**Formulation and Evaluation of Economical Extruded Fish Feed for GIFT Tilapia (Oreochromis niloticus) Fingerlings: A Study in Sustainable Aquaculture**

**ABSTRACT**

The purpose of this research was to find promising alternative ingredients which partially replace expensive GNOC for formulation of cost effective feed having better growth and feed efficiency of GIFT tilapia and optimise extruder parameters for the preparation of floating fish diets with appropriate physicochemical qualities. Accordingly, four iso-nitrogenous (crude protein 35%) experimental diets were formulated for GIFT tilapia advance fry by replacing 50% of GNOC protein of the control diet (T0) by sunflower oilcake (SFOC) (T1), by linseed oilcake (LSOC) (T2) and by fish based silage (T3) protein. The pellets extruded using a combination of 120 oC extruder barrel temperature, 25% moisture content of feed mix, 2.0 mm die diameter and 30 minute of pre-conditioning time. Results of the feeding trial using one hundred twenty number of GIFT tilapia advance fry of average weight 1.20 ± 0.34 g reared in twelve number of 200L FRP tanks under continuous aeration and fed with above four experimental diets in triplicate concluded that SFOC can be used to partially replace GNOC in floating fish feed without significantly (P<0.05) affecting the growth performance of GIFT tilapia, where as LSOC incorporation had a negative impact on the growth performance and feed efficiency parameters. However, fish silage when incorporated in the GIFT tilapia diet by partially replacing GNOC, it resulted in significantly higher growth rate (474.2 % weight gain) and better feed efficiency parameters of 2.27 FCR and 1.26 PER. Hence, fish silage and SFOC are promising alternative ingredients to partially replace expensive GNOC for formulation of cost effective feed for better growth and feed efficiency of GIFT tilapia.

**Key words:** Twin screw extruder, GIFT tilapia, sunflower oil cake, linseed oil cake, fish silage

1. **INTRODUCTION**

“Almost half of the fish consumed by people globally come from aquaculture” (Halden, et al., 2014). “Mushrooming growth of aquaculture activity has created a huge demand for fish feed for its sustenance. However, access to quality fish feed at affordable cost is a challenging task and also threatens the profitability and sustainability of aquaculture” (Munguti et al., 2014). “Thus, feed production is a key aspect to consider in both subsistence and commercial fish farming, since it affects both growth efficiency and resource utilization” (Tsevis et al., 2000). “Fishes generally require more protein in their diet then terrestrial farmed animals and therefore aqua-diets demand more protein rich ingredients” (Hasan, *et al.,* 2007). “Accordingly, the cost of protein source accounts for about 60% of the total cost of all the ingredients taken together. Generally protein from animal sources is the best, as it has better bioavailability. But animal protein, especially fish meal, is costlier and its availability is scanty”. (Arruda et al., 2009).

 “Among plant originated protein sources, groundnut oil cake (GNOC) and soybean meal are the most preferred conventional ingredients for preparation of aquafeed” (Barman and karim, 2007; Manomaitis, 2009). “However, their scanty availability and escalated cost has necessitated to find out suitable alternative source of protein, so as to replace these conventional feed ingredients and meet the protein requirement of the targeted fish at an affordable cost” (Rath *et al., 2014;* Lenka *et al.,* 2010; Danniel, 2016).

 “There are many types of oilcake available in our locality that are fairly rich in protein. Among them the oilcakes like Sunflower oilcake (SFOC), Linseed oilcake (LSOC) and Coconut oilcake (COC) are available in plenty at a cheap price. SFOC contains 27.8 – 37.4% crude protein (CP) with the limiting amino acids like methionine, arginine” (NRC, 1982). “Similarly, LSOC contains 31.5% CP, 9.0% crude fiber, 6% ash, 0.8% phosphorus and 0.4% calcium” (Declrq, 2006). “However, they are considered as non- conventional sources of protein as their use in animal feed has not yet been popularized and standardized.Use of such non-conventional feed ingredients have not gained suitable attention of the aquafeed industry due to the presence of a range of anti-nutritional factors (ANFs) e.g.- phytic acid, tannin, protease inhibitors, gossypol, saponin, lectins, haemoglutinis etc” (Francis *et al.,* 2001). “Non-ruminant organisms like fish are unable to break the phytate compound due to lack of phytase enzyme. A number of processing techniques e.g., roasting, germination, extrusion cooking, soaking, fermentation etc. have been suggested by many researchers for amelioration of ANFs and to increase their nutrient utilization” (Garg *et al.,* 2003). “Recently, extrusion cooking using twin screw extruder have been popularized to destroy anti-nutritional factors” (Nikmaram *et al.,* 2015; Moscicki, 2011), “increase nitrogen content and dietary fibre solubility, decrease lipid spoilage by denaturing deteriorative enzymes” (Alam *et al.,* 2016) and “destroy microbiological pathogen as well as to overcome problems associated with sinking pelleted feeds such as wastage of raw materials and water pollution, lower functional quality, particularly in terms of physicochemical qualities, etc”. (Munguti *et al.,* 2014).

“Extruded fish feed is getting increasingly popular, because it provides superior water stability, better floating properties, ease of digestion, growth, zero water pollution, optimised labour usage and zero wastage of raw materials” (Amalraaj *et al.,* 2010). “The nutrients are retained for a reasonable period of time while floating, allowing fish to consume the entire extruded ration” (Kearns *et al.,* 1989). “It is ideal for pelagic or surface feeders because the fish can easily access the feed and do not have to invest much energy searching for food at the bottom” (Balarin *et al.,* 1982). “The use of floating feed is safer because feed ingredients can be pasteurised or sterilised during the feed extrusion process, improving feed digestibility and reducing the negative effects of some feed materials on aquatic animal health” (Amalraaj *et al.,* 2010). Farmers can see how much and how actively the fish eat by using floating feed as a management tool.

GIFT tilapia (*Oreochromis niloticus*) are well-suited to a wide range of aquaculture systems because of their ease of propagation, adaptability to a broad range of climatic conditions, high growth rate, superior palatability, marketability, and nutrient benefits (Coddington et al.,,. 1997). Theses fishes particularly well-suited for developing countries like India because they can resist to stress and disease, capacity to breed in confined water, and accept artificial diet soon after yolk sac absorption (Sayed et al., 2006). Besides their hardy nature, they are inexpensive and simple for small-scale farmers to grow for food, nutrition, and income (Fitzsimmons 2006). Tilapias have progressed from being a low-prise, high-protein food fish to become popular as "aquatic chicken" (Pullin *at el*., 1985). Under this backdrop, present experiment has been designed to develop extruded fish feed using locally available ingredients for GIFT tilapia (*Oreochromis niloticus*) with the following objectives :

1. to prepare extruded fish feed for GIFT tilapia (*Oreochromis niloticus*) using various cost effective feed ingredients
2. to evaluate the growth performance of the fish through feeding trials
3. **MATERIALS AND METHODS**

**2.1. Formulation and Preparation of the Experimental diets**

Four iso-nitrogenous experimental diets with 35% crude protein (CP) recommended for advance tilapia fry (FAO, 2017) were formulated in this experiment by adding different ingredients as per their standard nutrient composition evaluated earlier (Barik, 2021). The control diet was prepared using conventional ingredients like fish meal, GNOC, soya meal, mustard oil cake, corn flour, vitabest and vitamin and mineral mixture. The experimental diets were formulated by replacing 50% GNOC protein with experimental oil cakes e.g., SFOC, LSOC and silage, while keeping all other ingredients constant. All the feeds were fortified with vitamin mineral premix. Accordingly, the different experimental diets were T0: control diet formulated with the conventional feed ingredients; T1: 50% of GNOC protein replaced with SFOC; T2: 50% of GNOC protein replaced with LSOC; T3: 50% of GNOC protein replaced with silage on dry weight basis (Table 1).

To prepare the experimental diets, all the powdered ingredients were weighed accurately one by one as per details given in Table-1 on to a tray. They were then hand mixed properly to get a uniform mixing. Vitabest, the lipid source and silage was then added as per requirement and the feed mix was mixed by repeated rubbing with hand. Then water was added at 25 % of the weight of the feed mix, hand kneaded thoroughly and kept pressed at one corner of the tray for half an hour for proper soaking. Feed mix were then used for preparation of extruder fish feed using instrument parameters as standardized.

**Table 1: Percentage composition of ingredients in the experimental diets**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Ingredients** | **T0** | **T1** | **T2** | **T3** |
| Fish meal | 8 | 8 | 8 | 8 |
| Ground nut oil cake | 40 | 20 | 20 | 20 |
| Mustard oil cake | 10 | 10 | 10 | 10 |
| Soya meal  | 18 | 18 | 18 | 18 |
| Corn flour | 20 | 19 | 16 | 20 |
| SFOC | - | 21 | - | - |
| LSOC | - |  - |  23 | - |
| Silage | - | - | - | 20 |
| Vitabest | 2 | 2 | 2 | 2 |
| Vitamin-mineral mixture | 2 | 2 | 2 | 2 |

**2.2. Feeding experiment**

**2.2.1. Site of the experiment**

This study was carried out in Aquaculture Laboratory of College of Fisheries (OUAT), Rangailunda. The feed preparation and chemical analysis were done in the Aquafeed Laboratory and Nutrition Laboratory of the College, respectably.
**2.2.2. Experimental animal**

GIFT tilapia (*Oreochromis niloticus*) advance fry were used as the experimental animals in this study. About two hundred fifty GIFT tilapia (*Oreochromis niloticus*) advance fry were procured from Govt. Fish Farm, Kausalyaganga Bhubaneswar. The fishes were transported to the laboratory in polythene bags with oxygen supply. They were disinfected using 1 ppm Porassium permanganate solution and released in three fiber re-enforced plastic (FRP) tanks of 500 litre, having clean borewell water. Introduced fishes were kept under aerated condition for two weeks for acclimatization to laboratory condition. During acclimatization period, they were fed with commercial carp feed twice a day up to satiation.

**2.2.3. Experimental set-up**

The experiment was done in 12 no. of FRP tanks of 200 litre capacity for four treatments in triplicate. After cleaning properly the tanks were soaked with potassium permanganate (KMnO4) solution and kept for overnight. On the next day, the tanks were thoroughly washed and filled with clean bore well water for stocking of the experimental fishes.

One hundred twenty fishes of uniform size (average initial weight of 1.2 ± 0.16 *g*) were randomly distributed into four experimental groups (named as T0, T1, T2, T3) in three replicate following a completely randomized design at the rate of ten fishes per tank. The fishes were subjected to 90 days of feeding trial under the provision of aeration for 24 hr except the period of feeding. 25% water from the experimental tank was siphoned out daily to remove uneaten food and fecal matter followed by refilling of same amount of clean bore well water.

**2.2.4. Feeding**

The fishes categorized in to four experimental groups were fed with the designated experimental diet (i.e., T0, T1, T2, T3) for a duration of ninety days. Initially fishes were fed @ 8% of the total biomass and subsequently feeding was adjusted according to the level of consumption. Daily ration was given in two equal parts in the morning at 8 AM and in the evening at 4 PM. The left over feed were siphoned out after 1 h of feeding. The siphoned water was passed through a strainer and dried to find out actual quantity of feed consumed by the experimental fishes. Periodical sampling was done in a three weeks interval to check the survival and growth of the experimental animals and to adjust the feeding ration.

**2.3. Water quality parameters**

Water quality standard of the research tanks like temperature, pH, total alkalinity, DO2 and hardness were estimated following standard methods (APHA, 2018).

**2.3.1. Temperature**

The temperature of the tank water were measured once a week before sunrise and at mid afternoon by using a mercury thermometer and represented in ˚C.
**2.3.2. pH range**

pH of the experimental tank water was measured once a week before and after water exchange by using a digital pH meter (ELICO-120).

**2.3.3. Total alkalinity**

Alkalinity is defined as the ability of water to neutralize acids. The acid base titration of phenolphthalein and methyl orange alkalinity was determined following standard methods (APHA, 2018). In a conical flask, 50 ml of tank water sample was collected, and 2 drops of the phenolphthalein indicator were added. The water turned pink due to the presence of carbonates in the sample. It was then titrated by adding 0.02 N of H2SO4 drop by drop till a pale pink end point was achieved. To the solution, 2 more drops of the methyl orange indicator were added, so that the solution turned yellow and the same process was followed upto orange end point was achieved. The phenolphthalein alkalinity (mg/l) value determines the carbonate content, while total alkalinity (mg/l) was obtained by subtracting the value of phenolphthalein alkalinity from that of the methyl orange alkalinity.

**2.3.4 Total hardness**

Hardness of the water is a measure of the amount of dissolved calcium and magnesium present. In a conical flask, 50 ml of the water sample was obtained, 1 ml of buffer (NH4Cl and conc. NH4OH) was added, and was carefully mixed followed by adding 4 drops of the EBT indicator. After that, a wine red color sample was obtained, which was then titrated against standard EDTA till the solution turns to blue and disappeared again. At that point the end point was noted down. The value of Mg (mg/l) hardness was measured by subtracting the value of Ca hardness (mg/l).
**2.3.5. Dissolved Oxygen**

Winkler's technique was used to calculate the water sample's dissolved oxygen (mg/l) level. The water sample was taken in BOD bottle, and it was fixed by using Manganous Sulphate and alkaline KI solution, so that a precipitation was formed and then conc. H2SO4 was slowly introduced to dissolve the precipitation, followed by titrating it against 0.025 N Sodium Thiosulphate solution till a pale yellow color was obtained. Following the addition of a few drops of starch indicator, the titration was continued by adding drops of thiosulphate solution until the solution turned colourless, at which point the final reading was recorded.

**2.4. Proximate composition**

The biochemical composition of the experimental diets was analyzed by following the standard procedures (AOAC, 2019) which are briefly described as follows:

**2.4.1. Moisture**

Moisture content in a given sample was quantified by drying the sample in a hot air oven at 100±2 °C for 14-16 hour till a constant weight was achieved. The difference in weigh of the sample before and after drying was calculated as the moisture content.

Moisture (%) = $\frac{Initial wt. of sample - Final wt. of sample }{Initial wt.of sample} $x 100

**2.4.2. Crude Protein**

The crude protein (CP) content of the samples was estimated as nitrogen content by micro-Kjeldahl method in KelPlus automatic Nitrogen estimation system (M/s Pelican Instruments, Chennai). In this method, about 0.4 g sample was taken in the Kjeldahl digestion tubes, to which 10 ml of concentrated Sulphuric acid and 3-4 gm of digestion mixture (Copper sulphate and anhydrous Potasium sulphate in the ratio of 1:5) were added. The flasks were then placed in the digestion chamber set at about 410 oC until complete digestion, indicated by clear green coloured solution, is achieved. About 30 ml of distilled water was then added to the digested samples to get a light green coloured solution. Then, the digestion flask containing sample was loaded on the KelPlus automatic distillation unit. Then distillate was collected in 250 ml conical flask containing two drops of mixed indicator. The distillate containing nitrogen as ammonium borate was estimated by titrating against 0.1 N Sulphuric acid. The quantity (ml) of 0.1N Sulphuric acid consumed for titration was recorded. Crude protein content was estimated as

Crude protein (%) = $\frac{Volume of 0.1N sulphuric acid used×0.0014×6.25×100×100}{Volume of aliquot\left(ml\right)x weight of dry sample (g) ×10}$

**2.4.3. Crude fat**

The amount of crude fat present in each sample was calculated as ether extract (EE) by automatic solvent extraction system (SOCS PLUS, M/s Pelican Instruments, Chennai). About 2 g of dry and powdered sample was accurately weighed into an extraction thimble. The thimble fitted with the thimble holder was then loaded into the fat extraction beaker and about 90 ml of petroleum ether was poured. After assembling the unit properly, extraction programme started. The extraction was allowed to continue as per the standardized and set programme. After finishing of the programme the extraction beakers with lipid were taken out and dried in a hot air oven set at 100oC for 1.0 hr. The flasks were then cooled using a desiccator and weighed. Lipid content was calculated as

 Crude fat (%) = $\frac{Weight of flask \left(after-before\right) fat extraction}{Weight of sample (g)}$ x 100

**2.4.4. Ash**

Total ash content was calculated from fat free dried samples taken in quartz crucibles and incinerated in a muffle furnace set for 3 hrs at 600 ˚C. Ash content was estimated as follows :

Ash content (%) = $\frac{Weight of ash (g)}{Weight of sample \left(g\right)}$ x 100

**2.4.5. Crude fibre**

Crude fibre content of experimental diet was estimated after successive treatment with boiling acid and alkali following standard protocol (Nambudiri, 1985). The fibre content (%) was then calculated as follows:

Crude fibre (%) = $\frac{Weight of fibre (g)}{Weight of sample (g)}$ x 100

**2.4.6. Total carbohydrate**

The total carbohydrate expressed as NFE (Nitrogen free extract) was calculated by subtracting the percentage of other nutrients on % dry weight basis from 100.

NFE = 100- {crude protein (%) + crude fat (%)+ crude fibre(%) + Ash (%)}

**2.4.7. Digestible Energy**

The digestible energy value of the experimental diets was calculated on the basis of standard physiological values (Halver, 1976) using the following formula.

Digestible energy (Kcal/ 100 g)= Protein (%) × 4 + lipid (%) × 9 + carbohydrate (%) × 4

**2.5. Assessment of growth performance**

Sampling for assessment of survival and growth was done at 15 days interval and the body length and weight were measured. The weights of experimental fishes were measured in an electronic balance and lengths were measured in a scale. After 90 days of feeding trial following growth parameters were calculated using the standard formulae.

**2.5.1. Daily weight gain (g)**

Daily weight gain (g) = $\frac{Final weight of fish\left(g\right)- Initial weight of fish(g)}{Total no. of experimental days}$

**2.5.2. Percentage weight gain**

Weight gain (%) = $\frac{Final weight of fish(g)-Initial weight of fish(g)}{Initial weight of fish(g)}$ x 100

**2.5.3. Specific growth rate (SGR)**

Specific growth rate = $\frac{(loglog e Final body weight- log e Initial body weight)}{Total no.of experimental days}×100$

**2.6. Assessment of feed efficiency and nutrient retention efficiency**
**2.6.1. Feed conversion ratio (FCR)**

Feed conversion ratio = $\frac{Total dry food intake(g)}{Total live weight gain(g)}$

**2.6.2. Protein efficiency ratio (PER)**

Protein efficiency ratio = $\frac{Total live weight gain (g)}{Total Protein fed (g)}$

**2.7. Statistical analysis**

The data were statistically analysed by statistical package SPSS version 20.0, in which data were subjected to one way ANOVA and Duncan’s Multiple Range Test to quantify the significance differences between mean values (at 5% probability level).

1. **EXPERIMENTAL RESULTS**

**3.1. Water quality parameters**

During the investigation period, the water quality parameters such as temperature (˚C), pH, dissolved oxygen (mg/l), total alkalinity (mg/l), hardness (mg/l) were noted at weekly interval and the average values of all treatments were given in Table-2.

 During the 90 days of feeding trial the average water temperature varied from 22.3˚C to 26.9˚C in the different experimental tanks. However, not much variation in water pH values were noted among the experimental tanks and ranged in between 7.3 to 8.3. Similarly, the dissolved oxygen concentration of water varied between 5.3 to 8.1 mg/l. The total hardness and alkalinity of all experimental tanks were also recorded within the range of 124 to 145 mg/l and 120 to 145 mg/l, respectively.

**Table-2:** Range of water quality parameters of the experimental tanks

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameters** | **T0** | **T1** | **T2** | **T3** |
| Temperature (0C) | 22.3-26.8 | 22.9-26.2 | 22.5-26.9 | 21.6-26.5 |
| pH | 7.8-8.2 | 7.7-8.3 | 7.3-8.1 | 7.4-8.3 |
| DO2(mg/l) | 5.5-7.2 | 5.3-8.1 | 5.5-7.2 | 6.0-7.9 |
| Total alkalinity (mg/l) | 120-142 | 118-146 | 125-145 | 120-136 |
| Hardness (mg/l) | 128-141 | 130-142 | 138-145 | 124-137 |

**3.2. Proximate composition of experimental diets**

Proximate compositions (on % dry weight basis and wet weight basis) of four experimental diets (e.g., T0, T1, T2, T3) were analyzed and the result is given in Table 3A & 3B. The moisture (%) of the experimental diets varied from 5.34 ± 0.007% to 6.98 ± 0.03%. The crude protein (%) as % of dry weight basis varied from 34.31 ± 0.11% to 34.75 ± 0.10%. The ether extract (%) and total carbohydrate (%) ranged from 7.11 ± 0.28% to 8.13 ± 0.36% and 33.44 ± 0.58% to 41.34 ± 0.77%, respectively in the experimental diets. The total ash and crude fibre contents were ranged from 11.73 ± 0.45% to 15.50 ± 0.20% and 6.05 ± 0.23% to 7.56 ± 0.21% respectively. The digestible energy (kcal/ 100g) of the diets was observed to be in the range of 363.17 ± 0.92 and 389.497± 0.1.74.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatments** | **Moisture** | **Total Dry matter** | **Parameters (as % of dry matter)** | **Digestible energy (Kcal/ 100g)** |
| **Crude protein** | **Crude fat** | **NFE** | **Crude fibre** | **Total ash** |
| **T0** | 5.54 ± 0.38  | 94.45 ± 0.38  | 34.31 ± 0.11 | 7.88 ± 0.17 | 38.39 ± 0.48  | 6.10 ± 0.34 | 13.32 ± 0.28 | 367.25±0.43 |
| **T1** | 6.50 ± 0.16  | 93.49 ± 0.16  | 34.36 ± 0.12 | 7.11 ±0.28 | 35.44 ± 0.58  | 7.59 ± 0.21 | 15.50 ± 0.20 | 363.17±0.92 |
| **T2** | 5.34 ± 0.07  | 94.66 ± 0.07  | 34.32 ± 0.11 | 7.5 ± 0.27 | 36.59 ± 0.39  | 7.26 ± 0.47 | 14.33 ± 0.08 | 363.57±1.20 |
| **T3** | 6.98 ± 0.03  | 93.02 ± 0.03  | 34.75 ± 0.10 | 8.13 ± 0.36 | 39.34± 0.77  | 6.05 ± 0.23 | 11.73 ± 0.45 | 389.49±1.74 |

**Table 3A:** Proximate composition of the experimental diets

**Table 3B:** Proximate composition (%) of the experimental diets (on wet weight basis)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | **Moisture** | **Crude protein** | **Crude fat** | **NFE** | **Crude fibre** | **Total ash** |
| **T0** | 5.54 ± 0.38 | 33.55 ± 0.72 | 7.49 ± 0.53 | 37.15 ± 0.15 | 5.77 ± 0.43 | 12.60 ± 0.13 |
| **T1** | 6.50 ± 0.16 | 33.29 ± 0.39 | 7.39 ± 0.39 | 31.30 ± 0.71 | 7.10 ± 0.33 | 14.51 ± 0.57 |
| **T2** | 5.34 ± 0.07 | 33.62 ± 0.42 | 7.46 ± 0.76 | 34.21 ± 0.56 | 6.88 ± 0.25 | 13.58 ± 0.69 |
| **T3** | 6.98 ± 0.03 | 33.50 ± 0.25 | 7.57 ± 0.41 | 38.50 ± 0.25 | 5.63 ± 0.27 | 10.93 ± 0.21 |

**3.3 Growth parameters**
**3.3.1 Body weight gain**

Body weight gain of tilapia advance fry in different treatment groups measured at three week interval during the experimental period has been given in Table 4. The initial and final body weight of different experimental groups is represented in the Fig.1. The average initial body weight of the experimental animals varied from 1.161 ± 0.214 (T3 group) to 1.256 ± 0.273 (T0). The final body weight varied from 5.101 ± 1.260 (T2 group) to 6.661 ± 1.602 g(T3 group). Hence, significantly (P<0.05) the highest average body weight gain after 90 days of feeding trial was found in T3 group and lowest was found in T2 group.

**Fig 1:** Initial and final body weight gain of GIFT tilapia with different diets

**3.3.2 Body length gain**

Average body length gains of different experimental groups measured at three week interval have been presented in Table 5. Initial body length among the experimental groups varied from 4.223 ± 0.327 cm (T3 group) to 4.553 ± 0.231 cm (T0 group). The final body length varied from 6.227 ± 0.355 cm (T2 group) to 7.058 ± 0.406 cm (T3 group).

**Table 4:** Average weight gain (g) and survival (%) of GIFT tilapia advance fry fed with different experimental diets

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Treatments | Initial | 1st sampling | 2nd sampling | 3rd sampling | 4th sampling | Survival (%) |
| T0 | 1.256 ± 0.273 | 1.852 ± 0.387 | 2.749 ± 0.603 | 3.901 ± 1.018 | 5.566 ± 1.570 | 95 |
| T1 | 1.238 ± 0.257 | 1.837 ± 0.495 | 2.783 ± 0.902 | 3.921 ± 1.165 | 5.519 ± 1.451 | 100 |
| T2 | 1.161 ± 0.214 | 1.778 ± 0.438 | 2.857 ± 0.733 | 3.878 ± 1.215 | 5.101 ± 1.260 | 100 |
| T3 | 1.160 ± 0.199 | 1.902 ± 0.408 | 3.133 ± 0.876 | 4.494 ± 1.178 | 6.661 ± 1.602 | 85 |

\* “±” indicates the standard deviation

**Table5:** Average body length gain (cm) of GIFT tilapia advance fry fed with different experimental diets

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatments | Initial | 1st sampling | 2nd sampling  | 3rd sampling  | 4th sampling  |
| T0 | 4.553 ± 0.231 | 4.835 ± 0.548 | 5.413 ± 0.247 | 5.749 ± 0.426 | 6.604 ± 0.349 |
| T1 | 4.432 ± 0.153 | 4.809 ± 0.177 | 5.485 ± 0.335 | 5.752 ± 0.358 | 6.601 ± 0.532 |
| T2 | 4.237 ± 0.342 | 4.759 ± 0.660 | 5.503 ± 0.146 | 5.716 ± 0.217 | 6.227 ± 0.355 |
| T3 | 4.223 ± 0.327 | 5.116 ± 0.633 | 5.597 ± 0.430 | 6.249 ± 0.571 | 7.058 ± 0.406  |

\* “±” indicates the standard deviation

**3.3.3. Percentage body weight gain**

Body weight gain of the experimental animals calculated in percentage is expressed in Table 6 and Fig 2. Analysis of variance (ANOVA) of data specified significant effect (P<0.05) of different experimental diets on percentage body weight gain of the experimental animals. Significantly (P<0.05) highest percentage body weight gain (474.2 %) was found in T3 group, where 50% of GNOC protein had been substituted by fish silage. There is no significant (P>0.05) difference in percentage body weight gain in between T0 and T1 group. The lowest weight gain percentage was found in T2 group (339.4 %).

**Fig 2:** Percentage weight gain of tilapia fry fed with different diet.

**3.3.4. Daily weight gain**

Daily weight gain (DWG) of the treatment groups fed with different experimental diets was calculated at the end the feeding trial and is presented in Table 6. Highest daily weight gain (0.092g) was found in T3 group, fed a diet where 50% of GNOC protein had been replaced by fish silage followed by T0 (0.072 g) and T1 (0.071 g) group, respectively.. Lowest DWG was found in T2 (0.066 g).

**3.3.5. Specific growth rate**

Specific growth rates (SGR) of different groups after completion of the feeding trial are shown in Table 6 and Fig. 3. The result showed significant (P<0.05) difference among the experimental groups. SGR showed the same trend as that of the weight gain percentage. Significantly (P<0.05) highest SGR (2.91) was observed in T3 group, where 50% of GNOC protein had been replaced by fish based silage. The lowest SGR was found in T2 (02.47)group.

Fig 3: Specific growth rate of tilapia fry fed with different diets

**3.3.6. Survival (%)**

During the course of feeding trial, the survivability (%) of tilapia advance fry in different treatment groups were found to be ranged in between 85 to 100%.

**3.4. Feed efficiency indices**

**3.4.1. Feed conversion ratio**

Effect of experimental diets on feed conversion ratio (FCR) of the different experimental groups after finishing the feeding trial is given in Table 6 and Fig 4. The average values of FCR varied significantly (P<0.05) among different experimental groups. A significantly (P<0.05) better FCR was recorded in T3 group (2.27 : 1) fed with the experimental diet, where 50% of GNOC protein was replaced with fish based silage, followed by T0 (2.68: 1) group fed with the conrol diet. Significantly (P<0.05) highest FCR (3.09: 1) was observed in T3 group. No significant (P>0.05) difference in FCR value was found in between the group T0 and T1.

**Fig 4:** Feed conversion ratio of different experimental diets

**3.4.2. Protein efficiency ratio**

The protein efficiency ratio (PER) values of different experimental groups has been given in Table 6. Analysis of the data indicated significant difference (P<0.05) among the experimental groups. Significantly (P<0.05) highest PER value (1.26) was recorded in T3 group followed by T0 group (1.06) and T1 group (1.05), respectively. No significant difference (P>0.05) was reported in between T0 group and T1 group. The experimental groups T2 (0.96) showed significantly lowest protein efficiency ratio.

**Table 6 :** Growth performance and feed efficiency parameters-

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameters** | **T0** | **T1** | **T2** | **T3** |
| Initial weight (g) | 1.256 ± 0.273 | 1.238 ± 0.257 | 1.161 ± 0.214 | 1.160 ± 0.199 |
| Final weight (g) | 5.566 ± 1.570 | 5.519 ± 1.451 | 5.101 ± 1.260 | 6.661 ± 1.602 |
| Weight gain (g) | 4.310b ± 1.223 | 4.281b ± 1.198 | 3.940c ± 1.046 | 5.501a ± 1.401 |
| Weight gain (%) | 343.2b ± 9.17 | 345.8b ± 20.6 | 339.4b ± 5.45 | 474.2a ± 24.6 |
| Daily weight gain  | 0.072 | 0.071 | 0.066 | 0.092 |
| SGR (%) \* | 2.48b ± 0.02 | 2.49b ± 0.06 | 2.47b ± 0.01 | 2.91a ± 0.06 |
| Total feed fed (g) | 11.57 ± 0.13 | 11.94 ± 0.13 | 12.17 ± 0.15 | 12.5 ± 0.13 |
| FCR \* | 2.68b ± 0.10 | 2.79b ± 0.29 | 3.09a ± 0.08 | 2.27c ± 0.23 |
| Protein fed (g) | 4.05 ± 0.05 | 4.06 ± 0.04 | 4.11 ± 0.05 | 4.38 ± 0.04 |
| PER \* | 1.06b ± 0.07 | 1.05b ± 0.08 | 0.96c ± 0.08 | 1.26a ± 0.09 |

SGR\*- Specific growth rate; FCR\*- Feed conversion ratio; PER\*- Protein efficiency ratio

# - Mean values sharing a common superscript did not differ significantly (P>0.05)

1. **DISCUSSION**

Feeding fish accounts for the highest cost in intensive and semi-intensive fish culture operation contributing to about 50-70% of the total recurring cost (Dayal *et al*., 2011). Increasing cost of the conventional feed ingredients, like fish meal and groundnut oil cake (GNOC), therefore remains the greatest challenge for the feed industry to produce cost effective diet. Because of its better nutrient and feed processing characteristics, GNOC is in great demand and hence the price is also escalating at a faster pace (Omoregie *et al*., 2014). Therefore, to make affordable and cost-effective diets, suitable alternative protein source to replace the GNOC, at least partially, is the demand of time. In this context, three non-conventional sources of protein such as sunflower oil cake (SFOC), linseed oil cake (LSOC) and fish dressing based silage have been used in this study by replacing 50% GNOC to develop cost effective diet for GIFT tilapia advance fry.

**4.1. Feeding trial experiment**

**4.2.1. Physico-Chemical Parameters of water**

The various water quality parameter such as temperature (0C), pH, dissolved oxygen (mg/l), total alkalinity (mg/l), hardness (mg/l) of different experimental tanks ranged from 23.30C to 28.9 0C, 7.4 to 8.6, 5.3 to 8.4 mg/l, 120 to 143 mg/l and 124 to 145 mg /l, respectively during the 90 days of feeding trial (Table 2). The observations indicated that the parameters of all the experimental tanks were well within the ideal range, providing a suitable environment for growth of a warm water fish (Saha and Ray, 2011; Ayyappan, 2011; Choudhary, 2002) and hence, not considered as a factor to affect the result of the experiment.

**4.2.2. Proximate composition of experimental diets**

“For feeding of GIFT tilapia fry, four isonitrogenous and isocaloric diets were prepared using the various conventional and non-conventional feed ingredients. After formulation (Table 1) of experimental diets proximate composition of these were found to be containing 34.31 ± 0.11% to 34.75 ± 0.10% CP, 7.11 ± 0.28% to 8.13 ± 0.36% EE, 6.05 ± 0.23% to 7.59 ± 0.21% crude fibre, 33.44 ± 0.58% to 41.34 ± 0.77% total carbohydrate, 11.73 ± 0.45 % to 15.50 ± 0.22% ash and 363.17 ± 0.92 to 389.49 ± 174 Kcal/100g digestible energy (Table-3 & 3(A)). Yaw (2014) has reported that maximum growth of tilapia fry under laboratory condition occurred at dietary protein content about 38%, but economically 35% of dietary protein content had been reported as optimum for growth of tilapia” (FAO, 2017). Mishra and Samantaray (2004) suggested that lipid requirement of fish is temperature dependent. The lipid requirement for tilapia fry at 21˚C was 6%, where as this requirement increases to 9% at 31˚C. Hence the experimental diets prepared in the present study can fulfil the lipid requirement of GIFT tilapia(*Oreochromis niloticus*) when the temperature of tank water ranged between 21.60C to 26.9 0C (Table 2).

**4.2.3 Growth performance of experimental animals**

The growth performance of the Tilapia advance fry segregated into four treatments and fed with four different experimental diets differed significantly (p<0.05) after ninety days of feeding trials (Table-4). The growth performance comprising of parameters like weight gain, percentage weight gain and specific growth rate was significantly (P<0.05) highest in the experimental group T3 (fed with the diet, where 50% of the GNOC protein was replaced by fish based silage, followed by T1 group (fed with the diet, where 50% of the GNOC protein was replaced with SFOC). This was followed by the T0 control group and T2 group (fed with diet where 50% of the GNOC protein was replaced with LSOC) (Table- 4). Better weight gain was reported in *Cyprinus carpio* with raw Sunflower meal than the diet containing soybean meal due to absence of some growth inhibitors in Sunflower meal (Khan *et al*., 2003). Significantly (P<0.05) better weigh gain and SGR has also been reported by Mahanty (2021) when GNOC was partially replaced by raw SFOC. Rehman *et al*. (2013) have reported that fish based silage and SFOC can be effectively incorporated in the feed formulation for fingerlings of *Labeo rohita* without any adverse affect on the weight gain percentage and SGR. Hanafy (2006) suggested that soybean meal can be completely replaced by LSOC with supplementation of *Yucca schidigera* in Nile tilapia.

In the present investigation, significantly (p<0.05) highest SGR and weight gain percentage was obtained in T3 group, which shows similarity with the research finding of Mukhopadhyay and Ray (2001) and Kamei *et al.* (2018), who fed Tilapia fingerlings with the diet containing 50% and 75% of the fish meal protein replaced with fish based silage.

At the same time the experimental group T2 (50% GNOC protein was replaced with raw LSOC) showed reduced growth performance including weight gain and specific growth rate (SGR) as compared to the control group (T0), though they are not significantly (P>0.05) different. This result is in agreement with the findings of Mukhopadhyay and Ray (1999), who concluded that the growth of tilapia fingerlings was declined due to the incorporation of raw LSOC by replacement of fish meal at 40%. Such a reduced growth rate of experimental fish with incorporation of raw LSOC may attributed be to its high amount of tannin content and fibre content (Edwards *et al*., 1985). Mahanty,(2021) has recommended to use solid state fermentation technology for amelioration anti-nutritional factors on raw LSOC, so that significantly (P<0.05) better growth rate can be achieved.

**4.3.4 Feed efficiency and nutrient retention parameters**

Feed efficiency and nutrient retentionparameters; including FCR, PER; differed significantly (P<0.05) among the experimental diets (Fig. 4 and Table 6). Significantly (P<0.05) lowest, i.e., the best, FCR was estimated in T3 (2.27 ± 0.031: 1) group followed by T0 (2.68 ± 0.016: 1) group. No significant (P>0.05) difference in FCR was noted among T1 (2.79 ± 0.043: 1) and T0 (2.68 ± 0.039: 1) group. Silage based experimental diets (T3), resulted in significantly (P<0.05) better FCR than the control (T0) group. A similar trend was observed in case of PER after 90 days of feeding trial in Tilapia advance fry. The study indicated significantly (P<0.05) better feed efficiency and nutrient efficiency parameters in T3 diet (where 50% GNOC protein had been replaced fish based silage) followed by T0 control diet. The findings of this study matches well with the findings of Nayak *et al,.* (2017), who has reported significantly (P<0.05) better FCR (2.052 ± 0.002: 1) and PER (1.603 ± 0.003) in Tilapiafed with the diet containing 39% fish based silage. Kamei (2018) and Rana (2019) also lad reported in the similar line when 75% of fish meal protein was replaced with silage based blended protein, it resulted in 126% better growth performance and feed efficiency parameters. Despite of high oil content in silage, it was proved as a good protein source after heat processed due to its good amino acid profile (Nayak *et al.,* 2017). Lowest PER (0.96 ± 0.010) were found in the experimental diet T2 (where 50% GNOC protein had been replaced with raw LSOC). The findings of this study is in agreement with Santos *et al.* (2009), who have reported significantly lowerest feed conversion efficiency in 45% LSOC inclusion in diet of Nile tilapia. Similarly, Mukhopadhyay and Ray (1999) reported reduced PER (1.09 ± 0.11) with inclusion of 30% raw LSOC in the diet of *Labeo rohita*. Olude *et al.* (2008) also found decline in growth rate of Nile tilapia with increase in inclusion of LSOC in the diet.

**CONCLUSION**

The purpose of this research was to find promising alternative ingredients and result concluded that SFOC can be used to partially replace GNOC in floating fish feed without significantly (P<0.05) affecting the growth performance of GIFT tilapia, where as LSOC incorporation had a negative impact on the growth performance and feed efficiency parameters. However, fish silage when incorporated in the GIFT tilapia diet by partially replacing GNOC, it resulted in significantly higher growth rate (474.2 % weight gain) and better feed efficiency parameters of 2.27 FCR and 1.26 PER. Hence, fish silage and SFOC are promising alternative ingredients to partially replace expensive GNOC for formulation of cost effective feed for better growth and feed efficiency of GIFT tilapia.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2.

3.

**8. REFERENCES**

Akdogan H. 1999. High moisture food extrusion. *International Journal of Food Science & Technology*, **34**(3), 195–207.

Alam MS, Kaur J, Khaira H and Gupta K. 2016. Extrusion and extruded products: Changes in quality attributes as affected by extrusion process parameters: *A review. Critical Reviews in Food Science and Nutrition*, **56**(3), 445–473.

Alves RML, Grossmann MVE and Silva RSSF. 1999. Gelling properties of extruded yam (*Dioscorea alata*) starch. *Food Chemistry*, **67**(2), 123–127.

Amalraaj S. 2010. Extruded floating fish feed a boon for farmers. *Online Publication The Hindu: Sci Tech / Agriculture*.

AOAC. 2019. AOAC Official Methods of Analysis. 21st Edn., *Association of Official Analytical Chemists*, Washington DC, USA.

APHA. 2018. Standard Methods for the Examination of Water and Waste water, 23rd Edn., *American Public Health Association*, AWWA, WPCE, Washington DC, USA.

Balarin JD and Haller RD. 1982. The Intensive culture of tilapia in tanks, receiving cases in recent advances in aquaculture. (Eds. J. F. Mur, R.K Roberts), *Lonon Croon Heln Ltd*, 260-360.

Barman BK and Karim M. 2007. Analysis of feeds and fertilizers for sustainable aquaculture development in India, *Food and Agriculture Organisation, Rome*, 113-140.

Dayal JS, Rajaram V, Ambasankar K and Ali SA. 2011. Sunflower oil cake as a replacement for fish meal in feeds of Tiger shrimp, *Penaeus* *monodon* reared in tank and in net cages, *Indian Journal of Geo-Marine Science*, **40**: 460-70.

Declercq DR. 2006. Quality of Western Canadian flaxseed, Canadian Grain Commission. [*www.graincanada.gc.ca*](http://www.graincanada.gc.ca).

Edwards P, Kamal M and Wee KL. 1985. Incorporation of composted and dried water hyacinth in pelleted feed for the tilapia *Oreochromis niloticus* (Peters), *Aquaculture & Fishery Management*, **1**:233-248.

FAO 2017. National aquaculture overview: Indian country profile fact sheets, FAO. Fisheries and Aquaculture Department, FAO, Rome.

Fitzsimmons K. 2006. Prospect and potential for global production In Tilapia: Biology, Culture, and Nutrition; Lim, C., Webster, C., Eds.; *Harworth Press*: Binghamton, NY, USA; pp. 51–72.

Garg SK, Alok K and Bhatnagar A. 2003. Oilcakes as protein sources in supplementary diets for the growth of (*Cirrihinus mrigala)* fingerlings: laboratory and field studies, *Bioresource Technology*, **86**: 283–291.

Halver JE. 1976. Formulating practical diet for fish, *Journal of the Fisheries Research*
*Board of Canada*, **33**: 1032-1039.

Hasan MR, Hecht T, De silva SS and Tacon AGJ. 2007. Study and analysis of feeds and fertilisers for sustainable aquaculture development, FAO Fisheries Technical Paper No. 49, pp. 510, *Food and Agriculture Organisation, Rome*.

Ilo S, Schoenlechner R, and Berghofe E. 2000. Role of lipids in the extrusion cooking processes. *Grasasy Aceites*, **51**(1–2): 97–110.

Kamei, M., **Sahu, B**., Raman, S., Nanda, S., Choudhury, D. and Dorothy, M.S., 2018. Use of fish silage based blended protein source for replacement of fish meal in Thai-Pangas diet. *Int. J. Curr. Microbiol. App. Sci., 7*(10): 2949-61

Kearns J. 1989. Key points in extruding fish feeds. Feed international, Nov. 9. Cited by Kerry W. T. et al. (1996), *Aquaculture Engineering* Vol. **15** (1): 53-65.

Lenka S, Giri SS and Paul BN. 2010. Nutrient digestibility and gastro-intestinal enzyme activity of *Cyprinus carpio* fingerlings fed water washed neem seed cake incorporated diets, *Indian Journal of Animal Science*, **80**: 96-100.

Manomaitis L. 2009. Improving South-east Asian aquaculture through feeds and technology, In: Proceeding of the 17th Annual ASAIM SEA Feed Technology and Nutrition Workshop, Vietnam, pp. 15-19.

Mukhopadhyay N and Ray AK. 2001. Effects of amino acid supplementation on the nutritive quality of fermented linseed meal protein in the diets for rohu (*Labeo rohita). International journal of fisheries and Aquatic studies,*51-67*.*

Munguti JM, Kim JD, Ogello EO. 2014. An overview of Kenyan aquaculture: Current status, challenges, and opportunities for future development. *Fisheries and Aquatic Sciences*, **17**(1): 1–11.

Nayak AP, Misra SK, Nanda S, Mohanta KN, Satpathy D, Mishra RN and Siddaiah GM. 2017. Evaluation of nutritionally enhanced feed through microbial fermentation on growth and survival of fingerlings of (*Labeo rohita)* (Hamilton), *International journal of fisheries and Aquatic studies,* **5**(4): 458-462.

Nikmaram N, Kamani MH and Ghalavand R. 2015. The effects of extrusion cooking on antinutritional factors, chemical propertiesand contaminating microorganisms of food. *International Journal of Farming and Allied Sciences*, **4**(4): 352–354.

Odunayo O and Ajiboye O. 2020. Optimization of Heat and Mass Transfer Process in Single Screw Floating Fish Feed Extruder, *Journal of Engineering Research and Reports,* **15***(1): 34-43.*

Ojo S, Olukunle O and Aduewa T. 2014. Performance Evaluation of Floating Fish Feed Extruder, *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS),* Volume 7, Issue 12 Ver. II (Dec. 2014), PP 103-113.

Omoregie E, Idoko DI and Ojobe TO. 2004. Replacement of groundnut meal by cottonseed meal in diets for the toothed carp (*Aphyosemion gairdneri*): Growth and feed utilisation, *Discovery and innovation*, **16**(1/2): 26-31.

Pullin R. 1985. Tilapia: Everyman’s fish. *Biologist* 1985, **32**: 84–88.

Rehman T, Asad F, Qureshi NA and Iqbal S. 2013. Effect of plant ingredients (Soybean and Sunflower meal) on growth and body composition *of Labeo rohita, American Journal of Life science*, **1**(3): 125-129.

Saha S, Ray AK. 2011. Evaluation of nutritive value of water hyacinth (*Eichhornia crassipes*) leaf meal in compound diets for rohu, *Labeo rohita* (Hamilton, 1822) fingerlings after fermentation with two bacterial strains isolated from
fish gut, *Turkish Journal of Fisheries and Aquatic Sciences*, **11**:199-207.

Sorensen M. 2012. A review of the effects of ingredient composition and processing conditions on the physical qualities of extruded highenergy fish feed as measured by prevailing methods. *Aquaculture Nutrition*, **18**(3): 233–248.

Tsevis AA and Azzaydi TA. 2000. Effect of feeding regime on selected species of fish. *Article publication of FISON*, Feb., 2000, Agora.