To standardise parameters for preparation of extruded feed using various conventional and non-conventional ingredients

ABSTRACT

The purpose of this research was to optimise extruder parameters for the preparation of floating fish diets with appropriate physicochemical qualities. The impact of temperature (90-120^{o}C), moisture content (15%-30%), die diameter (2.0-4.0 mm) and pre-conditioning duration (15-30 m) on physical properties of the pellets such as bulk density, floatability, expansion ratio and water stability, were examined. 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 (T_0) by sunflower oilcake (SFOC) (T_1), by linseed oilcake (LSOC) (T_2) and by fish based silage (T_3) protein. The pellets extruded using a combination of 120 ^{o}C extruder barrel temperature, 25% moisture content of feed mix, 2.0 mm die diameter and 30 minute of pre-conditioning duration gave most required pellet floatability (90-100%), expansion ratio (1.43-1.61), water stability (95-99%) and bulk density (0.8 - 0.9 g/cm^3) for T_1 and T_2. However, fish silage when incorporated in the feed mix, it affected negatively in all the physical characteristics of a floating feed and resulted in higher bulk density and lower floatability, expansion ratio and also water stability.

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

1. INTRODUCTION

Aquaculture accounts for almost half of all fish consumed by humans beings worldwide (Halden, *el 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, which limits small rural household participation, and also threatens the profitability and sustainability of aquaculture (Munguti *et al.,* 2014). Besides that the availability of ready-to-use or manufactured aqua-feeds is not widespread in rural areas (Ngugi *et al.,* 2007). Hence, production of feed is an important factor to be considered in both subsistence and commercial fish farming, as it has consequences on both growth efficiency and resources utilisation (Tsevis *et al.,* 2000).

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Protein is utilized for body growth, tissue repair, maintenance and other metabolic activities, besides being used to provide energy (Gan *et al.,* 2012; Yang *et al.,* 2002). Besides, the protein source must be capable enough to meet the amino acid requirement of the fish both quantitatively and qualitatively. Hence, in case of aqua feed, protein is the most important and expensive component to be incorporated. Accordingly, the cost of protein source accounts for about 60% of the total cost of all the ingredients taken together (Arruda *et al.,* 2009). It is therefore important to consider the source of protein selected for use as a feed ingredient.

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. 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.

Extrusion parameters such as feed composition, pre-conditioning, extruder temperatures, moisture, screw speed, and die diameter all have an impact on extrudate properties (Sorensen, 2012). Protein-rich components generate a plastic melt in the extruder barrel, which gives extrudes into a porous pellets with little expansion, whereas starch rich ingredients make a moderately elastic melt in the extruder barrel, resulting in expanded pellets with lower bulk density (Alve et al., 1999). Fat and fibre change the viscosity as well as behaviour of the melting feed in the barrel (Ilo et al., 2000). The material is made ready for extrusion by feed preconditioning. Die diameter controls melt flow and help the extrusion barrel create pressure (Akdogan, 1999). In order to acquire desired product quality, it is necessary to understand the relationship between components and processing variables on the

Commented [2]: In some references et al is written in italics and in another it is in normal. Please follow the any one reference style features of extruded pellets made from fish feed mix with locally available ingredients using a laboratory model twin screw extruder.

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 standardise the parameters for preparation of extruded fish feed
2) to prepare few extruded fish feed for GIFT tilapia (*Oreochromis niloticus*) using various cost effective feed ingredients

2. MATERIALS AND METHODS

This research was performed with a prime aim to standardize the parameters for preparation of extruded fish feed. Next part of the experiment aimed to develop extruded fish feed pellets using locally available cost effective feed ingredients for GIFT tilapia (*Oreochromis niloticus*). It was then aimed to conduct a feeding trial to determine the performance of such feeds on the basis of growth performance and feed efficiency parameters. The details of experimental setup, feed formulation, tests and analysis done and other materials and methods used have been described in this chapter

2.1. Standardization of parameters for preparation of extruded fish feed

2.1.1. Twin screw extruder

In this experiment, a laboratory type twin screw extruder (BPTL, Kolkata, West Bengal, India) with a process capability of 5-15 kg/hr was employed. Extruding system was made up of twin screws and a deblocking mechanism. The screw spinning speed, feeder channel speed, and other parameters can be adjusted via the control panel. Heat was regulated at the meter zone by band heaters installed on the extruder barrel. Die assembly was made up of replaceable circular discs, having central holes drilled to the disc and measuring 2, 3, and 4 mm in diameter, respectively.

2.1.2. Standardization of the extrusion process

Standardization of the parameter of twin screw extruder was done by varying one parameter at a time keeping others constant and use as many trial as possible to reach at the successful result. Four independent variables e.g., extrusion temperature, moisture, die-diameter, and conditioning duration was used for the standardization……

The feed mix prepared as per the formula was mixed with varying moisture content of 10, 15, 20 and 25% followed by blending and allowed for preconditioning for 15, 20, 25 and 30 min. The mixed ingredients were put into the feeder section manually. The feeder speed was set as per requirement from the control panel and the feed mix was channeled to barrel. Various heater temperature attempted were 90, 100, 110 or 120^{o}C, by setting at the control panel of extrusion machine. The extrudate was forced to exit through three different dies of 4.0, 3.0, or 2.0 mm die diameter. Selection of such feed pre-conditioning durations, extrusion temperatures, moisture, and die dimensions was selected as per recommendation of Ojo et al (2010). Pellets produced were then dried in hot air oven set at 60 ± 5^{o}C till moisture content was reduced to below 10%. The dried feed pellets were taken out of the oven and packed in dry air-tight containers, labeled suitably for analysis of their bulk density. The particular value of each parameter showing the lowest bulk density was selected for preparation of the experimental diets.

2.1.3. Selection of ingredient composition

With an aim to prepare cost effective extruded fish feed, attempt was made to study the effect of using low cost non-conventional feed ingredients by partially replacing costly groundnut oil cake (GNOC). Three number of non-conventional fish feed ingredients e.g., sunflower oil cake (SFOC), linseed oilcake (LSOC) and fish silage were used as the alternative protein source by replacing 50% GNOC to prepare the experimental diets for GIFT tilapia (*Oreochromis niloticus*) fry and their physical quality as well as nutritional quality was studied.

2.1.4 Procurement and processing of feed ingredients

Based on the basic dietary needs for GIFT tilapia (Oreochromis niloticus) fry, the feed composition was employed in this study. Conventional feed ingredients like fish meal, ground nut oil cake (GNOC), soya meal, mustard oil cake were used as the protein source along with other common ingredients for preparation of the control diet. Non-conventional feed ingredients like sunflower oil cake (SFOC), linseed oilcake (LSOC) and fish silage were used as the alternative protein source by replacing 50% GNOC to prepare the experimental diets.

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The fish meal prepared in Fish Nutrition Laboratory, College of Fisheries, Rangailunda in dry rendering method following standard protocol (Gopakumar, 1997). Briefly, fresh lesser Sardine, ribbon fish and anchovy were collected from Aryapalli fish landing centre, Gopalpur port. The fishes were cleaned properly under running tap water to remove the sand particles, then eviscerated and dried in hot air oven at 60 ± 5°C for 20 hours. After proper drying, the fishes were made into powder form in a pulveriser to get fish meal.

Silage was prepared in the Laboratory using fresh fish dressing waste collected from nearby Korapalli fish market. The dressing waste comprised of gut, gill, skin and fin etc. were washed under running tap water to remove the adhering sand and dirt. It was weighed and formic acid was added at 3.5% level for complete decomposition to a form of viscous fluid. The mixture was stirred once a day upto two week to get uniform liquid indicating the complete digestion. Excess oil present in the silage was extracted to get refined silage with the help of a table top centrifuge (REMI, R-8C BL).

Other ingredients such as GNOC, soyabean meal. mustard oil cake, SFOC and LSOC, were procured from the local market and were sun dried properly to remove excess moisture content followed by grinding to get their powder form and stored in air tied container. Corn flour was used as the binder; Vitabest (sunflower oil : cod liver oil::1:1) served as the lipid source and a commercially available vitamin and mineral mixture (a product of M/s Virbac Animal Health India Pvt. Ltd) was procured from local market.

2.2. 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 T_0: control diet formulated with the conventional feed ingredients; T_1: 50% of GNOC protein replaced with SFOC; T_2: 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 above.

Table 1: Percentage composition of ingredients in the experimental diets

Ingredients	**T_0**	**T_1**	**T_2**	**T_3**
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

2.3. Assessment of physical quality of the extruded feed

The experimental diets prepared as above were subjected to various tests for assessing the physical quality of extruded feed e.g., expansion ratio, bulk density, floatability, and water stability.

2.3.1. Determination of expansion ratio

The expansion ratio (ER) was measured according to Tumuluru (2013). For each sample, the diameter (D) of 10 pellets was measured at random, and the average value was calculated using a vernier caliper. The expansion ratio was determined by applying the formula D square/Di square, where Di is the die diameter.

2.3.2. Estimation of bulk density

The extruded pellets that had been produced in this way were then milled in a lab-grade grinder and pass through a strainer with a 1 mm orifice. A clean and dry 100 ml graduated measuring cylinder was gently filled with 100 g of the feed powder. The sample filled cylinder was softly taped in the bottom due to this sample volume was gradually decreased. Calculating the bulk density (BD) required dividing the sample weight by the volume (g/ml).

2.3.3. Estimation of floatability

Twenty random selected pellets were put in 250 mL glass beaker with 200 mL of distilled water at room temperature. This was done in three replication, and the average quantity of floating pellets after 20 minutes was noted. Floatability is estimated by the total number of pellets observed floated after 20 min divided by the number initially introduced to the water was multiplied by 100 (Umar, et al., 2013).

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2.3.4. Determination of water stability

The method described by (Umar et al. 2013) was used to assess the water stability. The feed pellets of four gram sample taken in duplicate, were weighed and put on a screen with a wire mesh size of 0.5 mm. The sample-containing screen was dip in a 200 ml glass beaker containing 150 ml of water for 25 minutes. The sample that was still on the sieve mess was then dried in a hot-air oven for 20 hours (100^{o}C). The weight of sample left in wire mesh was compared to the initial to estimate the water stability in a percentage.

2.4 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.

2.5. Proximate composition

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

2.5.1. 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.

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

2.5.2. 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 °C 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 (%) =

$$\frac{Volume\ of\ 0.1N\ sulphuric\ acid\ used \times 0.0014 \times 6.25 \times 100 \times 100}{Volume\ of\ aliquot(ml)\, x\ weight\ of\ dry\ sample\ (g) \times 10}$$

2.5.3. 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 100°C for 1.0 hr. The flasks were then cooled using a desiccator and weighed. Lipid content was calculated as

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

2.5.4. 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 :

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

2.5.5. 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:

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

2.5.6. 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 (%)}

2.5.7. 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

2.6. 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).

3. EXPERIMENTAL RESULTS

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The present experiment was conducted to standardize twin screw extruder for development of extruded floating fish feed from various locally available oil cakes such as Sunflower oil cake (SFOC), Linseed oil cake (LSOC) and fish silage. Also this experiment was carried out to know the efficacy of incorporation of these non-conventional feed ingredients in the diets of GIFT tilapia advance fry as expressed through their growth performance and survivability over ninety days of feeding trial. The control diet was prepared by using conventional feed ingredients like fish meal, groundnut oil cake (GNOC), soya meal, mustard oil cake (MOC) and corn flour. The experimental diets were prepared by replacing 50% GNOC protein with experimental feed ingredients. The findings of the experiment have been presented as tables and graphs in this chapter with appropriate statistical analysis.

3.1. Standardization of the extrusion process

Although the variables influencing the extrusion process are all interconnected, it may be challenging to discuss any variable in isolation. We discover this result in this experiment by taking into account one parameter at a time and maintaining the others constant.

3.1.1. Effect of moisture content on pellets

The effect of moisture content of feed mix on the finished feed pellets is expressed in term of its bulk density (Fig.1). The lowest bulk density noted was 0.78 g/cm^3, when moisture content was 25%, followed by 0.89 g/cm^3 and 0.95 g/cm^3, when moisture content was 20%, and 15%, respectively. When we went for further increasing the moisture content to 30 %, operational difficulty was noticed. Prior to this comparative study, it had been observed when the moisture content was below 10 %, the resulting pellets were not water stable and starch was not properly gelatinized. Therefore, most desirable pellets could be produced when the moisture content of the feed mix was kept at 25 %.

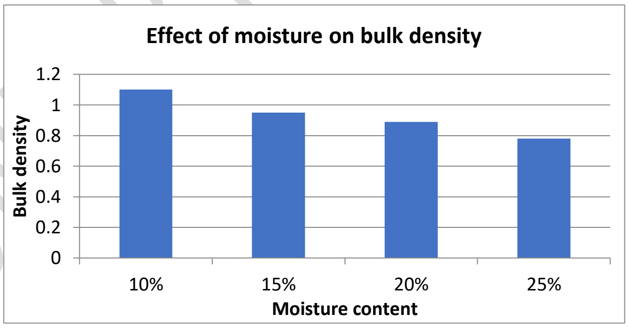

Fig1: Effect of moisture content of feed mix on pellet bulk density

3.1.2 Effect of die size on pellet quality

The bulk density of the pellets were the lowest at 0.84 g/cm^3 when 2 mm die was used, while 3 mm die resulted in pellets with bulk density of 0.97 g/cm^3 and 4 mm die resulted the pellets with the highest bulk density of 0.99 g/cm^3 (Fig. 2). The pellets produced with 3 mm and 4 mm die were found to be sinking, whereas, that produced using 2 mm die were found to be floating on the surface of water.

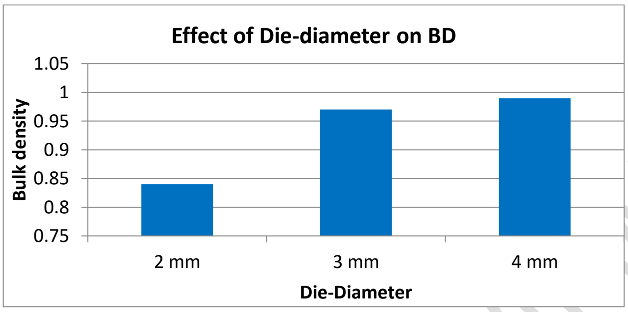

Fig 2: Effect of die-diameter on the pellet bulk density

3.1.3 Effect of soaking /pre-conditioning time on pellet quality

The influence of soaking or pre-conditioning time on the bulk density of the feed has been presented in Fig. 3. Observation showed that 30 minute soaking time resulted in the lowest bulk density 0.65 g/cm^3, followed by 25 min soaking that resulted in the bulk density of 0.88 g/cm^3. The highest bulk density 0.91 g/cm^3 was observed when the soaking time was 15 min.

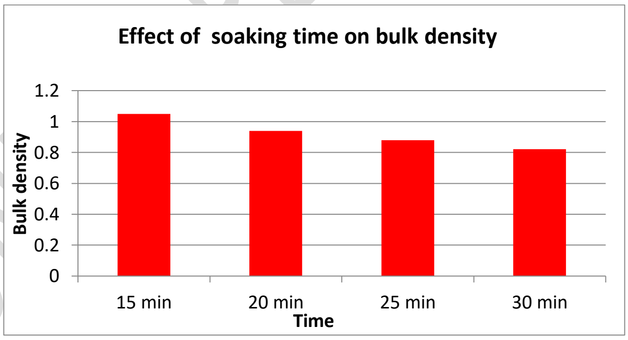

Fig 3: Effect of soaking time of feed mix on pellet bulk density

3.1.4. Effect of Temperature on pellet quality

The study on the effect of heater temperature on the pellet quality showed that the bulk density of the pellets decreased with the increase in heater temperature (Fig. 4) with the highest value of 1.03 g/cm^3 at 90°C to the lowest bulk density of 0.85 g/cm^3 when the heater temperature was 120°C.

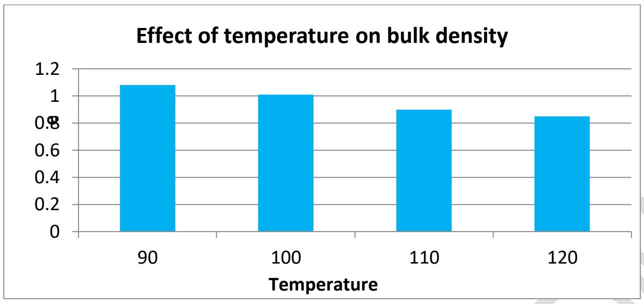

Fig 4: Effect of extruder barrel temperature on pellet bulk density

3.2. Assessment of physical quality of the extruded feed

The experimental diets as per the formula given in Table 1 was prepared using the twin screw extruder using the above standardized extrudion conditions of 120°C barrel temperature, 25% moisture content of feed mix, 2 mm die diameter, and 30 min pre-conditioning time resulting feed were subjected to various physical quality assessment and the findings have been given below.

3.2.1. Determination of expansion ratio

Expansion ratio is a measure of how extrudates puff while exiting the die. Expansion affects bulk density and floatability. Fig. 5 show expansion ratio of different experimental diets. Highest expansion ratio of 1.61 was obtained for T_2 experimental diet followed by 1.43 in T_1. Lowest expansion ratio of 1.08 was recorded for T_3.

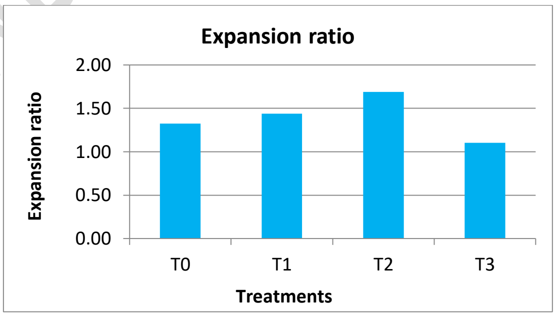

Fig 5: Expansion ratio of experimental diets

3.2.2. Determination of bulk density

When the machine was operated with the optimised extrusion parameter as suggested above, highest bulk density of 1.02 g/cm^3 was obtained for T_3 experimental diet followed by 0.9 g/cm^3 in both T_1 and T_0. There was a distinct difference in the bulk densities recorded for varying the feed ingredients composition. Lowest bulk density recorded was 0.8 g/cm^3 for T_3 experimental diet (Figure 6).

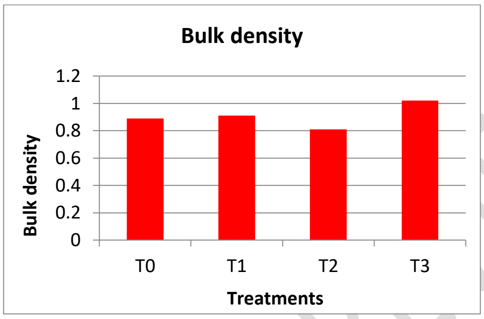

Fig 6: Bulk density of experimental diets

3.2.3. Determination of floatability

When the extrusion conditions were kept constant, the floatability of fish feed was probably due to the overall composition of the feed ingredients used in the study. Fig.7 show the floatability % of different experimental diet. Highest floatability of 100% was observed in both T_0 and T_2 experimental diets. Lowest floatability of 60% was recorded in T_3 experimental diet.

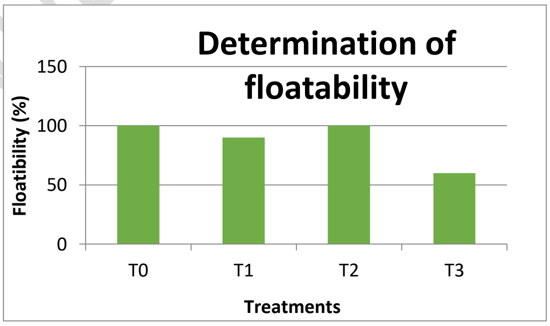

Fig 7: Floatibility (%) of experimental diets

3.2.4. Determination of water stability

Water stability is a measure of how strongly the extruded pellets will resist disintegration and thus prevent leaching of nutrients when placed in water. Fig. 8 shows the water stability of different experimental diet in percentage. Highest water stability observed was 99% in T_0 experimental diet followed by T_1 and T_2 having water stability of 95%. Lowest recorded was in T_3 experimental diet.

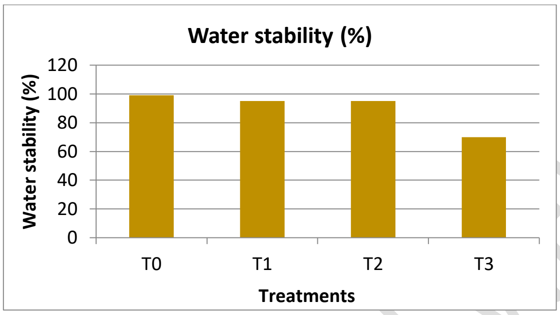

Fig 8: Water stability percentage of experimental diets

3.3 Proximate composition of experimental diets

Proximate compositions (on % dry weight basis and wet weight basis) of four experimental diets (e.g., T_0, T_1, T_2, T_3) were analyzed and the result is given in Table 2. 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± 0.1.7

Treatments	Moisture	Total Dry matter	Parameters (as % of dry matter)					Digestible energy (Kcal/ 100g)
			Crude protein	Crude fat	NFE	Crude fibre	Total ash	
T_0	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
T_1	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

T_2	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
T_3	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 2: Proximate composition of the experimental diets

4. DISCUSSION

The development of extrusion technology to produce floating fish feed has the potential to take care of a number of demerits associated with the traditional sinking pelleted fish feed. Therefore in this study attempt have been made to optimize extruder parameter to produce floating fish feed using above ingredients in a laboratory model twin screw extruder. The resulting experimental diets were analyse for their quality on a number of physical parameters and through feeding trails. The findings have been discussed in this chapter on the backdrop of previous studies.

4.1. Standardization of extrusion process

The effect of extrusion variables under study were quantified using bulk density and product quality parameters. Product quality parameters namely: expansion ratio, bulk density, floatability and water stability were used. The results have been discussed as follows:

4.1.1. Standardization of extruder parameter for production of floating pellets

Optimal conditions for processing good quality floating fish pellets were established using the response output method with the aim of obtaining extrudates with most desirable properties. The main criterion involved was maximizing floatability as estimated by bulk density. After a series of standardization reactions, the optimum extrusion conditions of temperature, die diameter, feed pre-conditioning time and moisture content was identified as 120 ^{o}C, 2 mm, 30 minutes, and 25% moisture respectively. The optimum values had a composite desirability to produce pellets of bulk density of 0.87 g/cm^3, which is acceptable for a extruded fish feeds. Our findings agree with other studies like Ojo *et al.* (2014), who reported die diameter of 2mm, and moisture content of 25% can produce pellets having bulk density of 0.94 g/cm^3. Irungu *et al*., (2018) also worked on optimization of extruder cooking conditions for the manufacture of fish feeds and suggested a combination of parameters like 120 ^{o}C extruder barrel temperature, 2 mm die diameter, and 100 s of feed preconditioning time gave most desirable pellet floatability (100%), expansion ratio (2.64), water stability (87%) and bulk density (0.479 g/ cm^3). The findings of the present study to corroborates with the previous findings and hence can be recommended for production of regular fish feeds.

4.2. Study on the physical qualities of the experimental diets

The experimental diets prepared in this study using conventional and non-conventional fish feed ingredients (Table 3) using the above standardized parameters in the laboratory model twin screw extruder were subjected to the physical quality evaluation for expansion ratio, bulk density, floatability and water stability (Fig. 5, 6, 7 and 8). Highest expansion ratio of 1.61 was obtained for T_2 experimental diet followed by 1.43 in T_1 experimental diet. Lowest expansion ratio was recorded 1.08 for T_3 experimental group (Fig. 5). The Lowest bulk density recorded was 0.8 g/cm^3 for T2 experimental feed and the highest bulk density of 1.02 g/cm^3 was obtained for T_3 experimental diet (Fig. 6). Highest floatability of 100% was observed in both T_0 and T_2 experimental diets while the lowest floatability of 60% was recorded in T_3 experimental diet. (Fig.7). Similarly, the highest water stability observed was 99% in T_0 experimental group followed by T_1 and T_2 having water stability of 95%. The lowest recorded water stability of 65% was in T3 experimental group (Fig. 8)

Expansion ratio is a measure of how extrudates puff at the die exit. Expansion affects bulk density and floatability. Pellet expansion ratio, bulk density, floatability and water stability are influenced by the instrument parameters like temperature, die diameter, moisture and feed pre-conditioning time (Singh & Muthukumarappan, 2016).. Increasing extrusion temperature resulted in higher extrudate expansion, whereas increasing die diameter decreased rate of expansion at constant feed pre-conditioning time of 30 minutes (Rosentrater *et al*., 2009). Likewise, increasing feed pre-conditioning time resulted in higher expansion (Rokey *et al*., 2010). The expansion of extrudates starts to occur at approximately 100^{o}C when starch has gelatinized and viscosity of the melt has considerably decreased (Majumdar & Singh, 2014). Furthermore, higher barrel temperature results in high pressure at the die, which leads to greater extrudate expansion (Meng, *et al.,* 2010). But as the temperature is increased further, viscosity of the melt continues to decrease and the material tends to expands more longitudinally while cross-sectional expansion decreases (Singh, *et al.,* 2014), which explains tendency of expansion ratio to decrease.

The moisture content of feed mix also has significant effect on the pellets quality as it helps in starch gelatizination that act as binder. This experiment showed that 25% moisture content in the feed mix was optimum, since the increasing moisture content more than 30 % resulted in operational difficulty (Fig.1). Similarly, it was observed that the pellets were not water stable and starch was not properly gelatinized when the moisture content was below 10 %. Increasing dextrinization as well as weakening of the dough structure could also lower expansion as processing temperature is increased further (Rosentrater *et al.,* 2009). Our findings agree with those of other studies that reported an increase in expansion ratio with increasing barrel temperature. The increase in expansion ratio with longer pre-conditioning times is due to moistening of the feed, which promotes starch gelatinization (Adeparusi & Famurewa, 2011; Rokey *et al*., 2010), whereas the decrease in expansion with increasing die diameter can be attributed to reduced pressure within the extrusion barrel (Singh & Muthukumarappan, 2014) as well as increase in melt viscosity (Akdogan, 1999). The two phenomena cause less puffing effect as the extrudate exits the die.

Bulk density accounts for expansion of the product in all directions and a low bulk density is desirable for the extrudates to float in water. Generally bulk density increases with temperature due to decreased melt viscosity that encourages bubble growth and greater expansion of the product (Giri & Bandyopadhyay, 2000; Meng *et al*., 2010; Singh & Muthukumarappan, 2016). Increasing extrusion temperature increased floatability, whereas increasing die-diameter decreased it. Increasing the feed pre-conditioning time increased floatability only marginally. Depending on composition, it has been found that extrudates do not typically expand until temperature approaches approximately 100 ^{o}C (Rosentrater *et al*., 2009). As with expansion, floatability of extrudates increased as the extruding temperature increased (Foley & Rosentrater, 2013). Saalah and Bono (2010) have, however, reported a trend where temperature did not affect floatability of fish feed, an observation that was probably due to the overall composition of the feed used in their study. With respect to die diameter, a small die diameter restricts extrudate exit, hence higher pressure is developed within the extrusion barrel resulting in greater expansion and therefore higher floatability (Vijayagopal, 2004).

Water solubility decreased with increasing temperature as well as with increasing feed pre-conditioning time but increased as the size of the die was made bigger. Water stability is a inverse measure of how strongly the extruded pellets will resist disintegration and thus disallow leaching of nutrients when placed in water (Ayadi et al., 2011). Water stability increased with increasing temperature. Generally, pellets produced under the experimental conditions of the present study exhibited high water stability ranging from 85–99%, which is the consequence of strong starch–protein matrix formation from the interaction of the gelatinized starch and denatured protein (Tumuluru, 2013). Increasing extrusion temperature enhanced the formation of such matrix (Vijayagopal, 2004). The curvilinear effects observed with respect to die diameter and conditioning time may be related to the interaction effects of temperature, pre-conditioning, and pressure within the barrel, which affect modification of the polymers, and the viscosity and integrity of the melt as it pushed through the die (Bandyopadhyay & Rout, 2001). Water stability of extruded pellets might decrease when die diameter is increased, partly because melt viscosity increases (Akdogan, 1999). Water stability may also increase with increased conditioning but decrease if the conditioning results in moisture level that diminishes cohesive strength of polymers due to excessive plasticization (Tumuluru, 2013).. The high water stability of pellets reported in this study implies that the products would exhibit minimum nutrient loss and environmental problems in fish ponds.

As expected there was significant direct correlation between expansion ratio and floatability (Umar *et al.,* 2013; Vijayagopal, 2004). From this observation, an inverse relationship between expansion ratio and bulk density would be expected (Majumdar & Singh, 2014). Water solubility was inversely related to water absorption as also reported by others (Fallahi *et al*., 2012; Singh & Muthukumarappan, 2014). Analysis of the findings on the backdrop of previous studies conclude that fish silage when incorporated in the feed mix, it affected negatively in all the characteristics of a floating feed like higher bulk density and lower floatability, expansion ratio and also water stability.

4.3. 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 2) 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-2). 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*).

5. CONCLUSION

This study was conducted to optimize extruder conditions for the manufacture of floating fish feeds with desirable physico-chemical properties. The optimum extrusion conditions of temperature, die diameter, feed pre-conditioning time and moisture content were identified as 120 ^{o}C, 2 mm, 30 minutes, and 25% moisture, respectively. The above extrusion conditions was used to prepare floating fish feed using conventional and non-conventional ingredients like LSOC SFOC and silage. However, fish silage when incorporated in the feed mix, it affected negatively in all the characteristics of a floating feed and resulted in higher bulk density and lower floatability, expansion ratio and also water stability.

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