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A comparative study on optimising the inclusion level of three different oilseed meals/cakes as a fishmeal substitute in the diet of *Penaeus monodon* Fabricius, 1798

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ABSTRACT

A 60-day indoor feeding trial was conducted in tanks to optimise the inclusion level of three oilseed meals/cakes, such as groundnut oil cake (GNC), rapeseed meal (RSM) and sesame oil cake (SOC), in the diet of *Penaeus monodon* Fabricius, 1798. Each test ingredient was included separately at the rate of 0 (control), 2.5, 5, 7.5 and 10% by substituting fishmeal (w/w). The shrimps were hand-fed the respective pellet feed thrice a day in a static condition at the rate of 8% of the total biomass and was adjusted based on the intake. Results revealed that RSM-fed groups showed a lower daily growth coefficient (DGC) (1.27% day^{-1}) than the other two groups (1.29-1.30% day^{-1}). The DGC decreased from 1.40 to 1.09% day^{-1} with increase in inclusion levels from 0 to 10%. Broken line regression indicated that the optimal inclusion level of GNC, SOC and RSM was 5.4, 2.9 and 2.4%, respectively. Digestibility of dry matter, crude protein, as well as of the amino acids, Arg and Met increased by 1.92, 0.72, 4.02 and 4.74%, respectively, at 2.5% inclusion, and the increase was extended up to 5% for His, Leu and Lys. The dietary change did not affect survival and carcass composition, while free amino acids in shrimp tail muscle varied among the treatments. Results concluded that all three oilseed meals/cakes are potentially viable and could substitute dietary fishmeal partially in *P. monodon.* In contrast, they were ranked as GNC > SOC > RSM according to the broken line model.

Keywords: Black tiger shrimp, Carcass composition, Digestibility, Plant proteins, Shrimp feed

Introduction

Aquaculture is the major food-producing sector that contributed 114.5 million t in live weight valued at USD 263.6 billion in 2018. It has grown by 527% from 1999 to 2018 (FAO, 2020), which simultaneously increased the growth rate of the aquafeed sector. Fishmeal is the major protein source used in aquafeed formulation (25-50%) as it is rich in essential nutrients and good in digestibility and palatability. The aquafeed sector consumed around 10% of the total fishmeal produced in the early 1990s, which increased from 34% in 2002 to >70% in 2018 (Jannathulla *et al.,* 2019). The sector is overly reliant on fishmeal, as evidenced by its consumption of more than 70% of the fishmeal produced globally, although accounting for only 4% of the global industrial feed production. On the other hand, about 28% decrease in global fishmeal production (7125 million t in 2000 to 5130 million t in 2018) has resulted in increasing the cost by 73% (USD 413 to 1546 per t) (Bae *et al.,* 2020). Unfavourable scenario and overdependence on fishmeal posed significant risks in terms of supply, price, quality and sustainability concerns. Therefore identifying suitable alternatives to fishmeal remains a high priority in nutrition research nowadays to escape the fishmeal trap in the future. Among the alternatives identified, researchers preferred protein sources originating from plants due to low cost and easy availability with desirable nutrient contents. Among the plant proteins, soybean meal is a predominant choice. A comprehensive database has already been developed regarding the growth, digestibility and nutrient utilisation of soybean meal in shrimp and fish feed. A similar effort was also made for other plant proteins in fish, but only a little on shrimp. Still, there is a restriction in utilising other plant proteins even at a lower level in the commercial shrimp feed formulations. This intended to thoroughly study the impact of other plant proteins on the actual practice to improve the knowledge on utilising these alternative ingredients. Consequent to this, the three most abundant oilseed meals/cakes available in India (USDA, 2021) such as groundnut oil cake (16,15,000 t), rapeseed meal (4657000 t) and sesame oil cake (7,46,000 t), were evaluated for their impact on growth, digestibility and carcass composition in black tiger shrimp, *Penaeus monodon* Fabricius, 1798, while substituting dietary fishmeal in the present investigation. This baseline data indicate how to

use and limit the selected plant proteins when formulating cost-effective commercial shrimp feed in future.

Materials and methods

Experimental diets

Three distinct defatted oilseed meals/cakes, including ground oil cake (GNC), rapeseed meal (RSM) and sesame oil cake (SOC) were acquired from different regions of Tamil Nadu and Andhra Pradesh, India (n=6) and were sundried. The coarse materials were hammer milled into fine particle (250 µm) and stored in plastic containers with proper labelling in the refrigerator at 4°C until further use. The nutrient composition (proximate and essential amino acids) of all three test ingredients, along with the fishmeal used in our study, is given in Table 1. In preparing experimental diets (Dayal *et al.,* 2020), the coarse ingredients listed in Table 2 were pulverised and passed through a 250 µm mesh screen. The control diet used in the present study contains 25% fishmeal and was substituted (w/w) at the rate of 2.5, 5, 7.5 and 10% using GNC (G-2.5, G-5, G-7.5 and G-10), RSM (R-2.5, R-5, R7.5 and R-10) and SOC (S-2.5, S-5, S-7.5 and S-10). The additives like vitamin-mineral mixture, vitamin-C (antioxidant), binder and chromic oxide (inert marker) were further added to the pulverised materials as per the quantity mentioned in Table 2. They were hand-mixed for 5 min and transferred to a domestic mixer for stirring for 10 min additionally. The homogenised mash was gradually supplemented with oil sources (fish oil and soy lecithin). De-ionised water was added to the mix at the rate of 500 ml kg^{-1} and kneaded into a soft dough. It was steamed under atmospheric pressure for 5 min before being pelleted in a tabletop pelletiser with a 1.4 mm diameter die. The pellets were dried at 60°C in a hot air oven to bring down the moisture content below 10%, cooled and packed in a plastic bag and stored in a refrigerator until use. The proximate and essential amino acid composition of experimental diets is presented in Table 3.

Experimental animals

P. monodon post-larvae (15-days old) obtained from a Govt. of India certified hatchery were transported to the Muttukadu Experimental Station, ICAR-Central Institute of Brackishwater Aquaculture (ICAR-CIBA), Chennai, India, in polythene cover under oxygen packing (95% purity). Approximately 1500 shrimps were raised in a net cage (2×2×1 m) erected in the lagoon and reared up to 0.30-0.40 g with a diet containing 40% crude protein. After reaching the desired size, they were transferred

Table 1. Nutrient composition of fishmeal and oilseed cake/meals used in the present study

Particulars	Fish meal	Oilseed cakes/meals		
		GNC[1]	RSM[2]	SOC[3]
Proximate composition (% dry weight basis)				
Crude protein	63.74	45.66	42.34	38.80
Ether extract	10.51	1.95	1.01	8.69
Crude fibre	0.53	8.85	12.34	8.74
Total ash	6.27	7.66	7.68	9.46
Nitrogen free extract[4]	18.95	35.88	36.63	34.30
Amino acid composition (g 16 g N^{-1})				
Arg	6.86	9.06	6.06	12.88
His	2.65	2.36	2.73	2.96
Ile	4.64	4.03	4.37	5.23
Leu	7.97	6.49	7.13	8.33
Lys	7.90	3.37	6.11	3.61
Met	2.98	1.04	1.30	3.50
Phe	4.31	5.14	3.98	5.74
Thr	4.53	2.88	4.53	4.58
Trp	1.11	1.08	1.2	1.79
Val	5.41	4.69	5.62	6.31
Anti-nutrients (mg 100 g dry $weight^{-1}$)				
Trypsin inhibitor	-	BDL[5]	BDL[5]	154.03
Phytic acid	-	1037.84	2489.47	18.91
Saponin	-	742.24	-	1984.26
Tannin	-	1739.48	846.37	4.29
Glucosinolates	-	-	341.86	-

[1]Groundnut oil cake; [2]Rapeseed meal; [3]Sesame oil cake
[4]Calculated by a difference: 100 - (% of crude protein + ether extract + crude fibre + total ash); [5]Below detectable limits

Table 2. Ingredient composition (% as fed basis) of experimental feeds used in the present study

Particulars	Experimental diets												
	Control	G-2.5	G-5	G-7.5	G-10	R-2.5	R-5	R-7.5	R-10	S-2.5	S-5	S-7.5	S-10
Fishmeal[1]	25.0	22.5	20.0	17.5	15.0	22.5	20.0	17.5	15.0	22.5	20.0	17.5	15.0
GNC[2]	-	2.5	5.0	7.5	10.0	-	-	-	-	-	-	-	-
RSM[2]	-	-	-	-	-	2.5	5.0	7.5	10.0	-	-	-	-
SOC[2]	-	-	-	-	-	-	-	-	-	2.5	5.0	7.5	10.0
Acetes[3]	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0
Squilla meal[4]	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4
Squid meal[5]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Soybean meal[6]	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Wheat gluten meal[2]	-	0.9	1.8	2.7	3.6	0.9	1.8	2.7	3.6	0.9	1.8	2.7	3.6
Wheat flour[2]	20.0	19.1	18.2	17.3	16.4	19.1	18.2	17.3	16.4	19.1	18.2	17.3	16.4
Fish oil[1]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Soy lecithin[6]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vit-min mix[7]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin C[8]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Binder[9]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Chromic oxide[8]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

[1]Bismi Fisheries, Mayiladuthurai, Tamil Nadu, India; [2]Local market, Chennai, India; [3]Om-Sai Aqua, Dandi, Gujarat, India; [4]Blueline Foods India Pvt. Ltd., Mangalore, Karnataka; [5]Khaja Mohammed Store, Chennai, Tamil Nadu, India; [6]Real Soy Enterprises, Indore, Madhya Pradesh, India. [7]Thiamine hydrochloride (25.50 g); Riboflavin (25.00 g); Pyridoxine hydrochloride Cyanocobalamin (0.10 g); Menadione (5.00 g); All-trans tocopherol acetate (99.00 g); Retinyl acetate (10.00 g); Vitamin D (50 g); Nicotinic acid (101.00 g); D-Ca-pantothenate (61.00 g); Biotin (25.00 g); Folic acid (6.25 g); Inositol (153.06 g); Ferric citrate (13.70 g); $ZnSO_4.7H_2O$ (28.28 g); $MgSO_4.7H_2O$ (0.12 g); $MnSO_4.H_2O$ (12.43 g); $CuSO_4.5\ H_2O$ (19.84 g); $CoC1_2.6H_2O$ (4.07 g); KIO_4 (0.03 g); KCl (15.33 g); Na_2SeO_3 (0.02 g); [8] Sigma-Aldrich, Missouri, USA; [9]Pegabind, Bentoli Agri-Nutrition Asia Pvt. Ltd., Singapore.

Table 3. Proximate and essential amino acid composition of experimental feeds used in the present study

Particulars	Experimental diets													R[1]
	Control	G-2.5	G-5	G-7.5	G-10	R-2.5	R-5	R-7.5	R-10	S-2.5	S-5	S-7.5	S-10	
Proximate composition (% fed basis)														
Moisture	8.83	9.54	9.87	9.96	8.47	9.09	8.84	8.97	9.12	8.68	8.57	9.54	9.02	
Crude protein	40.31	40.39	40.75	40.41	40.05	40.38	40.47	40.83	40.44	40.61	40.37	40.36	40.65	
Ether extract	7.11	7.34	7.81	8.02	8.03	7.62	8.36	7.75	7.94	7.89	7.56	8.51	8.34	
Crude fibre	3.15	3.72	3.98	4.35	4.98	3.57	3.99	4.67	5.12	3.29	3.89	4.25	5.10	
Total ash	16.74	15.90	15.68	15.76	15.52	15.08	14.49	14.68	14.98	16.78	15.56	14.93	15.07	
Nitrogen free extract[2]	23.86	23.11	21.91	21.50	22.95	24.26	23.85	23.10	22.40	22.75	24.05	22.41	21.82	
Essential amino acid composition (g 16 g N^{-1})														
Arg	5.78	6.12	6.01	6.31	6.54	6.34	6.03	6.02	5.96	6.97	7.21	7.46	7.50	5.3
His	2.53	2.33	2.26	2.35	2.52	2.30	2.32	2.52	2.50	2.34	2.33	2.35	2.29	2.2
Ile	3.20	3.76	3.78	3.76	3.90	3.62	3.76	3.85	3.83	3.30	3.49	3.52	3.52	2.7
Leu	5.76	5.89	5.84	6.01	6.29	7.63	7.36	6.71	6.80	7.07	7.03	6.34	6.22	4.3
Lys	6.47	6.31	4.96	4.21	4.22	6.36	6.20	6.07	5.96	5.37	5.08	4.44	3.99	5.2
Met	2.13	2.08	2.13	1.95	1.87	2.25	1.90	1.54	1.63	2.36	2.43	2.50	2.44	2.4
Phe	3.97	4.01	3.95	4.06	4.44	4.06	3.56	3.65	3.78	3.74	3.84	3.99	4.16	3.7
Thr	2.98	3.00	3.02	3.04	2.97	3.64	3.51	3.65	3.66	3.00	3.20	3.32	3.39	3.5
Val	3.55	3.64	3.63	3.64	3.75	4.41	4.50	4.58	4.72	4.51	4.61	4.73	4.62	0.5

[1]Dietary essential amino acid requirements of *P. monodon* (Rajaram *et al.,* 2021)
[2]Calculated by a difference [100 - (% of moisture + crude protein + ether extract + crude fibre + total ash)]

from the lagoon to fiberglass-reinforced plastic (FRP) tank in an indoor laboratory. Before this, the shrimps were screened for OIE-listed pathogens to rule out the disease risk. The shrimps were acclimatised for a week before the experiment began with the control diet in a 1000 l flat-base (43.3 inch bottom diameter) circular FRP tank. A 60-day feeding trial was carried out in 39 oval-shaped 500 l FRP tanks (1.30 x 0.64 x 0.73 m) with sand-filtered UV-treated

seawater and constant aeration. In a wholly randomised design, 780 shrimps with an average body weight of 0.35 g were distributed to have three replicates per treatment and twenty shrimp per replicate.The shrimps were fed thrice a day (at 7.30 , 12.30 and 17.30 hrs) in a static condition at the rate of 8% of the total biomass. The feed was adjusted later based on the intake and split by 40, 30 and 30%, respectively, throughout the experimental period. The shrimps were allowed to feed for an hour, and one hour after feeding, aeration was turned off. The uneaten pellets (if any) were siphoned to keep the tank bottom clean. The collected feed pellets were rinsed with de-ionised water and dried at 60°C to calculate the actual feed intake daily. Meanwhile, the freshly released faeces were collected using a clean Falcon tube in a silk fabric cloth. They were rinsed in deionised water, transferred to a filter paper with sterile forceps, dried, and promptly frozen at -20°C until analysis. The digestibility coefficient of dry matter, crude protein and essential amino acid were estimated as per Jannathulla *et al*. (2018). UV treated water, after filtering *via* a 5 m cartridge filter, was used throughout the experiment. Before the first feeding, about 80% of the water was exchanged daily and the water quality indices were monitored at regular intervals and were analysed using the standard method of APHA (2012). The mean values of the indices estimated are given in Table 4.

At the end of the experiment, growth performance in terms of daily growth coefficient (DGC), weight gain (WG%), feed conversion ratio (FCR), protein efficiency ratio (PER), apparent protein utilisation (APU) and survival were computed as:

DGC (% day^{-1}) = 100 x Final weight $(g)^{1/3}$ - Initial weight $(g)^{1/3}$ / No. of days

WG (%) = [Final weight (g) - Initial weight (g)] / Initial weight (g) x 100

FCR = Dry feed consumed (g) / Wet weight gain (g)

Table 4. Mean value of water quality parameters analysed during the experimental period of 60 days

Particulars	
Temperature (°C)	27.16±1.47
Salinity (‰)	24.16±1.31
pH	8.11±0.47
Dissolved oxygen (mg l^{-1})	7.86±0.84
Turbidity (NTU)	17.13±1.87
Total alkalinity (mg l^{-1} as $CaCO_3$)	147.77±4.13
Total hardness (mg l^{-1} as $CaCO_3$)	2137.14±59.47
Total ammonia (mg l^{-1})	0.08±0.01
Nitrite-N (mg l^{-1})	0.03±0.01
Nitrate-N (mg l^{-1})	0.04±0.01
Phosphate (mg l^{-1})	0.14±0.04

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

APU (%) = Protein gain (g) / Protein intake (g) x 100

Survival (%) = (Final number - Initial number) / Initial number x 100

Biochemical analysis

The standard AOAC (1997) method was used to determine the proximate composition in terms of moisture, crude protein, ether extract, crude fibre and total ash in experimental diets and the shrimp carcass. A difference [100 - (percentage of moisture + crude protein + ether extract + crude fibre + total ash)] was used to determine the level of nitrogen-free extract (NFE). Chromic oxide content in the feeds and faeces was analysed to calculate the nutrient digestibility (Furukawa and Tsukahara, 1966). Briefly, 50 mg of the sample was digested with 10 ml of the acid mixture (Nitric acid: Perchloric acid 4:1). The digested solution was made up to 50 ml in a volumetric flask, and the absorbance was read at 350 nm against reagent blank by using a UV visible spectrophotometer (UV-1800, Shimadzu, Japan). The samples were hydrolysed using 6-N hydrochloric acid in a sealed tube filled with nitrogen at 110°C in a vacuum oven for 22 h for the quantification of amino acids (Finlayson, 1964). Individual amino acids were separated on a Shimpack Column (ISC-07/S1504 Na) packed with a strongly acidic Na+ type cation exchange resin (Styrene-divinyl benzene copolymer with the sulfinic group) using buffer A (sodium citrate-perchloric acid, pH:3.2) and buffer B (boric acid sodium citrate-sodium hydroxide; pH:10.0) under a gradient elution at a rate of 0.3 ml min^{-1}. Amino acids were qualified and quantified by a fluorescent detector (FLD-6A) after post-column derivatisation with O-paraldehyde and 2-mercaptoethanol. Amino acid standard solution (Sigma-aldrich Inc., USA) for fluorescent detection was used as an external standard (Dayal *et al.,* 2011). Tryptophan, being labile to acid hydrolysis, was measured after alkali hydrolysis using a spectrophotometric method at 500 nm (Sastry and Tammuru, 1985). Anti-nutrients such as trypsin inhibitor (Kakade *et al.,* 1974), saponin (Wang *et al.,* 2007), phytic acid (Davies and Reid, 1979), tannin (Price *et al.,* 1978) and glucosinolates (McGhee *et al.,* 1965) were estimated using standard methods.

Statistical analysis

A wholly randomised design was adopted to analyse the data using two-way ANOVA (SPSS ver.16.0 for Windows). Tukey's test was used to compare mean differences with a probability of $p<0.05$. A broken-line regression analysis determined the optimal inclusion level of oilseed meals/cake. After determining the normal distribution, the data were examined for homogeneity

of variance before statistical analysis. The results are presented as the average of three replicates, along with the SEM (±), CV (%) and p-values.

Results and discussion

It is always important to consider not only the cost and availability, but also the nutrient content, digestibility and palatability of the economic protein sources used to substitute the dietary fishmeal. Soybean meal and its derivatives, like soy protein concentrate and isolate, are the most commonly used plant-based alternatives to fishmeal in aquafeeds due to their high protein content and low price compared to fishmeal. Likewise, other plant-based proteins such as GNC, RSM and SOC are also available with almost similar benefits as in soybean meal, even for still lower cost with abundant availability in India (Jannathulla *et al.,* 2018; 2019; USDA, 2021). But they are less utilised in shrimp feed than in fish feed, and other animal feeds. In our study, all three test ingredients were gradually incorporated (0, 2.5, 5, 7.5 and 10%) by substituting an equal volume of dietary fishmeal. This resulted in no significant difference (p=0.084) in survival among the dietary treatments. At the end of the experimental period, all dietary groups had a higher rate of survival irrespective of the ingredients (90.22-91.55%) and their inclusion level (85.93-93.33%). Furthermore, there was no sign of feed being rejected while including 10% of GNC, RSM and SOC in our study, indicating that shrimp in all the dietary treatments were in good health and that no nutrient deficiencies existed even at a higher level of inclusion. The results of growth performance (Table 5) revealed that there was no significant difference (p=0.071) in the final bodyweight of the shrimp (2.11-2.18 g) among the ingredients tested irrespective of the inclusion level, whereas the group fed on a diet containing RSM showed significantly lower DGC (1.27% day^{-1}) compared to other two ingredients (1.29-1.30% day^{-1}). All three ingredients used in our study are known to have a significant quantity of anti-nutrients (Table 1). However, the lower performance observed with RSM-fed groups than that of the other two groups might be due to glucosinolates. The RSM used in our study had 341.86 mg 100 g^{-1} of glucosinolates, which was absent in both GNC and SOC. The negative impacts of glucosinolates have been reported in various aquatic species when reared on a semi-purified practical diet containing RSM (Davies *et al.,* 1990; Gomes *et al.,* 1993). However, according to Shi *et al.* (2015), glucosinolates are primarily responsible for lowering palatability. In contrast, significantly a better FCR was observed with RSM-fed groups (1.88) than those fed on a SOC-based diet (1.95). Similarly, another anti-nutrient related to the palatability of feed is tannin, which was comparatively high in GNC (1739.48 mg 100 g^{-1}), followed by RSM (846.37 mg 100 g^{-1}) and SOC (4.29 mg 100 g^{-1}). This indicates that the presence of glucosinolates could be a possible reason for obtaining lower growth with an RSM-based diet. The protein efficiency measures such as PER and APU were significantly high in GNC-fed groups (1.63 and 31.02, respectively), irrespective of the inclusion levels.

Increasing the inclusion level of oilseed meals/cakes from 0 to 10% resulted in decreasing both final body weight (2.39-1.74 g) and DGC (1.40-1.09% per day) significantly irrespective of the test ingredients even with

Table 5. Growth performance of *P. monodon* fed different oilseed cakes/meal at varied inclusion levels by replacing dietary fishmeal

Particulars	Growth performance						
	IBW (g)	FBW (g)	DGC	FCR	PER	APU	Survival (%)
Oilseed cakes/meals							
GNC	0.35^{a}	2.14^{a}	1.29^{a}	1.73^{c}	1.63^{a}	31.02^{a}	91.55^{a}
RSM	0.35^{a}	2.11^{a}	1.27^{b}	1.88^{b}	1.46^{b}	28.53^{b}	90.67^{a}
SOC	0.36^{a}	2.18^{a}	1.30^{a}	1.95^{a}	1.45^{b}	28.03^{b}	90.22^{a}
Inclusion levels (%)							
0	0.35^{a}	2.39^{a}	1.40^{a}	1.64^{d}	1.68^{a}	32.12^{a}	93.33^{a}
2.5	0.34^{a}	2.31^{b}	1.38^{b}	1.66^{d}	1.65^{a}	31.57^{a}	92.59^{a}
5	0.36^{a}	2.20^{c}	1.31^{c}	1.84^{c}	1.50^{b}	29.33^{b}	89.63^{a}
7.5	0.36^{a}	2.04^{d}	1.24^{d}	1.94^{b}	1.43^{c}	27.60^{c}	92.59^{a}
10	0.36^{a}	1.74^{e}	1.09^{e}	2.18^{a}	1.31^{d}	25.35^{d}	85.93^{a}
Interactions							
Oilseed meals/cakes(A)	0.875	0.071	<0.001	<0.001	<0.001	<0.001	0.368
Inclusion level (B)	0.507	<0.001	<0.001	<0.001	<0.001	<0.001	0.061
A x B	0.268	0.001	<0.001	<0.001	<0.001	<0.001	0.084
SEM (±)	0.001	0.004	0.001	0.001	0.001	0.393	3.834
CV (%)	6.401	3.748	1.227	2.339	2.793	2.827	2.838

Means bearing the same superscript letters in a column within main effects and interactions between the categories do not differ significantly (p>0.05)

the increasing level of wheat gluten from 0.9 to 3.6% in test diets. Similar effects were reported in *P. monodon* (Richard *et al.,* 2011), *M. japonicus* (Bulbul *et al.,* 2014) and *P. vannamei* (Jannathulla *et al.,* 2018) when reared on plant-based materials. However, according to the broken line regression, the test ingredients were ranked as GNC > SOC > RSM, which revealed that GNC, SOC and RSM could be included at the rate of 5.4, 2.9 and 2.4%, respectively (Fig. 1). The lower final bodyweight with the higher inclusion irrespective of the test ingredients was mainly attributed to the intolerance of shrimp to the higher quantity of anti-nutrients (Sharawy *et al.,* 2016). Hindering digestibility, in particular, that of protein due to the presence of trypsin inhibitor (Jannathulla *et al.,* 2018), the formation of indigestible protein complexes due to tannin (Makkar and Becker, 1997) and phytic acid (Ravindran *et al.,* 1995) and other deleterious effects due to saponin (Chen *et al.,* 1996) and glucosinolates (Gomes *et al.,* 1993) would all be the possible reasons for poor growth with the higher inclusion of test ingredients.

The nutrient utilisation of feed differs according to its ingredients. As all the components of feed are not digested equally, determining apparent digestibility coefficients would help quantify the total amount of feed consumed and digested by the aquatic species (Jannathulla *et al.,* 2017). The results (Table 6) revealed that dry matter, crude protein, Met, Phe and Thr digestibility were significantly ($p<0.05$) high in GNC-based diets regardless of inclusion level. In contrast, it was Ile, Lys, Thr and Val in RSM-based groups and crude protein, Arg, His, Leu and Lys in SOC-based groups, in comparison to the control group, diets containing 2.5% of test ingredients, regardless of the type, increased digestibility by 1.92, 0.72, 4.02 and 4.74% for dry matter, crude protein, Arg and Met, respectively, and the increase was extended up to 5% inclusion for His, Leu and Lys. The digestibility of Phe, Thr and Val was lower even at a low inclusion level (2.5%). The trend was the same for all the nutrients at both 7.5 and 10% inclusions (Fig. 2). Apparent digestibility coefficients of test ingredients significantly varied among the tested ingredients, which might be due to the variation in nutrient composition of these ingredients. The values presented in our study were comparatively less than those reported in *P. setiferus* (Brunson *et al.,* 1997) and *P. vannamei*

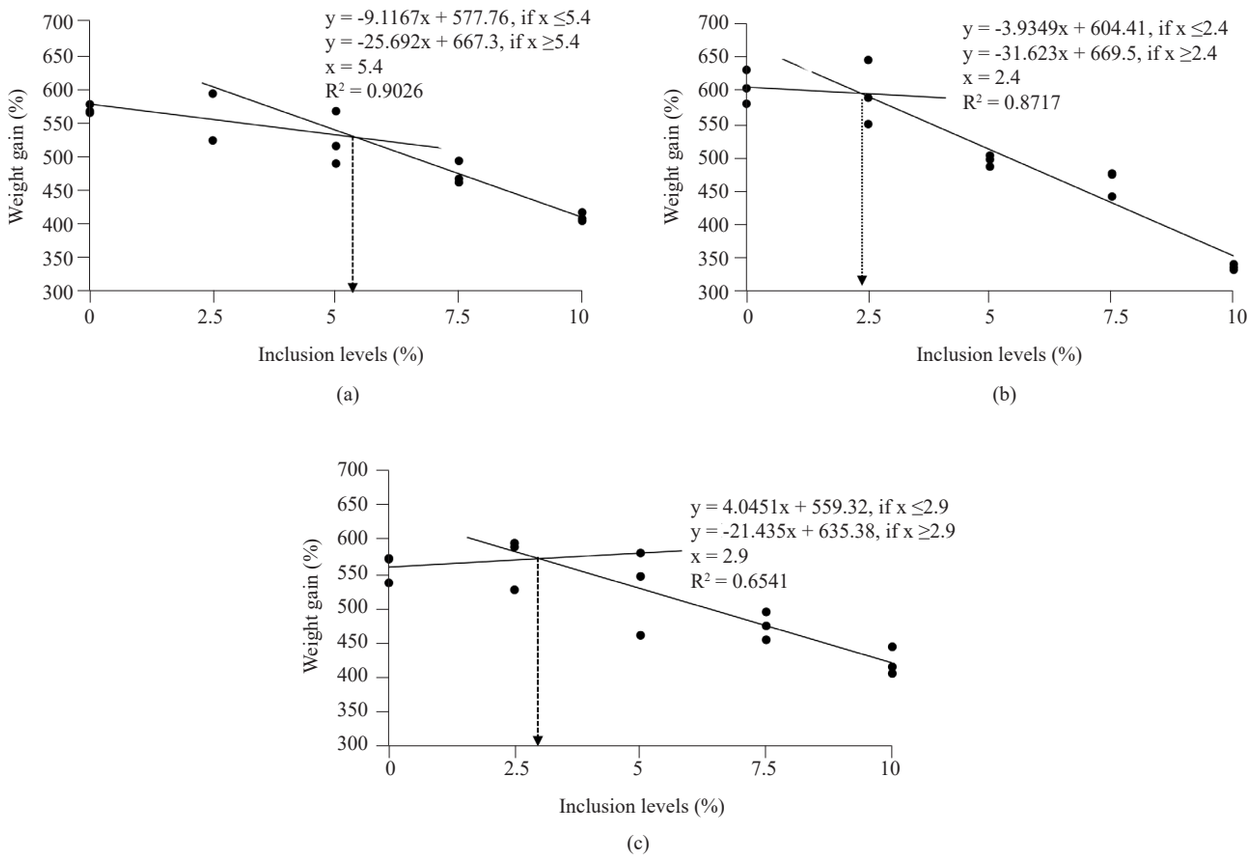

Fig. 1. Optimal inclusion level of GNC (a), RSM (b) and SOC (c) by replacing fishmeal in the diet of *P. monodon* using broken-line regression analysis

Table 6. Digestibility coefficients of experimental diets containing different oilseed cakes/meals at varied inclusion levels by replacing dietary fishmeal in *P. monodon*

Particulars	Digestibility of nutrients										
	DM	CP	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
Oilseed cakes/meals											
GNC	76.24^{a}	84.50^{a}	87.08^{c}	85.36^{c}	85.65^{b}	85.75^{b}	86.12^{b}	88.08^{a}	84.77^{a}	83.79^{a}	86.55^{b}
RSM	73.60^{c}	83.90^{b}	89.12^{b}	86.20^{b}	87.54^{a}	83.72^{c}	88.17^{a}	86.16^{c}	83.95^{b}	83.55^{a}	89.35^{a}
SOC	74.02^{b}	84.52^{a}	90.40^{a}	88.66^{a}	86.35^{b}	87.81^{a}	87.99^{a}	87.34^{b}	83.48^{c}	82.66^{b}	85.79^{c}
Inclusion levels (%)											
0	77.64^{b}	87.68^{b}	89.76^{b}	87.92^{b}	90.63^{a}	85.88^{b}	88.68^{b}	89.10^{b}	89.45^{a}	86.55^{a}	91.07^{a}
2.5	79.13^{a}	88.31^{a}	93.36^{a}	90.35^{a}	87.41^{c}	89.32^{a}	89.84^{a}	93.32^{a}	87.52^{b}	85.73^{b}	88.85^{b}
5	75.74^{c}	84.92^{c}	88.78^{c}	88.89^{b}	86.11^{d}	88.67^{a}	90.65^{a}	86.45^{c}	84.29^{c}	84.10^{c}	86.84^{c}
7.5	72.17^{d}	81.98^{d}	88.41^{c}	84.78^{c}	88.72^{b}	84.26^{c}	84.26^{c}	85.44^{d}	82.62^{d}	79.95^{d}	88.67^{b}
10	68.43^{e}	78.63^{e}	84.04^{d}	81.76^{d}	79.70^{e}	80.67^{d}	83.71^{c}	81.65^{e}	76.47^{e}	80.34^{d}	80.73^{d}
Interactions											
Oilseed meals/cakes(A)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
Inclusion level (B)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
A x B	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SEM (±)	0.143	0.174	0.254	0.585	0.672	0.733	0.578	0.330	0.079	0.359	0.531
CV (%)	0.667	0.651	0.747	1.160	1.247	1.313	1.145	0.867	0.440	0.946	1.100

Means bearing the same superscript letters in a column within main effects and interactions between the categories do not differ significantly (p>0.05)

(Cruz-Suarez *et al.,* 2009). The authors suggested that the size and age of the species, culture conditions and the digestive characteristics of species might be a reason for obtaining this difference. As a result of increasing the inclusion levels beyond 2.5%, irrespective of the ingredients tested, the digestibility of nutrients was reduced significantly. The authors suggested that the lower digestibility with the higher inclusion of oilseed meals/cakes could be attributed to the anti-nutrients. The essential amino acids like Met and Lys are the most limiting amino acids in shrimp feed, especially when the feeds are formulated with high content of plant proteins by substituting dietary fishmeal. The lower digestibility of these amino acids at high inclusion might be a reason for the lower growth in those treatments.

The carcass composition was not affected by the test ingredients and their inclusion levels in our study (Table 7). This result agrees with the findings of Yue *et al*. (2012) and Jannathulla *et al*. (2019) in *P. vannamei* when reared on a diet containing different plant proteins as a fishmeal alternative. Similarly, Khan *et al*. (2018) found that except for body lipids, there was no significant difference in the other components of *P. monodon* fed a diet containing partially substituted fishmeal and fish oil with plant-based origins. The authors suggested that including palm oil along with soy and lysolecithin might be a reason for obtaining the possible changes in the body lipid composition. Ogata (2002) documented that certain amino acids, particularly, His and their imidazole derivatives play a vital role in the taste and texture of aquatic species. No impact on amino acid composition due

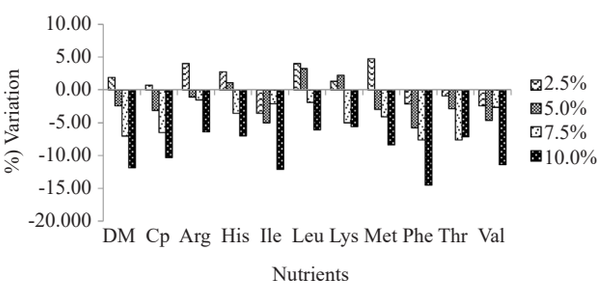

Fig. 2. Percent variation of digestibility parameters in the test diets containing oilseed cakes/meals by replacing fishmeal compared to the control in *P. monodon*

to the inclusion of oilseed meals/cakes in our study would also be an advantage to consider these plant proteins as a viable alternative to fishmeal. Apparent nutrient digestibility is a typical metric that assesses the quality of the dietary ingredients used in the feeds produced commercially for marine penaeid shrimp; nevertheless, it can only approximate overall bioavailability. However, the availability of nutrients for protein synthesis at the tissue level determines the overall retention efficiency. The use of radioisotope-labelling techniques is required to determine the level of nutrient deposition, which is highly complicated and time-intensive. Changes in tissue nutrient levels are another way to characterise the nutritional availability of shrimp and have previously been used to analyse post-feeding time-course changes in tissue-free amino acids (FAA) (Mente *et al.,* 2002; Fox *et al.,* 2009). Therefore, FAA concentration was determined in the tail muscle of the shrimp in our study. The results revealed

Table 7. Carcass composition (% dry weight basis) of *P. monodon* fed different oilseed cakes/meal at varied inclusion levels by replacing dietary fishmeal

Particulars	Carcass composition											
	Crude protein	Ether extract	Total ash	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
Oilseed cakes/meals												
GNC	72.73	8.17	15.05	5.01	1.10	2.66	7.27	4.99	1.79	2.95	2.91	3.13
RSM	72.73	8.17	14.95	4.93	1.22	2.57	7.31	4.85	1.75	2.91	2.85	3.11
SOC	73.40	8.09	14.84	4.72	1.26	2.53	7.19	4.72	1.71	2.83	3.06	2.90
Inclusion levels (%)												
0	72.88	8.26	14.92	4.91	1.23	2.55	7.30	4.83	1.74	2.87	2.70	2.89
2.5	73.03	8.20	14.89	4.83	1.19	2.58	7.29	4.88	1.68	2.96	3.00	3.13
5	73.07	8.01	15.09	4.74	1.06	2.58	7.16	4.88	1.80	2.85	2.94	3.13
7.5	72.85	8.01	14.93	5.12	1.21	2.60	7.35	4.89	1.75	2.90	2.97	3.04
10	72.93	8.23	14.90	4.83	1.27	2.62	7.18	4.77	1.76	2.90	3.08	3.04
Interactions												
Oilseed meals/cakes(A)	0.063	0.575	0.384	0.107	0.073	0.178	0.431	0.17	0.497	0.513	0.185	0.189
Inclusion level (B)	0.976	0.087	0.843	0.28	0.258	0.963	0.403	0.96	0.761	0.932	0.169	0.630
A x B	0.321	0.292	0.158	0.829	0.563	0.523	0.642	0.157	0.538	0.051	0.930	0.960
SEM (±)	0.421	0.034	0.101	0.081	0.022	0.022	0.035	0.084	0.019	0.041	0.06	0.078
CV (%)	1.171	2.998	2.795	7.682	16.454	7.547	3.405	7.846	10.276	9.223	10.98	12.038

No significant ($p>0.05$) difference within main effects and interactions between the categories

that the dietary change showed a significant difference in both essential (Table 8) and non-essential (Table 9) FAA composition in the tail muscle of the shrimp. The values of FAA reported in our study were almost similar to the earlier values reported in *P. kerathurus* (Torres, 1973), *P. esculentus* (Dall and Smith, 1987), *P. monodon* (Dayal *et al.,* 2011) and *P. vannamei* (Fox *et al.,* 2009). Though there was a variation in the free pool concentration of individual amino acids, the total remained stable irrespective of the dietary groups, indicating that the intracellular amino acids pools are regulated by active trans-membrane transport rather than passive transport (Mante *et al.,* 2002). Arg is the most predominant essential FAA in the shrimp tail muscle, which could be due to its crucial metabolic role as a precursor to the phosphagen phosphoarginine (Rajaram *et al.,* 2021), while Gly was the most abundant in non-essential FAA.

The feed formulation cost was computed based on the cost of ingredients prevailing in Indian markets. The formulation cost for the control formula was ₹73.86 per kg, which gradually decreased while replacing fishmeal,

Table 8. Free essential amino acid composition (µmol g tissue^{-1}) in the tail muscle of *P. monodon* fed different oilseed cakes/meal at varied inclusion levels by replacing dietary fishmeal

Particulars	Free essential amino acids								
	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
Oilseed cakes/meals									
GNC	42.01^{a}	3.14^{b}	4.28^{b}	11.56^{a}	8.81^{b}	3.99^{c}	2.19^{c}	8.75^{b}	9.68^{b}
RSM	40.70^{c}	3.33^{a}	4.38^{a}	11.18^{b}	9.99^{a}	4.12^{b}	2.41^{a}	9.49^{a}	9.36^{c}
SOC	41.26^{b}	3.17^{b}	3.97^{c}	10.64^{c}	8.73^{b}	4.30^{a}	2.27^{b}	8.66^{b}	10.20^{a}
Inclusion levels (%)									
0	44.12^{a}	3.53^{a}	4.68^{a}	12.94^{a}	10.63^{a}	4.51^{b}	2.58^{a}	8.94^{d}	9.95^{b}
2.5	42.51^{b}	3.57^{a}	4.36^{b}	10.50^{c}	10.00^{b}	4.70^{a}	2.35^{b}	7.92^{e}	10.45^{a}
5	42.83^{b}	3.08^{b}	4.25^{c}	11.11^{b}	9.00^{c}	4.22^{c}	2.33^{b}	9.28^{b}	9.47^{c}
7.5	39.68^{c}	3.13^{b}	4.01^{d}	10.95^{b}	8.65^{d}	3.90^{d}	2.17^{c}	9.12^{c}	10.28^{a}
10	37.50^{d}	2.78^{c}	3.76^{e}	10.13^{d}	7.62^{e}	3.36^{e}	2.01^{d}	9.58^{a}	8.60^{d}
Interactions									
Oilseed cakes/meals (A)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Inclusion level (B)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
A x B	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SEM (±)	0.297	0.002	0.004	0.039	0.015	0.004	0.001	0.013	0.029
CV (%)	1.737	1.647	1.848	2.348	1.726	2.030	1.528	1.677	2.279

Means bearing the same superscript letters in a column within main effects and interactions between the categories do not differ significantly ($p>0.05$)

Table 9. Free non-essential amino acid composition (µmol g tissue^{-1}) in the tail muscle of *P. monodon* fed different oilseed cakes/meal at varied inclusion levels by replacing dietary fishmeal

Particulars	Free non-essential amino acids						
	Ala	Asp	Glu	Gly	Pro	Ser	Tyr
Oilseed cakes/meals							
GNC	37.50^{a}	4.12^{a}	16.11^{a}	71.03^{b}	13.03^{a}	5.90^{b}	2.51^{c}
RSM	37.19^{a}	4.05^{b}	14.78^{b}	72.44^{a}	12.77^{b}	6.67^{a}	2.64^{b}
SOC	36.66^{b}	3.87^{c}	14.21^{c}	72.34^{a}	12.57^{c}	5.94^{b}	2.77^{a}
Inclusion levels (%)							
0	35.30^{c}	4.06^{ab}	14.59^{d}	72.22^{a}	12.44^{c}	5.86^{c}	2.53^{c}
2.5	30.98^{d}	3.84^{c}	14.52^{d}	72.25^{a}	12.68^{b}	5.55^{d}	2.58^{b}
5	35.91^{c}	3.99^{b}	15.37^{b}	71.33^{a}	13.08^{a}	6.72^{a}	2.46^{d}
7.5	40.51^{b}	4.08^{a}	15.78^{a}	71.65^{a}	13.10^{a}	6.40^{b}	2.57^{b}
10	42.90^{a}	4.10^{a}	14.91^{c}	72.24^{a}	12.65^{b}	6.32^{b}	3.09^{a}
Interactions							
Oilseed cakes/meals (A)	0.006	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Inclusion level (B)	<0.001	<0.001	<0.001	0.475	<0.001	<0.001	<0.001
A x B	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SEM (±)	0.251	0.004	0.024	1.035	0.023	0.007	0.001
CV (%)	1.775	2.084	1.351	1.861	1.558	1.726	1.374

Means bearing the same superscript letters in a column within main effects and interactions between the categories do not differ significantly (p>0.05)

irrespective of the oilseed meals/cakes tested in the present study. Optimum inclusion of GNC, SOC and RSM at the rate of 5.4, 2.9 and 2.4% by replacing dietary fishmeal reduced the total formulation cost by ₹1.27, 1.04 and 0.81 per kg, respectively.

In conclusion, all three test ingredients were found to be potentially viable in substituting fishmeal partially in the diet of *P. monodon.* However, the GNC resulted in better performance in terms of growth and feed utilisation compared to RSM and SOC. GNC could be included at the rate of 5.4% by substituting equal volume of dietary fishmeal. The optimised levels were 2.9 and 2.4% for SOC and RSM, respectively, thereby reducing the total formulation cost by ₹1.27, 1.04 and 0.81 per kg. According to the broken line regression, the test ingredients were ranked as GNC > SOC > RSM.

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