PROXIMATE ANALYSIS AND SELECTED MINERAL CONTENTS OF "2MM GRADE OF SELECTED FISH FEEDS", (Na, K, Cu, Fe, Zn, Cr, Co, Mn, Mg, Pb, Cd).

BY

EMMANUEL HAPPINESS CHINECHEREM

(PSC1707173)

PROJECT SUPERVISOR:

DR. EDWARD .N. DIBIE

CHEMISTRY DEPARTMENT

FACULTY OF PHYSICAL SCIENCES

UNIVERSITY OF BENIN, EDO STATE

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CERTIFICATION

We certify that this work was carried out by Emmanuel Happiness Chinecherem in the Department of Chemistry, Faculty of Physical Science, University of Benin, Benin City.

______________________ ____________________

Emmanuel Happiness Chinecherem **Date**
Student

______________________ ____________________

Dr. E.N. Dibie **Date**
Project Supervisor

______________________ ____________________

Prof. U.J Iyasele **Date**
Head of Department

DEDICATION

This work is dedicated to the Almighty God; the Author and finisher of our faith, who has made these work a success. I also dedicate this project to my mother. Without her, I will not be where I am today.

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Abstract

The present study was carried out to determine proximate analysis and selected mineral contents of "2mm grade of selected fish feeds", (Na, K, Cu, Fe, Zn, Cr, Co, Mn, Mg, Pb, Cd). The research was performed to qualify the nutrient and to assess the content of ash and moisture and the mineral contents. Proximate composition was determined on the homogenous basis. The percentage of protein in different feed ingredients ranged from 46.16 $\pm$ 0.18 to 51.50$\pm$ 0.02%. The highest protein content (51.50%) was recorded from the blue crown fish feed and the lowest value (46.18%) was recorded from the allerqua fish feed. The percentage of fibre, ash and moisture were recorded Fibre (0.34% to 0.54%), Ash (4.20 % to 6.20%) and Moisture (4.01% to 7.15%) respectively. The highest amount of moisture (7.15%) was found in Blue crown and the lowest amount (4.01%) was found in Aqualis and the determination of selected Mineral contents. To maintain the optimum nutrient content and ultimately rapid growth in the aquaculture farms, the farmer should take proper initiatives for measuring the nutrients in feed usually offered in farms.

CHAPTER ONE

1.0. INTRODUCTION AND LITERATURE REVIEW

1.1. INTRODUCTION

Modern commercial aquaculture relies heavily on manufactured feeds to give farmed fish the balanced nutrition they require. The fish are able to feed effectively and grow to their full potential thanks to the feeds, which come in the form of granules or pellets and provide the nutrition in a stable and concentrated form.

Today, many of the fish that are raised more intensively around the world, like Atlantic salmon, trout, sea bass, and turbot, are carnivorous. Fishmeal and fish oil were important ingredients in the feeds for these species during the contemporary aquaculture movement's development, which began in the 1970s. They are combined with additional ingredients like cereal grains, vitamins, minerals, and vegetable proteins before being formed into feed pellets. For instance, wheat is frequently used because it aids in the binding of the ingredients in the pellets.

Other forms of fish feed being used include feeds made entirely with vegetable materials for species such as carp, moist feeds preferred by some species (easier to make but more difficult to store), and trash fish that is fish caught and fed directly to larger species being raised in aquaculture pens.

Development of manufactured feeds.

The majority of fish hatcheries fed their trout raw meat—particularly horse meat—as a main diet until the conclusion of World War II. While employed by the New Mexico Game and Fish Department in the early 1950s, John E. (Red) Hanson started experimenting with dietary regimen and dry pellet formulations. At the Red River Hatchery near Taos, the first fish feed pellets were given to hatchery trout. Fish pellets were more widely used in hatcheries as a result of the pellets' better conversion rates of food intake to fish production.

Sustainability

Traditionally, fishmeal and fish oil have been two of the most crucial components. These are mostly produced by processing fish that was caught in the wild; these are often pelagic species that are typically not suitable for processing for human use. Fish used to create fishmeal are more expensive than those sold for human consumption. Reduction fisheries are another name for fisheries that target fishmeal. The government of Peru and Chile regulates the largest reduction fishery in the world, which is located in the Pacific Ocean off their respective coasts. Another significant source of fish for fishmeal and fish oil is the North Atlantic. The International Fishmeal and Fish Oil Organization has many significant suppliers as members.

Fish is cooked, pressed, dried, and ground to generate fishmeal, a dark, flour-like substance. The result of this process is fish oil, which is a rich source of energy and fatty acids for fish, including the crucial long-chain omega-3 fatty acids EPA and DHA, which are now connected to the health advantages of eating oily fish like salmon and mackerel. Governmental food agencies frequently advise including fish in a healthy diet because it is a good source of many vitamins and minerals in general.

The current focus of research and development is to enable aquaculture by adding vegetable proteins and oils to fishmeal and fish oil as a supplement.

Modern fish feeds

Fishmeal, vegetable proteins, and binding agents like wheat are among the materials that are ground and combined to create modern fish feeds. Extruders for fish feed are essential components of production lines in modern technology. Even though the extruder handles the majority of the creation of fish feed, mixing and grinding can have a significant impact on the end product's quality. A metal plate with holes is punched, water is added, and the resulting paste is extruded. The diameter of the holes, which can vary from less than a millimeter to more than a centimeter, is typically the most crucial factor in determining the pellets' diameter. The feed is chopped into the necessary length pellets as it is extruded. Oils are added once the pellets are dry. Manufacturers can create pellets that are suitable for various fish farming techniques, such as feeds that float or sink slowly and feeds suitable for recirculation systems, by adjusting variables like temperature and pressure. The dry feed pellets are stable for a fair amount of time, making storage and delivery simple. Feeds are given in enormous, bulk bags that typically weigh one tonne or 25 kg. Specialist feeds are provided in smaller quantities for use in fish hatcheries.

1.1.1. Background of Study

Plant or animal matter meant for consumption by pet fish kept in aquariums or ponds is known as aquarium fish feed. Fish feeds typically include the macronutrients, trace elements, and vitamins needed to maintain the health of captive fish. About 80% of hobbyists who keep fish only feed their pets foods that are typically produced in the form of flake, pellet, or tablet. Pelletized foods, some of which sink quickly, are frequently used for larger fish or bottom-feeding species like loaches or catfish. Additionally, some fish meals include substances like sex hormones or beta carotene to modify the color of ornamental fish (Wikipedia).

Fish oil and fish meal are two marine substances that have traditionally been used in fish feed. A new breakthrough is the partial replacement of marine ingredients of animal origin (fish oil and meal) with vegetable ingredients to lower feed costs and increase the sustainability of fish farming. (B.T. Lunestad, J.T. Rosnes, 2011).

1.1.2. Statement of Problem (S)

Commercial fish and other aquatic creatures need an adequate amount of nutrients, both in terms of quantity and quality, in order to develop, stay healthy, and reproduce.

The aquaculture industry will be impacted, maybe leading to the failure of the firm, if a fish feed does not satisfy the nutritional requirement value and is fed to the fish. Families will be impacted, which will then have an impact on the economy. There could be a serious health catastrophe if the improper chemicals are being used on these animals and these fish are sold for general human consumption.

To assure good fish flesh and stop environmental deterioration, feed analysis is primarily done for these reasons. Feed should provide the necessary nutrients and energy in accordance with what the body requires to maintain physiological processes including growth, reproduction, and wellness.

Feeds are evaluated using "proximate analysis" so that a determination may be made on its nutritional value and the role that it can play in satisfying an animal's requirements for various nutrients.

1.1.3. Justification/Relevance of the Research Work

Regardless of the culture method in which they are grown, commercial fish and other aquatic animals need an adequate supply of nutrients in terms of both quantity and quality for good growth, health, and reproduction. To meet the nutrient and energy needs of the species being cultivated as well as the system's production objectives, input supplies (such as feeds and fertilizers) must be provided (Hasan, 2001). Commercial fish meals are frequently utilized nowadays to increase aquaculture production. The primary growth-promoting component of diet is protein. Commercial fish size, water temperature, feeding rate, the availability and quality of natural sources, and overall digestibility all have an impact on how much protein they need.

The goal of the current study is to compare the actual proximate composition to the nutrient content stated by various enterprises in Nigeria. The majority of the crude protein analysis data were found to be more or less comparable to the firm reported values, according to the chemical analysis. Most of the feeds from the various commercial fish businesses analyzed will have crude protein contents that are within the permissible limit for commercial fish (NRC, 1983). According to Wilson (2000), the majority of commercial fish feeds, such as catfish meals, contain 32% crude protein. According to Boonyaratpalin (1988), the protein needs of fry, grow-out, and brood stock for tropical catfish are 35–40%, 25–35%, and 28–32% respectively.Fish output rose by using high amounts of protein, or 35% and above, in their diet, according to Watanabe et al. (1990), and phase feeding may be more profitable. In formulated diets, lipids are predominantly used as an energy source to maximize their protein sparing impact (Hassan). According to Cowey and Sargent (1979), who indicated that 10–20% of lipid in the majority of freshwater fish diets provides ideal development rates without producing an overly fatty carcass, the measured lipid values are consistent with their findings. In contrast, Wilson (2000) stated that the lipid content in catfish meals should be between 5 and 6%. Additionally, Luquet (2000) noted that the tilapia diet frequently uses dietary fat levels of 5 to 6%.

A certain quantity of fiber is included in every plant-based substance. Fiber gives the feeds physical mass. The movement of feed through the alimentary canal is moderated by a specific quantity of fiber in feed, which also helps with better binding. De Silva and Anderson (1995) pointed out that it is not advisable to have a fibre content in fish diets above 8–12% since doing so would always lead to a decline in the quality of an unusable nutrient in the diet.

A high fiber content reduces the ability of nutrients to be digested. With the exception of Adolf Calyx, all of the meals under consideration have analysed crude fiber contents that are under the acceptable dietary limit for fish.

Despite discrepancies between manufacturers' claims and the findings of the current investigation, the research has shown that all feeds—aside from Adolf Calyx—have crude protein concentrations above 40%, making them all safe for catfish culture.

1.1.4. Scope of Work

The University of Benin's chemistry is the site of this study. to determine the specified mineral concentrations and the proximate analysis (Na, K, Cu, Fe, Zn, Cr, Co, Mn, Pb and Cd). utilizing the Atomi absorption spectroscopy, Kjedahl method, and digestion process.

1.1.5. Aim and Objectives

Aim

The aim of the research is to analyse the minerals and proximate content of 2mm graded fish feed in order to explore its nutritional values in fish.

Objectives

The objective of the present study is to determine the proximate analysis of ingredients and minerals available in 2mm graded fish feed.

2mm graded fish feed will be collected and analyzed for proximate composition viz. moisture, crude protein, total ash, crude fiber, crude fat and nitrogen free extract (NFE) followed by the determination of the selected minerals content using Atomic Adsorption Spectroscopy preparation.

The knowledge of proximate analysis of feed ingredients will help for the safety aspects of the product.

Determination of proximate analysis, fatty acid, and mineral content profile of 2mm graded fish feed.

1.2. Literature Review

Fish must be fed a balanced diet for healthy growth and wellbeing. Their health will be directly impacted by the food they consume. Fish kept in captivity must get their nutrition from their feed, unlike fish in their natural habitat. The fish might not be able to survive or develop to their full capacity if the diet is deficient in essential nutrients. The quality of the diet becomes even more crucial if you want to effectively reproduce your fish.

Second, fish feed is important economically. Fish feed can account for more than half of the operating expenses in aquaculture. Finding high-quality feed at an affordable price is becoming more difficult for many commercial farmers as feed costs continue to rise.

Last but not least, fish feed, particularly in large-scale aquaculture, can have an effect on the marine environment. Through the NOAA-USDA Alternative Feeds Initiative, the National Oceanic and Atmospheric Administration (NOAA) has been attempting to lessen its reliance on marine fish as a source of aquaculture feed since 2007. Alternatives like plant-based substances are being investigated as feed ingredients rather than continuing to largely rely on fishmeal and fish oil. According to a general observation, compared to feed ingredients of an animal origin, vegetable feed ingredients are more prone to the growth of fungus due to inherent or storage- and transport-related factors (Moss, 2002).

1.2.0 Types of Fish Feed

Prepared or Artificial feeds

These might be both full and supplemental. Fish fed a complete diet receive all the vitamins, minerals, proteins, carbs, and lipids required for optimum growth and health. The typical farmer consumes a diet that contains 18 to 50 percent protein, 10 to 25 percent lipids, 15 to 20 percent carbohydrates, 8 to 10 percent water, and trace amounts of vitamins and minerals.

Depending on the fish species and life stage being raised, the feed's nutritional value will vary. When fish are raised in high-density indoor settings and are unable to eat naturally occurring foods (such as algae, aquatic plants, aquatic invertebrates, etc.), a complete diet must be given to them. Supplemental (i.e., incomplete or partial) meals are solely meant to supplement the natural food that fish in ponds or outside raceways often have access to. Supplemental diets are often utilized to assist reinforce the naturally available diet with additional protein, carbohydrate, and fat since they do not contain the entire complement of vitamins and minerals.

There are different types of fish food in the market today. The types of fish food can broadly be categorized into processed feed, dried food, fresh food, frozen food, and live fish food.

Processed Fish Feed

The greatest category of fish feed for both ornamental and farmed fish is processed or manufactured fish feed. It lasts a very long time. For example, pellet feed will last longer than flake food on the shelf.

Pellet Feed

A common type of fish food is pellet feed, which is typically used to feed farmed fish and larger aquarium fish. Pellets come in a range of sizes. While some are designed to float on water, others are built to sink. Compared to conventional fish feed, pellet food takes longer to break down in water. Its shelf life is greater.

Flake Food

Another common kind of fish food is flake food, particularly for aquarium fish. Food flake will float for a while before gradually sinking to the bottom. For communal fish tanks where various fish species coexist, flake feed works excellent.

Powdered Food

In fish hatcheries, powdered food is frequently used to feed young fish. The dry powder can be given to the fish straight away or blended with water first. Powdered feed can quickly contaminate the tank water.

Other categories of processed fish feed include: specialized feed such as color enhancing, medicated, vacation and species specific fish food.

Dried Fish Feed

Fish feeds that have been dried or frozen It is a minimally processed, natural fish feed. In this dried state, the majority of the nutritious value is still present. It may be an excellent method to round up a basic diet. This form is accessible for a variety of invertebrates, including blood worms, brine shrimp, plankton, krill, and many more.

Fresh and Frozen Fish Feed

Fish food is both fresh and frozen. Fresh fish feed can be very beneficial for fish with unique nutritional requirements. For instance, a fresh food diet high in protein is beneficial when attempting to train fish for breeding.

Live Fish Feed

One of the best sources of fish feed is live fish. Fish are flexible feeders. In the wild, many fish regularly consume worms and insects.

Color-Boosting Fish Food

For the feeding of tropical fish and koi fish, color enhancers are popular. Carotenoids are provided as an enhancer to assist the fish to exhibit the most vivid color. In aquaculture, carotenoids like astaxanthin and canthaxanthin are frequently added to salmon feed as a supplement.

Medicated Fish Feed

Bacterial infections are treated using medicated fish feed.

Vacation Fish Feed

Fish feeds for trips are made to release the food gradually over the course of one to two weeks. As a result, aquarium fish will be able to survive while the owner is on vacation.

1.2.1 Fish Feed Ingredients

Processed feed can be formulated to have all the essential nutritional components in the right ratio. Ingredients of a processed fish feed often consist of the following:

Proteins

The most expensive component in the majority of fish feeds will be the protein.

Fishmeal, which is often made from other fish, is a source of protein. Legumes like soyabean are additional sources of protein.

Lipids

Typically, lipids or fats make up 10-15% of the meal. It offers roughly twice as much energy as carbohydrates and proteins.

Fish oil is commonly used as a source of lipid. Sardines are one type of fish from which the oil is derived. Vegetable oils made from canola and sunflower provide an alternative.

Carbohydrates

For fish, carbohydrates are a cost-effective source of energy. It aids in lowering the price of feed. When making feed, especially feed that is intended to float, it can be helpful as a binding agent. 20–30% of many commercial feeds are made largely of carbohydrates.

Some fish can't handle it in large doses. Fish may show symptoms of illness if the carbohydrate concentration is too high.

It has been demonstrated that some fish species use carbohydrates more effectively than others, including koi fish, different catfish, and african tilapia.

Vitamins

Organic substances known as vitamins are essential for the growth and wellbeing of fish. A, B1, B2, B3, B5, B6, B12, Biotin, C, Choline, D3, E, Folacin, Inositol, and K are essential vitamins for fish.

Minerals

Fish require minerals, which are inorganic substances, to function properly. Minerals are necessary for the upkeep of bones, the immune system, and cells. The essential minerals that fish require are calcium and phosphorus. Additionally required are minute amounts of sodium, magnesium, iron, iodine, chloride, copper, potassium, sulfur, and zinc.

Fortunately, minerals last longer than vitamins in the body.

Crude Fibre and Ash: This is an estimation of the feed's portion that is either indigestible or slowly digestible. These substances give the digestive system the volume it needs for appropriate peristaltic activity. All the minerals in the feed are contained in the ash content. Knowing how much of various minerals (such as potassium, phosphorus, copper, zinc, manganese, etc.) the feed contains is helpful. Ass fish can only digest mineral content, using too much ash can have a negative impact because it will merely pollute the water.

Moisture Content:

A crucial nutrient that is frequently overlooked is the feed's moisture content. Feeds with a high moisture content are more likely to spoil quickly, primarily due to fungal contamination. The animals may become ill or even die as a result of the molds and, more specifically, the toxins that many molds produce, which make the feed unpleasant. However, despite being stable, dry feeds are less appealing.

1.2.2 Why fishmeal and fish oil is used in the diets of farmed fish.

Fishmeal is a natural and well-balanced source of high-quality protein. As ingredients in aquaculture feed, fishmeal and fish oil supply essential amino acids and fatty acids reflected in the normal diet of fish. Fish oil is a major natural source of the healthy omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These fatty acids are not made by the fish, but become concentrated in fish further up the food chain from the marine phytoplankton (microscopic marine algae and microbes) that do synthesize them.

Through research, we are learning that other combinations of ingredients can achieve the balance of the 40 essential nutrients. Affordable replacement ingredients for fishmeal and fish oil are becoming increasingly common, which is leading to declining percentage on those ingredients in farmed fish diets.

Including ingredients like oils from algae or marine microbes maintain the nutrient requirements of the final product without depending on fish oil. The economics of using blended oils is improving as fish oil prices rise and the technology to produce algae (and other replacement ingredients) improves.

1.2.3 Origin of fish meal and oil and their uses.

About 70% of the fishmeal and oil are produced from the harvest of small, open-ocean (pelagic) fish such as anchovies, herring, menhaden, capelin, anchovy, pilchard, sardines, and mackerel. These fish have short life cycles and are capable of rapid reproduction and stock replenishment. The other 30% is generated from the scraps produced when fish are processed for human consumption.

Fishmeal and fish oil supply several major industries because they are natural ingredients of high nutritional value. While they have been major ingredients of swine and poultry feeds for many decades, a growing percentage of these resources have been used to manufacture aquatic feeds. This is due to the worldwide growth of aquaculture over the past two decades. Demand for fish oil in the supplement industry is also rising rapidly.

1.2.4 Feed care and Storage

Large farms typically buy commercial fish feed in truckloads of bulk feed, which is then kept in outside containers. Feed is frequently purchased in 50-pound bags by smaller farms. Bagged feed should be stored as coolly as possible and out of direct sunshine. Particularly susceptible to heat and easily denatured by high storage temperatures include proteins, lipids, and vitamins. High moisture encourages the formation of mold and the degradation of feed. Avoid handling the feed bags excessively or damaging them, since this could cause the pellets to break and produce fines (powder) that the fish won't eat.

Feed should not be kept for more than 90 to 100 days without routine inventorying. More than ten bags should not be piled on top of one another since the added weight from the higher bags will crush the pellets in the lower bags, resulting in further fines (dust). It is best to use older feed first, and all feed should be routinely checked for mold before feeding. All rotten feed needs to be thrown away right away. In the feed storage, mice, rats, roaches, and other pests need to be tightly controlled (David D. Kuhn et al, 2017).

1.2.5 Feeding Rate, Frequency, and Timing

Fish size influences feeding rates and frequency to some extent. Fish fry and larvae need to be frequently and typically in excess fed a high-protein diet. Due to their high energy requirements, little fish must be fed almost continuously and every hour. Because little fish only need a modest amount of feed in relation to the amount of water in the culture system, overfeeding them is not as problematic as overfeeding larger fish. Feeding frequencies, rates, and protein content should all be decreased as fish grow. However, feeding less can enable the grower to use the same feed (protein level) throughout the grow-out stage rather than moving to a lower protein diet, simplifying feed inventory.

Fish feeding is time-consuming and expensive. The availability of workers, the size of the farm, the production system, and the types and sizes of fish raised all affect how often they are fed. Due to labor and time constraints, large catfish farms with numerous ponds typically only feed once day, whereas smaller farms may feed twice daily. In general, feeding frequency increases growth and feed conversion. Fish may be fed up to five times daily in indoor, intensive fish culture systems in order to maximize growth at ideal temperatures. Fish feeding rates are influenced by numerous factors. These factors include age, day of the week, season, water temperature, dissolved oxygen concentrations, and other aspects of water quality. For instance, it is not advisable to feed fish raised in ponds in the morning when the levels of dissolved oxygen are at their lowest. Fish can be fed at almost any time in recirculating aquaculture systems, where oxygen is continuously provided. Warm-water fish in ponds feed less frequently in the winter and at low water temperatures, and this should happen proportionately.

The ingredients and feed quality have an impact on the acceptability, palatability, and digestibility of the feed. In order to estimate feed acceptance, compute feed conversion ratios and feed efficiencies, keep tabs on feed prices, and track feed demand throughout the course of the year, fish farmers pay close attention to feeding activity.

For the majority of fish species that are frequently cultivated, published feeding rate tables are available. Using the average length or weight of the fish in the tank, raceway, or pond as well as their number, farmers may determine the best feeding rates (see New, 1987). Fish raised in farms are routinely fed 1 to 5 percent of their body weight each day (David D. Kuhn et al, 2017).

1.2.6 Managing Fish Wastes

The most crucial fish nutrition guideline is to avoid overfeeding. A waste of expensive feed is overfeeding. Additionally, it causes bacterial loads to grow, low dissolved oxygen levels, low biological oxygen demand, and water pollution. Fish should often only be fed as much food as they can ingest fast (in less than five to 10 minutes). Approximately 80% of the feed they desire to consume is a decent general rule of thumb when feeding fish (satiation). According to this method, you feed the fish for one day's worth of food on a regular basis—perhaps twice a month. Following that, feed roughly 80% of that ration for the following few weeks, then repeat.

To monitor feeding activities and determine whether to provide more or less feed, many growers utilize floating (extruded) feeds. Even under the best management, some feed is wasted. For instance, out of 100 units of feed given to fish, 40 to 50 percent is frequently wasted: fish produce 10 to 15 units of solid waste and 30 to 35 units of liquid waste for every unit of feed that is consumed. About 25 units of the leftover feed are used for metabolism, while another 25 units are used for growth (heat energy for life processes).

With respect to species, sizes, activity, water temperature, and other environmental factors, these numbers can vary significantly (David D. Kuhn et al,s2017).

"Proximate analysis" is a major commercial concern since food manufacturing companies must make sure that their products adhere to legal declaration requirements, applicable regulations, and product safety standards before releasing them to consumers.

The suitability of various raw materials used as feed ingredients as a substitute source for fish meal has been estimated and evaluated through extensive research utilizing proximate analysis. Proximate analysis include examining foods and feeds for nitrogen (protein), ether extract (fat), crude fiber, and ash (mineral salts), as well as calculating the amount of soluble carbohydrates by deducting these values from the total. The objective of this study is to evaluate the nutrient content of 2mm graded fish feed. (Modupe O. Dawodu et al 2012).

1.2.7 Related Work

Previous research had established useful techniques for determining the total mineral element amounts in a sample of fish feed. The materials will be dissolved in concentrated mineral acids at high temperatures to perform these tests. The digestion of concentrated nitric acid, dry ashing, nitric perchloric acid, and sulfuric acid will all be major topics of discussion (Bader, 2011; Hseu, 2004).

When examining the mineral composition of fish feed, AOAC's official method 999.10 (Association of Analytical Communities) offered a microwave digestion approach. Weighing 0.2-0.5g of sample into the digestion tube, adding 5 ml of HNO_3 and 2 ml of 30% H_2O_2, and then doing the digestion will be discussed. By taking the power/temperature program into account, this will be positioned under the microwave (Arvidsson et al., 2000).

By employing the dry ashing method, AOAC official method 999.11 will be provided to determine the mineral element levels of fish feed, including Na, K, Co, Mn, Mg, Pb, Cr, Cu, Cd, Zn, and Fe. A furnace that can be controlled will be used for ashing. The fish feed sample will be placed in the furnace at a beginning temperature of no more than 100 °C, and the temperature will be raised at a maximum rate of 50 °C/h up to 450 °C. This will be heated for eight hours or over night. Until the sample is completely reduced to ash, the temperature will be changed at predetermined intervals by removing the crucible and adding distilled water. There will be a minimum of two blanks required for each batch (Arvidsson et al., 2000).

As opposed to the AOAC 999.11 official procedure, another technique uses dry ashing as a digestion method and adds HCl to the crucible after the sample has been entirely reduced to ash. It will then be again dissolved in nitric acid (Sarojam, 2009).

Under wet digestion, aqua regia digestion will be a successful method of digestion. In this procedure, the digestion process will be carried out using an aqua regia mixture, which is a 3:1 mixture of HCl acid and HNO3. The digestion tube's sample will be combined with the aqua regia mixture and allowed to sit overnight with reflux. Once the mixture has reached 180 °C, the samples will be processed until only 1ml of acid is left. When there is no distinct, colorless endpoint, 4 ml more of aqua regia solution will be added, evaporated off, and the extracts will then be filtered (Black et al., 2013).

Using HNO_3 and H_2O_2, the USEPA (United States Environmental Protection Agency) proposed an acid digestion process in 1998. The sample (1.0 g) will be weighed for this method before being placed to the digestion tube and 10 ml of HNO_3. The samples will be cooked to 95°C for 10 minutes after being placed in a digestion block. 5 ml of HNO_3 will be added after cooling and evaporated off at 100 °C for an additional two hours. After cooling the samples, 3 ml of 30% H2O2 and 2 ml of double-distilled water will be added. After that, the samples will remain in the block for an additional two hours at 100 °C. After filtering, the digests will be packaged in plastic (Black et al., 2013).

You can also use concentrated sulphuric acid (H_2SO_4) for wet digestion. H_2SO_4 will be heated along with the sample until the sample is burned and changes into a thick, dark liquid resembling oil. To finish the digestive process, two to three drops of analytical grade concentrated HNO_3 will be carefully added. When the end result is seen, the digestion will be finished (Noik, Sakari, Seng & Tuah, 2015).

One popular approach for sample preparation is pressure digestion. The digestion will take place at a certain external temperature since in this procedure, heating takes place in customized heater blocks rather than a laboratory oven. These devices are able to totally digest practically any sample and transfer it into solution, claims ZUNDEL Holding Enterprise. Another way is to mix H_2SO_4 and potassium permanganate with a little piece of fish feed, which is then digested on a hot plate. Following digestion, hydroxylamine hydrochloride will be used to decrease the excess permanganate (Evans, Johnson & Leah, 2010).

CHAPTER TWO

2.0 MATERIALS AND METHOD(S)

2.1 Materials

2.1.0 Reagents

Potassium Sulphate + Copper Sulphate

Selenium dioxide

Sodium hydroxide (NaOH)

Boric acid solution

Hydrochloric acid

Methyl red indicator

Distilled water

Sulfuric acid H2SO4

Nitric acid

Deionized water

Acetone

Perchloric acid.

2.1.1 Apparatus

Spatula

Metal tong

Gloves

Weighing balance

Dessicator

Crucible

Muffle furnace

Petri dish

Wash bottle

Hot air oven

Cotton cloth

Volumetric flask

Funnel pipette filler

Pipette

Measuring cylinder

Kjeldah's flash

Conical flask

Hot plate

Muffle furnace

Beaker.

2.1.2 Collection of samples:

The fish feeds were collected from different feed value chain actors like ingredient suppliers, retailers, fish farmers etc., from Edo state Agricultural development program (Edo ADP), Adolor, Ringroad, Faculty of Agric, Areas of Benin city, Edo state.

2.1.3 Preparation of samples:

The feed ingredients were collected as bulk quantity from different lots that supplies fish feeds.

Each fish feed was grounded using marble mortal and pistle from the laboratory into small particle size.

Proximate analysis:

The analysis of proximate composition was done in the Chemistry laboratory of University of Benin, Benin city. On each chemical analysis, duplicate determinations were carried out.

2.2 Methods/Methodology

The experimental samples were analyzed proximally in accordance with the Association of Official Analytical Chemist's Standard procedures (AOAC 2005).

2.2.0 Ash Content:

Ash content was calculated by igniting samples of known weight (3g) at 700 oC for two hours, followed by cooling the samples in a desiccator and weighing them. The following calculation was used to determine the proportion of ash:

$$\text{Ash content (\%)} = \frac{\text{Weight of ash - Weight of the crucible X 100}}{\text{Weight of sample}}$$

2.2.1 Moisture Content:

The samples with known weight (3g) were dried in an oven at 100–135°C for two hours to ascertain its moisture content.

$$\text{Moisture \%} = \frac{\text{Weight of sample – (Weight of dish after drying - Weight of dish) X 100}}{\text{Weight of sample}}$$

2.2.2 Crude Protein:

The standard semi-micro Kjeldahl technique was used to calculate the crude protein content. This formula was used to obtain the total nitrogen percentage:

$$\text{\% Total Nitrogen} = \frac{V_1 \text{ X } n_1 \text{ X } F_1 \text{ X MWn}}{\text{WS X 1000}}$$

where N = Normality of the acid

The percentage crude protein was calculated by multiplying the total nitrogen by conversion factor of 6.25.

Crude protein = N% X factor X F_2

V_1 = Volume of HCL

N_1 = Normality of HCL

F_1 = Acid factor

F_2 = Dilution factor

MWn = molecular weight of itrogen

Ws = Sample wight

2.2.3 Crude Fiber:

After being digested with 0.255 N H_2SO_4 and 0.313 NaOH, dried lipid-free residues were tested for loss of ignition, which is a measure of crude fiber. Any organic component was dissolved with the addition of 10 ML of acetone. The following formula yielded the proportion of fiber:

$$\% \text{ Crude fibre} = \frac{\text{Weight of crucible with fiber - Weight of crucible with ash X 100}}{\text{Sample weight}}$$

2.2.4 Determination of Crude lipid

Fat is examined with low boiling organic solvent (petroleum ether/ diethyl ether, xylem) by soxhlet extraction and the extract thus obtained weighed after recovery of the solvent. Crude fat was determined through Soxhlet extraction technique and hexane (65 ^{o}c-70 ^{o}c) as the solvent.

Calculation

% of crude fat = (corrected weight of fat ÷ weight of sample) ×100

% of carbohydrate = 100 – (moisture + ash + protein + fat)

Nitrogen-free extract (% carbohydrate) was determined by subtracting sum of (moisture % + % crude fat + % crude protein + % ash) from 100.

The gross energy content of feed samples was determined by using adiabatic bomb calorimeter using the Gallenkamp Auto bomb system. A 1g of dried sample was placed into a crucible.
Nickel firing wire was fixed between the electrodes and then a cotton string was wound from the pellet around the firing wire and the shorter electrode. The electrode assembly was placed into the calorimeter bomb. The water jacket of the bomb was filled with tap water and the calorimeter vessel was filled with water at 21-23 ºC and weighed to 2-3kg. The calorimeter vessel was placed into the water jacket. Before firing the calorimetry bomb the thermometer reading was recorded as the initial temperature. The bomb was fired and when the temperature was stabilised, it was recorded (final temperature).
The energy content (KJ/g) of the sample was calculated as:

$$\%\ \text{Gross Energy} = \frac{\text{Final Temperature} - \text{Initial Temperature X } 10.82 - 0.0896}{\text{Sample Weight (g).}}$$

Where 10.82 was the factor of heat capacity of the system and 0.0896 represent the combined energy value (expressed in KJ) for the wire and the cotton thread that was used on the analysis (Karalazos 2007).

2.2.5 Determination of Minerals

The mineral elements to be determined are: Zn, Fe, Mn, Mg, Cd, Cu, Co, Pd, Na, K and Ca . This was achieved by digesting the sample using 5 mL of nitric acid and perchloric acid (Almoaruf et al. 2003). The digested samples was later been centrifuged and the digested solutions were analyzed by using atomic absorption spectrophotometry and flame photometer for Potassium and Sodium. (Modupe O. Dawodu, Godwin O. Olutona, Funmilola Ajani, O.A. Bello - Olusoji 2012).

CHAPTER THREE

3.0 RESULTS AND DISCUSSION

Proximate Analysis

The protein content of the different 2mm graded fish feed was calculated. Each sample contains a different amount of crude protein, ranging from 46.16 $\pm$ 0.18 to 51.50 $\pm$ 0.02%.

Bluecrown crude protein content is relatively high when compared to the crude protein content of Topfeed, Aqualis and Allerqua fish feed.

Bluecrown, Topfeed, Aqualis and Allerqua graded fish feeds, according to the laboratory analysis, contained roughly 51.50, 50.05, 47.13%, and 46.18 protein, respectively.

Buecrown fish feed had a crude protein content of 51.50%, Topfeed fish feed had a crude protein content of 50.05%, Aqualis fish feed had a crude protein content 47.13%, and Allerqua fish feed had a crude protein content of 46.18% on a dry weight basis.

For the best results, Blue crown fish feed is the best fish feed for an early stage fish (e.g 2mm fishes). The necessary quantities of vital vitamins and minerals should be added, as well as the proper energy to protein ratio for each stage of the fish's development.

The samples of 2mm graded fish feed ingredients after analysis had an average ash value ranging from 4.20 ± 0.06% to 6.20 ± 0.05. 4.80 ± 0.02 in bluecrown, 4.20 ± 0.06 in topfeed, 6.20 ± 0.05 in Aqualis, 6.17 ± 0.03 in allerqua.

Narayon T., Rana., Sazzad, Md., Hossan., Masud R., Preparation of salt-smoked-dried fish products from SIS., (2019).

The percentage of crude fibre was recorded in different fish feed ranged from (0.34% to 0.54%). The highest fibre content (0.54%) was recorded from Aqualis (Table) Bluecrown has the lowest carbohydrate concentration 0.34%.

The percentage of moisture content was recorded in different fish feed ranged from (4.01% to 7.15%). The highest moisture content (7.15%) was recorded from Aqualis (Table) Bluecrown has the lowest carbohydrate concentration (4.01%).

The range of feeds constituents differed significantly between the four different 2mm graded fish feeds. With the four different 2mm graded fish feeds containing a crude lipid content that was analysed. The average crude lipid concentration ranged from 0.21 0.08 to 12.06 0.03%. Bluecrown had a crude lipid of 12.06%, topfeed had a crude lipid of 11.15%, aqualis had a crude lipid of 0.81%, and allerqua had a crude lipid of 0.21%.

3.1 Results

Table 1 The Proximate composition of four types of 2mm fish feed

Samples	% Moisture	% Ash	% Protein	% Crude Fat	% Carbohydrate	% Crude Fibre	Energy (KJ/g)
Blue Crown	7.15± 0.05	4.80± 0.02	51.50± 0.02	12.06± 0.03	23.68± 0.16	0.34± 0.05	0.33± 0.01
TopFeed	7.01 ±0.03	4.2 0±06	50.05± 0.02	11.15 ±0.075	24.76± 0.03	0.43 ±0.03	0.43± 0.03
Aqualis	4.01± 0.05	6.20 ±0.05	47.13± 0.07	0.81± 0.03	21.61± 0.06	0.54± 0.01	0.46± 0.02
Allerqua	5.05± 0.05	6.17± 0.03	46.18± 0.08	0.21± 0.08	25.05± 0.03	0.48± 0.08	0.43± 0.03

Table 2 Mineral composition of experimental 2mm graded fish feed evaluated (mg/kg).

Samples	Na	K	Cu	Fe	Zn	Cr	Co	Mn	Mg	Pb	Cd
BlueCrown	26.49	321.46	0.05	5.2	4.99	0.12	0.06	0.91	1.09	0.03	0.03
TopFeed	47.31	386.96	0.02	4.6	5.06	0.09	1.04	0.69	0.98	0.02	0.02
Aqualis	26.08	262.98	0.03	3.8	4.73	0.06	0.66	0.72	0.88	0.07	0.02
Allerqua	28.0	271.0	0.01	2.3	3.84	0.08	0.05	0.64	0.84	0.15	0.018

Table 2 displays the dietary and main mineral composition of the samples.

Compared to aqualis and allerqua 2mm graded fish feeds, the value of Fe and Zn is significantly higher in bluecrown and topfeed 2mm graded fish feeds.

According to Allaway, the concentrations of these metals in agricultural products range from 4 to 15 ppm for Cu and 15 to 200 gg-1 for Zn. However, all of the samples' concentrations of these components fell within this range.

Tacon states that the concentration of Zn for feed formulation is 75 gg-1, the concentration of Cu is 4.5 gg-1, and the content of Mn is 37.5 gg-1.

The samples' Mn concentrations, which ranged from 0.72, 0.91, 0.69 and 0.64 gg-1, fell within Tacon's recommended range.

Based on ADI for Pb, the acceptable limit of Pb for plants is 10 gg-1.

All of the samples in this investigation had a mean Pb range of 0.02 to 0.15gg-1, and these levels fell within this range.

The samples for K concentration, ranged from 262.98 to 386.96. The K value for bluecrown was 321.46, the K value for topfeed was 386.96, the K value for aqualis was 262.98 and the K value for allerqua was 271.0.

The samples for Na concentration, ranged from 26.08 to 47.31. The Na value for bluecrown was 26.49, the Na value for topfeed was 47.31, the Na value for aqualis was 26.08 and the Na value for allerqua was 28.0.

3.3 CONCLUSION

This study used proximate analysis to evaluate the feed composition of four different fish feeds. It is important to pay attention to how Nigeria's fish feed sector is developing. Consequently, regular feed analysis is required.

It can be concluded that a balanced mixture of feed ingredients will provide more balance nutrients than only use of inadequate feed components to formulate the fish feed.

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APPENDIX

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