59.78^{a}	56.17^{b}	0.162	0.002	0.923
C18:0	1010.15^{a}	839.49^{b}	793.53^{c}	20.023	0.000	0.668
C20:0	24.95^{a}	20.20^{b}	17.53^{c}	0.013	0.000	0.731
C22:0	9.61^{a}	8.08^{b}	9.88^{a}	0.017	0.000	1.885
C23:0	10.76^{c}	15.13^{b}	20.86^{a}	0.152	0.000	3.293
Mono unsaturated fatty acids (MUFAs)						
C14:1	28.53^{a}	19.90^{b}	18.60^{c}	0.094	0.000	1.809
C16:1	176.15^{a}	119.56^{b}	116.46^{b}	2.327	0.000	1.461
C17:1	20.11^{b}	14.02^{c}	41.67^{a}	0.117	0.000	1.785
C18:1	1016.16^{a}	859.48^{b}	756.03^{c}	14.185	0.000	0.565
C20:1	14.47^{a}	6.45^{b}	14.60^{a}	1.980	0.009	15.642
C22:1	14.34^{a}	9.97^{b}	11.73^{b}	0.498	0.011	7.734
C24:1	22.98^{a}	14.87^{b}	22.09^{a}	0.144	0.000	2.503
Poly unsaturated fatty acids (PUFAs)						
C18:2	974.17^{a}	761.86^{b}	629.38^{c}	36.529	0.000	1.009
C20:2	17.03^{a}	18.69^{a}	17.71^{a}	1.849	0.569	10.049
C22:2	16.93^{a}	17.18^{a}	16.56^{a}	0.441	0.705	5.173
C18:3	83.81^{a}	77.88^{b}	64.48^{c}	0.060	0.000	0.426
C20:3	10.53^{b}	9.14^{b}	17.02^{a}	1.373	0.007	12.610
C20:4	419.49^{a}	313.51^{b}	277.72^{c}	32.992	0.000	2.244
C20:5	833.16^{a}	817.49^{a}	811.57^{a}	644.341	0.734	4.070
C22:6	505.07^{a}	451.22^{a}	517.53^{a}	309.994	0.050	4.717
All the values are means of three replications Means bearing the same superscript in the same row do not differ significantly (p>0.05)						

Shrimp also had a considerable quantity of minerals which are beneficial to humans and other animals. Among the analyzed elements, the level of calcium was high (6.60 to 7.68 g kg^{-1}). The values are similar to those reported earlier by Jannathulla *et al.* [17] in *P. vannamei*. However, its quantity was significantly ($p<0.05$) increased with increasing fishmeal substitution with fungal fermented oilseed cakes/meals. Increasing calcium is beneficial since it plays various important roles, especially bone formation, muscle contraction, blood clotting, etc., [17]. However, other elements were not affected in shrimp (Table 5). Jannathulla *et al.* [17] suggested that this could be due to the extraction of most of the minerals from the rearing system (water) by the shrimp.

Table 5: Macro mineral composition of *Penaeus vannamei* fed experimental diets having fermented ingredients by replacing fishmeal (g kg^{-1} wet basis)

Particulars	Experimental diets			SEM	p-value	CV (%)
	Control	FGNC 100	FRSM 75			
Ca	6.60^{c}	7.31^{b}	7.68^{a}	0.010	0.001	1.815
K	2.98^{a}	2.92^{a}	2.85^{a}	0.004	0.295	2.987
Mg	0.60^{a}	0.63^{a}	0.62^{a}	0.001	0.462	3.860
Na	1.63^{a}	1.53^{a}	1.54^{a}	0.001	0.114	2.811
P	2.41^{a}	2.39^{a}	2.39^{a}	0.002	0.858	2.284
All the values are means of three replications Means bearing the same superscript in the same row do not differ significantly (p>0.05)						

5. Conclusion

From the present investigation, it could be concluded that the inclusion of fermented groundnut oil cake and rapeseed meal by replacing fishmeal (w/w) did not influence nutritional constituents in *Penaeus vannamei* in the present condition. Hence, the considerable quantity of fishmeal could be replaced by the fungal fermented ingredients in the diet of *Penaeus vannamei*.

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