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# Dietary replacement of fish meal with soybean meal for the optimal growth of Juvenile Milkfish, *Chanos chanos* (Forsskal, 1775) in seawater tanks

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## Abstract
The core of the current study is to find out a different source of diet that stimulates better growth rate and low-cost feed for commercial fish milkfish Chanos chanos. This study was to assess the impact of partial replacement of fish meal by soybean meal in the diet of juvenile milkfish, Chanos chanos (initial weight 25 ± 0.6 g). The juveniles were fed with two types of isonitrogenous diets (40% crude protein). Plant protein source diet (D1) comprised of soybean meal 45% and 10% fish meal, while animal protein source diet (D2) contained fish meal 25% and soybean meal 30%. The trial was conducted with two replications for 60 days. Fish juveniles (10 each) were stocked in outdoor fiberglass seawater tanks (5000 Liters). Results revealed that final weight gain (WG), and specific growth rate (SGR) were significantly lower in fish fed D2 diet. Fish fed D1 had better feed efficiency, weight gain (21.88 ± 1.9 g), and SGR (1.05 ± 0.3), while D2 had a lower growth rate, WG (18.71 ± 2.3 g) and SGR (0.93 ± 0.2). The maximum growth rate found on plant diet which is significantly higher weight gain than D2. In D2 feed conversion ratio (0.54 ± 0.21) was significantly higher ($P \leq 0.05$). Condition factor among both treatments did not differ significantly ($P \geq 0.05$). The survival rate remained constant (100%) in both treatments. Based on the obtained results, it is recommended that 45% soybean meal with addition of 10% fishmeal (40% protein) is more effective than 30% soybean meal with addition of 25% fish meal to the omnivorous milkfish for aquaculture.

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## Introduction
A decent aquaculture production does not only depend on water variables or reproduction but also profoundly on the good quality of feeds. A good quality feed not only supports fingerlings to grow rapidly into adults but also helps brooders for better production. Good quality and appropriate feed is key for commercial farming. Since fish meal is a valuable and important source of protein in fish feed, it is the most expensive of all other ingredients because it is the main source of protein and contains maximum nutrients. The selection of feed ingredients and their costs affect fish farming (Craig and Helfrich, 2002; Khan *et al*., 2014). To reduce feed costs, alternatives to protein sources must be find out (Richard *et al*., 2011; Singh *et al*., 2014). A good protein source should be considered to avoid rejection by the fish, a variety of food attractants and essence are being continuously introduced in the market without any concentration of anti-nutrient reduction, which has high remarked in fish growth (Mokolensang *et al*., 2003).

The feed formulation stipulates a mixture of different ingredients and alternative sources, and its best quality needs to be known and clear to achieve desired feed (Khan *et al*., 2004). Interactive effects of feed ingredients cause negative growth which makes feed unaffected and costly (Shioya *et al*., 2011). However, the information is rare for the milkfish artificial diet.
The fish feed has a few main assets; like fishmeal, fish oil, slaughterhouse wastes, and plant sources. The two main components of fishmeal (FM) and fish oil have importance in aquaculture feed. FM is commonly being used because it contains primary nutrients like essential amino acids and fatty acids, vitamins, minerals, and unidentified growth factors (Samocha *et al*., 2004; Amaya *et al*., 2007). Nevertheless, high demand and limited availability make fishmeal a costly component. FM is continuously studied for an alternative source that promotes growth rate, fish health, and as well as delivers comparable nutrients to human utilization. The most common substituent for fishmeal is a plant-sourced diet. FM has been replaced completely or partially by various alternative protein sources in fish feed. In addition, FM and plant meal (PM) sources were used by a number of researchers for different species like, *Salmo salar* Atlantic salmon (Torstensen *et al*., 2008), *Pagrus major* red sea bream (Kader *et al*., 2012), *Salmon salar* L. Atlantic salmon (Carter and hauler, 2000), *Sparus aurata* Gilt-headed bream (Gomeze-Requeni *et al*., 2004), *Dicentrarchus labrax,* European bass (Kaushik *et al*., 2004), *Oreochromis niloticus* L. Nile tilapia (El-Saidy and Gaber, 2003), *Megalobrama amblycephala*, Wuchang bream (Ahmed *et al*., 2018), *Mugil liza* Mullet (Rosas *et al*., 2019) and *Litopenaeus vannamei*, white shrimp (Amaya *et al*., 2007). These kinds of diets substitute fishmeal by including soybean, corn, canola, wheat, and rice. Generally, in artificial fish feed sunflower, cottonseed, linseed, and soy meals have been used individually to replace fishmeal (El-Sayed, 1999). These plant protein sources have a high level of amino acid profiles, although some have one or more essential amino acid deficiencies (NRC, 1993). It is necessary to examine plant protein's nutritional value in order to replace fishmeal. Amongst plant sources of protein, soybean meal has highly preferred in the replacement of fishmeal because it has balanced amino acids, nutritional value, worldwide accessibility, and a low price (Tacon, 2000; Divakaran *et al*., 2000; Samocha *et al*., 2004). The practices of supplementary ingredients in the feed are used to acquire better-balanced nutrient assessment and increase nutrient utilization and feed efficiency (Hernandez *et al*., 2004).
White meat can renovate growth, but it is also necessary to regulate fish health and meat quality. The exceptional quality of meat is expressed, by the intensity of color tinges (Cheng *et al*., 2015), the quality of the fatty acids (Torcher, 2015), or carotenoid content (Choubert, 2010). All of these qualities' appearance, oxidative stability, nutritional aspects, and preservation are acceptable for the consumers to live a healthy life (Lie, 2001). The elevated subject of fatty acids (omega 3, 6 and 9), minerals, and vitamins make milkfish most acceptable all over (Swastawati, 2014). Promotion of this species *Chanos chanos* needs the lowest cost appropriate diet, boosting his economic and social demand. This study's objective is to find different sources of nutrition assessment that may directly affect growth factors. An appropriate diet design for milkfish juveniles according to the protein profile of species with additional nutrition.

## Materials and Methods

### *Fish specimen*

The milkfish juveniles with preliminary mean weight (25 ± 0.6 g) were caught by cast net on Sonmiani coast and fetched into (CEMB) aquaculture lab. The collected juveniles were acclimatized for seven days before stocking. Ten juveniles per tank were distributed into four outdoor treatment fiberglass tanks. Each tank with capacity of 500 L of seawater. A chemical and physical assessment like salinity, temperature, and pH were observed on regular basis whereas ammonia ($NH_3$) by ammonium HAB kit and dissolved oxygen (DO) were examined weekly. Salinity managed to 16-18 ppt and the water temperature was controlled in between 23-29 ºC for 60 days.

### *Diet formulation and preparation*

The mixture was blended with fish oil, soybean oil, and water to produce a paste. The paste was passed through a pelleted machine to produce 1 mm in diameter pellets. The obtained pellets dried under high temperature (105 ºC) and kept in polythene bags until use.

### *Feeding management*

All fish were measured for length and weight and transferred to experimental outdoor fiberglass tanks. Feeding management was set according to the average body weight of total fish in treatment tanks. 2.5 % of body weight in three times intervals in 24 hours. Unfed pellets were siphoned down and dried for estimation of the feed intake ratio.

### *Water quality parameter*

The temperature, salinity, pH, and DO of both treatments were monitored on daily basis. Salinity was checked by salinometer, Temperature and pH were verified by a digital thermometer, and DO by YSI (model 57) meter. Ammonia ($NH_3$) was controlled between 0.05 – 0.02 by daily siphoning and exchanging 50% of water.

### Growth parameters

Primary seven parameters were used to determine growth indices, these are:

Weight gain = $\frac{final\ body\ weight - intial\ body\ weight}{intial\ body\ weight} \times 100$

Specific growth rate = $\frac{Ln(final\ weight) - Ln(initial\ weight)}{period\ culture} \times 100$

Condition factor = $\frac{final\ weight}{final\ length3} \times 100$

Feed intake = $diet\ given - remaining\ unfed\ pellets$

Feed conversion ratio = $\frac{diet\ given}{weight\ gain}$

Protein efficiency ratio = $\frac{weight\ gain}{proteofintake}$

Survival rate = $final\ number\ of\ fish - initial\ number\ of\ fish \times 100$

### Biochemical and physiological analysis

At the end of the experiment, fish meat was analyzed for chemical composition. Such as lipids, proteins, moisture, fiber, and ash contents studied by standard methods. Crude lipid determined by the Folch method (methanol/chloroform by 1:2 ratio); crude protein calculated by the Kjeldahl method (N*6.25); crude fiber estimated by acid detergent analysis; moisture calculated by dehydrating in the oven at 105 ºC for 24 hours and ash assessed by burning in a muffle furnace at 550 ºC for 18 hours. All samples were presented and studied in duplicate.

**Table 1. Feed formulation for growing milkfish in seawater tanks.**

| **Experimental diets** | D1 (% soybean meal-based diet) | D2 (% fish meal-based diet) |
|---|---|---|
| Fish meal | 10 | 25 |
| Soybean meal | 45 | 30 |
| Wheat bran | 5 | 5 |
| Rice bran | 10 | 10 |
| Topica | 6 | 6 |
| Fish oil | 7.5 | 7.5 |
| Mustured oil cake | 13.7 | 13.7 |
| *Vitamin mix | 2.8 | 2.8 |
| ***Composition of essential amino acid*** | | |
| Arginine | 2.11 ± 0.43 | 2.24 ± 0.4 |
| Histidine | 1.28 ± 0.21 | 1.21 ± 0.21 |
| Isoleucine | 1.77 ±0.08 | 1.81 ± 0.05 |
| Leucine | 3.21 ± 0.15 | 3.29 ± 0.23 |
| Lysine | 2.94 ± 0.75 | 3.04 ± 0.79 |
| Methionine | 0.93 ± 0.04 | 0.88 ± 0.02 |
| Phenyalanine | 1.67 ± 0.07 | 1.74 ± 0.08 |
| Threonine | 1.63 ± 0.3 | 1.62 ± 0.29 |
| Tryptophan | 0.42 ± 0.02 | 0.41 ± 0.02 |
| Valine | 2.14 ± 1.09 | 2.17 ± 1.21 |

*Abbas et al (2011).

**Table 2. Proximate composition of experimental feed percentage (%)**

| | *D1* | *D2* |
|---|---|---|
| *Crude Protein* | 39.9 ± 1.32 | 39.7 ± 1.49 |
| *Fat* | 22.3 ± 1.2 | 22.4 ± 1.23 |
| *Crude Fiber* | 7.8 ± 1.13 | 8.1 ± 1. 16 |
| *Ash* | 8.1 ± 1.41 | 8.2 ± 1.48 |
| *Moisture* | 12.3 ± 1.64 | 13.4 ± 1.73 |

### Statistical analysis

Statistical values and calculations were shown as the mean ± SD. Growth rate, feed utilization efficiency, nutrient digestibility, and proximate analysis were performed by one-way analysis of variance for significant differences. Probability tests were determined according to Duncan's multiple range test, as defined by Steel and Torrie (1980). Linear regression analyses between fish and plant source diet determined by a coefficient ($R^2$).

## Results

The result showed that the two different sources of feed notably favored the growth rate of milkfish juveniles. In the 1st week, the fishmeal diet (D2) responded with a better growth rate than soybean meal (D1). D2 which contains 25 % fishmeal, may fill the gap of the starvation period loss in the acclimation period. The combination of soybean and fish meal in the right proportion resulted in an excellent growth rate in starting but after two weeks the growth was much slower than soybean meal (D1) (Fig 1). Conversely, D1 progressively affected the growth rate more prominent in the 3rd to 5th week. However, in the sixth week, the weight gain of milkfish in D1 was not significantly different ($P \geq 0.05$) from D2. The maximum growth rate found on the plant diet with 21.88 ± 1.9 weight a gain, specific growth rate 1.05 ± 0.3 which is significantly higher ($P<0.05$) than D2. The feed conversion ratio (FCR) in D2 (0.54 ± 0.21) was significantly higher ($P \leq 0.05$) and condition factors among both treatments did not significantly differ ($P \geq 0.05$). The survival rate between the two treatments remains constant (100%).

Proximate examination of wet-weight milkfish showed a significant ($P <0.05$) enhance protein, fat, ash, and moister level which is higher in D1 as shown in (Table 4). Body composition in both diets contains equal proportions of nutrients with slight differences which reveals well utilization of feed. The regression analysis of both treatments showed an allometric growth rate which is a positive indication of feed utilization and good growth.

**Table 3. Growth performance of milkfish (Chanos chanos) fed soybean meal (D1) and fish meal (D2) based diets.**

| Growth Factors | D1 | D2 |
|---|---|---|
| **M.W0 (g)** | 25.04 ± 3.2 | 25.1 ± 2.4 |
| **M.L0 (cm)** | 13.69 ± 2.7 | 13.63 ± 3.0 |
| **M.Wt (g)** | 46.91 ± 1.7 | 43.81 ± 1.8 |
| **M.Lt (cm)** | 25.63 ± 1.7 | 23.54 ± 1.3 |
| **WG (g)** | 21.88 ± 1.9 | 18.71 ± 2.3 |
| **SGR** | 1.05 ± 0.3 | 0.93 ± 0.2 |
| **CF** | 0.28 ± 0.03 | 0.34 ± 0.02 |
| **FI** | 0.023 ± 0.01 | 0.024 ± 0.02 |
| **FCR** | 0.85 ± 0.3 | 0.91 ± 0.21 |
| **PER** | 1.11 ± 0.02 | 1.04 ± 0.02 |
| **SR (%)** | 100% | 100% |

The values are denoted as means ± SD. Related letters in each line express as M. W0 = mean initial weight; M. L0 = mean initial length; M.Wt = mean final weight; M.Lt = mean final length; WG = weight gain; SGR = specific growth rate; CF = condition factor; FI = feed intake; FCR = feed conversion ratio; PER = protein efficiency ratio; SR = survival rate

The comparative study of both treatments exhibited better feed utilization, FM source diet growing at a uniform rate from starting to the endpoint of the trial but the PM source diet regularly gained weight in different stages of the trial. The increment was very corresponded ($P < 0.05$) to the aggregate of PM source diet and FM source diet replacement ($R^2 = 0.995$; $R^2 = 0.9649$) and the comparative correlation between the two was ($R^2 = 0.8392$).

**Table 4. Proximate composition of wet milkfish**

| | (D 1) | (D 2) |
|---|---|---|
| **Protein** | 20.2 ± 1.2 | 19.8 ±1.6 |
| **Fat** | 3.3 ± 1.4 | 3.01 ±1.5 |
| **Ash** | 1.8 ± 1.3 | 1.6 ± 1.1 |
| **Moisture** | 72.8 ± 1.7 | 72.5 ± 1.9 |

Moreover, the combination of a small portion of fish meal and a major portion of soybean meal gets a better growth rate ($R^2 = 0.995$).

## Discussion

Feeding resources, plant meals and animal meals have raised fish farming attention to substituent cheap and qualitative sources which may well replace these components (Tocher, 2015) however fish requirements invigorate it (especially in omnivorous fish) (Oliva-Teles *et al*., 2015). Based on our results,

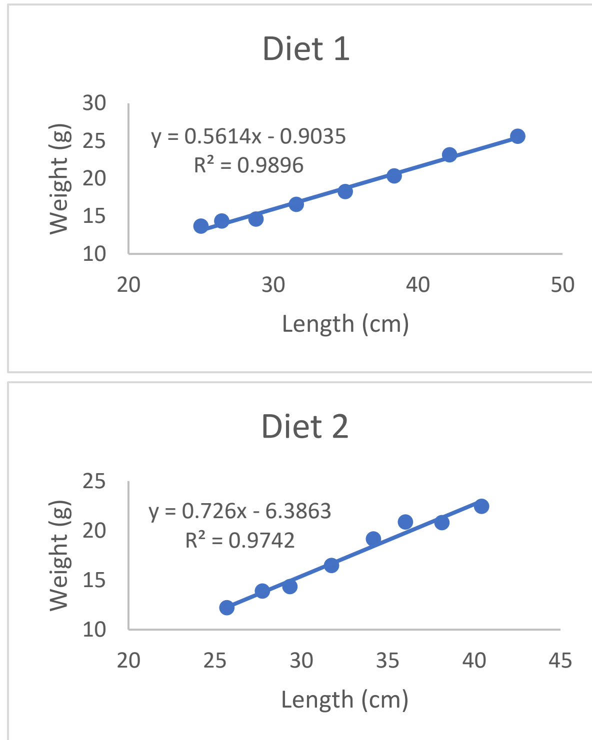


**Figure 1.** Length and weight relationship among fish fed soybean meal (D1) and fish meal (D2) based diets.

we suggest that 45% soybean meal partial-replace in a fish meal can be used without significant modifications in the parameters mentioned above. The dual modification of PM and FM in milkfish feed had the top performance in terms of growth, SGR, FCR, CF, and survival. The partial substitution of FM treatment had an adverse effect on the growth, SGR, FCR, and CF than PM treatment. Differences between the two diets can be suggested as the combination of PM and FM with the percentage of 45 and 10 is contained a variety of amino acids in the diet which promotes a better growth rate. On the other hand, an FM diet with 25 and 30 percent may not meet the required amino acids and nutrients profile for milkfish juveniles. It may because of the lowest quality of fishmeal or minimal nutritional trash fish used in the meal. The survival rate of both treatments was observed 100% without any physical symptoms or differences. It indicates both feed property and efficiency are almost in a superior range. A similar result was found by Go *et al*., (2018) on a PM diet with a combination of various plant sources obtain (WG, 130.1g) better growth performance in milkfish juveniles under a controlled environment. Moreover, Magondu *et al*., (2016) obtained the highest growth rate on commercial feed (WG, 27.9g) than non-formulated feed. The result of Nochera and Ragone, (2016) proposed that 55.1% protein from breadnut flour in a diet is a nutritive and high-energy diet. 35% of fishmeal and 15% of soya cake in the diet of milkfish, resulted in a better immune system and faster growth rate. Different percentages of eel meal, soybean meal, wheat germ, vitamins, and minerals were performed on brooders to get an effective result (Azad *et al*., 2007). As for as the overall protein percentage in a diet is concerned, Borlongan and Coloso, (1993) also suggested 40% protein is enough for a milkfish diet. However, we overly used 40% protein in a diet to promote a better growth rate. Subsequently, Borlongan *et al*., (2000) formulated a diet where the main protein source kept in the diet was fish meal and defatted soybean meal with the addition of cod liver oil and bread flour. The result of combining different sources of protein delivered the highest growth and survival rate than exclusively prepared feed.

Fatty acids are as essential as protein in a diet. Deficiency of fatty acids results in abnormalities such as one-eyed fish, scoliosis, lordosis, slow growth, and low FCR (Alava and Kanazawa, 1996), 22.4% of lipid was set in both diets to support better health and achieve the exclusive result. (Go *et al*., 2018) used the sundry percentage of plant source lipid for milkfish fingerlings and best result found in 24.4 % lipid diet. Similar research was observed by utilizing different sources of lipid (soybean oil, coconut oil, fish oil, cod liver oil, and marine source) with a combination of two or more than two ingredients had the highest SGR, FCR (Alava,1998). The optimal dietary level of soybean lecithin for milkfish is 33.56g/100g a diet that improved growth, nutrient utilization, and survival (Balito-Liboon *et al*., 2018). Subsequently, soybean is the most effective component in the diet of milkfish by means of protein as well as lipid constituent which simultaneously fulfilling the requirement of the necessary nutrients.

High levels of FM substitution with PM (soybean) have been nurtured to obtain a better growth rate. Earlier research suggested that complete FM replacement in the feed had low body weight, in the diet of omnivorous fish species such as *Oreochromis niloticus*, *Siluriformes*, and *Chanos chanos*, fishmeal should be partially replaced rather than completely surrogate (Oyelese, 2007; Ogunji *et al*., 2008; Sing *et* al., 2013). Some authors also indicated that partially fish meal (50%) delivered optimal level in FM feed. However, 45% of PM and 10% of FM in the diet of milkfish is much more effective. The expected result is significantly more efficient to support the partial replacement of fish meal in omnivorous milkfish *Chanos chanos* diet under controlled environment.

## Conclusions

45 % of plant meal is the appropriate concentration for the optimal growth of milkfish juveniles. However, milkfish is omnivorous species, but it moderately tends to feed primary production. The appropriate combination of different sources of protein is significantly effective for milkfish farming.

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## Conflict of interest

The authors declare that no conflict of interest exists.

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