

# EVALUATION OF ALTERNATIVE PROTEIN SOURCES TO REPLACE FISH MEAL IN PRACTICAL DIETS FOR TILAPIA (*OREOCHROMIS MOSSAMBICUS*) ADVANCE FRY

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## ABSTRACT

The effects of partial replacement of dietary fish meal (FM) with plant protein sources on the growth performance and feed utilization of Tilapia *Oreochromis mossambicus*, were investigated. Two feeding experiments were conducted in which cotton seed meal (CSM) and soybean meal (SBM) replaced FM. Triplicates were maintained for separately for each experiment. The six isonitrogenous experimental diets were prepared which included a control diet (100% fish meal) and five treatment diets viz. fish meal replaced with cotton seed meal (Experiment-I) and soybean meal (Experiment-II) at 10%, 20%, 30%, 40% and 50% of dietary protein levels respectively. The diets were formulated to provide 40% crude protein on a dry weight basis. Fish were fed experimental diets *ad libitum* for 60 days. The water quality parameters were found in the permissible range during entire experimental period. After the end of Experiment-I it was found that higher weight gain ( $788.83 \pm 44.61$ ), SGR ( $1.58 \pm 0.04$ ), PER ( $1.06 \pm 0.06$ ), survival ( $100 \pm 00$ ) and lowest FCR ( $2.36 \pm 0.12$ ) were obtained by feeding the fish with the diet T3 containing 30% Cotton seed meal and in experiment-II higher weight gain ( $711.88 \pm 47.16$ ), SGR ( $1.51 \pm 0.04$ ), PER ( $0.96 \pm 0.03$ ), survival ( $95.00 \pm 2.89$ ) and lowest FCR ( $2.61 \pm 0.09$ ) were obtained by feeding the fish with the diet T3 containing 40% Soybean meal. The present study suggests that, the Cotton seed meal replacing 30% of fish meal and Soybean meal replacing 40% of fish meal in the diet of *O. mossambicus* advance fry gave better growth

## INTRODUCTION

Aquaculture has been one of the fastest growing food sector for over 25 years, with APR (annual percentage rate) of 8.2% per year, compared with 1.3% for capture fisheries and 2.6% for total agricultural meat production (FAO, 2013). The reported global production of food fish from aquaculture, including fin fishes, crustaceans, molluscs and other aquatic animals for human have reached 62.7 million tonnes in 2011, up by 6.2% from 59 million tonnes in 2010. The estimated value of farmed food fish is USD 130 billion (FAO, 2013). Due to the expansion of global aquaculture production, there has been a significant increase in the demand for aquaculture feeds. Globally, 708 million tonnes of industrial compound animal feeds were produced in 2008, of which 29.2 million tonnes were aquafeeds (4.1 percent of all animal feeds). Feed cost is the largest production cost for commercial aquaculture (Akiyama, 1989).

Fish meal is the major protein source in aqua feeds, globally averaged about 15.0 million tonnes in 2010 (FAO, 2012). Fish meal is the first choice as a raw material in aquafeed production for its high quality of protein with a well-balanced amino acid profile (Gatlin *et al.*, 2007). With continued growth in demand, fish meal prices have risen in real terms in the past three decades and are likely to increase further. Also, fish meal production is heavily localized in some regions of the world resulting in becoming more expensive and difficult to

obtain in many countries practicing aquaculture (Tacon and Jackson 1985, Hossain *et al.*, 2001, Adebayo *et al.*, 2004). The protein component in aquaculture diet is the single most expensive portion and important dietary nutrient (Nalawade and Bhilave, 2011). Significant progress has been made over the past decade in reducing levels of fishmeal in commercial feeds for farmed fish. Despite these advances, the quantity of fishmeal used by the aquafeed sector has increased as aquaculture production has expanded. Thus, further reduction in percentages of fishmeal in aquafeeds is necessary (Hardy, 2010). As the supply of fishmeal is finite, it becomes necessary for the aquafeed sector to seek alternative cheaper sources of protein with sufficient global production to meet the requirements of aquafeeds for the foreseeable future. The quest to reduce the quantity of fish meal while maintaining the protein quality in fish feed has been the focus of fish nutritionists (Bhilave *et al.*, 2010). These may be in the form of plant based or animal based conventional protein sources.

Cottonseed meal is the residue produced after oil extraction from cottonseed (Hertrampf and Piedad-Pascual, 2000). Cottonseed meal ranks third in tonnage among the plant protein concentrates produced globally (Swick and Tan 1995.). Cottonseed meals are among the most available plant protein sources in the world. Besides being relatively cheap, cottonseed meal contains good protein contents (26-54%, depending on processing methods) and amino acid profile.

However, it contains relatively low levels of lysine, cystine and methionine and processing conditions may also have a negative effect on the amino acid content (Lovell, 1998). Crude fiber is a limiting factor in the use of cottonseed meal as feed. It is around 10% in decorticated meals and expellers while its content in corticated meal can exceed 20%. The digestible energy of cottonseed meal is relatively low due to the high crude fiber content (Hertrampf and Piedad - Pascual, 2000).

Soybean meal is regarded as an economical and nutritious feedstuff with high crude protein (44% – 48%) content (Cheng *et al.*, 2003) and a reasonably balanced amino acid profile compared to other plant proteins (Gatlin *et al.*, 2007). Soybean meal has significantly less phosphorous (6.5 g kg<sup>-1</sup>) than fish meal (17.42 g kg<sup>-1</sup>) (Cheng *et al.*, 2003). For this reason, it may be the most promising alternative protein source to fish meal (Yesilayer *et al.*, 2011). Soybean meal (SBM) was used as the sole protein source with the addition of supplemental amino acids in diets of common carp *Cyprinus carpio* (Viola *et al.*, 1982) and constituted 67% and 50% of diets for tilapia *Oreochromis spp.* and channel catfish *Ictalurus punctatus*, respectively (Storebakken *et al.*, 2000). Soybean meal is widely used as the most cost-effective alternative for high-quality fish meal in feeds for many aquaculture species (Storebakken *et al.*, 2000).

Tilapia is a generic term which is used to designate a group of commercially important food fish belonging to the family Cichlidae. Tilapias are among the most successful cultured finfish species in the world. This has been largely because of their fast growth rate and ability to feed low on the aquatic food chain. Thus, they have become excellent candidates for aquaculture, especially in tropical and subtropical regions. Tilapia species are used in commercial farming systems in almost 100 countries and are developed to be one of the most important fish for aquaculture in this century (Fitzsimmons, 2000). The primary genus reared for aquaculture is *Oreochromis* which includes Nile Tilapia (*O. niloticus*), Mozambique Tilapia (*O. mossambicus*), and Blue Tilapia (*O. aureus* and *O. urolepis hornorum*) (Fitzsimmons, 1997). Production of tilapias has a wide distribution and 72 percent are raised in Asia. By volume, industrial compound aquafeeds used by tilapias is 13.5 percent of the total aquafeeds (FAO, 2012). Protein requirement of tilapia (2.4-3.50 g) is reported as 30-35% of the diet (Wang, *et al.*, 1985, Abdelghany, 2000). It is, therefore, of interest to replace part of fish meal with protein-rich alternative feed ingredients.

With this consideration, two consecutive feeding experiments were conducted to determine the optimum dietary level of cotton seed meal and soybean meal in order to replace fish meal (FM) in diets for tilapia (*O. mossambicus*) advance fry.

## MATERIALS AND METHODS

Advance fry of *O. mossambicus* (tilapia) weighing 0.62 ± 0.04 g (mean ± SE) and 0.8 ± 0.03 g (mean ± SE) were selected for the experiment-I and II respectively. The experimental setup in each individual experiment consisted of 18 plastic tanks of 30 L capacity. Three hundred sixty (360) fishes were randomly distributed in six distinct experimental groups. Proximate composition of ingredients used in

experiment-I and II for preparation of treatment diets are shown in Table 1.

In experiment-I, six isonitrogenous (40% crude protein) experimental diets were formulated to replace fish meal with cottonseed meal at 10, 20, 30, 40 and 50% of dietary protein levels respectively. The diets were designated as T0 (control, 100% fish meal as primary protein source; zero% CSM), T1 (90% of fish meal and 10% CSM), T2 (80% of fish meal and 20% CSM), T3 (70% of fish meal and 30% CSM), T4 (60% of fish meal and 40% CSM) and T5 (50% of fish meal and 50% CSM). Formulation and proximate composition of treatment diets used in experiment-I are shown in Table 2.

In experiment-II, six isonitrogenous (40% crude protein) experimental diets were formulated to replace fish meal with shrimp head meal at 10, 20, 30, 40 and 50% of dietary protein levels respectively. The diets were designated as T0 (control, 100% fish meal as primary protein source; zero% SBM), T1 (90% of fish meal and 10% SBM), T2 (80% of fish meal and 20% SBM), T3 (70% of fish meal and 30% SBM), T4 = (60% of fish meal and 40% SBM) and T5 (50% of fish meal and 50% SBM). Formulation and proximate composition of treatment diets used in experiment-II are shown in Table 3.

During both the experiments, fishes were fed *ad libitum* till the end of the experiment. Observations for increase in length and weight were recorded by sampling 5 fishes from each replicate tank fortnightly. The biomass was estimated every 15 days by collectively weighing all the fishes from each tank. Water quality parameters viz. temperature, pH, dissolved oxygen, alkalinity, nitrate, nitrite were recorded daily during the experiment period.

### Moisture

The moisture content of the sample were determined by taking a known weight of sample in petri-dish and drying in hot air oven at 100-105°C till a constant weight was achieved.

$$\text{Moisture (\%)} = \frac{\text{Wet weight of sample} - \text{Dried weight of sample}}{\text{Wet weight of sample}} \times 100$$

### Crude protein

Nitrogen content of the sample was estimated by Kjeldahl method and the crude protein was estimated by multiplying nitrogen percentage by a constant factor 6.25.

$$\text{Crude protein (\%)} = \text{Nitrogen (\%)} \times 6.25$$

### Ash

Ash content was estimated by taking a known weight of sample in silica crucible and placing it in a muffle furnace heated at 600°C for 6 hours.

$$\text{Ash (\%)} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100$$

### Fat

Fat was estimated by Soxhlet apparatus using petroleum ether as a solvent.

$$\text{Fat (\%)} = \frac{\text{Weight of initial sample} - \text{Weight after extraction}}{\text{Weight of initial sample}} \times 100$$

**Percentage weight gain**

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

**Specific growth rate (SGR)**

$$\text{SGR} = \frac{\text{Log}_e W_2 - \text{Log}_e W_1}{T_2 - T_1} \times 100$$

$T_1$  and  $T_2$  are 0 and 60<sup>th</sup> day of the experiment

$W_2$  = weight of fish at time  $T_2$

$W_1$  = weight of fish at time  $T_1$

**Food conversion ratio (FCR)**

$$\text{FCR} = \frac{\text{Amount of feed intake (g)}}{\text{Wet weight gain (g)}}$$

**Survival**

$$\text{Survival} = \frac{\text{No. of fish survived after rearing}}{\text{No. of fish stocked}} \times 100$$

**Statistical analysis**

All treatments were assigned by a completely randomized

design. One way analysis of variance (ANOVA) was applied to test the level of significance amongst the treatments. Least significant difference (LSD) test was applied to find out the best treatment (Snedecor and Cochran, 1967) Data are presented as mean  $\pm$  standard error (SE).

**RESULTS****Experiment-I**

The effects of protein sources on fish performance are given in Table 4. All diets produced excellent growth rates throughout the study. The specific growth rates of fishes fed with 30% of cottonseed meal was found to be significantly higher as compared to the other treatments ( $p < 0.05$ ). While the FCR and PER were significantly retarded ( $p < 0.05$ ). Moreover, diet containing 50% of cottonseed meal produced the lowest fish performance.

Fish survival for all treatments ranged between 73 and 100% (Table 4). Water quality parameters such as temperature, pH, dissolved oxygen, salinity, nitrate, nitrite, alkalinity was found to be within the optimum range during the experimental period.

**Experiment-II**

The observations on effect of different protein sources on fish growth performance are shown in Table 4. All the treatment diets produced excellent growth rates throughout the study. The specific growth rate (SGR) of fish fed with diet T4 (60% fish meal: 40% of soybean meal) was found to be significantly

**Table 1: Proximate composition of ingredients used in the experiment-I and II**

Ingredients	Moisture (%)	Dry matter (%)	Ash (%)	Protein (%)	Fat (%)
Fish meal <sup>1</sup>	3.85	96.15	26.75	56.00	5.56
Cotton seed meal	7.10	92.90	37.35	60.00	4.45
Soybean meal	6.60	93.40	6.65	51.00	3.60
Wheat flour <sup>3</sup>	9.15	90.85	1.70	12.41	1.01
Rice flour <sup>3</sup>	10.50	89.50	6.15	8.02	1.05
Wheat bran <sup>3</sup>	10.85	89.15	4.65	12.00	2.99

<sup>1</sup>Star fish meal plant, Veraval, Gujarat India; <sup>2</sup>Heena Marine Group of Industries Ltd. (HMG), Veraval, Gujarat; <sup>3</sup>Local market, Veraval, Gujarat India

**Table 2: Formulation and proximate composition of diets used in experiment-I**

Ingredients	Diets					
	T0(CONTROL)	T1	T2	T3	T4	T5
Cottonseed meal(60 CP)	0.00	6.90	13.80	20.70	27.60	34.50
Fish meal(56 CP)	69.00	62.10	55.20	48.30	41.40	34.50
Wheat bran(12 CP)	11.00	9.00	8.00	6.00	5.00	3.00
Rice Flour	3.00	5.00	6.00	8.00	9.00	11.00
Wheat Flour	6.00	6.00	6.00	6.00	6.00	6.00
CMC <sup>1</sup>	2.00	2.00	2.00	2.00	2.00	2.00
Sun flower oil <sup>2</sup>	4.00	4.00	4.00	4.00	4.00	4.00
FISH OIL <sup>3</sup>	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin Mixture <sup>4</sup>	1.00	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Proximate analysis (determined on dry matter basis)						
Crude protein (CP) (%)	39.96	40.00	40.15	40.19	40.34	40.38
Ether extract (%)	10.40	8.8	10.35	9.18	9.20	10.13
Ash (%)	13.15	18.15	16.70	16.00	14.05	16.03
Moisture (%)	10.83	10.90	11.04	11.07	11.09	11.03

<sup>1</sup>CMC- carboxy methyl cellulose (HiMedia Laboratories Ltd.); <sup>2</sup>Gemini sunflower oil; <sup>3</sup>seven sea cods; <sup>4</sup>Vitamin and mineral mixture/Kg premix: Vitamin A-7,00,000IU, Vitamin D3-70,000IU, Vitamin E-250 mg, Nicotinamide-1000 mg, Cobalt-150mg, Copper-1200mg, Iodine-25g, Iron-1500 mg, Magnesium-6000 mg, Manganese-1500 mg, Potassium-100mg, Sodium- 5.9mg, Sulphur-0.72%, Zinc-9600 mg, Calcium-25.5%, Phosphorus-12.75%

**Table 3: Formulation and proximate composition of diets used in experiment-II**

Ingredients	Diets					
	T0(CONTROL)	T1	T2	T3	T4	T5
Soybean meal (51 CP)	0.00	7.00	14.00	21.00	28.00	35.00
Fish meal(56 CP)	70.00	63.00	56.00	49.00	42.00	35.00
Wheat bran(12 CP)	9.00	10.00	12.00	14.00	16.00	18.00
Rice Flour	4.00	5.00	4.00	3.00	2.00	0.00
Wheat Flour	6.00	4.00	3.00	2.00	1.00	1.00
CMC <sup>1</sup>	2.00	2.00	2.00	2.00	2.00	2.00
Sun flower oil <sup>2</sup>	4.00	4.00	4.00	4.00	4.00	4.00
FISH OIL <sup>3</sup>	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin Mixture <sup>4</sup>	1.00	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Proximate analysis (determined on dry matter basis)						
Crude protein (CP) (%)	40.28	40.05	39.94	39.83	39.72	39.61
Ether extract (%)	10.40	9.10	8.40	8.95	8.70	8.50
Ash (%)	14.50	16.85	14.30	16.35	11.25	13.90
Moisture (%)	9.83	9.93	10.70	10.80	10.90	10.40

<sup>1</sup>CMC - carboxy methyl cellulose (HiMedia Laboratories Ltd.);<sup>2</sup>Gemini sunflower oil;<sup>3</sup>seven sea cods;<sup>4</sup>Vitamin and mineral mixture/Kg premix: Vitamin A-7,00,000IU, Vitamin D3-70,000IU, Vitamin E-250 mg, Nicotinamide-1000 mg, Cobalt-150mg, Copper-1200mg, Iodine-25g, Iron-1500 mg, Magnesium-6000 mg, Manganese-1500 mg, Potassium-100mg, Sodium-5.9mg, Sulphur-0.72%, Zinc-9600 mg, Calcium-25.5%, Phosphorus-12.75%

**Table 4: Growth, food conversion ratio and protein efficiency ratio of *O. mossambicus* fed the test diets for 60 days**

Parameters	Treatment					
	T0	T1	T2	T3	T4	T5
L1	3.60±0.06	3.67±0.03	3.60±0.06	3.64±0.03	3.66±0.02	3.63±0.03
L2	8.30±0.12	8.47±0.35	8.43±0.19	8.57±0.09	8.23±0.43	7.73±0.28
W1	16.29±0.14	16.27±0.19	16.31±0.23	16.18±0.13	16.36±0.15	16.14±0.09
W2	131.13±9.33	136.16±5.63	139.26±8.73	143.73±6.79	106.97±6.52	89.87±8.88
SGR	1.51±0.06	1.54±0.04	1.55±0.05	1.58±0.04	1.36±0.04	1.24±0.06
FCR	2.58±0.16	2.51±0.12	2.37±0.12	2.36±0.12	3.20±0.26	3.30±0.08
PER	0.98±0.06	1.00±0.05	1.06±0.06	1.06±0.06	0.79±0.07	0.76±0.02
SURVIVAL	98.33±1.67	100.00	96.67±3.33	100.00	96.67±1.67	73.33±14.53
Weight gain (%)	705.56±60.27	737.85±41.69	755.37±62.10	788.83±44.61	553.64±38.71	456.32±51.59

\*L1- Initial length (cm); L2- Final length (cm); W1- Initial weight (g); W2- Final weight (g); SGR- Specific Growth Rate; SGR =  $\text{Loge W2} - \text{Loge W1} / \text{T2-T1} \times 100$ ; FCR- Food Conversion Ratio; FCR = Amount of feed intake (g) / Wet weight gain (g); PER- Protein Efficiency Ratio; PER = Increment in body weight (g) / Protein intake (g); Survival - survival (%) = No. of fish survived after rearing / No. of fish stocked  $\times 100$ ; Weight gain (%) = Final weight - Initial weight / Initial weight  $\times 100$

higher as compared to the other treatment diets ( $p < 0.05$ ). Similarly, the food conversion ratio (FCR) was significantly low in the fish fed with diet T4 ( $p < 0.05$ ), while the protein efficiency ratio (PER) was significantly high in the fish fed with diet T4 ( $p < 0.05$ ).

Fish survival for all treatments ranged between 83.33-100%. Water quality parameters such as temperature, pH, dissolved oxygen, salinity, nitrate, nitrite and alkalinity were found to be within the optimum range during the experimental period.

## DISCUSSION

According to Jauncey *et al.* (1983) and Jauncey and Ross (1982), the protein requirement for *O. mossambicus* fry is in the range of 40-50%. Therefore, the percentage of dietary crude protein was kept at 40% level in the present study which is in the recommended range to maximize the growth of *O. mossambicus* advanced fry.

### Experiment-I

The present study clearly demonstrated that there was a significantly higher growth reported in fish fed with the diet containing 30% cottonseed meal, as compared to the control diet which contained fish meal (100%) as a major protein

source. On the other hand, Diet T5, which included maximum level (50%) of cottonseed meal showed significantly lower growth as compared to control diet. Similar results have been reported by Meric *et al.* (2011). The authors found that diets containing 45% cottonseed oil cake resulted in slightly reduced growth (*O. niloticus*) while 15-30% of fishmeal could be replaced by cottonseed oilcake without impact.

Significantly higher SGR was observed in all the diets containing cottonseed meal as the source of protein (T1, T2 and T3) as compared to control (T0). The Highest SGR was found in T3 diet treatment on the other hand lowest SGR was found in T5 and T4. The present results agreed with that of Mbahinzireki *et al.* (2001) who reported that SGR for *O. niloticus* was in the range of 1.0 to 1.4 when dietary fish meal was replaced by cottonseed meal.

In the present study, the highest FCR was observed in diet containing 50% of cotton seed meal. The present results agreed with study conducted by El-Saidy and Gaber (2003) when fish meal was replaced by cottonseed meal in juvenile Nile tilapia, (*O. niloticus*). The PER was recorded in the present investigation was higher in diets containing 30% of cottonseed meal as protein source compared to control diet. On the other hand,

the lowest PER was recorded in T5 diet (0.76) treatment. Similar observations were recorded by Meric *et al.* (2011) who recorded significant changes in PER for Nile tilapia (*O. niloticus*) when up to 30% fish meal was replaced with cottonseed oil cake in the diets.

### Experiment-II

In the present study, significantly higher growth was obtained in fish fed with the diet containing 40% soybean meal, as compared to the control diet (100% fish meal). However, in case of *O. niloticus*, El-Sayed (2005) concluded that growth in fish fed with diets supplemented with soybean meal at 25% fish meal substitution level were not significantly different from those of fish fed the control diet (containing 40% fish meal as a sole protein source).

In the present study, significantly higher SGR was observed in all the diets containing soybean meal as the major protein source compared to control (T0). The highest SGR was found in T4, while the lowest SGR was found in control (T0). The present results agreed with that of Koumi *et al.* (2009) who reported that SGR was in the range of 1.4 to 1.6 when soya protein was used as an alternative protein source in *O. niloticus* diet. However, the present results differ from the study conducted by El-Sayed (2005) who reported that SGR was higher (2.05 - 2.31) when soybean meal was used as partial substitutes for fish meal in Nile tilapia (*O. niloticus*) fingerling diets.

In the present study, the highest FCR was observed in control (T0) followed by T2, T5, T1, T3 and T4 treatments respectively. All the treatments did not significantly differ from each other. The FCR obtained in the present study was comparatively higher which may be due to low winter temperatures, higher fish stocking density per tank and water used for the experiment being saline with 10 ppt salinity. The present results are in agreement with the report of El-Saidy (2003) where FCR ranging from 1.94 to 2.57 was reported when soybean meal was used as partial substitute for fish meal in the Nile tilapia (*O. niloticus*) fingerling diets. The PER recorded in the present investigation was higher in diets containing 30% (T3) of soybean meal compared to control diet. On the other hand, the lowest PER was found in the fishes fed with T0 diet (0% soybean meal) compared to rest of the treatments. Similar observations were recorded by Fernandes *et al.* (1999) who reported significant changes in PER for Nile tilapia (*O. niloticus*) when fish meal was replaced by plant protein diet.

In conclusion, the Experiment-I and Experiment-II revealed that cottonseed meal and soybean meal could successfully replace fish meal up to 30% and 40% level in practical diets for tilapia (*O. mossambicus*) under the experimental conditions employed. Use of such locally available protein source would help in reduction of fish feed price due to replacement of costly fish meal protein. Nevertheless, more field level experiments are required to ascertain the viability of cottonseed meal as well as soybean meal are excellent plant protein source as better and cheaper source for replacement of fish meal protein.

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