



Partial replacement of fishmeal with soybean meal and distillers dried grain solubles (DDGS) as alternative protein sources for milkfish *Chanos chanos* (Forsskal, 1775) fingerlings

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ABSTRACT

A 60 days feeding trial was conducted to investigate partial replacement of fishmeal (FM) with either soybean meal (SBM) or distillers dried grain solubles (DDGS) as alternative protein sources for milkfish, *Chanos chanos* fingerlings. Nine experimental diets were formulated with either fishmeal or with two alternative protein sources such as SBM or DDGS at three protein levels (35, 40 and 45%). Milkfish fingerlings (270 nos.) with mean length and weight of 5.24±0.03 cm, 1.25±0.05 g were selected for the study and in total there were 9 treatments in triplicates. The experimental diets were: F35 (35% CP - FM diet), F40 (40% CP - FM), F45 (45% CP - FM), FS35 (35% CP - FM + SBM), FS40 (40% CP - FM + SBM), FS45 (45% CP - FM + SBM), FD35 (35% CP - FM + DDGS), FD40 (40% CP - FM + DDGS), FD45 (45% CP - FM + DDGS). Diets incorporated with fishmeal alone served as control diet for this experiment. We observed that, final weight was significantly ($p<0.05$) higher for fishmeal based diet irrespective of the crude protein level. The same trend was observed for body weight gain for FM based diet. Specific growth rate (SGR) was found to be more than 2% per day for all the experimental diets fed fish and FD45 showed the lowest SGR compared to other treatment groups. Similarly FD45 had poor feed conversion ratio (FCR) when compared to other dietary groups. Survival rate of the fingerlings was found to be significantly higher for all FM diets (80%), FS35 (80%) and FD35 (80%) diet fed groups, whereas FD45 dietary group showed significantly lower survival rate (60%). Higher amylase activity was observed in F40 diet, higher lipase enzyme activity in FS 35 diet, while higher protease activity was recorded in F35 diet and the values showed significant difference ($p<0.05$) between different treatment groups. Results of the study clearly indicate that soybean meal (FS35) could be used to partially replace the fishmeal in the diet of milkfish fingerlings.

Keywords: Alternative protein source, *Chanos chanos*, DDGS, Fingerlings, Milkfish, Partial replacement, Soybean meal

Introduction

Digestibility and amino acid profile are the most important factors which decide quality of the protein sources (Kaushik *et al.*, 1991). Fishmeal is the most vital protein source, which is used in fish feeds and other animal diets. This is because of its balanced amino acids, essential fatty acids, high digestibility and palatability (Tacon *et al.*, 2006; Cruz-Suarez *et al.*, 2009). Therefore, the demand for fishmeal in global markets increased, which conjointly hiked price of fishmeal and fish feed cost which in turn increased the production cost (Tacon *et al.*, 2011; Valle *et al.*, 2014). In this context we need to find an alternative protein source for aqua feed formulation.

Among the plant protein sources, soybean meal (SBM) is the most commonly used alternative protein source for fishmeal replacement. It has high protein content, well balanced amino acid (AA) profiles and hence its nutrient profile, lesser cost and availability make this ingredient more feasible for fishmeal replacement in fish feed. In general, for every 100 kg of soya beans, approximately 82 kg of soybean meal (SBM) and 18 kg of soya oil are obtained (Hertrampf and Piedad-Pascual, 2000; Storebakken *et al.*, 2000). The AA profile of SBM is good enough for FM replacement, but it has limitations of methionine and taurine. Apart from AA limitations in SBM, other issues are also reported such as presence of anti-nutritional factors (ANFs) viz., protease inhibitors, lectins, antigenic proteins, phenolic compounds,

oligosaccharides and phytates. However, these ANFs could be destroyed by proper heat treatments (Tacon, 1993; Gaylord *et al.*, 2007).

Besides SBM, Distiller's Dried Grain Solubles (DDGS) is also being used as an alternative ingredient to FM in fish feed. DDGS is a byproduct from ethanol production industry. It contains good protein (28-32%), lipid (9%), trace minerals, vitamins and fibre. Also, no anti-nutrient is reported in DDGS. The nutrient profile of DDGS is varied based on the sources of grains used and distillation process (Shiau *et al.*, 1987; Webster *et al.*, 1993). Cellulose and hemicellulose are the major drawbacks in DDGS, which is not easily digestible by the fishes (Krogdahl *et al.*, 2005). Good nutritional profile, lesser cost as compared to SBM and easy availability, make this ingredient too as a good source of replacement for FM in fish feed (Metts *et al.*, 2011).

Milkfish (*Chanos chanos*) is a potential candidate brackishwater fish species, which is popularly cultured in South-east Asian countries including India. It is an euryhaline species (Pullin, 1981) and can be farmed in wide salinity ranges. This species is an omnivore which requires both animal and plant protein sources in their diet. This feeding habit of the species helps us to utilise both fishmeal and plant protein sources as ideal feed ingredient for milkfish farming. The success of milkfish culture in different culture systems are basically dependent on cost of the feed and its nutritional profile (Apines-Amar *et al.*, 2015). To reduce the feed production costs, replacement of expensive fishmeal ingredient with more affordable plant protein ingredient sources without any compromise on the fish physiology and growth and its nutritional quality are highly counselled (Tidwell *et al.*, 1993). It has been well established that milkfish require 40% crude protein in their diet (Lim *et al.*, 1979). Hence the study attempted three protein levels (optimal level, 5% above and 5% below optimal level) of protein in the milkfish diet. The present study was conducted to evaluate the growth performance and digestive enzyme activity of milkfish fingerlings under partial replacement of fishmeal with either soybean meal or DDGS at different protein (35, 40, 45%) levels in the diets.

Materials and methods

Experimental fish and sampling

Milkfish *Chanos chanos*, fingerlings collected from Pamban lagoons, Ramanathapuram, Tamil Nadu, India,

were transported live in oxygenated polythene bags to the Centre for Sustainable Aquaculture, Mandapam, Ramanathapuram, Tamil Nadu, India. The fingerlings were stocked in fibre reinforced plastic tanks (FRP) (1000 l) and acclimatised for 10 days. The acclimatised fingerlings (1.25 ± 0.05 g) were randomly stocked (10 fish per tank) in 27 experimental tanks (60 l per tank). The tanks were facilitated with proper aeration and covered with small mesh sized net, to prevent jumping out of the fish. Feeding was done twice a day (09:00 and 17:00 hrs) and fish were fed *ad libitum* until satiation. The water quality parameters, such as dissolved oxygen, temperature, pH and salinity were measured on daily basis, and ammonia ($\text{NH}_4^+/\text{NH}_3$), once in a week, following standard methods (APHA, 1995).

Experimental diets

Nine experimental diets were prepared with three different protein levels such as 35, 40 and 45% using three different protein sources *viz.*, fishmeal (FM), soybean meal (SBM) and DDGS. The following diets were prepared, F35 (35% crude protein (CP) - FM diet), F40 (40% CP - FM), F45 (45% CP - FM), FS35 (35% CP - FM + SBM), FS40 (40% CP - FM + SBM), FS45 (45% CP - FM + SBM), FD35 (35% CP - FM + DDGS), FD40 (40% CP - FM + DDGS), FD45 (45% CP - FM + DDGS). Feed pellets prepared were of 1 mm dia, which were dried in sunlight, ground to appropriate size and stored in cold room. Details of proximate composition of the feed ingredients and experimental diet formulation are given in Table 1 and 2.

Growth study

The growth performance of fish was assessed fortnightly by measuring length and weight of fish. In each sampling, all the fishes from each tank were collected and sampled. The following growth parameters were calculated, such as:

$$\text{Weight gain (g)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{Weight gain (\%)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}} \times 100$$

$$\text{Specific growth rate (SGR) (\% day}^{-1}\text{)} = \frac{\ln \text{ final weight (g)} - \ln \text{ initial weight (g)}}{\text{Days of experiment}} \times 100$$

Table 1. Proximate composition of the feed ingredients used

Ingredients	Moisture%	Crude protein%	Crude lipid%	Crude fibre%	Ash%	Gross energy (kcal kg ⁻¹)
Fish meal	12.61	58.56	7.62	< 1	19.04	4116
Soybean meal	5.39	47.17	1.52	5.30	7.46	4347
DDGS	5.68	31.5	5.98	6.01	4.32	4251

Table 2. Diet formulation and proximate composition

Feed ingredients (%)	Experimental diets								
	F35	F40	F45	FS35	FS40	FS45	FD35	FD40	FD45
Fishmeal	27	32	43	13	13	13	13	13	13
Soybean meal	0	0	0	15	15	15	0	0	0
DDGS	0	0	0	0	0	0	15	15	15
Cassava starch	5	5	5	5	5	5	5	5	5
Corn gluten meal	21	25	25	23	31	40	27	35	44
Corn flour	27	21	12	27	18	13	19	20	13
Rice bran	15	12	10	12	13	9	16	7	5
Soy lecithin	1	1	1	1	1	1	1	1	1
Vitamin premix	2	2	2	2	2	2	2	2	2
Mineral premix	2	2	2	2	2	2	2	2	2
Proximate composition									
Moisture (%)	5.94	7.48	6.01	5.88	5.95	5.87	5.59	6.55	5.90
Crude protein (% dry matter, DM)	35.33	40.67	44.61	34.87	40.64	45.17	36.69	40.09	45.17
Crude lipid (%DM)	3.21	4.66	4.88	2.22	2.54	2.89	3.31	3.41	3.21
Ash (%DM)	11.46	11.15	11.09	10.37	10.20	10.10	10.56	9.90	8.71
Crude fibre (%DM)	3.51	3.49	3.60	3.20	3.53	3.40	3.75	3.65	3.15

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total feed intake (Dry weight, g)}}{\text{Total weight gain (Wet weight, g)}}$$

$$\text{Feed efficiency (FE)} = \frac{1}{\text{FCR}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{Final number of survived fish}}{\text{Initial number of fish}} \times 100$$

Digestive enzyme analysis

At the end of feeding trial, two fishes from each tank (n=6) were sacrificed and their intestine was collected for digestive enzyme analysis. The dissected intestine was transferred to chilled sucrose solution (0.25 M) and homogenised (5% homogenate) using poly pestles. The homogenate was then centrifuged at 5000 g for 10 min at 4°C. After centrifugation, the supernatant was collected and used for further analysis. All the enzymatic activities were expressed as specific activity (U mg protein⁻¹).

The protease assay was done following the method of Kunitz (1947). The reaction mixture consisted of 1% casein (prepared in 0.01 N NaOH), 0.05 M tris phosphate buffer (pH 7.8) and 0.1 ml tissue homogenate, which was incubated at 37°C for 15 min. Trichloroacetic acid (TCA, 10%) was added to stop the reaction. Later, the whole content was filtered and the final OD was measured at 280 nm in UV spectrophotometer (UV-1800, Shimadzu Corporation, Japan). Tyrosine standard curve was used

to determine the protease enzyme activity and expressed as number of micromoles of tyrosine released (U mg protein⁻¹).

Amylase activity was determined as done by Bernfeld (1955). Soluble starch (1%) was used as a substrate. The reaction was initiated by adding 1 ml of tissue homogenate into 1 ml of starch (prepared in 0.1 M phosphate buffer, pH 7.0) solution, in a test tube and incubated for 15 min. Two ml of 3, 5 dinitrosalicylic acid (DNS) was used to stop the reaction and the tubes were placed in a boiling water bath for 5 min. Then the tubes were cooled and the volume was made upto 10 ml using distilled water and the intensity of the colour developed was recorded in a UV spectrophotometer at 560 nm. Maltose standard curve was used to determine amylase activity and expressed as the number of micromoles of maltose released (U mg protein⁻¹).

Lipase activity was determined as per Cherry and Crandell (1932) using a stabilised emulsion of olive oil. The reaction mixture consisted of 3 ml of distilled water, 1 ml tissue homogenate, 0.5 ml phosphate buffer (0.1 M, pH 7.0) and 2 ml olive oil emulsion, which was incubated for 24 h. Then 3 ml of alcohol (95%) and 2 drops of phenolphthalein indicator were added and it was titrated against alkali (0.05 N NaOH), until the appearance of permanent pink colour. A control was prepared using enzyme source and the enzyme activity was inactivated by keeping it in a boiling water bath for 15 min, prior to the addition of buffer and olive oil emulsion and expressed as numbers of fatty acid released (U mg protein⁻¹).

Estimation of diet production cost

Details of the cost of feed ingredients used in the experiment is provided in the Table 4, for comparison between the diets used in the study, which considers only the ingredient cost not including manufacturing and other costs. All costs are provided in Indian rupees (₹) and converted to US dollar (US\$) and conversion values of ₹70 for 1 US\$ was taken.

Statistical analysis

Data were expressed as mean±standard error (SEM) and the data variables were checked for normality and homogeneity. All the parameters were analysed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests to determine the significant differences between the means using IBM-SPSS statistics version 20. The p values of <0.05 and <0.01 were considered to represent the significance level between treatment groups.

Results and discussion

All the water quality parameters recorded in the experimental tanks were within the acceptable range: Dissolved oxygen - 5.9 mg l⁻¹; Temperature - 32°C; pH - 8.5; Salinity - 34‰ ppt and Ammonia-N - <0.1 mg l⁻¹. Around 20% of water was exchanged daily to maintain water quality under optimum level.

Data on growth performance of milk fish fingerlings is given in the Table 3. Weight gain, weight gain percentage, specific growth rate and survival rate showed significant difference (p<0.05) among different experimental diets. Significantly higher weight gain was observed in fishmeal based diets such as F35, F40 and F45 followed by SBM contained diet FS35. The same trend was observed for body weight gain for FM based diet. SGR was found to

be more than 2% per day for all the experimental diet fed fish and FD45 showed the lowest SGR compared to other treatment groups. Survival rate of the fingerlings was found to be significantly higher (p<0.05) for all FM diets (80%), FS35 (80%) and FD35 (80%) diet fed groups, whereas FD45 dietary group recorded significantly lower survival rate (60%).

Feeding performance of fishes fed the experimental diets is given in Table 3. Significant differences were observed in FCR, FE and PER of fish fed different dietary treatments. Best FCR was observed in F35, F45, F40 and FS35 with FD45 registering the poorest FCR as compared to other dietary groups. Best FER was observed in F35, F40, F45, FS35, F45 and FD45. PER was observed higher for FS35 dietary group.

Enzymatic activity recorded for different experimental groups is given in Fig. 1. Higher amylase enzyme activity was observed in F40, F35 and F45 while highest lipase activity was observed in FS35. Highest protease activity was observed in F35 followed by FS35, F40, F45. All values showed significant difference among the experimental diets (p<0.05).

Generally the fish stages (fry, juveniles, fingerlings) require more protein level than adult ones and in earlier days this was met using fishmeal. Because of high price and more demand, fishmeal needs to be completely or partially replaced from fish diet (Thompson *et al.*, 2008; Webster *et al.*, 2015). In the present study, F35 diet gave better growth performance compared to other experimental groups. This study was supported by Pongmaneerat *et al.* (1993), who found, among diets with different inclusion levels of soybean meal, a diet with 36.13% crude protein (100% fishmeal diet) showed better growth performance compared to other diets in carp. In another study,

Table 3. Growth performance of milkfish fingerlings fed with different diets

Parameters	Diet									p value
	F35	F40	F45	FS35	FS40	FS45	FD35	FD40	FD45	
Initial weight (g)	1.25 ^a ±0.02	1.24 ^a ±0.02	1.24 ^a ±0.02	1.25 ^a ±0.02	1.25 ^a ±0.02	1.23 ^a ±0.01	1.25 ^a ±0.01	1.24 ^a ±0.01	1.24 ^a ±0.02	0.982
Final weight (g)	5.63 ^a ±0.02	5.61 ^a ±0.01	5.58 ^a ±0.01	5.47 ^b ±0.06	5.35 ^c ±0.02	5.31 ^c ±0.05	5.39 ^{bc} ±0.01	5.31 ^c ±0.02	4.81 ^d ±0.04	<0.001
Weight gain (g)	4.38 ^a ±0.03	4.37 ^a ±0.02	4.34 ^a ±0.01	4.22 ^b ±0.06	4.10 ^c ±0.03	4.08 ^c ±0.05	4.13 ^{bc} ±0.02	4.06 ^c ±0.02	3.57 ^d ±0.05	<0.001
Weight gain (%)	350.83 ^{ab} ±6.84	351.42 ^a ±7.08	350.38 ^{ab} ±4.59	338.90 ^{abc} ±7.58	328.13 ^c ±5.69	332.01 ^{abc} ±5.37	330.21 ^{bc} ±6.24	327.20 ^c ±5.44	289.19 ^d ±7.56	<0.001
SGR (% day ⁻¹)	2.51 ^a ±0.02	2.51 ^a ±0.03	2.51 ^a ±0.01	2.46 ^{ab} ±0.03	2.42 ^b ±0.02	2.44 ^{ab} ±0.02	2.43 ^{ab} ±0.02	2.41 ^b ±0.02	2.26 ^c ±0.03	<0.001
FCR	1.96 ^{cd} ±0.02	1.96 ^c ±0.02	1.96 ^{cd} ±0.01	1.99 ^{bcd} ±0.02	2.01 ^{bc} ±0.01	2.00 ^{bcd} ±0.01	2.00 ^{bcd} ±0.01	2.01 ^b ±0.01	2.11 ^a ±0.02	<0.001
FER	0.51 ^a ±0.00	0.51 ^a ±0.00	0.51 ^a ±0.00	0.50 ^{ab} ±0.00	0.50 ^b ±0.00	0.50 ^{ab} ±0.00	0.50 ^{ab} ±0.00	0.50 ^b ±0.00	0.47 ^c ±0.01	<0.001
PER	1.44 ^a ±0.01	1.25 ^c ±0.01	1.14 ^c ±0.01	1.44 ^a ±0.01	1.22 ^d ±0.01	1.10 ^d ±0.01	1.36 ^b ±0.01	1.24 ^{cd} ±0.01	1.05 ^e ±0.01	<0.001
Survival rate (%)	80.00 ^a ±0.00	80.00 ^a ±5.77	80.00 ^a ±0.00	80.00 ^a ±0.00	70.00 ^{ab} ±5.77	76.67 ^a ±3.33	80.00 ^a ±5.77	76.67 ^a ±3.33	60.00 ^b ±5.77	0.038

All data are expressed as mean±SE. Mean values within the same row with different superscripts are significantly different (p<0.05, One-way ANOVA, Duncan Post-Hoc)

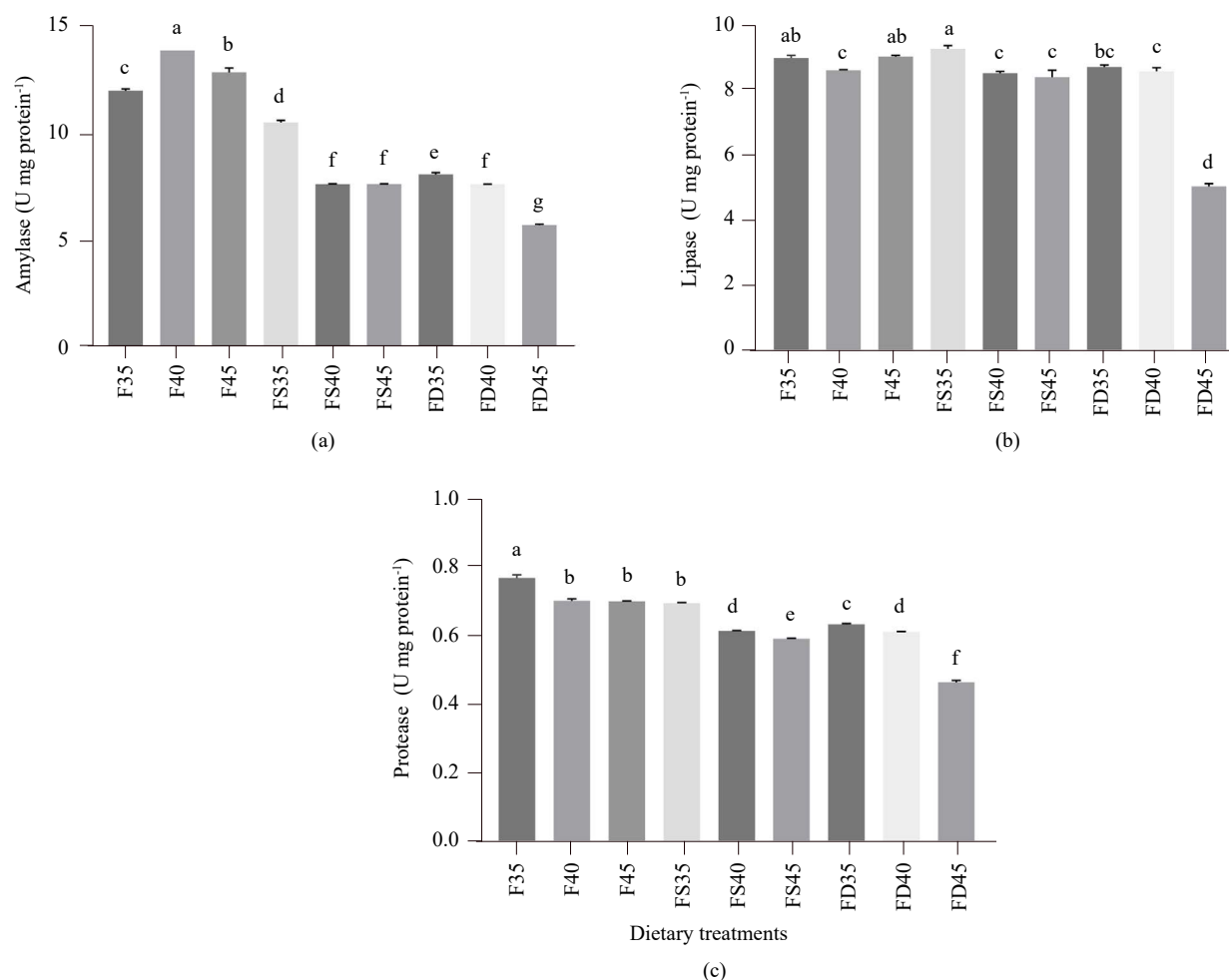


Fig. 1. Enzymatic activity of (a) Amylase, (b) Lipase and (c) Protease in intestine of milkfish fed experimental diets for 60 days. Values are mean $\pm$ SE represented by vertical error bars for each treatment. Different letters indicate significant ($p < 0.05$) differences among treatments determined by Duncan's test

Shiau *et al.* (1987) observed highest weight gain in tilapia fed diet containing 32% protein with inclusion of fishmeal compared to FM+SBM supplemented diets. Similar results were observed in red seabream *Pagrus major* (Biswas *et al.*, 2007) and parrot fish *Oplegnathus fasciatus* (Lim and Lee, 2009). FM+SBM diet showed higher growth rate than FM+DDGS diets at 35% protein level in milkfish (Mamauag *et al.*, 2017). Chatvijitkul *et al.* (2016) also reported that fishmeal partially replaced with SBM diet showed high growth performance compared to fishmeal replaced with DDGS at 35% protein level in hybrid tilapia. A combination of 40% SBM and 30% FM in diet resulted in better growth and feed utilisation in amberjack *Seriola dumerili* than the complete fishmeal diet (Dawood *et al.*, 2015). Similar result was also observed in tiger puffer, *Takifugu rubripes* (Lim *et al.*,

2011). In channel catfish, 35% protein diet, prepared using 12% of FM and 48% of SBM, showed good weight gain percentage and survival (Webster *et al.*, 1992). Fishmeal diet showed higher growth rate than DDGS based diets in black sea bream (Rahman *et al.*, 2013). Coyle *et al.* (2004) reported FM+SBM diet gave better weight gain and specific growth rate than FM+DDGS in hybrid tilapia. In rainbow trout, FM+SBM diet gave better growth performance than FM+DDGS diet (Cheng and Hardy, 2004). Barnes *et al.* (2012) observed control diet had higher weight gain than FM+DDGS diet in rainbow trout. However, protein requirements of species may differ based on differences in water temperature, salinity, diet composition, quality and biological value of protein sources and source of non-protein energy (NRC, 1983).

The digestive performance depends on the availability of digestive enzyme in fish digestive tract (Phillips, 1989). Amylase, lipase and protease are the major digestive enzymes, which express feed utilisation capacity and growth performance of the particular animal (Li *et al.*, 2017). Feeding behaviour, feed formulation, feed preference and anti-nutritional factors may affect the enzyme secretion in fishes (Hidalgo *et al.*, 1999; Pavasovic *et al.*, 2007). In the present study, higher amylase activity was observed in FS40. The present study was supported by Zhang *et al.* (2018), reported that fishmeal supplemented diet with 42% protein showed higher amylase activity compared to other SBM diets in Japanese seabass (*Lateolabrax japonicas*). Similar result was also observed in juvenile jian carp (*Cyprinus carpio* var. *jian*) (Li *et al.*, 2017), red seabream (*P. major*) and yellowtail (*Seriola quinqueradiata*) (Murashita *et al.*, 2015). In the present study, higher lipase enzyme activity was observed in FS35. Tok *et al.* (2017), reported that FM can be partially replaced with SBM based 35% crude protein diet and showed higher lipase activity in *Pangasianodon hypophthalmus*. Similar results were

gilthead seabream (*Sparus aurata*) (Diogenes *et al.*, 2018) who revealed higher protease activity in SBM based diets than DDGS based diets.

Results of the study clearly indicated that, milkfish fed with FM+SBM having 35% crude protein is the best alternative protein source for milkfish fingerlings. Upon selection of FS35 diet as a best alternative for partial fishmeal replacement, we calculated feed formulation cost for the milkfish fingerlings (Table 4).

The findings of our present study showed that fishmeal based diet resulted in higher growth and survival of milkfish fingerlings. However, taking into consideration, the level of fishmeal incorporation and its high cost, fishmeal replacement with alternative protein sources needs to be thought about. Based on the results obtained in the present study, we recommend that soybean meal is an ideal replacement for fish meal rather than DDGS as a protein source in milkfish diet. In brief, The outcome of this study will be useful for fish nutritionists to formulate cost efficient feed for milkfish production.

Table 4. Formulation cost of experimental diets

Feed ingredients	Cost of the ingredients		Formulation cost (₹) (1 US\$ = ₹70)								
	Price/Kg (INR)	USD (1 US\$ = INR. 70)	F35	F40	F45	FS35	FS40	FS45	FD35	FD40	FD45
Fishmeal	120	1.71	32.4	38.4	51.6	15.6	15.6	15.6	15.6	15.6	15.6
Soybean meal	32	0.45	-	-	-	4.8	4.8	4.8	-	-	-
DDGS	28	0.4	-	-	-	-	-	-	4.2	4.2	4.2
Cassava starch	39	0.55	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95
Corn gluten meal	43	0.61	9.03	10.75	10.75	9.89	13.33	17.2	11.61	15.05	18.92
Corn flour	26	0.37	7.02	5.46	3.12	7.02	4.68	3.38	4.96	5.20	3.38
Rice bran	16	0.22	2.4	1.92	1.6	1.92	2.08	1.44	2.56	1.12	0.8
Soy lecithin	70	1	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Vitamin premix	800	11.4	16	16	16	16	16	16	16	16	16
Mineral premix	275	3.9	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
Total (₹)			75	81	91	63	65	67	63	65	67
Total (US\$)			1.07	1.15	1.30	0.90	0.92	0.95	0.90	0.93	0.96

observed in rohu fingerlings (*Labeo rohita*) (Debnath *et al.*, 2007) and juvenile jian carp (*Cyprinus carpio* var. *jian*) (Li *et al.*, 2017). Highest protease activity in this present study was observed in fingerling milkfish fed F35 dietary treatment. These results are in agreement with the study conducted by Zhang *et al.* (2018), who observed higher protease activity in Japanese seabass (*Lateolabrax japonicas*) fed FM included diet than SBM supplemented diets to replace FM. Other attempts were carried out to replace FM with SBM and DDGS in grey mullet (*Mugil cephalus*), milkfish (*C. chanos*) (Jana *et al.*, 2012) and

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