Effect of Dietary Banana Peel on the Growth Performance and Digestive Enzymatic Activity of *Oreochromis mossambicus*

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, "Effect of Dietary Banana Peel on the Growth Performance and Digestive Enzymatic Activity of *Oreochromis mossambicus*" is based on the results of investigations carried out by me in the MSc. Zoology Discipline at The School of Biological Sciences and Biotechnology, Goa University, under the supervision of Ms. Gandhita V. Kundaikar, and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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COMPLETION CERTIFICATE

This is to certify that the dissertation report "Effect of Dietary Banana Peel on the Growth Performance and Digestive Enzymatic Activity of *Oreochromis mossambicus*" is a bonafide work carried out by Ms. Arya Anil Pokle under my supervision in partial fulfilment of the requirements for the award of the degree of Masters of science in the Discipline Zoology at the School of Biological Sciences and Biotechnology, Goa University.

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CHAPTER 1: INTRODUCTION

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1.1 BACKGROUND

1.1.1 Aquaculture Industry

Aquaculture, a practice also known as aquafarming, is an intricate and fascinating process of cultivating and breeding aquatic organisms, such as fish, crustaceans, molluscs, algae, and other valuable species. This process is carried out in controlled conditions, including freshwater, brackish water, and saltwater populations in semi-natural or controlled environments. Aquaculture is not only a profitable and sustainable industry, but it also plays a crucial role in restoring and rehabilitating marine and freshwater ecosystems. By utilizing advanced technologies and scientific techniques, aquaculture helps in meeting the growing demand for seafood consumption while preserving our planet's natural resources. Fish is a valuable natural resource for the world, and it is particularly important to the state of Goa, where it is an integral part of the culture and daily life. Around 5-10% of the population is engaged in fishing and related activities, such as marketing, drying, processing, and small-scale vending, which provide livelihoods for a significant number of people. Despite the benefits of the fisheries sector, it faces various challenges, including overexploitation, indiscriminate fishing practices, barriers to fish migration, pollution, habitat degradation, invasive species, climate change, and a lack of proper management. These issues must be addressed to ensure the sustainability and prosperity of the fisheries industry in Goa.

Aquaculture is a rapidly growing food industry due to the increasing demand for fish and fishery products worldwide. With a global fish production of 178.5 million tonnes (mt) in the year 2018, the aquaculture sector contributed a share of 45.99% (82.1 mt) (Handbook of Fisheries Statistics, 2020). There has been a significant increase in the inland fisheries sector's contribution to the total national fish production of 14.18 mt in

the year 2019-20, propelled by freshwater aquaculture. At the national level aquaculture production has increased to 6.2 million metric tons in FY 2017-18 from 1.9 million metric tons in 2000-01and about 88% of the farmed fish comes from freshwater aquaculture (NFP, 2020). Globally, the aquaculture sector is also trying to fulfill the enormously increasing demand for fish to combat against malnutrition and enhance food and nutritional security (FAO, 2018). With an objective to increase fish production, aquaculture practices have been shifted from extensive to semi-intensive and intensive culture systems where good nutrition plays a critical role in influencing fish growth, health, waste production, and production costs (Gatlin, 2002). In semi-intensive and intensive fish farming systems, fish feed contributes about 60-70% of operational cost (FAO, 1999). Incorporating cost-effective plant sources into fish feed can reduce expenditure and increase profits for farmers. The use of these natural resources will further be instrumental in ensuring sustainable aquaculture development in terms of environmental, social, and economical returns (FAO, 2017).

The cost of the feed is one of the major contributors to the operational cost of fish farming, accounting for 50-70% of the production costs. Commercial feeds are too expensive for small-scale fish farmers in most states, limiting their ability to intensify aquaculture production. As the cost of commercial feeds increases, many fish farmers are searching for alternative feeds. The aquaculture sector in many states generally depends on the use of imported fishmeal. Formulating aquafeeds using locally available ingredients could decrease costs. India is primarily an agricultural country, with vast land areas dedicated to cultivating crops such as mango, banana, pineapple, citrus, and others. After processing, these crops produce significant quantities of waste and byproducts, which can pose a threat to the environment if not disposed of properly. Hence, there is a need to explore ways to repurpose these waste materials in aquafeeds.

Additionally, agricultural byproducts like sugarcane bagasse and soybean curd residues have the potential to be utilized as sources of energy and protein for aquaculture feeds. While some of these byproducts have been used for producing fertilizers and livestock feeds, others have found their way into the production of functional ingredients or products.

With the rapid growth of the aquaculture industry, the demand for high-quality fish feed has continued to increase. Providing quality fish feed has become the primary focus of every aquaculturist. Although fish feed accounts for around 50% of production costs, it plays a crucial role in production and yield outcomes (Mzengereza et al., 2014). Due to the increase in demand for fish, the price of fish feed has also increased. One of the main reasons for this increase is the rise in demand for fish meal, which is a crucial source of protein in the feed. The supply of fish meal is dwindling with stagnating marine catches and alternative use for livestock and human consumption, while the demand is increasing (Fasakin et al., 1999). The search for alternative protein sources to replace complete/partial fish meal in animal feed became a priority (Magouz et al., 2008).

Plant sources are a possible alternative to expensive animal protein sources in fish feed, without compromising nutritional quality (El-Sayed, 1999; Francis et al., 2012). Moreover, the use of cheaper and locally available plant sources to substitute the expensive fish meals would mean a reduction in the production cost and thereby enhance the profit (Osman et al., 1996, Munguti et al., 2006). The most important factor to consider when selecting fish feed ingredients and formulating the feed is the availability, digestibility, palatability, and cost of the ingredients (Lovell, 1991; Rodriguez et al., 1996; De Silva and Anderson 1994). The addition of plant-based ingredients in fish feed is often limited due to the low protein content, as well as the

presence of anti-nutritional factors such as alkaloids, glycosides, oxalic acids, phytates, protease inhibitors, haemagglutinin, saponin, mimosine, cyanoglycosides, and other imbalances in essential amino acids, fatty acids, and micronutrients (Wee, 1991; Abowei et al., 2011). The potential of the feedstuffs to be used in fish diets can be established based on their proximate chemical composition (Mzengereza et al., 2014). Efforts are made to remove the anti-nutritional factors in the plant sources (Anderson and Wolf, 1995; Bairagi et al., 2002). Before use in diets, leaf meals were soaked, dried, and ground (Lochmann et al., 2011). In the realm of fish feed, many studies have been conducted on the utilization of various plant-based meals including soybean, rapeseed (canola) meal, cottonseed meal, sunflower seed meal, wheat and corn gluten, peanut meal, and moringa leaves meal (Makkar and Becker., 1996; Francis et al., 2012; Egwui et al., 2013; Mondal and Payra, 2015).

1.1.2 Use of alternative plant sources in fish feeds

Plant products contain huge amounts of protein, different amino acids, and fatty acids which are not available in animal protein (Mondal and Payra, 2015). The use of plant protein in the fish feed industry has been experimented with for various commercially cultured fish species. This is because the feed formulation is specific to each species based on their unique requirements. The benefits of using plant protein are not only in its availability and economic advantages but also in the fact that plant-based products contain less phosphate and nitrogen than animal protein. This helps in reducing the chances of pond eutrophication. Vhanalakar, (2009) has reported that certain plant-based foods are suitable for use in fish feed formulations. These foods include pods, seeds, leaves, fruits, grains, and oilcake such as linseed, safflower, sunflower, soybean,

and more. Additionally, aquatic weeds, grasses, vegetables, and plant extracts are commonly used in the fish feed industry. Other examples of suitable feedstuffs include roots, cereals, cereal by-products, broken rice, rice polish, tubers of sweet potato, wheat bran, maize, and sorghum (Mondal and Payra, 2015).

1.1.3 Nutritional Requirement of Fish

Quality nutrition in animal production systems is essential to the economical production of a healthy, high-quality product especially in aquaculture, where approximately 50-60 percent of the variable production cost is invested in feed (FAO, 2009). Nutrient requirements of fish are reported as minimum dietary levels needed to support the maximum performance of fish under experimental conditions when fed diets typically made using semi-purified ingredients (Small et al., 2016). The nutritional needs of fish can vary depending on various factors such as species, developmental stages, feeding habits, and water conditions including primary productivity and availability of natural food. For semi-intensive culture practices, a recommended diet for fish consists of a combination of oilcake, fish meal, meat meal (as a protein source), and rice bran, wheat bran, or maize (as a carbohydrate source) to meet their energy and growth requirements. A vitamin-mineral premix is also suggested to supplement the basic diet.

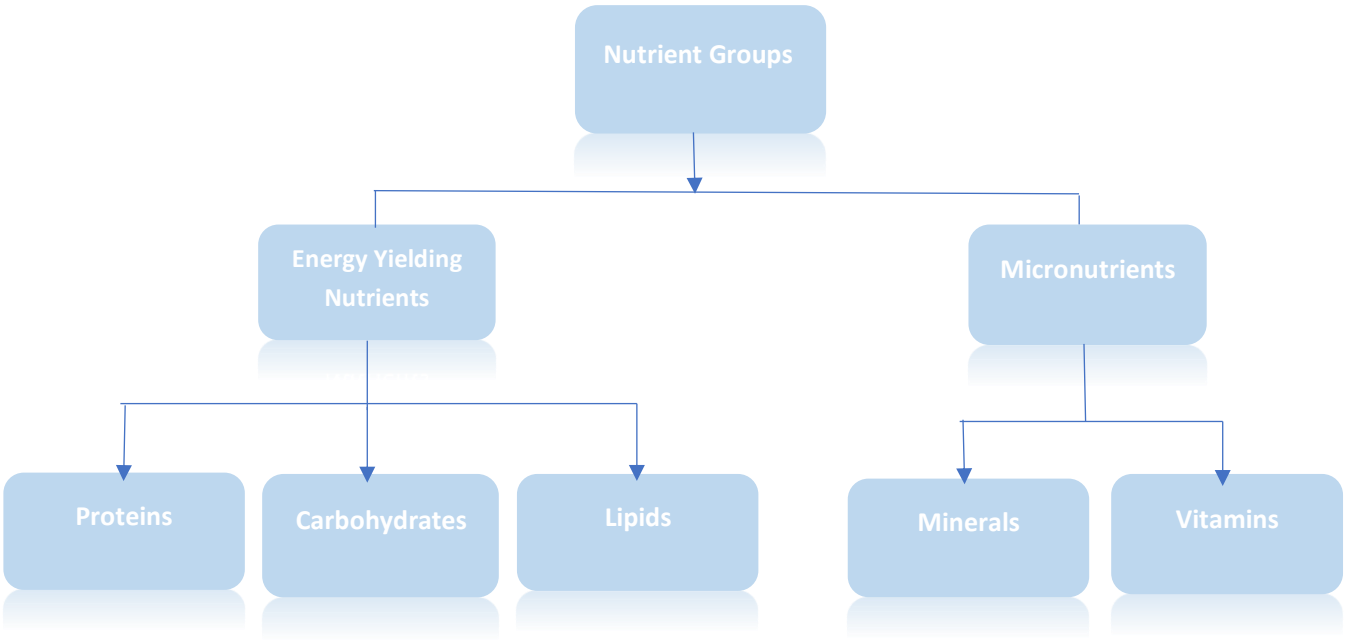

Fig 1.1: Flow chart showing different categories of major nutrient groups.

1.1.3.a Macronutrients:

Proteins

Proteins are macromolecules composed of amino acids, which are essential for the proper functioning of living organisms. They play a crucial role in animal nutrition as they are the building blocks of tissues and organs. A majority of animal tissues, including muscles, skin, and bones, are made up of proteins and other elements. These building blocks are necessary for the growth and repair of tissues, as well as the production of enzymes, hormones, and other vital molecules in the body. Therefore, a diet that is rich in proteins is crucial for the overall health and well-being of fish.

Carbohydrates

Carbohydrates are a crucial source of energy for farmed fishes, and they are widely used in commercial fish feed formulations. However, the ability of fish to utilize energy from digestible forms of carbohydrates varies significantly based on various factors such as their feeding habits, anatomical–physiological features, and rearing conditions. Among the different types of fish, carnivorous fish are considered to be less efficient in utilizing dietary carbohydrates due to their low intestinal glucose uptake rates and slow blood glucose clearance. As a result, they have been the subject of most studies focusing on carbohydrate nutrition and metabolism. Understanding the factors that influence the ability of fish to metabolize carbohydrates can help to optimize the formulation of fish feeds and improve the overall health and productivity of farmed fish.

Fats

Essential fats are crucial components of human nutrition as they help in reducing cholesterol and phospholipids levels in the blood. They are also responsible for forming the structure of cell membranes, affecting the function of enzymes and transport

systems, and modulating the cellular immune response. The significant metabolites of essential fatty acids include prostaglandins, prostacyclins, and thromboxane, which are active compounds in various body systems. Triglycerides, phospholipids, and sterols also play a crucial role in nutrition as they are involved in transporting and absorbing fat-soluble vitamins like vitamins A, D, E, and K. It is recommended to include more beneficial fats in our diet as they have a positive impact on our health. On the other hand, it is advised to avoid harmful fats in our diet as they can lead to various health issues (Gibney et al., 2009). The dietary reference intake for fats is 20%-35% of the total calories taken in from fat.

Fibre

Fibre plays a crucial role in the feeding process of animals, as it affects their feed intake, nutrient utilization, and overall feed efficiency. In addition to its impact on fish health and productivity, feed efficiency is also a vital factor when discussing the sustainability of the entire animal feed industry. By improving feed efficiency, farmers and producers can reduce waste, save resources, and minimize the environmental impact of animal agriculture. Therefore, optimizing fibre intake and utilization is a key strategy for promoting both animal welfare and environmental sustainability in the animal feed industry. The composition of fibre in fish feed can significantly influence growth and overall well-being. The presence of fibre in feed promotes healthy digestion and supports the movement of feed through the digestive system.

Lipids

Dietary lipids are essential for the proper functioning of animal tissues and play a crucial role in energy production processes. In addition to providing energy, they also serve as a source of essential fatty acids (EFA) that are necessary for the growth and

development of various organs and systems in the body. However, their dietary significance goes beyond these functions. Dietary lipids also act as carriers for certain non-fat nutrients, including the fat-soluble vitamins A, D, and K, which are critical for maintaining good health. Recent studies on EFA in fish have found that the requirements of fish for these essential fatty acids vary significantly from one species to another. This highlights the importance of understanding the specific dietary needs of different species of fish to ensure their optimal growth and development.

1.1.3.b Micronutrients

Vitamins

Vitamins are very necessary for the proper functioning of the body. They act as coenzymes in several metabolic processes and are involved in the synthesis of various compounds. Based on their solubility, vitamins are classified into two types: fat-soluble (A, D, E, and K) and water-soluble (eight B complex and C). Water-soluble vitamins are not stored in the body and are easily excreted if taken in excess, making them non-toxic. However, fat-soluble vitamins are stored in fatty tissues and can become toxic if taken in excessive amounts. Vitamins are crucial for embryo development, reproduction, growth, immune response, building of connective tissues, and bone metabolism. Among all vitamins, vitamin D is of utmost importance. The body has vitamin D receptors located throughout it, including in the immune cells. Vitamin D is essential for proper calcium absorption, and it is known to reduce the risk of fractures by strengthening bones, improving balance, and preventing falls. Additionally, it plays a role in maintaining healthy lungs due to its anti-inflammatory effect. Vitamin D also

regulates kidney function and is vital in the treatment of kidney diseases (Amen D., 2018).

Minerals

Minerals are inorganic compounds that can be found in our body as ions or as part of complex molecules. They play an essential role in maintaining normal metabolic processes. Major minerals and trace minerals are two categories of minerals. Major minerals, such as calcium, phosphorus, sulphur, sodium, chloride, magnesium, and potassium, are required in amounts of more than 100mg. On the other hand, trace minerals, such as iron, zinc, copper, manganese, iodine, selenium, fluoride, molybdenum, chromium, and cobalt, are present in small amounts and are needed in lesser quantities. Each mineral has a specific function. Calcium helps maintain bone health, potassium keeps muscles and the nervous system strong, and sodium assists in regulating fluid-alkali balance. Zinc helps boost the immune system, providing defense against infections (Mary L. et al., 2015).

Nutritional Requirements of Fishes

	Protein	Carbohydrates	Lipids
Labeo rohita			
Fingerling	**40%**	**35-40%**	**12-15%**
Adult	**25-30%**	**30%**	**9-10%**
Catla catla			
Fingerling	**30-40%**	**22-26%**	**8-10%**
Adult	**30%**	**26%**	**7-9%**
Oreochromis niloticus			
Fingerling	**35-40%**	**35-40%**	**5-10%**
Adult	**28-32%**	**35-40%**	**10-15%**
Chanos chanos			
Fingerling	**30-40%**	**25-30%**	**7-10%**
Adult	**24-27%**	**30%**	**5-7%**
Cyprinus carpio			
Fingerling	**30-38%**	**30-35%**	**5-15%**
Adult	**35-40%**	**30-35%**	**5-15%**

		Protein	Carbohydrates	Lipids
Lates calcarifer	Fingerling	50%	25-30%	15-18%
	Adult	45-50%	20%	18%
Oreochromis mossambicus	Fingerling	40%	30%	5-7%
	Adult	40-45%	35%	8-10%
Clarias batrachus	Fingerling	50%	21%	9%
	Adult	40-42%	15-35%	10-17%
Pangasionodon hypophthalmus	Fingerling	34-36%	30-45%	8.5%
	Adult	30-30%	20-30%	8.5%

(FAO, 1987)

Fig 1.2: Nutritional requirements of different fishes.

1.1.4 Need for Alternative Feed Supplements

The increased demand for supplementary feed due to the rapid growth of aquaculture has led to a rise in the cost of feed ingredients. Fish feed accounts for a substantial amount in the variable expenditure of fish farming enterprises (Falaye, 1993), accounting for more than 60% of the total cost in a commercial aquaculture operation. Among the major ingredients of formulated feed, protein is the most expensive one, and the major sources of protein in aqua feed are fish meal and de-oiled cakes. However, due to high cost, poor quality, and limited availability, the replacement of fish meal with plant protein sources is of great interest (Rumsey, 1993). Hence, fish farmers from all over the world need to explore the usage of alternate cost-effective, and easily available plant-based feed ingredients (Bhosale et al., 2010). Incorporating animal protein into aquaculture production can potentially cause contamination issues over time. Additionally, the use of anabolic products, hormones, antibiotics, and synthetic growth promoters are common practices to increase aquaculture production. Since the last decade, several countries have restricted the use of antibiotics to combat the disease problem in aquaculture due to its serious consequences such as bacterial drug resistance, breakage of the animal intestinal micro-ecological balance, and the presence of antibiotic residues in resultant fish/shrimp products (Smith, 1962) and efforts are underway to discover effective environmentally-friendly alternatives. All these artificial feed additives ultimately affect the health of consumers resulting in decreased consumer acceptance of fish and fishery products (Hua et al., 2019). Nowadays, most fish farmers prefer herbal or plant-based products instead of artificial growth enhancers to make aquaculture activities more environmentally friendly and economically viable. In this direction, the use of plant-derived resources in fish feed has gained significant attention in recent years, owing to their potential to improve

various desirable traits in aquaculture. These resources have been found to promote growth in fish and stimulate their appetite, while also aiding in gonadal maturation and immuno-stimulation. Additionally, they possess anti-stress and antimicrobial properties, which can help maintain a healthy and disease-free environment for fish. Furthermore, certain plant-based resources have been known to enhance the colour of fish, making them more attractive and marketable. In light of these benefits, plant-derived resources have emerged as a promising alternative to conventional fish feed, and are being explored extensively for their potential to revolutionize the aquaculture industry (Kaur, 2017).

1.1.5 Agro-waste as a potential alternative

The increasing amount of fruit and vegetable waste generated as agro-waste is exerting immense pressure on the environment. In 2019, India was the second major producer of fruits and vegetables in the world after China, and around 16% of the total production of fruits and vegetables is wasted every year (Sharma, 2019). The waste produced from processing fruits and vegetables is a valuable source of energy and protein that is often discarded and ends up polluting the environment. Utilizing this waste in fish feed could be a promising alternative that would help clean the environment in an eco-friendly way, and also reduce production costs. The plant resources can provide the necessary nutrients to produce high-quality and safe fisheries products while having minimal impact on the environment. Fruit processing waste and vegetable processing wastes are potential sources of energy and have been established to stand as good sources of numerous polyphenols that help in digestion and growth promotion (Peschel et al., 2006). The use of non-food parts from agricultural products as animal/fish feed will not

only enhance food security but also contribute to the alleviation of environmental problems associated with their disposal (Bakshi et al., 2016).

1.1.6 Banana

Banana is an evergreen herbaceous plant that belongs to the Musaceae family and *Musa* genus, which are both part of the Zingiberales order. Bananas are known for their high calcium, magnesium, and nitrogen assimilation emissions, which make them an essential part of many people's diets. The fruit itself is quite unique in its appearance, with variable qualities based on size, colour, and firmness. It's generally curved and fleshy, covered with green skin that turns yellow after maturation and brown when ripe. Bananas are also known for their growth patterns; they grow in cones from the plant's top, and it can take anywhere from 80 to 180 days for the fruit to fully mature. When it comes to classification, bananas have more than 50 species and dozens of hybrids. They have large and rhizomatous underground stems, which produce large leaves with powerfully spirally arranged pods. This gives the appearance of a false stem, also known as a pseudostem. Overall, bananas are a fascinating and essential fruit that has been enjoyed by humans for centuries. They are not only delicious but also packed with nutrients that make them an excellent addition to any healthy diet.

1.1.7 Banana Peel as a potential feed source

The banana-based food industry produces a lot of banana peel waste. Banana peels are often discarded without being used to their full potential. Banana peel waste can be used as an alternative to fish feed ingredients because it still contains nutrients and is cheap. Nutritional contents in banana peels are carbohydrates 11.27%, protein 1.71%, fat 3.28%, and vitamin C 0.30% (Susanto, 2016). Several studies on the use of banana peel waste as alternative feed ingredients have been carried out. Jeharu et.al (2015) conducted a study to determine the effect of feeding with banana peels on growth and feed efficiency for tilapia fish. The results of the study showed that feeding containing 20% of banana peels gave the highest absolute growth value of 2.93. Pratamaningrum (2013) researched fermented banana peel as an alternative feed for tilapia juveniles (*Oreochromis niloticus*). The result showed that 20% banana peels as feed for tilapia gave the highest growth 8,06%, and feeding efficiency ranged from 10,31% - 13,65%. Other research on banana peels as alternative feed was also carried out by Utari (2019). Utari (2019) used banana peel waste and chicken feather flour silage as feed ingredients for sangkuriang catfish (*Clarias gariepinus*). The results showed that giving 75% banana peel flour and 25% chicken feather silage gave an absolute daily growth rate of 142.85%, absolute length increase11.86 ± 0.15, absolute weight growth of 12.99 ± 0.15, the utilization efficiency of 2.2% of feed, relative growth of 333.3% and feed conversion (FCR) 1.73.

1.1.8 Banana Processing and Waste Production

The banana industry is known for generating a significant amount of waste, including unused plant parts such as peels, roots, stems, and leaves during the processing of bananas. Despite being unused, these plant parts contain valuable components such as lignin, cellulose, pectin, and hemicellulose. These compounds are essential for various industrial and agricultural applications and have the potential to be converted into useful products through sustainable and innovative processes.

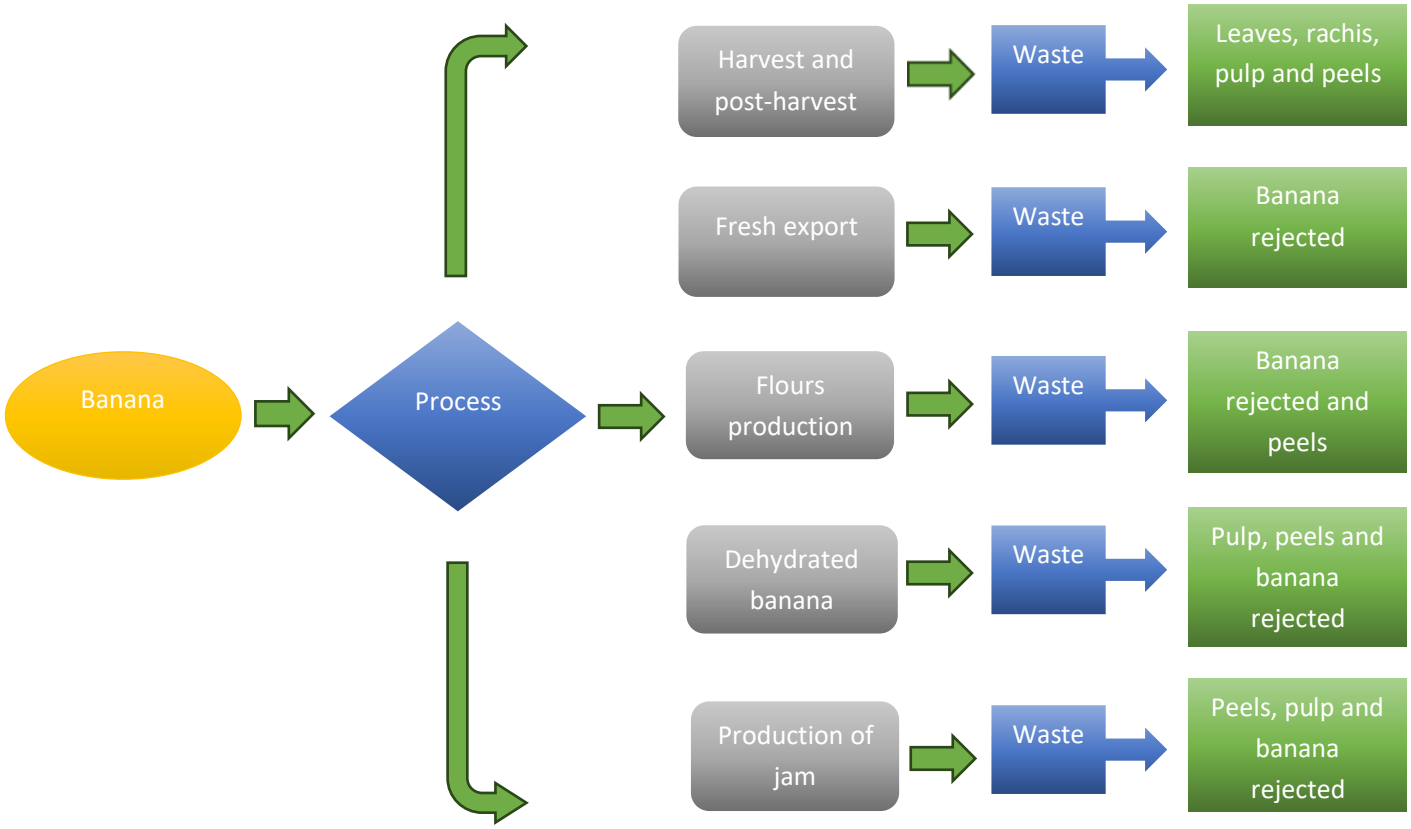

Fig 1.3: Processing of banana and waste production.

The banana peel is one of the most significant byproducts generated from banana processing. It's interesting to note that bananas are composed of 60% pulp and 40% peel. However, the peel contains a high concentration of carbon-rich organic compounds, such as cellulose, hemicellulose, pectin, lignin, chlorophyll pigments, and other low molecular weight compounds. If these wastes are not treated correctly, they can produce an annoying odour due to natural decomposition and release gases that contribute to the greenhouse effect. With the ever-increasing cost of fish feed, we have attempted to incorporate fruit wastes into fish diets to reduce the cost of fish feed without reducing the quality of the feed. The feed given to cultivated fish plays a crucial role in determining their growth and survival. It is, therefore, essential to manage alternative feed that is both effective and efficient, while also reducing production costs. Banana peels, which are derived from agricultural waste, are emerging as a promising alternative material that can be used as fish feed. These peels are rich in nutrients and can provide a balanced diet for fish, leading to better growth and development. Moreover, using banana peels as fish feed can help reduce waste and promote sustainability in the aquaculture industry.

Fish feed formulation plays a crucial role in the aquaculture industry as it directly affects the growth, health, and overall performance of fish. The search for alternative feed ingredients that are cost-effective, sustainable, and nutritionally balanced has led to the exploration of various unconventional sources. One such potential ingredient is banana flour, derived from banana peels. This study aimed to evaluate the use of banana flour in fish feed formulation and its impact on fish growth and overall health.

1.2 AIMS AND OBJECTIVES

To the best of our knowledge, no study has been conducted in the state of Goa or elsewhere in India to assess the impact of dietary administration of banana peel on the growth performance and digestive enzymatic activity of *Oreochromis mossambicus*. Given the aforementioned knowledge gap, this current study has been undertaken to accomplish the following objectives:

1. To evaluate the proximate content of Banana peel.

2. To Formulate Fish Feed Supplemented with Banana peels.

3. To investigate the effect of feeding a Banana peel–based formulated diet on the growth, well-being, and digestive enzymatic activity of Tilapia.

1.2.1 Importance of the study

- Improved feed composition and better feed conversion efficiency increase fish production and lower feed cost.
- A balanced diet for fish is important in ensuring fast-growing, healthy, and disease-free fish.
- Giving feed that supplies all the components of good nutrition is essential in good aquaculture practices.

1.3 HYPOTHESES

The incorporation of Banana peel flour in the formulated fish diets will induce positive effects on the growth and digestive enzymatic activity of Tilapia. The dietary administration of Banana peel flour will maximize the growth performance and better survival rate in fish. Furthermore, this study may provide information that the Banana peel flour inclusion into fish diets will increase digestive enzymatic activity and will aid in good metabolism.

1.4 SCOPE

The world's growing demand for seafood has resulted in an increase in the exploitation of fish to produce fishmeal, which is not sustainable in the long term. Therefore, it is essential to develop a plant-based fish diet that can be equally effective as fishmeal. To cater to this need, we have invested in identifying cost-effective plant-based fish feed options that can replicate the appropriate nutritional content for fish.

One promising source for plant-based fish feed is banana peel. Once the formulated fish feed from banana peel is carried out, we can conduct an economic analysis and distribute it to fish farms. This can help enhance the nutritional value of farmed fish and improve the field of Aquaculture and fisheries. Furthermore, formulating fish feed supplemented banana peel and evaluating its effect on other farmed fish can help identify alternative sources of fish feed ingredients, which can motivate other seafood communities and aquaculture producers to improve fish feed sustainability.

CHAPTER 2: LITERATURE REVIEW

Banana peel waste is a valuable resource that can be utilized in various applications, including animal feed production. This literature review aims to explore the effect of banana peel feed on the growth and body functions of fish. The research findings discussed in this review highlight the potential benefits of incorporating banana peel in fish diets, as well as the broader applications of banana peel waste.

2.1 Studies on the nutritional content of banana peel

In recent years, there has been a growing interest in exploring the nutritional content of banana peels. Several studies have investigated the potential health benefits and nutritional value of banana peels. Traditional feed ingredients, such as fishmeal and soybean meal, are expensive and have limited availability. Therefore, there is a need to explore alternative and sustainable sources of feed ingredients. One such potential source is banana peel, which has been found to contain significant amounts of nutrients and minerals necessary for healthy fish growth (Dong et al., 2010).

Additionally, there is a need for further research to determine the appropriate processing methods for incorporating banana peels in fish feed and to assess the long-term effects on fish growth and health (Hamre et al., 2012).

According to Kousoulaki et al. (2015), banana peels contain the required amounts of nutrients, including proteins, carbohydrates, lipids, vitamins, and minerals. These nutrients can contribute to the nutritional requirements of fish larvae and potentially replace or supplement traditional feed ingredients.

Hassan et al. (2018) conducted a study to determine the proximate and mineral levels of banana peel and found that it contains significant amounts of nutrients and minerals

needed for the healthy growth of animals. This suggests that banana peel can be incorporated into the formulation of animal feed to reduce waste and provide essential nutrients for animals.

Additionally, banana peels are rich in minerals such as potassium, calcium, magnesium, and phosphorus, which are essential for bone development and overall health (Hassan et al., 2018).

Recently Ndarubu et al. (2021) found that banana peels could be considered good source of nutrients for production of human and animal feeds, and their utilization for this purpose should be encouraged, as this will also help in reducing the menace of nutrient deficiencies.

Yossa et al. (2021) conducted a study on the apparent digestibility coefficients of banana peel, cassava peel, cocoa husk, copra waste, and sugarcane bagasse in the GIFT strain of Nile tilapia and this experiment generated basic information for the use of these ingredients in diet formulation for tilapia.

These findings suggest that banana peels have the potential to be a valuable source of nutrition and antioxidants. However, more research is still needed to fully understand the extent of the nutritional benefits and potential applications of banana peels in food and other industries. In conclusion, the nutritional content of banana peels includes minerals such as potassium, calcium, sodium, iron, manganese, bromine, rubidium, strontium, zirconium, and niobium. The peels also contain a significant amount of protein, crude lipid, carbohydrate, and crude fibre.

2.2 Studies on the incorporation of Banana Peel in Fish Diets

Fish feed formulation is a critical aspect of aquaculture, aiming to provide balanced nutrition to fish while optimizing feed costs and sustainability. Banana peels, often considered as waste, have been explored as a potential ingredient in fish feed due to their nutritional content and potential benefits. This literature review discusses studies on the inclusion of banana peel in fish feed formulation, focusing on its effects on growth performance, nutrient utilization, and feed quality.

The studies collectively suggest that banana peels possess significant nutritional content, including minerals, protein, carbohydrates, and antioxidants. However, it is important to note that further research is still needed to fully understand the nutritional benefits and potential uses of banana peels in food applications (Li et al., 2014).

Similarly, Giri et al. (2016) conducted a study on Rohu, *Labeo rohita*, and investigated the effects of dietary administration of banana peel flour on growth, antioxidant status, cytokine responses, and disease susceptibility. The abstract of this study also does not provide specific information on the effects of banana peel flour on fish feed formulation.

Studies have investigated the effects of incorporating banana peel flour (BPF) in fish diets on growth performance and immune functions. Ngugi et al. (2017) found that the inclusion of BPF at 5% in the diet significantly improved the growth performance and immune functions of *Labeo rohita* fish. The study demonstrated that BPF can be a beneficial ingredient in fish feed formulations, leading to enhanced growth and improved immune responses in fish.

Banana peel has been shown to be a good source of energy and fiber for fish. A study by Oluwaniyi et al. (2018) demonstrated that African catfish fed diets containing banana peel had higher digestibility coefficients for crude protein and crude fibre compared to those fed a control diet. This indicates that banana peel can be effectively utilized by fish for nutrient uptake and energy production.

Additionally, banana peels have been found to possess antioxidant activity and higher levels of antioxidants compared to other parts of the plant (Som et al., 2019).

The literature suggests that banana peel can be a viable ingredient in fish feed formulation, offering benefits in terms of growth performance, nutrient utilization, and feed quality. However, further research is needed to optimize inclusion levels and to assess long-term effects on fish health and product quality. Overall, banana peel shows promise as a sustainable and cost-effective ingredient for aquafeed production.

2.3 Studies on the Effect of Banana Peel on Fish Growth

Banana peel, a byproduct of banana consumption, has been explored as a potential ingredient in fish feed due to its nutrient content and potential benefits for fish growth. Several studies have investigated the effects of incorporating banana peel in fish feed formulations, focusing on different fish species and varying inclusion levels.

Studies have shown that banana peel can be included in fish feed formulations as a source of energy, fibre, and various nutrients. The inclusion level varies depending on the fish species and the specific goals of the study. Generally, inclusion levels range from 5% to 30% of the total feed composition.

Several studies have investigated the use of banana peel in fish feed and its impact on growth performance. For example, a study by Kpogue et al. (2017) found that Nile tilapia fed diets containing banana peel meal showed similar growth performance to those fed a commercial diet.

Mones et al., (2017) conducted a study to evaluate the dietary effect of fermented banana peel at different stages of ripeness on growth, antioxidant capacity, metabolic response, and survival of Red tilapia and these results indicated that FBP at different stages of ripeness, especially enhances growth performance, stabilizes both antioxidant capacity and metabolic response, improves the resistance of Red tilapia against infection and provides better cost-benefit ratio. FBP could be therefore considered as a potential alternative to synthetic growth promoters and antioxidant products used in the aquaculture industry.

Incorporating banana peel into fish feed formulations has also been shown to improve feed quality and palatability. A study by Oluwaniyi et al. (2018) reported that fish fed diets containing banana peel had higher feed intake and feed conversion efficiency compared to those fed a control diet. This suggests that banana peel can enhance the palatability of fish feed and improve overall feed utilization.

Similarly, another study by Olude et al. (2020) reported that African catfish fed diets containing banana peel had comparable growth rates to those fed a commercial diet.

Aisyah et al., (2021) investigated the effect of commercial feed substitution with fermented banana peel flour and fish meal on feeding rate, specific growth rate, feed efficiency, fat, and energy retention and observed that the commercial feed substitution using fermentation of banana peel flour and fish meal up to a dose of 20% in feed has the same feed consumption, specific growth rate, feed efficiency, fat retention and energy retention values as well as the control feed.

Similarly Gonzales et al. (2022) conducted a study on the growth performance of tilapia fingerlings fed with phytoadditives from fruit wastes (pineapple, citrus, and banana) for aquaculture. Results showed that the weight gain was significantly affected when fed with fruit waste. Better FCR and survival rate were observed when fingerlings were fed with 2% banana peel but had no significant difference with 2% citrus or pineapple.

Several studies have reported positive effects of banana peel inclusion on weight gain in fish, fed with a diet containing 10% banana peel which showed significantly higher weight gain compared to those fed a control diet. Similarly, improvements in FCR have been reported in some studies, indicating better feed utilization efficiency.

Overall, the inclusion of banana peel in fish feed has shown promising results in improving growth performance, particularly in terms of weight gain and feed conversion efficiency. However, further research is needed to optimize inclusion levels, understand nutrient utilization, and explore potential health benefits for fish.

2.4 Study on the Digestive Enzymatic Activity

According to a study conducted by (Susanto et al., 2023), incorporating Ambon banana peel flour into the diet of Nile tilapia at a rate of 2% kg-1 feed resulted in significant improvements in the fish's overall health, survival, and growth performance. The researchers attribute these positive outcomes to an increase in the number of lactic acid bacteria and digestive enzyme activity, which were observed in the fish.

2.5 Lacunae In Study

Currently, there is a lack of research on the effects of formulated feed on the digestive enzymatic activity of Tilapia. Therefore, it is essential to conduct a thorough investigation to determine how formulated feed impacts the digestive enzyme activity of Tilapia.

Moreover, there is a need to study the optimal inclusion levels of banana peel in fish feed formulations to maximize growth performance and overall functions. This would require extensive experimentation to determine the most effective concentration of banana peel flour that provides the most significant benefits without any adverse effects on fish health.

To fully comprehend the potential of banana peel as a feed ingredient, a detailed analysis of its nutritional composition is necessary. This would include a comprehensive evaluation of its protein, fat, carbohydrate, and mineral content, among others. Such an analysis would provide a better understanding of the nutrient profile of banana peel and its potential contribution to the formulation of balanced and nutritionally complete fish feeds.

Thus, the present study was aimed at fulfilling this literature gap by analyzing the proximate composition of BPF and its inclusion in fish feed formulation and determining its impacts on the growth and digestive enzymatic activity in Tilapia.

CHAPTER 3:

MATERIALS AND METHODS

3.1 Study Animal

The Mozambique tilapia, scientifically known as *Oreochromis mossambicus*, is a freshwater fish belonging to the cichlid family. This species is highly sought after for aquaculture purposes due to its adaptability to varying environmental conditions. Originally native to southeastern Africa, the Mozambique tilapia has been introduced by humans to numerous tropical and subtropical habitats across the world. This popular fish is commonly referred to as Tilapia and is recognized for its significant contribution to the aquaculture industry.

Fig. 3.1: Study animal: Oreochromis mossambicus

3.2 Classification of the study animal

Domain: Eukaryota

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Cichliformes

Family: Cichlidae

Genus: *Oreochromis*

Species: *O. mossambicus*

3.3 Study Area

Goa is a small state located along the western coast of the Indian Peninsular, located at 28° 38’ N latitude and 72° 12' E longitude, Goa covers an area of 3,700 km². The Western Ghats run along its Eastern boundary while the Arabian Sea flanks its western coast. The climate in Goa is tropical, with an average temperature ranging between 28-33°C, and humid weather throughout the year. It receives an annual rainfall of 330cm. The major industries in Goa are Fishing, Agriculture, and Tourism. Fish is a crucial natural resource, and it holds greater significance in the state of Goa due to its integral role in Goan life and culture. It is considered a staple food for over 90% of the population in Goa. The average per capita consumption of fish in Goa is between 15-17 kg per year. Around 5-10% of the entire population of Goa is involved in fishing and related activities.

Selection Criteria: A preliminary questionnaire-based survey was conducted across fish farms in Goa to study the various types of feeds given to fish. The farm workers were interviewed about the following parameters: type of feed given (Pellets, Flakes, Granules), type of feed concerning the moisture content (Dry fish feed, Semi-moist fish feed, Moist fish feed), Feeding habits in fishes (Surface feeders, Column feeders, Bottom feeders), Type of feed (Sinking fish feed, Floating (Extruded) fish feed), Type of fish (herbivore, Carnivore, Omnivore). Based on the collected data feed type to be administered was decided.

3.4 Sample Collection and Preservation

For the present study, banana peel samples were used: **banana peel** samples were collected to analyze the nutritional content. In addition to this, banana peels were used to obtain **Banana Peel Flour (BPF)** which was used in the formulation of fish feed.

1. **Banana Peel:** The raw materials, Banana fruits (Safed Velchi Musa) were collected from the fruit vendor in a local market. The banana fruits were peeled and peels were gathered. The collected peels were washed thrice using tap water and distilled water was used for final wash. The washed fruit peels were dried using a hot air oven at 60°C for seven days till they were completely dried. The dried peels were ground into a fine powder using a porcelain mortar and pestle and sieved using a fine mesh net.

2. **Groundnut Oil Cake:** The groundnut oil cake was collected from the local market and was ground into fine powder. The fine powder was then utilized for determining the proximate content and was used in feed formulation. The inclusion of 100% GOC was used as a control to feed the fishes.

3. **Sampling of fish:** Tilapia fish were obtained from the Green Lake Farm at Majorda, Salcete, Goa. The fish specimens were brought to the study site and housed for further study purposes.

3.5 Experimental Setup

A randomized design was used in setting four tanks (diameter 36 inches and height 12 inches) that were used for the experiment. The experiment was performed in four glass aquariums. A group of 10 fish of *Oreochromis mossambicus* (Tilapia) juveniles was stocked in each aquarium. Tanks and air stones that were used in the experiment were disinfected prior to the conduct of the feeding trial. Tanks were filled with 70-80 litres of water and stocked with 10 fish. The fish were fed with groundnut oil cake supplemented with different percentages of banana peel flour (10%, 20%, and, 30%). The treatment design was GOC 100%, BPF 10%, BPF 20% and, BPF 30%. The fish were fed at 5% feed of fish wet body weight. The fish were subject to a complete randomized design, and subsequently, a sample of fish was selected for further analysis in the laboratory.

3.6 Quality Assurance

It is necessary to maintain sterile conditions and prevent contamination by other substances to get accurate readings. To avoid this, the glassware and crucibles were thoroughly cleaned and sterilized. Prior to conducting the experiment, they were washed with distilled water to clean the surface. Experiments were conducted in clean and sterile conditions, wearing clean lab coat, gloves, and masks. Samples were constantly covered using aluminium foil to avoid contamination.

Fig 3.2: Safed Velchi Musa (Musa sp.)

Fig 3.3: Experimental Setup

3.7 Apparatus and Instruments

General laboratory wares like measuring cylinders, beakers, test tubes, glass rods, micropipettes, reagent bottles, mortar and pestle, porcelain crucibles, conical flasks, petri dish, test tubes, funnels, and condensing flasks were used. Instruments like Muffle furnace (i-therm AI-7981), Hot air oven (MIC-165), Hot plate, Weighing balance (PGB,200), Centrifuge (R-24), and UV- Visible spectrophotometer (BL 1073) were used.

3.8 Biochemical Analysis

All the chemicals and reagents used in the present study are of analytical grade.

The following biochemical parameters of the banana peel sample and groundnut oil cake were studied:

1. Crude Protein
2. Carbohydrates
3. Crude Fibre
4. Total Lipids
5. Ash and moisture

3.8.1 Estimation of Protein (Lowry et al., 1951)

Principle

The peptide nitrogen reacts with the copper ions under alkaline conditions and copper catalyzes the oxidation of the aromatic acids and converts the Folin's Ciocalteau phosphomolybdic phosphotungstic acid to hetero molybdenum blue. The blue colour obtained is read at 660 nm.

Reagent preparation

1. **Lowry's reagent**

   a. Solution A - 4g of sodium carbonate was added in 100 ml of distilled water.

   b. Solution B - 0.1g of copper sulfate was added to 5 ml of distilled water.

   c. Solution C – 0.2g of Sodium-Potassium Tartrate was added in 5 ml of distilled water 98 ml of solution A + 1 ml of solution B + 1 ml of solution C was mixed to prepare the Lowry's reagent.

2. **Folin's Ciocalteau reagent**

   10 ml of Folin's reagent was added in 10 ml of distilled water to make 1:1 Folin's solution.

Estimation

The BSA standard solution was added in serially increasing concentrations in 6 of the test tubes. This was then diluted up to 1 ml with distilled water. Then 5 ml of Lowry's reagent was added and incubation was done for up

minutes. This was followed by the addition of 0.5ml of Folin's reagent and again incubation for 10 minutes. Finally, the absorbance was read against the blank at 660 nm. For the sample, 1ml of the sample was taken and in that 5ml of Lowry's reagent was added and this was followed by incubation for 10 minutes. Then 0.5ml of Folin's reagent was added and again incubation was done for about 10 minutes. The absorbance was then finally read at 660 nm. The concentration of the proteins was determined from the unknown OD by using the standard curve.

3.8.2 Estimation of Carbohydrates (Hedge and Hofrreiter, 1962)

Principle

Formation of furfural takes place when carbohydrates are dehydrated with concentrated sulphuric acid. Then condensation of furfural with that of anthrone takes place to form a green coloured complex which is measured with the help of the spectrophotometer at 620 nm.

Preparation of reagents

Anthrone reagent: 0.2g of anthrone powder was added in 100 ml of concentrated sulphuric acid.

Estimation

A Standard solution of glucose was added in a serially increasing manner in 6 test tubes (0.2-1ml). This was diluted up to 1 ml by using distilled water. The blank test tube contained just 1 ml of distilled water. After this 5ml of anthrone reagent was added to test tubes and then they were kept in a boiling water bath for about 20 minutes. The OD was then read at 620 nm. For the sample, 1ml of sample was taken and then 5ml of anthrone reagent was added. This was followed by incubation in a boiling water bath for 20 minutes and finally, absorbance was read against the blank at 620 nm.

3.8.3 Determination of Total Lipids (AOAC International 79:487-492)

Principle

The sample is homogenized and the solvent is added. The homogenate is filtered and NaCl is added. The solvent evaporates after drying and the weight of the lipid extracted is measured.

Reagents:

The following reagents were used: chloroform, methanol, and sodium chloride.

Estimation:

First, cut the sample into small pieces and grind it. Then, homogenize the sample in a blender and weigh out 3g of the resulting paste. Next, prepare a solvent mixture of 30ml of CHCI3 and 1MeOH in the correct ratio by measuring out each solvent accurately and blending them together for 1 minute at a moderate speed.

After that, filter the homogenate through a coarse filter paper and then funnel it into a 100ml glass stoppered graduated cylinder. Add 12 ml of a 0.5% NaCl solution to the cylinder, gently shake it by tilting it five times, and then let it stand until a visible separation occurs.

Next, using a 10 ml pipet, remove an aliquot of more than 3ml of the chloroform (lower) layer and transfer exactly 3ml into a pre-weighed beaker. Evaporate off the solvent on a hot plate at a setting between low and 2, avoiding excessive heating and drying. Finally, weigh the beaker to determine the weight gain as the weight of the extracted lipid.

Lipid content (%)

$$= \frac{\text{Lipid extracted (g)}}{\text{Sample weight (g)}} \times \frac{\text{(chloroform layer + amounts lost) (ml)}}{3\ \text{ml}} \times 100$$

3.8.4 Determination of crude fibre (AOAC)

Principle

The crude fibre content of the feed is determined by subjecting the feed sample to acid and alkali digestion, weighing the remaining residue, and measuring the loss of weight after ashing.

Reagents

7 ml of conc. H_2SO_4 is dissolved in distilled water to get one litre of solution.

12.78 g of sodium hydroxide is dissolved in distilled water to get one litre of solution.

Estimation

First, weigh precisely two grams of feed. Then, accurately measure 200ml of H_2SO_4 into a beaker. Place the beaker on an electric heater and position a round bottom condensing flask over it, filling the flask with cold water. Once it is ready, turn on the heater.

Digestion in acid

A beaker is first heated to bring the acid to its boiling stage. Next, a sample of 2 grams of substance is carefully added to the boiling acid. The acid then boils and reacts with the sample, breaking it down and digesting it. This boiling and digestion process is continued for a duration of 30 minutes.

Filtration

First, fix a linen cloth over the funnel. Then, transfer the contents from the beaker to the filtering funnel. Once all the acid and acid digested residues have been transferred to the linen cloth, wash the beaker with distilled water and transfer the contents to the filtering funnel. Keep washing the residue with distilled water until it becomes acid-free.

Test

To check if the residue is free of acid, catch one or two drops of filtrate over blue litmus. If the blue litmus remains blue, that means the residue is washed clean. Once the washing is complete, take the filter cloth along with the residue and squeeze out the water. Next, place the filter cloth on a porcelain slab and gently scrape off the residue from the cloth. Finally, keep the residue in the centre of the filter cloth.

Digestion in alkali

To subject the acid digested residue to alkali digestion, 0.313 N sodium hydroxide solution is used. Pour 200 ml of sodium hydroxide into a lipless beaker. Place the beaker on the heater and fix a condensing flask over it. Heat the alkali solution until it starts boiling. Once it reaches the boiling stage, remove the condensing flask and transfer the acid digested residue to the boiling alkali.

Then replace the condensing flask and continue with the heating process. The residue should then be digested in boiling NaOH for a period of 30 minutes. After 30 minutes, you can remove the condenser and transfer the contents of the beaker to a filtering funnel. The residue should be washed repeatedly with distilled water until it is completely free of alkali.

Test

To check if the residue is free from alkali, you can perform a simple test. Catch one or two drops of the filtrate over red litmus. If the litmus remains red, it indicates that the residue is free from alkali. Once you have confirmed that the residue is free from alkali, you should squeeze the cloth well to dry it and transfer the residue, without any loss, to a clean silica crucible.

Drying and Washing

The process involves placing the crucible in a hot air oven preheated at 110ºC overnight to remove all moisture. Once completely dry, the crucible is cooled in a desiccator and weighed with the residue. Next, the residue is ashed by heating the crucible with an electrical bunsen. Continue heating until a whitish ash is obtained. After ashing, the crucible is cooled to room temperature and weighed again to obtain the weight of the residue.

Calculation for Crude fibre

The difference in weight of the crucible before and after ashing is reported as the crude fibre content of the feed taken.

Weight of the sample = c

g Weight of crucible with dry residue = a

g Weight of crucible with ash = b

$$\text{g Per cent crude fibre} = \frac{a - b}{c} \times 100$$

3.8.5 Ash And Moisture Content (AOAC, 2000)

Small crucibles were obtained and then the weight of the empty crucible was taken and this was recorded as (1). 1gm of the sample was weighed separately and put into the crucible. The crucible with the sample was then weighed and this weight was noted as (2). The crucible containing the sample was then placed in the hot air oven at a temperature of 105 degrees Celsius for about an hour. The crucible was then weighed after cooling and this weight was recorded as (3). The crucibles were placed in the muffle furnace for 1 hour at 550 degrees Celsius for the ash content. Then the crucibles were cooled and weighed and this weight was noted as (4).

Calculations for ash and moisture

Wet weight (A) = (2) – (1)

Dry weight (B) = (3) – (1)

Moisture = [A – B / A] × 100

C = (4) – (1) [required for calculating ash]

Ash = [C/A] × 100

Fig 3.4: Protein estimation

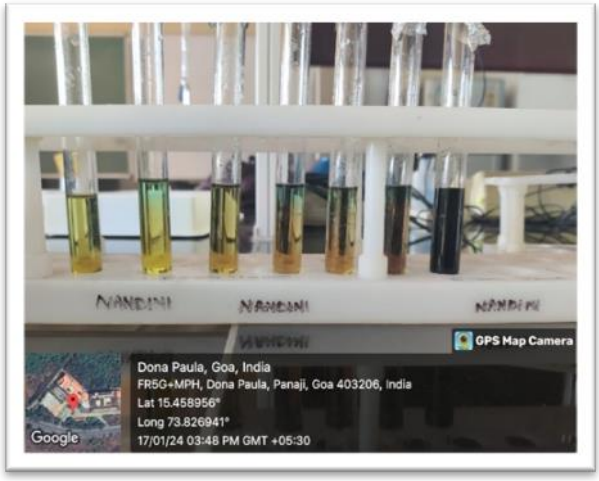

Fig 3.5: Carbohydrate estimation

Fig 3.6: Total lipids estimation

Fig 3.7: Crude fibre estimation

Fig 3.8: Ash and moisture estimation

3.9 Formulation of fish feed

The proximate analysis of feed ingredients such as banana peel flour and groundnut oil cake was carried out first. The banana peels were collected and dried using hot air oven at 60 °C for seven days. Once the peels were fully dried they were ground into powder using a porcelain mortar and pestle and sieved through a fine mesh net to obtain fine powder. The obtained banana peel flour (BPF) was used for performing proximate analysis and was supplemented to the groundnut oil cake feeds at 10%, 20%, and 30% of the total feed weight. The feeds added with BPF were fed to the Tilapia juveniles at an initial rate of 5% of the body weight for 30 days during the month of February to March in the year 2024.

Selecting fish feed Ingredients

The use of locally available raw materials as ingredients in aquaculture feed is a crucial step towards achieving sustainable and responsible resource utilization. This approach not only contributes to potential growth in aquaculture production but also minimizes the environmental impact of the industry. In order to develop a successful feed, it is important to conduct a thorough evaluation of the feed ingredients. This evaluation allows for the identification of the most suitable raw materials for the feed, ensuring that the final product meets the nutritional requirements of the target species while also being environmentally responsible.

- Banana Peel Flour (BPF)
- Groundnut Oil Cake (GOC)
- Palm Oil
- Vitamin and mineral pre-mix

When it comes to feeding cultured fish, it's important to note that no one ingredient can meet all their nutritional needs. Each type of feedstuff in a diet formulation should serve a specific purpose, such as providing energy, protein, essential amino acids, essential fatty acids, and so on. Furthermore, every feedstuff should be the most cost-effective option available for its function in the diet. By having a good understanding of the composition of the available ingredients and the basic nutritional requirements of the fish being raised, it's usually possible to create a diet that will promote optimal growth and survival. Diet formulation is an essential process that involves carefully selecting appropriate feed ingredients to be blended together.

3.9.1 Compound feed for Tilapia:

Sinking fish feed Preparation:

Grinding and Mixing: Grinding is the first important step in the sinking fish feed production process, it requires fine powder. To produce high-quality sinking fish feed, grinding becomes the pivotal first step in the production process. The ingredients selected must be finely ground into a powder, as this ensures a uniform mixture and facilitates easy digestion. The grinding process is essential to achieve homogeneity, which is vital for the nutritional value of the feed and the optimal health of the fish.

Mixing: The process of mixing ground materials with oils and additives is an essential step that requires thoroughness and attention to detail. This process aims to produce a homogeneous blend that exhibits consistency and uniformity in its properties and characteristics.

Extrusion Process

Preconditioning: To create a smooth and consistent mixture, the ingredients are carefully blended and then heated to a specific temperature. Once the mixture is heated, it is moisturized to achieve the optimal level of moisture content. This prepares the mixture for the next step in the process, which is extrusion.

Extruding: The process of producing fish feed involves several crucial steps, but none are as important as the cooking and shaping of the feed mixture into pellets. This step requires a careful balance of pressure, heat, and moisture to ensure that the mixture is cooked thoroughly and shaped into pellets that are the ideal size and texture for the fish to consume. Without this step, the fish feed would not be suitable for consumption by fish, which could have serious consequences for their health and well-being.

Cutting: The process of preparing fish feed involves cutting pellets into specific lengths and shapes, which are carefully chosen based on the size of the fish. This step is crucial to ensure that the fish can easily consume the feed and obtain the necessary nutrients for their growth and development.

Sinking Process

Density Control: The density of the pellets should be controlled to make them sink.

It involves adjustments in ingredients and temperature.

Water Stability: One of the important steps in ensuring the quality of pellets is to conduct water stability testing. This involves checking if the pellets are able to maintain

their form and structure when submerged in water for a certain period of time, without dissolving or disintegrating quickly. It is a crucial process that helps to ensure that the pellets are durable and can withstand the environmental conditions they are meant to be used in.

Cooling and Drying:

Cooling: The process of cooling the finished pellets to room temperature involves reducing their temperature to the same level as the surrounding environment, which is typically around 20-25°C. This is done to ensure that the pellets are safe to handle and store, as they can be quite hot when they first come out of the production process.

Drying: The process of reducing the moisture content of the final pellets through the method of drying is crucial in enhancing the durability and longevity of the pellets, thereby increasing their shelf life.

Quality Assurance:

Laboratory testing: The process involves conducting various tests and evaluations on the products to examine their nutritional value, size, density, and other relevant parameters. The purpose of these tests is to ensure that the products meet the specified standards and requirements.

Sensory evaluation: Evaluating the appearance, smell, and texture.

Packaging and Storage:

Packaging: The process of packaging the final pellets involves delicately placing them inside sturdy bags or containers. These packages are then appropriately labeled with crucial information, such as the ingredients used in the pellets, their nutritional values, and the expiration dates. This ensures that the end product is safe and healthy.

Storage: It is important to ensure that the appropriate storage conditions are maintained in order to preserve the quality of the feeds. This may include factors such as temperature, humidity, lighting, and ventilation. By taking steps to maintain optimal storage conditions, you can help prolong the lifespan and quality of feeds.

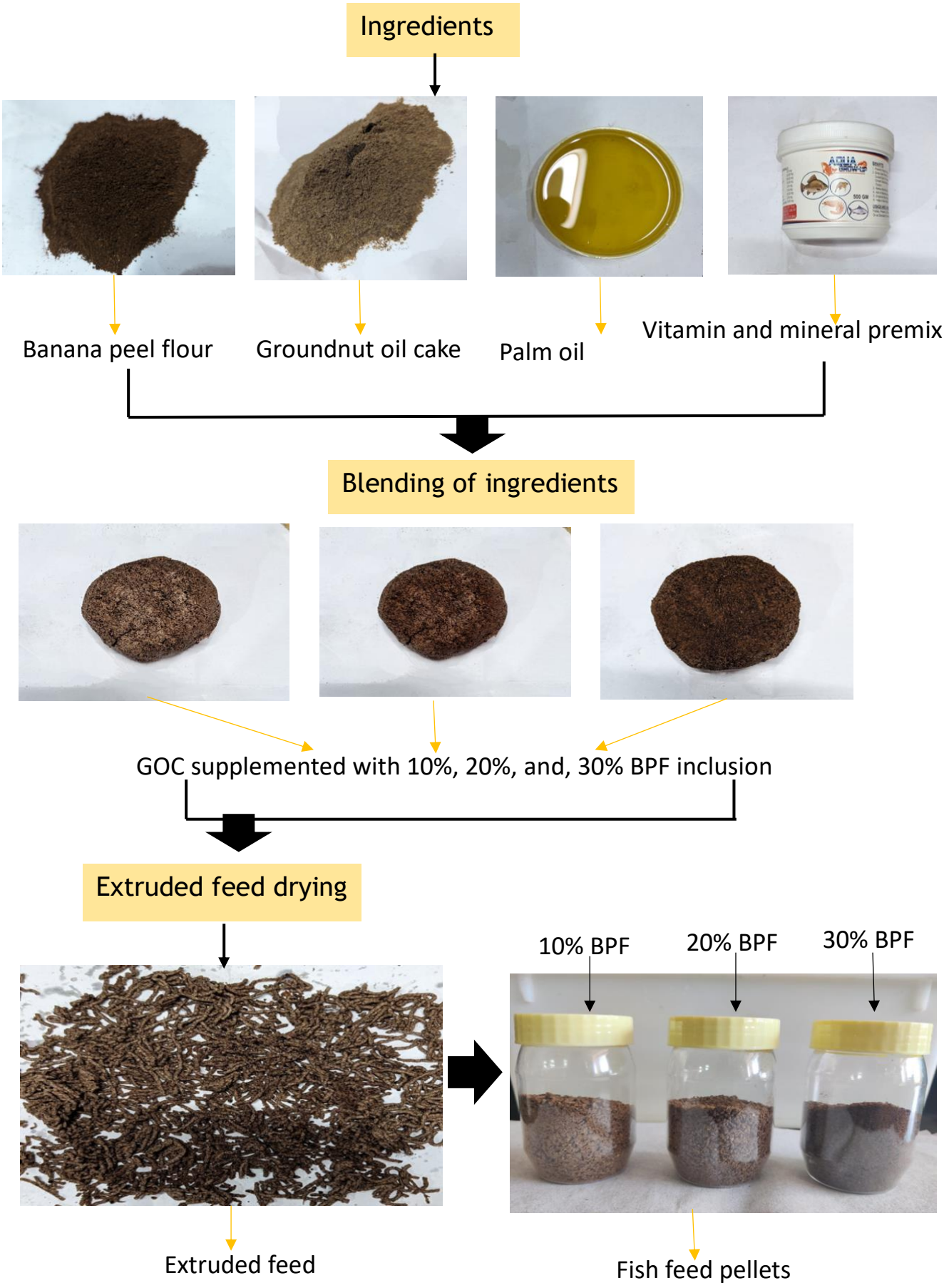

Fig 3.9: Formulation of fish feed

3.10 Fish rearing, feeding, and sampling

In this study, a total of 40 fish were carefully selected from a fish farm and assigned to four glass aquaria based on a complete randomized design. The fish were distributed evenly across the four aquaria to ensure that each aquarium had an equal number of fish. Prior to the start of the 30-day rearing experiment, the fish were acclimatized to the aquarium conditions for a week, which allowed them to adjust to their new environment.

During the rearing experiment, the fish were fed a once-daily ration of 5% of their body weight to ensure they received sufficient nutrients for healthy growth and development. The aquariums were well aerated to maintain optimal conditions for the fish. The temperature, pH, and water quality were closely monitored and kept within safe levels to prevent any adverse effects on the fish's health.

To maintain a clean and hygienic environment, feces and uneaten feed were siphoned out regularly. A third of the water was replaced daily to ensure that the water quality remained high. These measures were vital to maintaining a healthy living environment for the fish and preventing any potential health issues.

Fig 3.10: Fish feeding experiments: groundnut oil cake (control)

Fig 3.11: Fish feeding experiments: Formulated fish feed supplemented with BPF (experimental)

3.11 Growth performance and analysis

The weight sampling was conducted once every 7 days using a weighing balance. Additionally, the length was measured using a scale to ensure accurate measurements. To maintain consistency, the quantity of feed given was precisely measured before feeding. Furthermore, the survival rate in each aquarium was actively monitored on a daily basis to ensure the safety and well-being.

Weight gain (WG), Specific Growth Rate (SGR), and Feed Conversion Ratio (FCR) were measured. Body Lengths were used as indices for the growth performance of fish.

After completing the feeding trial fish was weighed to calculate the percentage weight gain, net weight gain, SGR% (specific growth rate) and FCR (feed conversion ratio) were calculated using a standard formula.

Net weight gain = Average final weight (g) - Average initial weight (g)

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

$$\textbf{Specific growth rate (SGR\%)} = \frac{[\text{(Final wet body weight)} - \text{(Initial wet body weight)}]}{\text{Number of days}} \times 100$$

$$\textbf{Feed conversion ratio} = \frac{\text{Feed Intake (g)}}{\text{Weight gain (g)}}$$

$$\textbf{Survival rate (\%)} = \text{Total fish } \frac{\text{survived}}{\text{number of fish stocked}} \times 100$$

3.12 Estimation of enzyme activity

3.12.1 Amylase Activity Assay

Estimation:

Preparation of Samples:

For the extraction of enzymes from fish intestine samples, homogenize them in a suitable buffer like phosphate buffer. Then, centrifuge the homogenate at low speed to remove debris and collect the supernatant for analysis.

Preparation of Assay Mixture:

Prepare an assay mixture with fish intestine extract, maltose substrate, and buffer. Concentrations depend on assay conditions and can be optimized experimentally.

Incubation:

Allow the amylase enzyme to hydrolyze the maltose substrate by incubating the assay mixture at an appropriate temperature (e.g., 37°C) for a suitable amount of time (e.g., 10-30 minutes).

Stopping the Reaction:

Add DNSA reagent in equal volume to stop the reaction and develop colour.

Colour Development:

Develop the colour reaction by heating the reaction mixture in a boiling water bath for 5 minutes.

Cooling and Neutralization:

Cool down the reaction mixture, then add a few drops of NaOH solution and sodium potassium tartrate solution to neutralize it.

Measurement:

Using a spectrophotometer, measure the absorbance of the reaction mixture at 540 nm. The intensity of the colour is proportional to the amount of reducing sugars produced, indicating amylase activity.

Calculations:

Use a standard curve with known maltose concentrations to determine reducing sugars and enzyme activity in fish intestine samples.

Controls:

Include appropriate controls in the assay, such as a blank (containing no enzyme) and a positive control (with a known amount of enzyme) to obtain accuracy.

3.12.2 Pepsin Activity Assay

Estimation:

Preparation of Samples:

First, homogenize fish intestine samples in a suitable buffer such as phosphate buffer to extract enzymes. Remove debris by centrifuging the homogenate at a low speed and collect the supernatant for analysis.

Preparation of Assay Mixture:

Prepare a mixture of fish intestine extract, hemoglobin (pepsin substrate), and buffer. Optimize concentrations based on assay conditions.

Incubation:

Allow the pepsin enzyme to digest the hemoglobin substrate by incubating the assay mixture at an appropriate temperature (e.g., 37°C) for a suitable amount of time (e.g., 30 minutes to 1 hour).

Stopping the Reaction:

Add 10% TCA in equal volume to the reaction mixture to precipitate undigested hemoglobin and stop the reaction.

Centrifugation:

Separate the precipitated hemoglobin from the supernatant by rapidly centrifuging the mixture, which contains the digested peptides.

Colour Development:

Transfer a portion of the supernatant into a new tube and add NaOH to raise the pH to around 8 to 9. The resulting alkaline hematin complex will develop a pink color.

Measurement:

Use a spectrophotometer to measure absorbance at 280 nm. Pink color intensity indicates pepsin activity.

Calculations:

Use a standard curve with known pepsin concentrations to determine enzyme activity in fish intestine samples.

3.12.3 Lipase Activity Assay

Estimation:

Preparation of Samples:

Extract enzymes from fish intestine samples by homogenizing them in a suitable buffer (e.g. phosphate buffer). Remove debris by centrifuging the homogenate at a low speed and collect the supernatant for analysis.

Preparation of Assay Mixture:

Prepare an assay mixture with fish intestine extract, olive oil emulsion (lipase substrate), and buffer. Optimize concentrations based on assay conditions.

Incubation:

Hydrolyze the olive oil emulsion substrate by incubating the assay mixture at an appropriate temperature (such as 37°C) for a suitable amount of time (for example, 30 minutes to 1 hour).

Stopping the Reaction:

Add a stop solution with an equal volume of chloroform and isopropanol to halt the hydrolysis reaction and extract the fatty acids.

Centrifugation:

Separate the fatty acids in the chloroform layer from the aqueous layer by centrifuging the mixture.

Colour Development:

Transfer a portion of the chloroform layer to a new tube and add phenolphthalein indicator solution and NaOH solution to develop a pink colour due to the formation of fatty acid salts.

Measurement:

Using a spectrophotometer, measure the solution's absorbance at a specific wavelength (550 nm). The intensity of the pink colour is proportional to the amount of released fatty acids, indicating lipase activity.

Calculations:

Use a standard curve with known concentrations of fatty acids to determine lipase activity in fish intestine samples.

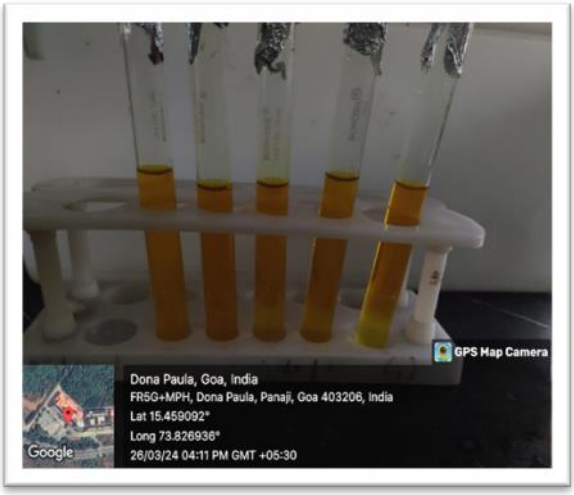

Fig 3.12:Amylase estimation

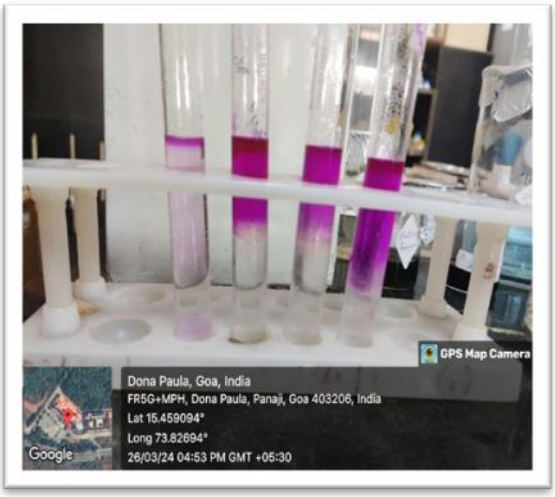

Fig 3.13:Lipase estimation

Fig 3.14: Pepsin estimation

3.13 Statistical analysis

The collected data was subjected to statistical analysis using Excel and GraphPad Software. All the samples were analyzed and were expressed as Mean ± SD (Standard Deviation). Statistical analysis of data was performed by Analysis of Variance (ANOVA) to estimate the difference in mean concentrations of growth parameters and digestive enzymatic activity and the f value and P value were determined. * $P < 0.05$, **$P < 0.01$, ***$P < 0.001$, NS = Non significant. Pearson correlation was used to measure a linear correlation between the variables.

CHAPTER 4:

ANALYSIS AND CONCLUSIONS

4.1 Proximate Analysis

The nutritional value of banana peel flour (BPF), groundnut oil cake (GOC), GOC supplemented with 10% BPF, GOC supplemented with 20% BPF, and GOC supplemented with 30% BPF used in the study are presented in Table 4.1, 4.2, 4.3, 4.4, and, 4.5 respectively, which is estimated through proximate analysis. The proximate analysis provides important information on the nutritional composition of the sample and helps to assess its quality by determining its moisture, protein, lipid, ash, fibre, and carbohydrate content.

4.1.1 Proteins

The protein content mean values of different samples were analyzed in this study. The BPF sample showed a mean protein content of 3.95 ±0.50. The sample of GOC had a mean protein content of 40.68 ±0.45. Furthermore, the GOC sample was supplemented with three different percentages of BPF: 10%, 20%, and 30%. The GOC sample supplemented with 10% BPF showed a mean protein content of 37.11 ±0.92, while the sample supplemented with 20% BPF had a mean protein content of 37.88±0.04. Finally, the sample supplemented with 30% BPF had a mean protein content of 32.53±0.69. The analysis shows that the protein content in GOC was significantly higher compared to BPF. When GOC was supplemented with 10% and 20% BPF, the protein content was found to be similar, with no differences observed. However, when GOC was supplemented with 30% BPF, the protein content was comparatively lower than that of 10% and 20% BPF.

4.1.2 Carbohydrates

The present study aimed to analyze the mean carbohydrate content of various samples. The BPF sample exhibited a mean carbohydrate content of 43.96 ±0.47. The GOC sample, on the other hand, had a relatively lower mean carbohydrate content of 34.53±0.58. Furthermore, the GOC sample was supplemented with three different percentages of BPF: 10%, 20%, and 30%. The sample supplemented with 10% BPF revealed a mean carbohydrate content of 39.46±0.65, while the sample supplemented with 20% BPF exhibited a mean carbohydrate content of 47.53±0.15. Finally, the sample supplemented with 30% BPF displayed a mean carbohydrate content of 46.55±0.17. It has been observed that the level of carbohydrates in BPF is relatively higher than that in GOC. Additionally, the results of the study indicate that the sample which was enriched with 20% BPF had a higher carbohydrate content than the samples containing 30% and 10% BPF.

4.1.3 Fibre

The samples, BPF and GOC were analyzed for their mean fibre content. The BPF sample had a mean fibre content of 6.1±0.1, whereas the GOC sample had a relatively lower mean fibre content of 4.51±0.33. The GOC sample was further supplemented with three different percentages of BPF, namely 10%, 20%, and 30%. The sample supplemented with 10% BPF showed a relatively higher mean fibre content of 4.80±0.02, while the sample supplemented with 20% BPF exhibited a further increase in mean fibre content to 4.87±0.3. Finally, the sample supplemented with 30% BPF displayed the highest mean fibre content of 5.21±0.1.

4.1.4 Lipids

The study analyzed and compared the mean lipid content of different samples. Upon analysis, it was found that the BPF sample exhibited a mean lipid content of 1.65±0.05, whereas the GOC sample had a higher mean lipid content of 7.47±0.35. To study the impact of BPF supplementation on the lipid content of GOC, three different percentages of BPF were added to the sample: 10%, 20%, and 30%. The sample that was supplemented with 10% BPF demonstrated a mean lipid content of 5.42±0.5. Similarly, the sample supplemented with 20% BPF exhibited a mean lipid content of 3.17±0.3, indicating a decrease in lipid content. Finally, the sample supplemented with 30% BPF displayed a mean lipid content of 4.04±0.05.

4.1.5 Ash

The study investigated the ash content of different samples. The BPF sample displayed a mean ash content of 1.23 ±0.08, while the GOC sample had a higher mean ash content of 5.43 ±0.19. To improve the nutritional quality of GOC, it was supplemented with three different percentages of BPF: 10%, 20%, and 30%. The sample enriched with 10% BPF showed a mean ash content of 3.20 ±0.05, while the sample containing 20% BPF exhibited a lower mean ash content of 2.63 ±0.03. Finally, the sample supplemented with 30% BPF revealed a mean ash content of 2.85 ±0.04.

4.1.6 Moisture

The study involved the analysis of the mean moisture content of different samples. The BPF sample exhibited a mean moisture content of 13.32 ±0.6, which signifies that it contains a higher amount of moisture content compared to the GOC sample. On the other hand, the GOC sample had a mean moisture content of 7.23 ±0.3, indicating that it contained a lower amount of moisture content as compared to the BPF sample. Furthermore, the GOC sample was supplemented with three different percentages of BPF, including 10%, 20%, and 30%. The sample supplemented with 10% BPF revealed a mean moisture content of 6.82 ±0.4, while the sample supplemented with 20% BPF exhibited a mean moisture content of 6.04 ±0.2. Finally, the sample supplemented with 30% BPF displayed a mean moisture content of 5.92 ±0.2, indicating a reduction in the moisture content.

4.2 Growth Parameters

The results of all the growth parameters (7 days) are presented in Table 4.6. The initial weight of stocking fish (*Oreochromis mossambicus*) showed statistically non-significant ($P > 0.05$). For the control, the fish were fed with GOC (groundnut oil cake) which showed a lower weight gain (1.46±0.64). The fish were fed with different treatments of BPF in 10%, 20%, and 30% which was Supplemented to GOC. The treatment BPF 20% (2.43±0.43) displayed the highest weight gain as compared to all other treatments, GOC (1.46±0.64), BPF 10% (1.85±0.57), and BPF 30% (2.04±0.52). Treatment BPF 30% (2.04±0.52) showed higher weight gain when compared to GOC (1.46±0.64) and BPF 10% (1.85±0.57). Treatment BPF 20% (1.08±0.03) showed

highest net weight gain as compared to other treatments GOC (0.33±0.02), BPF 10% (0.63±0.04), and BPF 30% (1.01±0.02). BPF 30% showed high net weight gain when compared to GOC (0.33±0.02) and BPF 10% (0.63±0.04). Treatment BPF 30% (96.15±0.78) showed the highest percentage weight gain as compared to GOC (20.20±0.41), 10% (52.89±0.64), and 20% (80.00±0.27). In treatment GOC (0.44±0.04) FCR was exceptionally higher than treatment BPF 10% (0.35±0.02), BPF 20% (0.26±0.01), and BPF 30% (0.31±0.01). In treatment, BPF 10% (0.35±0.02) the FCR is high as compared to BPF 20% (0.26±0.01), and 30% (0.31±0.01). The lowest value of FCR was recorded in BPF 20% (0.26±0.01), which is best. The highest SGR (specific growth rate) value was observed in BPF 20% (15.42±0.27). The low-value SGR was observed in GOC (4.71±0.21), and BPF 10% (9.14±0.35). In treatment, BPF 30% the SGR value rate (14.28±0.51) was observed to average as compared to other treatments shown in Table 4.6. No significant difference was observed in the survival rate of fish. The correlation test was used to determine the relation between the weight and length parameters. The results indicated a strong positive correlation (r=0.7688, P= 0.2312) between the two variables.

The results of all the growth parameters (14 days) are presented in Table 4.7. The initial weight of stocking fish (*Oreochromis mossambicus*) showed statistically non-significant ($P > 0.05$). For the control, the fish were fed with GOC (groundnut oil cake) which showed a lower weight gain (1.79±0.40). The fish were fed with different treatments of BPF in 10%, 20%, and 30% which was Supplemented to GOC. The treatment BPF 20% (3.39±0.30) displayed the highest weight gain as compared to all other treatments GOC (1.79±0.40), BPF 10% (2.85±0.48), and 30% (2.29±0.73). Treatment BPF 30% (2.29±0.73) showed higher weight gain when compared to GOC (1.79±0.40) and BPF 10% (2.85±0.48). Treatment BPF 20% (2.04±0.02) showed

highest net weight gain as compared to other treatments GOC (0.66±0.03), BPF 10% (1.64±0.04), and BPF 30% (1.25±0.03). BPF 10% showed high net weight gain when compared to GOC (0.66±0.03) and BPF 30% (1.25±0.03). Treatment BPF 10% (54.05±0.46) showed the highest percentage weight gain as compared to GOC (22.60±0.21), 20% (39.50±0.56), and 30% (12.25±0.58). In treatment GOC (0.36±0.04) FCR was exceptionally higher than treatment BPF 10% (0.22±0.02), BPF 20% (0.19±0.01), and BPF 30% (0.28±0.01). In treatment, BPF 30% (0.28±0.01) the FCR is high as compared to BPF 10% (0.22±0.02), and 20% (0.19±0.01). The lowest value of FCR was recorded in BPF 20% (0.19±0.01), which is best. The highest SGR (specific growth rate) value was observed in BPF 20% (14.57±0.73). The low-value SGR was observed in GOC (4.71±0.28), and BPF 30% (8.92±0.48). In treatment, BPF 10% the SGR value rate (11.71±0.65) was observed to average as compared to other treatments shown in Table 4.7. No significant difference was observed in the survival rate of fish. The correlation test was used to determine the relation between the weight and length parameters. The results indicated a very strong positive correlation (r=0.9767, P= 0.0233) between the two variables.

The results of all the growth parameters (21 days) are presented in Table 4.8. The initial weight of stocking fish (*Oreochromis mossambicus*) showed statistically non-significant (P > 0.05). For the control, the fish were fed with GOC (groundnut oil cake) which showed a lower weight gain (2.50±0.37). The fish were fed with different treatments of BPF in 10%, 20%, and 30% which was Supplemented to GOC. The treatment BPF 20% (3.59±0.48) displayed the highest weight gain as compared to all other treatments GOC (2.50±0.37), BPF 10% (3.02±0.52), and 30% (2.80±0.34). Treatment BPF 10% (3.02±0.52) showed higher weight gain when compared to GOC

(2.50±0.37) and BPF 30% (2.80±0.34). Treatment BPF 20% (2.24±0.04) showed highest net weight gain as compared to other treatments GOC (1.37±0.03), BPF 10% (1.81±0.05), and BPF 30% (1.76±0.02). BPF 10% showed high net weight gain when compared to GOC (1.37±0.03) and BPF 30% (1.76±0.02). Treatment GOC (39.66±0.74) showed the highest percentage weight gain as compared to 10% (5.96±0.67), 20% (5.89±0.56), and 30% (22.27±0.52). In treatment GOC (0.26±0.06) FCR was exceptionally higher than treatment BPF 10% (0.21±0.02), BPF 20% (0.18±0.05), and BPF 30% (0.23±0.04). In treatment, BPF 30% (0.23±0.04) the FCR is high as compared to BPF 10% (0.21±0.02), and 30% (0.23±0.04). The lowest value of FCR was recorded in BPF 20% (0.18±0.05), which is best. The highest SGR (specific growth rate) value was observed in BPF 20% (10.66±0.47). The low-value SGR was observed in GOC (6.52±0.51), and BPF 30% (8.38±0.61). In treatment, BPF 10% the SGR value rate (8.61±0.42) was observed to average as compared to other treatments shown in Table 4.8. No significant difference was observed in the survival rate of fish. The correlation test was used to determine the relation between the weight and length parameters. The results indicated a very weak positive correlation (r=0.1315, P= 0.8685) between the two variables.

The results of all the growth parameters (28 days) are presented in Table 4.9. The initial weight of stocking fish (*Oreochromis mossambicus*) showed statistically non-significant (P > 0.05). For the control, the fish were fed with GOC (groundnut oil cake) which showed a lower weight gain (3.45±0.21). The fish were fed with different treatments of BPF in 10%, 20%, and 30% which was Supplemented to GOC. The treatment BPF 20% (4.19±0.26) displayed the highest weight gain as compared to all other treatments GOC (3.45±0.21), BPF 10% (3.95±0.16), and 30% (3.66±0.17). Treatment BPF 10% (3.95±0.16) showed higher weight gain when compared to GOC

(3.45±0.21) and BPF 30% (3.66±0.17). Treatment BPF 20% (2.84±0.02) showed highest net weight gain as compared to other treatments GOC (2.32±0.04), BPF 10% (2.74±0.03), and BPF 30% (2.62±0.04). BPF 10% showed high net weight gain when compared to GOC (2.32±0.04) and BPF 30% (2.62±0.04). Treatment GOC (38.00±0.48) showed the highest percentage weight gain as compared to 10% (30.79±0.66), 20% (16.71±0.57), and 30% (30.71±0.62). In treatment GOC (0.18±0.03) FCR was exceptionally higher than treatment BPF 10% (0.16±0.02), BPF 20% (0.15±0.03), and BPF 30% (0.17±0.04). In treatment, BPF 30% (0.17±0.04) the FCR is high as compared to BPF 20% (0.15±0.03), and 10% (0.16±0.02). The lowest value of FCR was recorded in BPF 20% (0.15±0.03), which is best. The highest SGR (specific growth rate) value was observed in BPF 20% (10.14±0.28). The low-value SGR was observed in GOC (8.28±0.25), and BPF 10% (9.78±0.47). In treatment, BPF 30% the SGR value rate (9.35±0.21) was observed to average as compared to other treatments shown in Table 4.9. No significant difference was observed in the survival rate of fish. The correlation test was used to determine the relation between the weight and length parameters. The results indicated a moderate positive correlation (r=0.5524, P= 0.4476) between the two variables.

4.3 Enzyme Activity

The results of the digestive enzyme activity of amylase are represented in Table 4.10. The amylase activity was showed statistically non-significant (P > 0.05). The amylase activity was observed highest in BPF 20% (33.98±0.39) when compared to other treatments, BPF 10% (30.98±0.32) and BPF 30% (32.22±0.60) however, were higher than GOC (11.87±0.55). The results of the digestive enzyme activity of lipase are represented in Table 4.11. The lipase activity was showed statistically significant (P<0.05). The lipase activity was observed highest in BPF 20% (31.52±0.59) as

compared to 10% (28.12±0.39) and 30% (29.87±0.55) and was observed lowest in GOC (20.33±0.63). The results of the digestive enzyme activity of pepsin are represented in Table 4.12. The pepsin activity was showed statistically significant ($P<0.05$). The pepsin activity was observed highest in GOC (21.38±0.69) as compared to BPF 10% (14.43±0.53), BPF 20% (13.63±0.59), and BPF 30% (13.23±0.57) treatment.

Analysis of the variance of all parameters is presented in Table 4.13.

LIST OF TABLES

Table 4.1 Proximate composition of Banana Peel (Mean±SD).

Parameters	**Values**
Crude protein	3.95 ±0.50
Carbohydrates	43.96 ±0.47
Moisture (%)	13.32 ±0.6
Ash (%)	1.23 ±0.08
Crude Fibre (%)	6.1±0.1
Total lipids (%)	1.65±0.05

Table 4.2 Proximate composition of Groundnut Oil Cake (Mean±SD).

Parameters	**Values**
Crude protein	**40.68 ±0.45**
Carbohydrates	**34.53 ±0.58**
Moisture (%)	**7.23 ±0.3**
Ash (%)	**5.43 ±0.19**
Crude Fibre (%)	**4.51±0.33**
Total lipids (%)	**7.47±0.35**

Table 4.3 Proximate composition of GOC supplemented with 10% BPF (Mean±SD).

Parameters	**Values**
Crude protein	37.11 ±0.92
Carbohydrates	39.46 ±0.65
Moisture (%)	6.82 ±0.4
Ash (%)	3.20 ±0.05
Crude Fibre (%)	4.80±0.02
Total lipids (%)	5.42±0.5

Table 4.4 Proximate composition of GOC supplemented with 20% BPF (Mean±SD).

Parameters	**Values**
Crude protein	37.88 ±0.82
Carbohydrates	47.53 ±0.15
Moisture (%)	6.04 ±0.2
Ash (%)	2.63 ±0.03
Crude Fibre (%)	4.87±0.3
Total lipids (%)	4.81±0.02

Table 4.5 Proximate composition of GOC supplemented with 30% BPF (Mean±SD).

Parameters	**Values**
Crude protein	32.53 ±0.69
Carbohydrates	46.55 ±0.17
Moisture (%)	5.92 ±0.2
Ash (%)	2.85 ±0.04
Crude Fibre (%)	5.21±0.1
Total lipids (%)	4.04±0.05

Table 4.6: Growth parameters of Tilapia juveniles in treatment fed with different inclusion of banana peel in the diet for 7 days (Mean±SD).

Parameters	GOC (100%)	BPF (10%)	BPF (20%)	BPF (30%)
Initial Weight (g)	1.13±0.24	1.21±0.43	1.35±0.52	1.04±0.41
Final Weight (g)	1.46±0.64	1.85±0.57	2.43±0.43	2.04±0.52
Length (cm)	4.45±0.59	4.85±0.45	5.01±0.39	5.25±0.49
Weight Gain (%)	20.20±0.41	52.89±0.64	80±0.27	96.15±0.78
Net weight gain (g)	0.33±0.09	0.63±0.14	1.08±0.17	1.01±0.10
FCR	0.44±0.15	0.35±0.08	0.26±0.10	0.31±0.16
SGR (%)	4.71±0.21	9.14±0.35	15.42±0.27	14.28±0.51
Survival Rate (%)	100±0.00	100±0.00	100±0.00	100±0.00

Table 4.7: Growth parameters of Tilapia juveniles in treatment fed with different inclusion of banana peel in the diet for 14 days (Mean±SD).

Parameters	GOC (100%)	BPF (10%)	BPF (20%)	BPF (30%)
Initial Weight (g)	1.13±0.24	1.21±0.43	1.35±0.52	1.04±0.41
Final Weight (g)	1.79±0.40	2.85±0.48	3.39±0.30	2.29±0.73
Length (cm)	5.3±0.41	5.75±0.78	6.2±0.64	5.3±0.54
Weight Gain (%)	22.60±0.21	54.05±0.46	39.50±0.56	12.25±0.58
Net weight gain (g)	0.66±0.13	1.64±0.20	2.04±0.19	1.25±0.14
FCR	0.36±0.07	0.22±0.06	0.19±0.08	0.28±0.04
SGR (%)	4.71±0.28	11.71±0.65	14.57±0.73	8.92±0.48
Survival Rate (%)	100±0.00	100±0.00	100±0.00	100±0.00

Table 4.8: Growth parameters of Tilapia juveniles in treatment fed with different inclusion of banana peel in the diet for 21 days (Mean±SD).

Parameters	GOC (100%)	BPF (10%)	BPF (20%)	BPF (30%)
Initial Weight (g)	1.13±0.24	1.21±0.43	1.35±0.52	1.04±0.41
Final Weight (g)	2.50±0.37	3.02±0.52	3.59±0.48	2.80±0.34
Length (cm)	6.88±0.21	6.81±0.42	6.96±0.31	7.05±0.20
Weight Gain (%)	39.66±0.74	5.96±0.67	5.89±0.56	22.27±0.52
Net weight gain (g)	1.37±0.23	1.81±0.15	2.24±0.17	1.76±0.22
FCR	0.26±0.06	0.21±0.09	0.18±0.05	0.23±0.10
SGR (%)	6.52±0.51	8.61±0.42	10.66±0.47	8.38±0.61
Survival Rate (%)	100±0.00	100±0.00	100±0.00	100±0.00

Table 4.9: Growth parameters of Tilapia juveniles in treatment fed with different inclusion of banana peel in the diet for 28 days (Mean±SD).

Parameters	GOC (100%)	BPF (10%)	BPF (20%)	BPF (30%)
Initial Weight (g)	1.13±0.24	1.21±0.43	1.35±0.52	1.04±0.41
Final Weight (g)	3.45±0.21	3.95±0.16	4.19±0.26	3.66±0.17
Length (cm)	7.03±0.27	7.35±0.45	7.30±0.23	7.40±0.24
Weight Gain (%)	38±0.48	30.79±0.66	16.71±0.57	30.71±0.62.
Net weight gain (g)	2.32±0.24	2.74±0.13	2.84±0.18	2.62±0.20
FCR	0.18±0.04	0.16±0.07	0.15±0.05	0.17±0.04
SGR (%)	8.28±0.25	9.78±0.47	10.14±0.28	9.35±0.21
Survival Rate (%)	100±0.00	100±0.00	100±0.00	100±0.00

Table 4.10: Amylase activity in fish species fed with different percentages of BPF for 30 days (Mean±SD).

Feed Ingredients	Inclusion level	*Oreochromis mossambicus*
		Intestine
Groundnut Oil Cake	100%	11.87±0.55
Banana Peel Flour	10%	30.93±0.39
	20%	33.98±0.32
	30%	32.22±0.60

Table 4.11: Lipase activity in fish species fed with different percentages of BPF for 30 days (Mean±SD).

Feed Ingredients	Inclusion level	*Oreochromis mossambicus*
		Intestine
Groundnut Oil Cake	100%	20.33±0.63
Banana Peel Flour	10%	28.12±0.39
	20%	31.52±0.59
	30%	29.87±0.55

Table 4.12: Pepsin activity in fish species fed with different percentages of BPF for 30 days (Mean±SD).

Feed Ingredients	Inclusion level	*Oreochromis mossambicus*
		Intestine
Groundnut Oil Cake	100%	21.38±0.69
Banana Peel Flour	10%	14.43±0.53
	20%	13.63±0.59
	30%	13.23±0.57

Table 4.13: Analysis of variance of different parameters.

Growth Parameters	***F value***	***P-value***	
Initial Weight (g)	30.72	1.22	NS
Final Weight (g)	10.18	0.001	***
Length (cm)	58.63	1.93	NS
Weight Gain (%)	3.17	0.06	NS
Net Weight Gain (g)	15.14	0.0002	***
FCR	6.84	0.006	**
SGR (%)	0.34	0.79	NS
Digestive Enzyme Activity			
Amylase	36.53	8.14	NS
Pepsin	6.44	0.001	***
Lipase	3.24	0.038	*

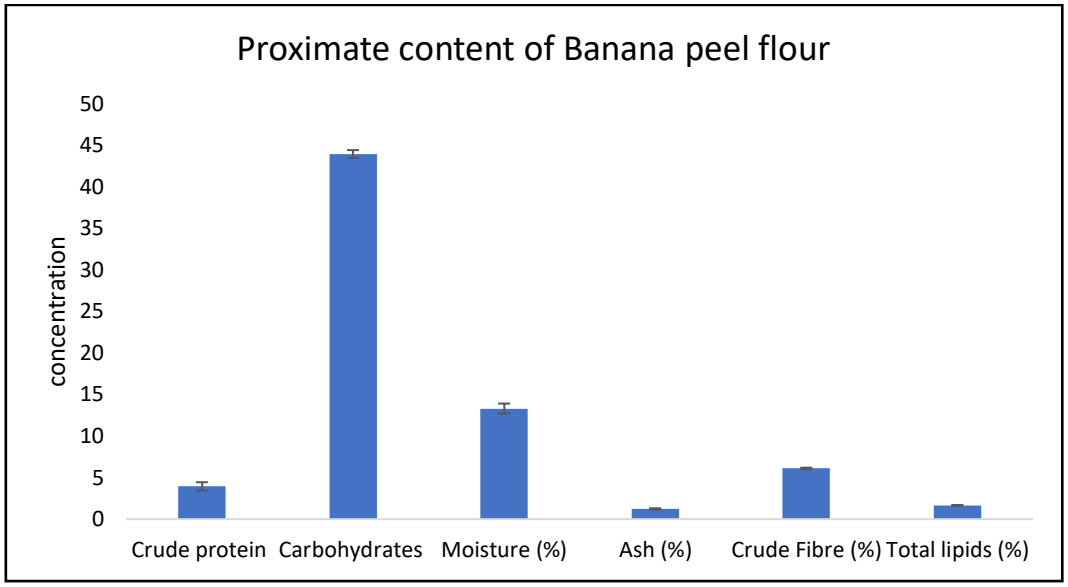

Fig. 4.1:Proximate content of banana peel

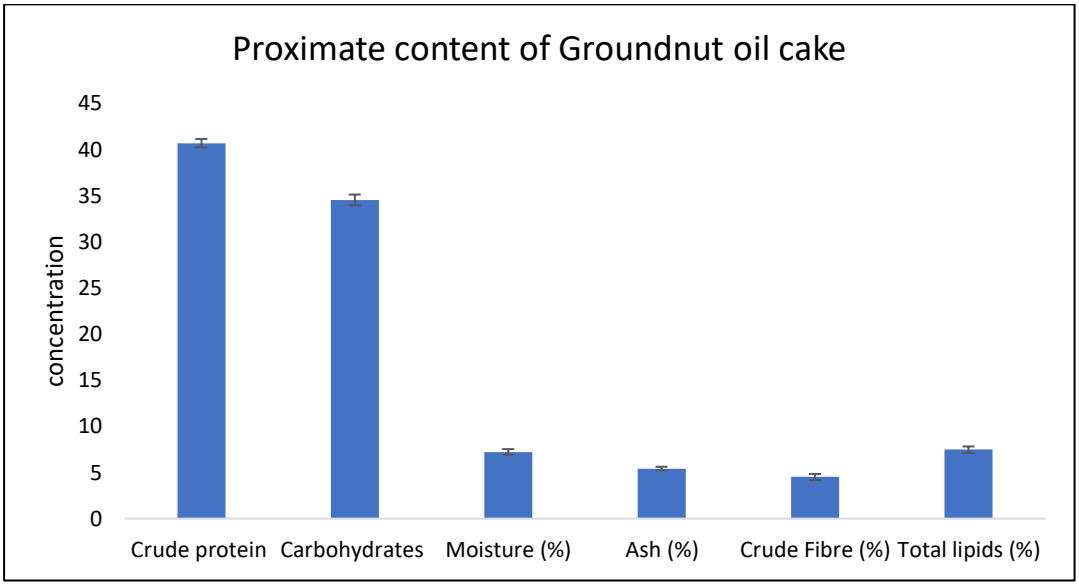

Fig. 4.2:Proximate content of Groundnut oil cake

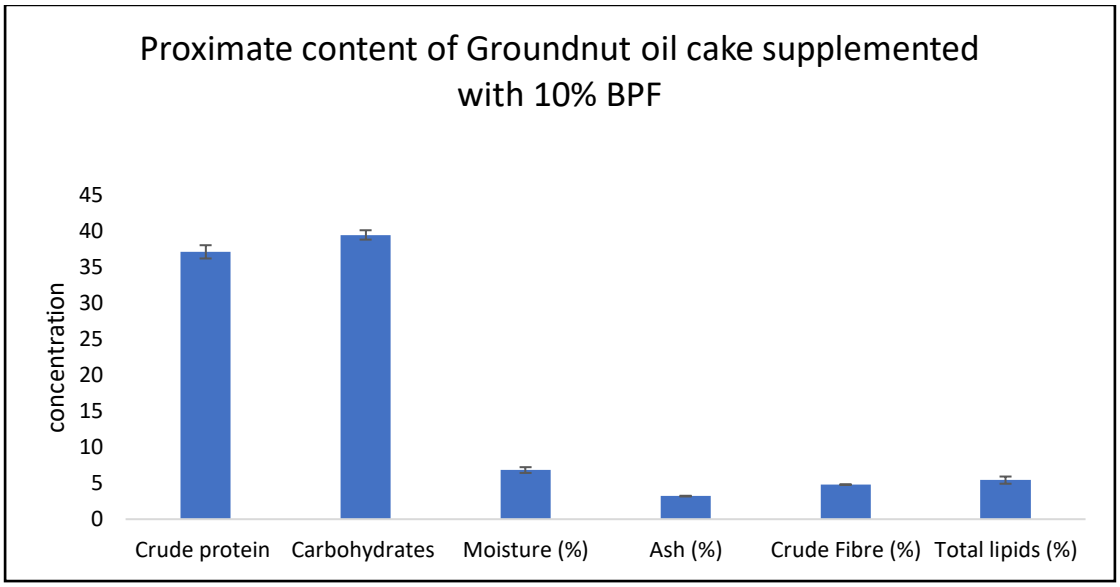

Fig. 4.3: Proximate content of GOC supplemented with 10% BPF

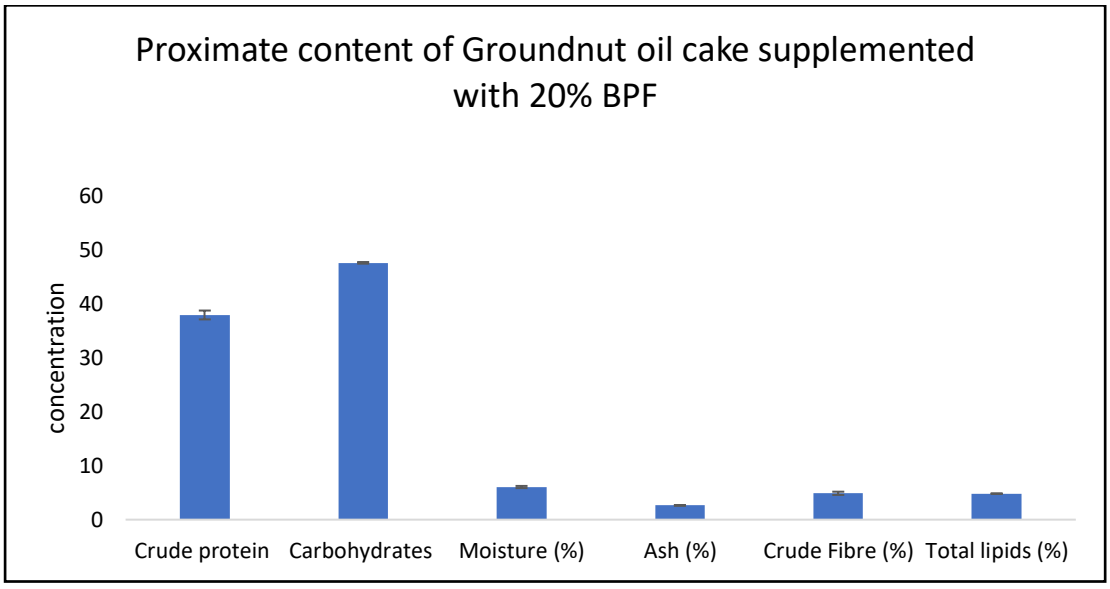

Fig. 4.4: Proximate content of GOC supplemented with 20% BPF

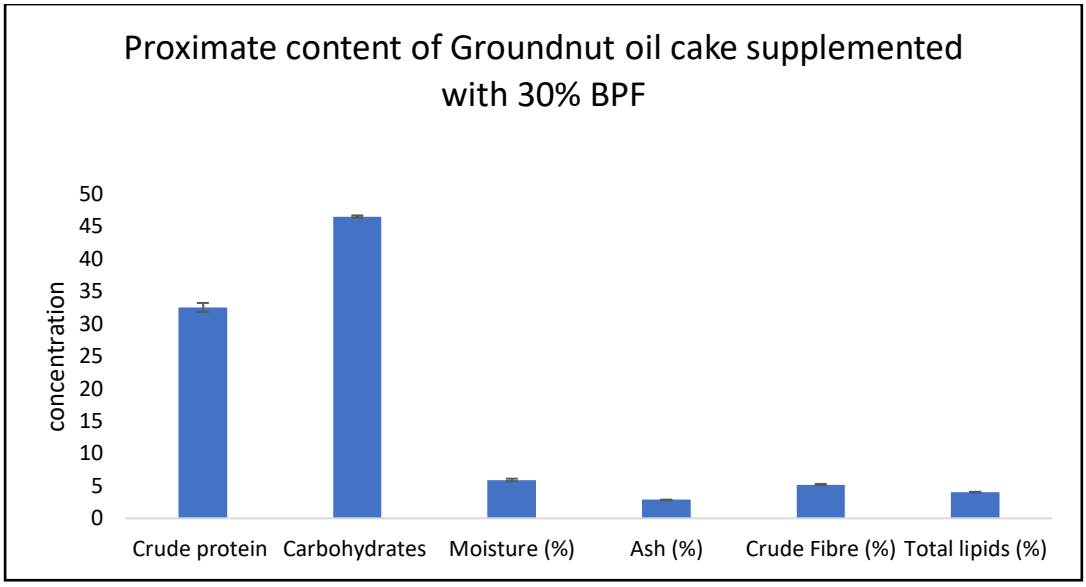

Fig 4.5: Proximate content of GOC supplemented with 30% BPF

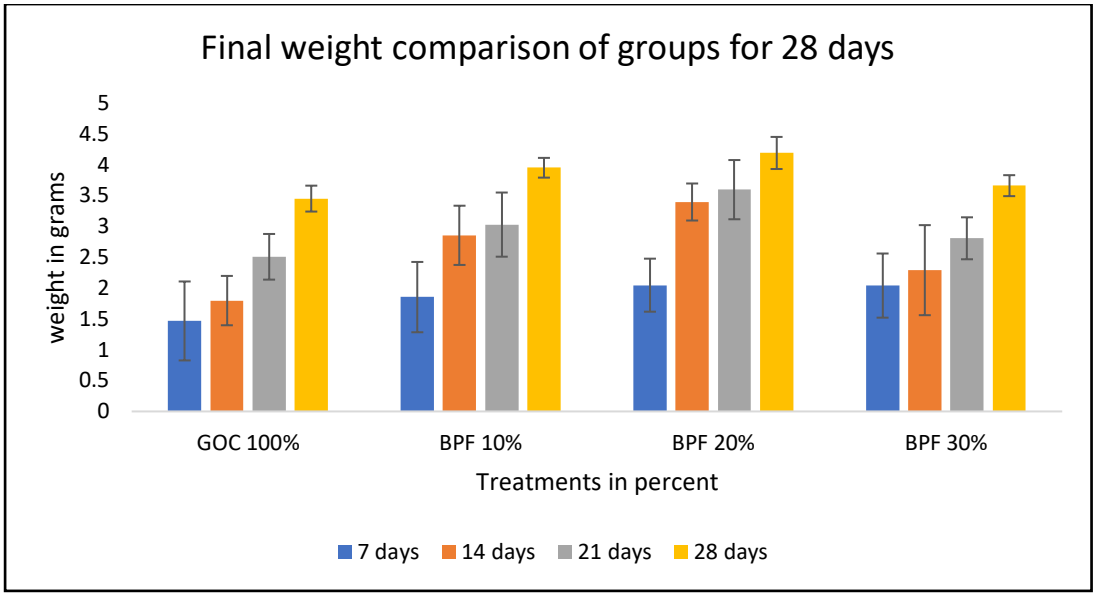

Fig 4.6: Comparison of final weight in different groups for 28 days.

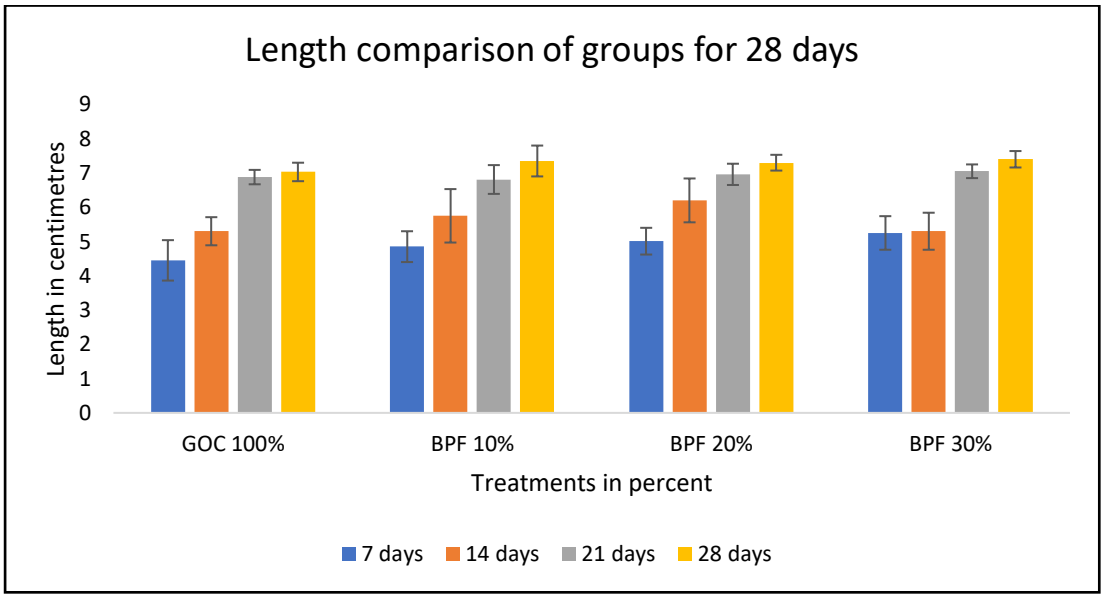

Fig 4.7: Comparison of length in different groups for 28 days.

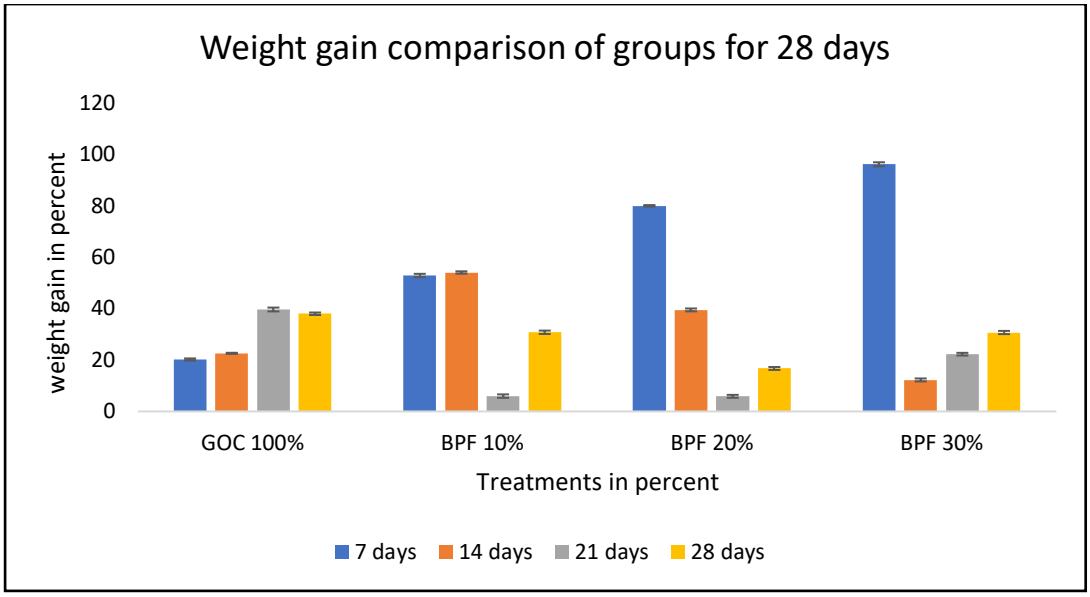

Fig 4.8: Comparison of weight gain in different groups for 28 days.

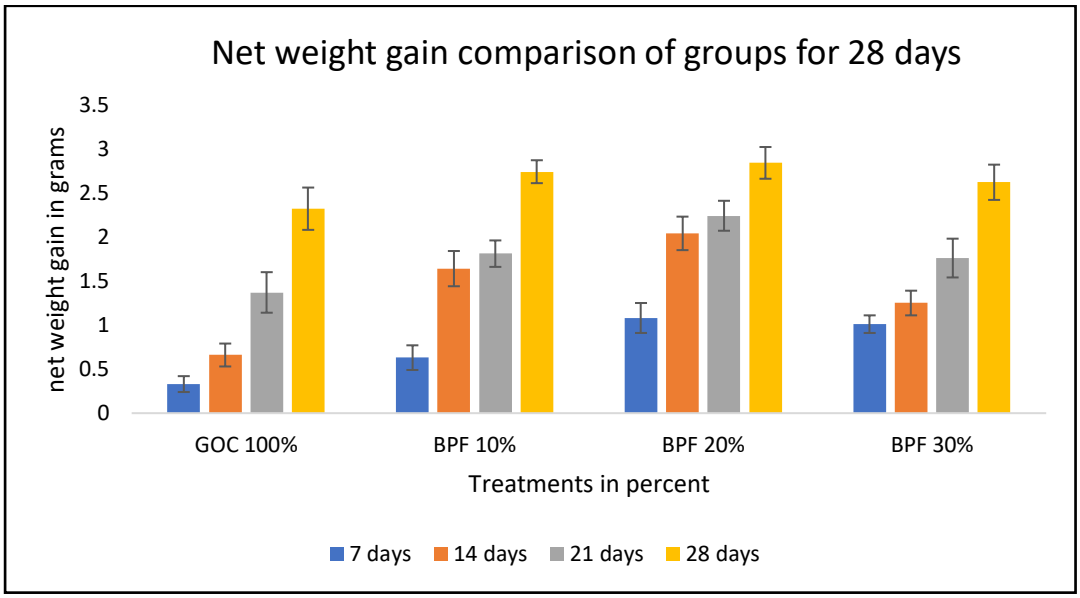

Fig 4.9: Comparison of net weight gain in different groups for 28 days.

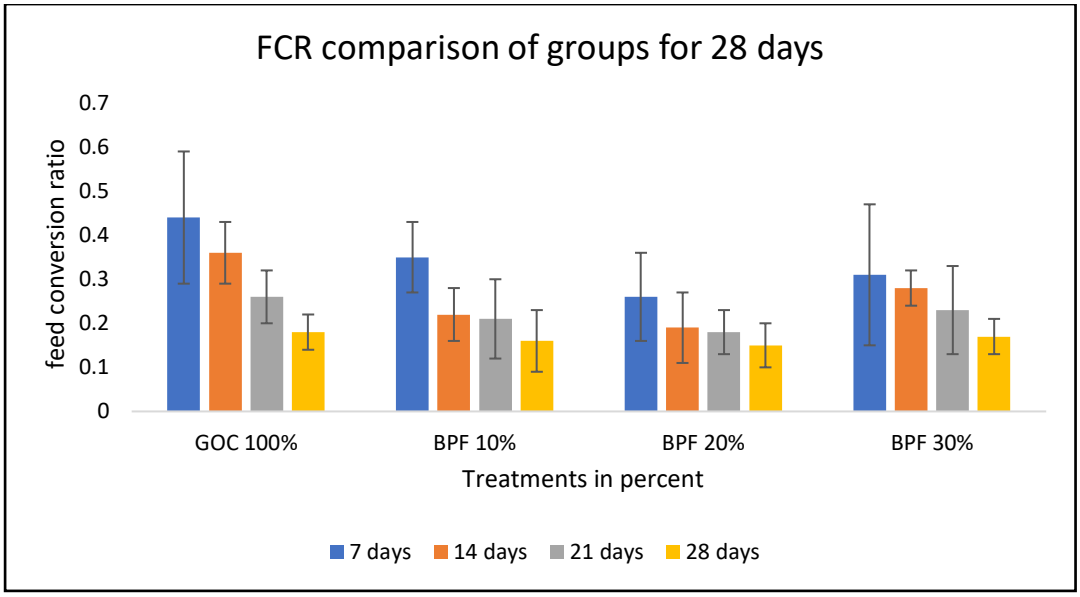

Fig 4.10: Comparison of FCR in different groups for 28 days.

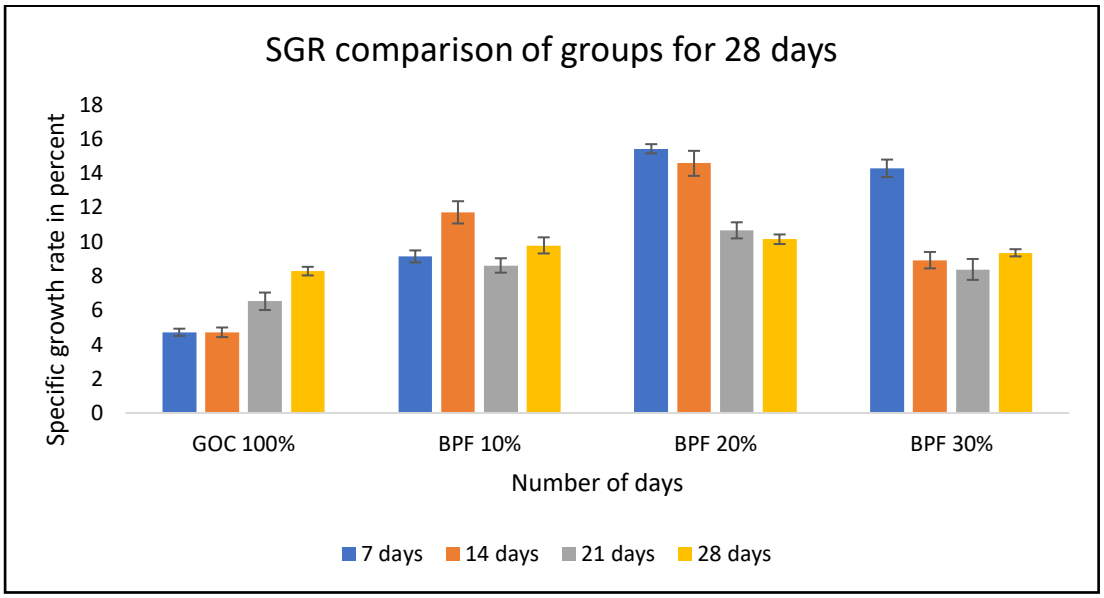

Fig 4.11: Comparison of SGR in different groups for 28 days.

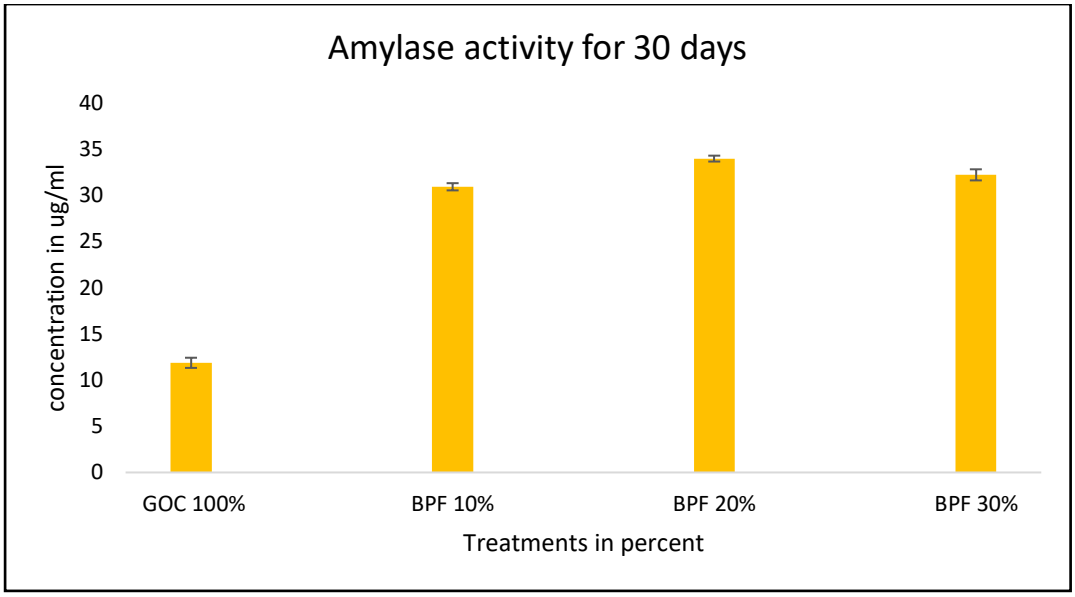

Fig 4.12: Estimation of amylase activity in fishes fed with different feed treatments for 30 days.

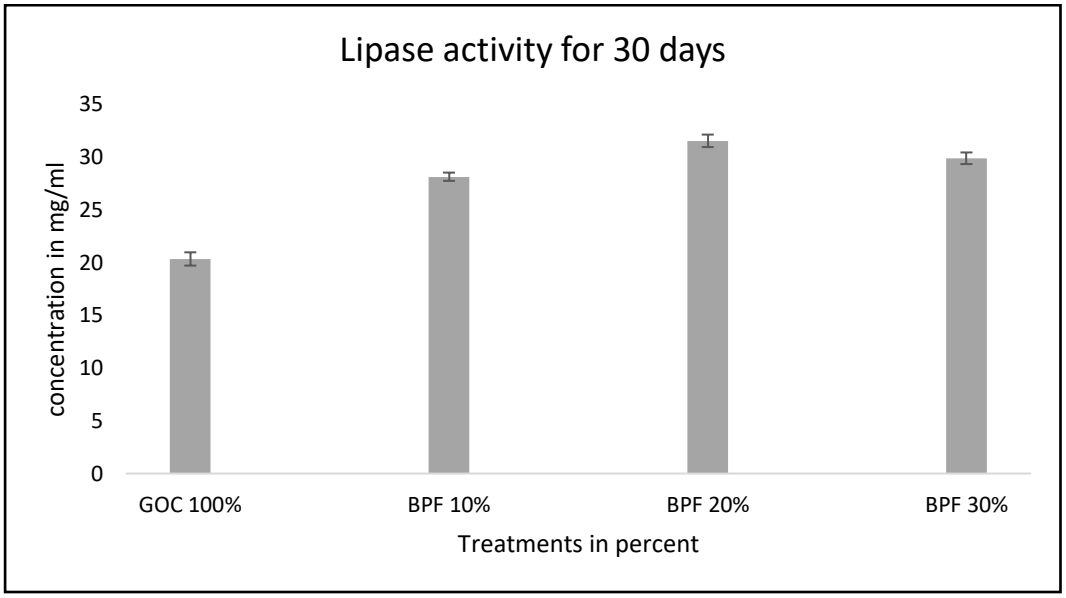

Fig 4.13: Estimation of lipase activity in fishes fed with different feed treatments for 30 days.

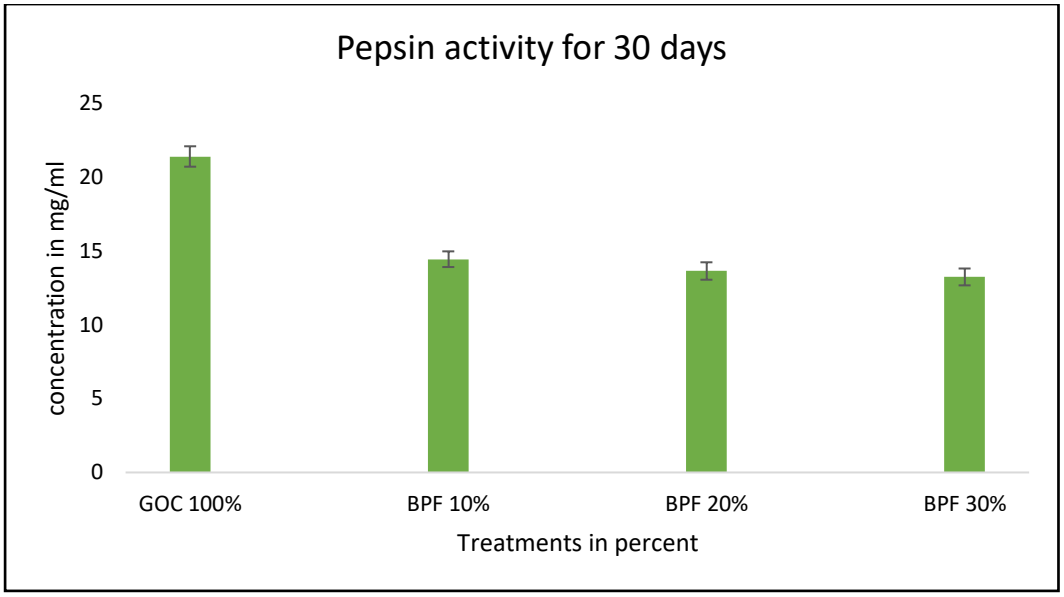

Fig 4.14: Estimation of pepsin activity in fishes fed with different feed treatments for 30 days.

DISCUSSION

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As of today, no research study has been published that has estimated the impact of feeding BPF (Banana Peel Flour) on the growth and digestive enzymatic activity of Tilapia fish in Goa. The present study aims to bridge this gap by revealing some important findings. Firstly, the proximate analysis of BPF was estimated, which includes the measurement of various nutritional parameters such as protein, fat, ash, and moisture content. Ndarubu et al., (2021) found that banana peels could be considered a good source of nutrients for the production of human and animal feeds, and their utilization for this purpose should be encouraged, as this will also help in reducing the menace of nutrient deficiencies.

Banana Peel Flour is a highly nutritious food ingredient that contains a significant amount of carbohydrates, proteins, lipids, fibre, and other essential nutrients. It is a rich source of essential amino acids that are needed for better fish growth and overall performance. When used in fish feed, Banana Peel Flour has been found to improve the nutritional quality of the feed and enhance the growth rate and health of fish. Additionally, Banana Peel Flour is a sustainable food ingredient that can be produced from banana peels that would otherwise be discarded as waste. Its use in fish feed can help reduce the environmental impact of fish farming and promote a more sustainable food system.

The results showed that the feed containing 20% banana peel flour demonstrated a greater increase in growth parameters as compared to the feeds containing 10% and 30% banana peel flour. The study results suggest that the feed containing 20% banana peel flour is more nutritionally stable and can meet all the requirements of tilapia fish including lower feed conversion ratio. A study by Oluwaniyi et al. (2018) reported that fish fed diets containing banana peel had higher feed intake and feed conversion

efficiency compared to those fed a control diet. This suggests that banana peel can enhance the palatability of fish feed and improve overall feed utilization.

Incorporating banana peel flour in the diets provided to tilapia fish resulted in a marked improvement in their digestive enzymatic activity. Moreover, the use of banana peel flour in fish feed is a cost-effective and eco-friendly alternative to traditional feed ingredients, and could potentially benefit the aquaculture industry in terms of sustainability and profitability. Also, the incorporation of banana peel flour in tilapia fish feed could be a viable strategy for enhancing fish health and productivity, while also promoting sustainable and environmentally-friendly aquaculture practices.

Biochemical characteristics of banana peel

In order to evaluate the nutritional value of banana peel flour (BPF) and assess its suitability for its use in fish feeds, its proximate analysis was conducted. Banana peels contain the required amounts of nutrients, including proteins, carbohydrates, lipids, vitamins, and minerals. These nutrients can contribute to the nutritional requirements of fish and potentially replace or supplement traditional feed ingredients (Kousoulaki et al., 2015).

Along with the estimation of the proximate content in banana peel flour, the proximate content of Groundnut oil cake which is mostly used as a complete feed in most of the fish farms to feed tilapia fish was also determined. The study aimed to determine the proximate content of groundnut oil cake, which was supplemented with different percentages of banana peel flour (BPF) - 10%, 20%, and 30%, to make it nutritionally stable and fulfill the nutritional requirements of fish. The experiment investigated the

impact of these feed supplements on the growth performance and enzymatic activity of the fishes.

Upon analysis, the results showed a significant increase in the growth performance and enzymatic activity of the fishes that were fed with the feed supplemented with different percentages of banana peel flour (BPF) as compared to those only fed with groundnut oil cake. This finding suggests that the use of banana peel flour as a feed supplement can significantly enhance the nutritional value of groundnut oil cake and can be an effective approach to meet the nutritional requirements of fishes.

Proteins

In order to make fish feed more nutritionally balanced, banana peel flour has been included as a supplement to groundnut oil cake. According to Guo et al. (2020), the balance of protein and energy in feed is essential to optimize the use of protein for fish growth and maximize fat and carbohydrates as an energy source. The protein content in banana peel was found to be (3.95 ±0.50). Despite being an excellent source of potassium and fibre, banana peel does not contain the necessary amount of proteins required by fish but is rich in essential amino acids. Therefore, when combined with groundnut oil cake, which is rich in proteins (40.68 ±0.45) and other nutrients, banana peel can help to enhance the nutritional value of the fish feed. Banana peels are rich in minerals such as potassium, calcium, magnesium, and phosphorus, which are essential for bone development and overall health (Hassan et al., 2018). Daudpota et al. (2014), the growth performance of the fingerlings of tilapia was found to be significantly fed with the dietary protein level.

Carbohydrates

Including carbohydrates in the diet of fish can play a significant role in supporting the feed formulations that are cost-effective, and help maintain their growth at a lower cost per unit gain. In addition to this, carbohydrates can improve the binding, stability, and floatability of pellets used in fish feed and also aid in the removal of fecal matter due to their binding properties. Upon conducting a proximate analysis, it was found that banana peel is rich in carbohydrates, with a measured amount of (43.96 ±0.50). This carbohydrate content is of great importance for the healthy growth of fish, as it serves as a crucial source of energy and aids in the maintenance of various physiological functions. This finding highlights the potential of banana peel as a valuable and sustainable ingredient in fish feed formulations. Hemre et al. (2002), found that the inclusion of carbohydrates in fish diets can prove to be highly beneficial for fish farmers in terms of maintaining healthy growth and overall productivity.

Fibre

Through the proximate analysis it was found that banana peel contains the significant amount of fibre (6.1±0.1) content necessary for healthy growth of fish. Fibre can aid in the absorption of nutrients and minerals by providing a conducive environment for the growth of beneficial gut bacteria. Depending on the specific circumstances and the type of fish, the effects of fibre composition on fish growth may vary. However, it is generally acknowledged that fibre is an essential component of animal feed that provides numerous benefits to the animal's digestive health. Dietary fibre is essential for the health of many species of fish (particularly herbivores and omnivores) and may

be beneficial for intestinal motility and health in some carnivorous fish (Davies, 1985; Li et al., 2021).

Lipids

Lipids are crucial for the healthy growth and development of fish as they play a vital physiological role in providing energy, essential fatty acids, and fat-soluble nutrients. Through proximate analysis it was found that banana peel contains (1.65±0.05) lipid content. A deficiency in dietary lipids may lead to an increase in the utilization of protein for energy, which can result in higher levels of ammonia excretion, ultimately leading to water pollution. This is because, in the absence of lipids, fish may resort to breaking down protein for energy, which leads to the release of ammonia as a waste product. Similarly, the results of De Borba et al. (2003); Siddiqui and Khan (2009) were in accordance with our experiment results. Their results also evidenced that the increase in the dietary level of protein can enhance the fish's body crude protein level. Therefore, it is important to ensure that fish are provided with an adequate amount of dietary lipids to maintain their health and well-being and to prevent pollution (Kaushik and Cowey, 1991).

Ash and Moisture

Moisture content is a crucial factor that plays a significant role in determining the shelf life of various foods. It is an essential indicator that can provide an estimate of the product's longevity, regardless of the sample properties in a wet or dry state. Proper moisture levels also help to maintain the texture, flavour, and overall aesthetics of the food. Through proximate analysis it was found that banana peel contains (13.32 ±0.6)

of moisture content. On the other hand, ash content was found to be (1.23 ±0.08), which is equally crucial for a food's nutrition and longevity. It is a measure of the inorganic matter present in a food item, which can include minerals like calcium, potassium, and magnesium. Adequate ash content is vital for the proper functioning of the body and can help prevent various health issues. A food's ash content also plays a significant role in determining its shelf life, as it can affect the food's stability, texture, and overall quality.

Growth Determination

Growth is influenced by dietary nutrients such as protein; the higher the protein content in feed ingredients, the more expensive the operational costs for cultivation are (Sutili et al., (2018). As such, growth is a multifaceted phenomenon that is essential for the development and survival of organisms. The effect of supplementing fish feed with varying percentages of banana peel flour (BPF) on the growth of fishes was studied. The study involved feeding fishes with diets containing 10%, 20%, and 30% BPF, and the results were compared with those of fishes fed with (control) groundnut oil cake (GOC). The findings revealed that fishes fed with diets containing BPF exhibited a much better growth rate including higher weight gain, specific growth rate, and better FCR when compared to those fed with GOC, as BPF contains the vital nutrient contents needed by fish and is also rich in essential amino acids necessary for fish growth. Rao et al. (2006) reported an elevated specific growth rate of *L. rohita* when fed with medicinal plants as compared to the control. Interestingly, the growth rate was observed to be the highest in fishes that were fed with the 20% BPF containing diet, followed by those fed with 10% and 30% BPF containing diets. This suggests that feed containing

20% BPF is nutritionally complete and can eventually meet all the nutritional requirements of fishes. This indicates that the inclusion of banana peel flour in fish diets at varying percentages can have a significant positive impact on the growth parameters of fishes. In fish, growth rate and survival data are important parameters to estimate the effects of feed quality on growth and related metabolic reactions (Wang et al., 2006).

Enzyme Activity

The determination of digestive enzyme activities in different groups of fish can be incredibly useful in selecting the most suitable low-cost feedstuffs with maximum digestibility. It is generally observed that the total intestinal enzyme activity increases with the age of the fish, primarily due to the increase in the size of the intestine. Klahan et al. (2008) concluded that different fish had different levels of enzymes activity. The utilization of nutrients in fish is heavily dependent on the activities of various digestive enzymes present in their digestive organs (Natalia et al., 2004). Hence, studies on digestive secretions in fish can elucidate certain aspects of its nutritive physiology and help resolve nutritional problems, such as artificial diets (Xiong et al., 2011). Numerous studies have focused on the definition of digestive enzyme activity profiles, which can provide a more comprehensive understanding of the digestive processes in fish and aid in the development of better feeding strategies for aquaculture. The study investigated the effects of banana peel flour (BPF) supplementation on the digestive enzymatic activity of fish. Three different percentages of BPF, namely 10%, 20%, and 30% were used. The results showed that the amylase activity was significantly higher in fishes fed with GOC supplemented with BPF compared to those fed with GOC alone. The highest amylase activity was observed in fishes fed with a diet containing 20% BPF. This can be due to the higher carbohydrate content and also due to good acceptability of feed. Pavasovic et al. (2007) documented higher amylase activity in fish when fed with diets

containing plant-based ingredients. On the other hand, the pepsin activity was slightly higher in fishes fed with GOC alone compared to those fed with BPF inclusions. Pepsin facilitates the breakdown of proteins into smaller peptides and amino acids. Amylase and pepsin are crucial enzymes in cellular metabolism. It is noted that the decrease in dietary protein and consequent reduction of some amino acids are responsible for the reduction in the amylase expression. Dietary levels are found to affect the protease activity of fish bodies in the intestinal segment (Phadate, 1987; Gangadhar et al., 1997). In addition, an increase in dietary lipids also impacts the reduction of amylase activity in fish (Bhilave et al., 2014). These enzymes play a crucial role in breaking down complex macromolecules into simpler building blocks, which are then utilized by cells to generate energy or create other biomolecules. Amylases are enzymes that break down starch and glycogen into sugars. However, the lipase activity was significantly higher in fishes fed with BPF containing diets than in those fed with GOC. The results suggest that BPF has a beneficial effect on the digestive enzymatic activity of fish. Therefore, it can be concluded that the inclusion of banana peel flour in the diet of fish could enhance their digestive enzyme activity, particularly amylase and lipase. This study aims to understand the digestive enzymes present in tilapia fish. The study will not only help promote sustainable fish management practices but also aid in the improvement of fish feed formulas. This, in turn, will help increase the growth rate of fish, reduce feed costs, and shorten the cultivation time required in aquaculture.

CONCLUSIONS

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The present study aimed to assess the effect of banana peel flour (BPF) based fish feed on the growth performance and digestive enzyme activities of tilapia juveniles (*Oreochromis mossambicus*). Four different treatments were evaluated, including groundnut oil cake (GOC) as a control, and BPF at 10%, 20%, and 30% levels. Growth parameters, including weight gain (WG), length, specific growth rate (SGR), feed conversion ratio (FCR), and survival, as well as digestive enzyme activities, were examined. The findings indicated a significant variation in fish performance across different BPF percentages when compared to GOC. The treatment with BPF 20% feed demonstrated the highest weight gain, specific growth rate, and best FCR, compared to other treatments. Similarly, digestive enzyme activity was significantly higher in fishes fed with BPF 20% supplement than those on other treatments. It could be inferred that feeding tilapia with GOC supplemented with 20% BPF enhances growth rate and enzyme activity, thereby yielding a promising outcome.

Future Prospect

The findings of this study have established that banana peels can serve as a viable substitute ingredient in fish feeds. Nonetheless, it would be intriguing to explore the potential differences in impact when compared to various commercially available fish feeds.

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