EFFECT OF *LEMNA MINOR* IN CULTURE WATER ON THE PROXIMATE AND MINERAL COMPOSITION OF *OREOCHROMIS NILOTICUS*

BY

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DEPARTMENT OF AQUACULTURE AND FISHERIES MANAGEMENT.
FACULTY OF AGRICULTURE
UNIVERSITY OF BENIN
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SUBMITTED TO

THE DEPARTMENT OF AQUACULTURE AND FISHERIES MANAGEMENT, FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE.

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF AGRICULTURE DEGREE B. AGRIC (AQUACULTURE AND FISHERIES MANAGEMENT)

SUPERVISOR: DR. M. EGWENOMHE

NOVEMBER, 2025

CERTIFICATION

This is to certify that this project titled "**Effect of *lemna minor* in culture water on the Proximate and mineral composition of *oreochromis niloticus***" was carried out by **Miracle Abieyuwa JESUOROBO** with matriculation number **AGR2004378** of the department of Aquaculture and Fisheries Management, Faculty of Agriculture, University of Benin, Benin City, Edo State, Nigeria.

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DR. M. EGWENOMHE **DATE**
Head of Department

DEDICATION

This research work is dedicated to Almighty God for His love, care, guidance and protection throughout my years in the university and to all my family members. Thank you and God bless you.

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ABSTRACT

The quality of meat is affected by the nutrition of the fish. Recently, most Tilapia farmers have developed the habit of feeding fish with *Lemna minor* in order to reduce the quantity of feed used to rear fish. This study investigated the effect of *Lemna minor* (duckweed) supplementation in culture water on the proximate and mineral composition of *Oreochromis niloticus* (Nile tilapia). Fingerlings were cultured for 12 weeks in a completely randomized design with four treatments: T_0 (control, commercial feed only), T_1 (1/2 commercial feed fed in T_0 + duckweed), T_2 (same quantity of commercial feed fed in T_0 + duckweed), and T_3 (duckweed only). Each treatment was replicated three times, with 50 fish per 2000 L tank. Fish were harvested at the end of the 12 weeks culture period, filleted, and analyzed for proximate composition (moisture, protein, fat, ash, crude fibre, and carbohydrate) and mineral content (Ca, P, Mg, Na, K, Fe, Zn) using standard AOAC and ISO procedures.

The results of the study showed significant difference ($P<0.05$) across all proximate and mineral parameters. Moisture (77.86±0.06%) and carbohydrate (0.96±0.02%) were highest in T_3, while crude protein (18.99±0.04%) and fat (6.36±0.04%) were highest in T_1. Ash and fibre were moderately increased in duckweed treated groups. Mineral analysis revealed the highest concentrations in T_3 for Ca (3.2065±0.0053 mg/g), P (9.8102±0.0053 mg/g), Mg (1.1670±0.0036 mg/g), K (3.1050±0.0055 mg/g), Na (0.8926±0.0047 mg/g), Fe (0.0338±0.00008 mg/g), and Zn (0.0232±0.00004 mg/g)

The results demonstrate that Lemna minor serves as a natural bioenhancer, improving the nutritional and mineral quality of *O. niloticus* through nutrient recycling and water enrichment. The integration of duckweed into aquaculture systems presents a sustainable, cost effective, and eco-friendly strategy to enhance fish growth, flesh quality, and overall nutritional value for consumers.

CHAPTER ONE

1.0 INTRODUCTION

Fish production is an important constituent of global food security and aquatic ecosystem management. Fish farming, on the other hand, is considered a key agricultural sector of food production all over the world. It is viewed as a crucial agricultural activity that can reduce nutritional deficiencies considerably on a global scale and therefore reduce poverty effectively. Interestingly, aquaculture or fish cultivation offers a major source of animal protein and contributes heavily to household diets, livelihood, and economic development in many countries and regions of the world. Meeting the diverse consumption demands of the population through increased production of goods and services presents a continuous challenge, especially in light of environmental issues such as climate change. Figge and Thorpe (2023). Eating fish regularly offers a variety of health benefits due to its high content of essential nutrients. Fish like salmon, mackerel, and sardines are excellent sources of omega-3s, which help reduce inflammation, lower blood pressure, and support heart and brain health.

Lemna is a globally distributed, free-floating aquatic flowering plant ingredient alternatives to replace wild fish meal and fish oil, which are becoming increasingly unsustainable. The *Lemna*ceae family consists of 36 species grouped into five genera: *Landoltia, Lemna*, *Spirodela*, *Wolffia*, and *Wolffiella*. These genera are further categorized into two subfamilies: *Lemnoideae*, which includes the rooted species *Landoltia*, *Lemna*, and *Spirodela*, and *Wolffioideae*, which consists of the rootless species *Wolffia* and *Wolffiella* (Pagliuso *et al.,* 2020). All *Lemna*ceae species are small, free-floating freshwater plants found worldwide. They represent the most structurally simplified higher plants (Cheng and Stormp, 2009). Nonetheless, *Lemna* primarily propagates through vegetative reproduction, budding from two lateral reproductive sacs.

In the early 21st century, researchers acknowledged the crucial role of aquatic and semi-aquatic plants in shaping the structure, function, and ecosystem services of water based environments. These plants influence hydrological, geomorphological, and physico-chemical conditions while also interacting with various organisms, ranging from microbes to vertebrates. They contribute to the ecosystem by providing habitat and food. Aquatic plants, also known as hydrophytes, include both vascular and non-vascular species that have adapted to thrive in freshwater and saltwater environments. Found in lakes, rivers, and wetlands. These plants provide shelter for aquatic creatures like fish, amphibians, and insects, support benthic invertebrates, generate oxygen through photosynthesis, and serve as a food source for herbivorous animals. Common examples of aquatic vegetation include water lilies, lotus, duckweed, mosquito fern, floating heart, water milfoils, mare's tail, water lettuce, water hyacinth, and algae. The advancement of aquaculture towards intensive aquaculture and higher productivity requires innovative feed ingredient alternatives to replace wild fish meal and fish oil, which are becoming increasingly unsustainable.

The Nile tilapia (*Oreochromis niloticus*) is a freshwater fish that is easy to cultivate. As production continues to grow, maintaining high quality tilapia remains a key commercial priority. Fish feed plays a crucial role in enhancing both production and quality, and in freshwater aquaculture, it accounts for over 50% of total production costs (Appenroth *et al.,* 2018; Chakrabarti *et al.,* 2018; Pramana *et al.,* 2019). One of the primary ingredients used in tilapia feed is soybean meal. However, soybean prices have been steadily increasing, currently reaching 4,500 rupiah per kilogram (Handajani, 2011). As a result, there is a need for alternative feed ingredients that are cost effective and environmentally friendly. One of such alternative is duckweed (*Lemna minor*) meal, a commonly overlooked aquatic plant with a high protein content. Duckweed (*Lemna minor*) also enhances water quality by naturally filtering out nutrients such as nitrogen and phosphorus, as well as certain heavy metals.

1.1 Justification of the Study

One of the common challenges in intensive aquaculture is the high cost of commercial feed, which can account for 60-70% of total production expenses. This high cost is primarily due to the reliance on imported raw materials for fish feed, resulting in elevated prices. To address this issue, a more affordable and readily available alternative feed is necessary. One approach to reducing costs is the use of forage feed, like *Lemna sp.*

Lemna minor exhibits continuous population growth until it reaches an equilibrium, forming a dense floating mat (Driever *et al.,* 2005). Under controlled and sterile laboratory conditions, it demonstrates rapid vegetative growth and can double its biomass in just two days if conditions are favorable (Iqbal *et al.,* 2019). Due to these characteristics, *L. minor* is frequently used as a model plant in environmental studies and fundamental plant research (Van Hoeck *et al.,* 2015).

In nature, various fish species consume these plants, often making them a fundamental part of their diet. A study conducted by (Goswami *et al.,* 2022) on *Cyprinus carpio* tested five different levels of *Lemna minor* in their feed (0% as the control, 5%, 10%, 15%, and 20%). The results showed that fish fed with 15% and 20% *L. minor* had greater final weight, improved growth rates, and higher amino acid content. It was concluded that incorporating duckweed (*Lemna minor*) into the diet enhanced the nutritional profile of carp by increasing protein, lipid, amino acid, and omega-3 content. A separate study by (Irabor *et al.,*2022) involving tilapia (*Oreochromis niloticus*) and freshwater shrimp (*Macrobrachium rosenbergii*) found that replacing 50% of their feed with *L. minor* over four months led to greater weight gain and accelerated growth. Another study by (Irabor *et al.,* 2022) determined that up to 50% replacement of fish feed with *L. minor* was optimal, as exceeding 60% resulted in reduced growth in catfish (*Clarias gariepinus*). Additionally, research conducted in India by (Devi *et al.,* 2022) aimed to evaluate the protein content of locally available plants, including *L. minor*, by simulating the digestion process in *Anabas testudineus* and *Channa punctata*. *L. minor* exhibited the highest crude protein content, exceeding 30%, and demonstrated superior digestibility for *C. punctata* at approximately 54%. Although its relative protein digestibility was lower compared to other plant proteins in the study, it remained above 50%, suggesting that *L. minor* could serve as a viable fish protein substitute due to its protein content and digestibility. *Lemna minor* is not only a staple in fish diets but can also be utilized as a dried food supplement. It can be combined with soybeans, organic acids, and various fishmeal formulations to enhance nutritional value. There is paucity of knowledge in use of plastic tank in culturing *Oreochromis niloticus* and especially its effect on the proximate and mineral composition of *Oreochromis niloticus*. That is why this course is studying the effect of *Lemnar minor* in culture water on the proximate and mineral composition of *Oreochromis niloticus*.

1.2 AIM AND OBJECTIVES OF THE STUDY

The aim of this study was to examine the effects of *Lemna*r minor on the proximate and mineral composition of *Oreochromis niloticus.*

The specific objectives of this study were to determine:

1. The proximate composition (nutritional value) of *Oreochromis niloticus*

2. Mineral constituents (macro and micro minerals)

CHAPTER TWO

2.0 LITERATURE REVIEW

Lemna minor is a floating freshwater aquatic plant, with one, two, three or four leaves each having a single root hanging in the water. As more leaves grow, the plants divide and become separate individuals. The root is 1–2 cm long. Leaves are oval, 1–8 mm long and 0.6–5 mm broad, light green, with three (rarely five) veins and small air spaces that assist flotation. It reproduces mainly vegetatively by division. Flowers are rarely produced and measure about 1 mm in diameter, with a cup-shaped membranous scale containing a single ovule and two stamens. The seed is 1 mm long, ribbed with 8-15 ribs. Birds are important in dispersing *L. minor* to new sites. The sticky root enables the plant to adhere to the plumage or feet of birds and can thereby colonize new ponds. (Kaur *et al.,* 2021). *Lemna minor* has a sub cosmopolitan distribution and is native throughout most of Africa, Asia, Europe and North America. It is present wherever freshwater ponds and slow moving streams occur, except for arctic and subarctic climates. It is not reported as native in Australasia or South America, though it is naturalised there (Patil and Kumar, 2022; Rahman *et al.,* 2023).

2.1 Botanical and Nutritional Profile

Duckweed (family *Lemnaceae*) is a tiny flowering plant that offers enormous potential for bioregenerative space life support. It is one of the fastest growing plants in the world, growing on slow flowing or still shallow water bodies. It thrives on wastewater rich in nutrients and dissolved organic compounds (i.e. waste). There are four genera: *Lemna*, *Spirodela*, *Wolffia*, and *Wolffiella*, which include about 40 different species in total (Journey *et al.,* 2022**).**

Duckweed, is a free floating aquatic plant that is common in lakes, ponds, canals, rice fields, and ditches (Zhuang, 2023). It occur in most still or slow flowing water bodies under a wide range of conditions (except in nutrient poor or highly acidic water) (Zhuang, 2023). It can even grow in a trickle of water over vertical surfaces, such as seepages in cliffs (Zhuang, 2023), on mud, or on water that is only millimeters deep (Leng, 2021). Though it is a flowering plant, duckweed primarily reproduces through vegetative budding. Over a 10-day period, an individual mother frond may produce up to 10 generations of daughter fronds before dying (Journey *et al.,* 2022). Only occasionally does it produce tiny flowers and seeds (Journey *et al.,* 2022). Duckweed has leaf-like ovoid parts, called fronds, with hair-like roots (thought to provide stability). Fronds range from 1-20 mm across, depending upon the species. Since little structural tissue is required for this buoyant floating plant, fronds contain < 5% fiber (Journey *et al.,* 2022).

2.1.1 Applications in Aquaculture

In natural environment, *L. minor* grows in shallow nutrient rich water bodies and are protected from wind (Nafea, 2016; Chakrabarti *et al.,* 2018). In previous study, *L. minor* performed well in fertilized tanks with an optimum depth of 30 cm (Chakrabarti *et al.,* 2018) which was maintained and any water lost through evaporation was replaced. In deeper waters, *L. minor* lacks ability to fully utilize the available nutrients in the culture units resulting in decreased multiplication rate and development of longer roots. Furthermore, in such cases, *L. minor* will appear whitish in color due to nutrients deficiency thus produces lower biomass (Appenroth *et al.,* 2018). According to Nafea (2016) and Xu *et al.,* (2011), *L. minor* grows rapidly in waste water but lack of essential nutrients namely nitrogen, potassium and phosphorous affects its growth significantly. The need for fertilization relies on the water source and nutrient richness. For instance; rain water needs balanced NPK for *L. minor* growth unlike waste water. Previous studies have reported a direct relationship between nutrients availability in the culture units and crude protein of *L. minor*. Earlier studies reported that unlike inorganic manure, organic manure from livestock and poultry is highly effective for mass culture of *L. minor* (Srivastava *et al.,* 2006; Chakrabarti *et al.,* 2018). However, nutrients availability is dependant on animal's diet, housing and bedding, storage as well as handling. High doses of manure above the recommended amount will lead to production of excess ammonia which might affect both growth, quality and overall *L. minor* multiplication. Therefore, the nature of the culture medium in terms of nutrients availability influence the physiological well-being of the plant which in turn affects its production (Lees *et al.,* 2002). In addition, it requires relatively low inputs for cultivation, which qualifies it to be cost effective and eco-friendly to be used in integrated aquaculture systems. *L. minor* biomass is harvestable throughout, hence qualifying it to be a circular economy supporting renewable resource used in aquaculture (Afolayan *et al.,* 2021).

Recent studies also highlighted its role in integrated multi-trophic systems of aquaculture (IMTA) where its occurrence helps achieve overall system equilibrium by recycling nutrients and alleviating waste discharge (Gao *et al.,* 2020). Its role as both biological filter and nutritional supplement places *L. minor* as an important element of eco-friendly development of aquaculture under rising environmental and economic demands.

2.1.2 Phytoremediation Capabilities

Besides its application in aquaculture, *Lemna minor* is also well investigated for its phytoremediation potential, particularly in uptake of emerging contaminants from aquatic environments (U.S. Environmental Protection Agency, 2024; Wang *et al.,* 2022). *L. minor* is highly tolerant of polluted environments and effectively sequesters pollutants within its biomass using root and frond uptake. The large surface area, structural simplicity, and mode of continuous growth of *L. minor* enhance its capacity to sequester pollutants such as PFOA. The U.S. EPA (2024) has identified *L. minor* as one of the most promising candidates for application to phytoremediate wetland ecosystem and wastewater treatment plants where PFAS and related persistent organic pollutants prevail. Subsequent studies between 2020 and 2025 have shown that the plant can also alleviate levels of pharmaceutical waste, pesticides, and endocrine disrupting compounds (Singh *et al.,* 2023; Li *et al.,* 2021). Its application in constructed wetland systems and decentralized treatment plants is becoming increasingly prominent as it requires little maintenance and is capable of thriving in various climates. Researchers recommend its expanded use in eco-remediation, especially in developing countries where conventional water treatment infrastructure is not well established (Musa *et al.,* 2022).

2.2 Meat Quality of Fish with Scales and Mineral Composition

2.2.1 Importance of Fish Scales

Fish scales not only protect from environmental threats and pathogens physically but also provide an effective biological index of fish condition and health. In terms of structure, scales consist of collagen and hydroxyapatite, which provide fish locomotion and protection's mechanical strength and flexibility. All of this is in accordance with the circular bioeconomy strategy, which intends to valorize fish by-products instead of discarding them as waste (Ahmed *et al.,* 2022).

2.2.2 Mineral Composition of Fish Flesh

Fish flesh is also an important source of minerals. The contents of K, Na, Cl, Mg, P and Ca are up to 1 mg/100 g, whereas those of Fe, Zn, Cu and I are less than 1 mg/100g (Kietzman *et al.,* 1974, Navarro, 2022, Paul and Southgate, 2023). However, it is difficult to generalise and to establish the mean mineral values, because they depend on several factors such as species, sex, biological cycle and on the portion of fish analysed (Pérez-Martín, 2023), and also on ecological factors, such as season, place of development, nutrient availability, temperature and salinity of the water.

It is well known that fish flesh is a good mineral source of Ca and P, however, it is interesting to take into account that the splinters as well as bony fish skeleton are richer in these elements. Bone tissue, in its stage of highest crystallisation, it is constituted by the hydroxyapatite salt, which has Ca and P in a 2.15/1 ratio (Russell *et al*., 2023), its addition to the flesh could contribute to high Ca and P contents. Fish can not be eaten with bone in its natural state, as it can not be chewed or digested. However, it is technically possible to process some fish with bone by careful prior homogenization, obtaining a fish puree which could be incorporated in some manufactured foods, increasing the Ca and P contents and the Ca/P ratio of the meal. Examples are the homogenized fish-based weaning foods, which are sold in jars and are the main meal of babies from 6 to 12 months old and young children from 1 to 3 years old.

Ca and P are necessary to maintain an optimal bone development (Cruz and Tsang, 2023), more of both minerals being required during childhood and growing stages (NRC, 2023) to prevent rickets and osteomalacia. Related to the diet, it is necessary to take into account the Ca/P ratio, because it has important consequences for bony development. Theoretically, the same amount of both minerals should be ingested, though ratios between 1 and 1.5 could be accepted. However, when this ratio changes from 1 to 2 (Ca/P: 2/1), harmful effects can appear (Aranda and Llopis, 2023). Moreover, it is known that several aberrations in bone mineral homeostasis and bone metabolism result as a consequence of severe Mg deficiency in experimental rats. These include reduced bone growth and bone volume (Carpenter *et al*., 2023, Wallach, 2023), and incresed skeletal fragility (Creedon *et al*., 2021, Kenny, *et al*., 2023). Although Ca, Mg and P are important in bone metabolism and development, other minerals such as Fe, Cu, Zn and Mn are considered to be essential for normal growth and for avoiding several pathologies (Prassed, 2022, Sherman, 2023).

In previous studies, we concluded that the addition of bone to homogenized fish-based weaning foods increased the content and availailability of Ca and P (Martínez, *et al*., 1998). For this reason, it could be interesting to prepare fish pasta as a food ingredient. The aim of the study was to ascertain the influence of incorporating fish bone on the content and nutritional significance of Fe, Zn, Cu, Mn, Mg, Ca, P, Na and K in four commercial types of fish added as ingredients of weaning foods. A current study set out to look into mineral content of few different species of fish of mean values in (mg/100g) are as follows; Minerals analyzed and of importance as recommended by Ali *et al*.,(2024).

Table 1: Average values of macro elements in fish species samples under examination (n = 25 per sample)

Fish species	Calcium	Phosphorus	Magnesium	Sodium	Potassium
Oreochromis niloticus	494.34^{a}±60.94	151.41^{b}±5.19	22.04^{a}±2.23	1462.47^{b}±55.23	217.21^{c}±14.38
Bargus Bayad	156.34^{b}±26.41	159.20^{b}±9.52	15.12^{b}±0.68	1465.27^{b}±57.58	394.28^{a}±55.99
Lates niloticus	159.99^{b}±20.38	133.98^{b}±6.77	19.37^{ab}±1.30	1395.11^{b}±58.93	151.88^{c}±9.81
Clarias gariepinus	249.85^{b}±39.19	232.13^{a}±18.34	19.29^{ab}±2.03	1993.46^{a}±139.71	347.72^{ab}±37.34
Mormyrus kannume	187.11^{b}±29.30	265.30^{a}±19.83	13.81^{b}±1.61	1673.54^{ab}±78.16	239.79^{bc}±11.22

The means in the same column show significant differences (P<0.05) with distinct superscripts.

Table 2: Micro element means values of fish species samples analyzed (n = 25 per sample)

Fish species	Iron	Zinc
Oreochromis niloticus	$2.70^{c}\pm0.28$	$4.57^{a}\pm0.003$
Bargus Bayad	$3.48^{bc}\pm0.31$	$2.48^{b}\pm0.36$
Lates niloticus	$4.58^{b}\pm0.29$	$3.67^{ab}\pm0.28$
Clarias gariepinus	$6.88^{a}\pm0.66$	$3.41^{ab}\pm0.49$
Mormyrus consume	$2.63^{c}\pm0.22$	$3.79^{ab}\pm0.43$

The means in the same column show significant differences (P<0.05) with distinct superscripts.

2.2.3 Proximate composition of fish flesh

Proximate composition refers to the nutritional composition of a substance, such as protein, lipid, and moisture content, which can vary depending on factors like species, diet, and environmental conditions.

The proximate composition of fish refers to the percentage of its main components: moisture (water), protein, fat, and ash (which consists mostly of minerals). The chemical makeup of fish can differ significantly between species and even among individuals of the same species, depending on factors such as age, gender, habitat, and season. While protein and ash levels tend to remain relatively stable, fat content can vary greatly and often has an inverse relationship with water content. When one is high, the other is typically low. Current studies shows the proximate composition of fish analyzed by Ali *et al.,(*2024)

Table 3: The following is the average values of the fish species samples' proximate composition

Fish species	Moisture	Dry matter	Fat	Ash
Oreochromis niloticus	79.40^{ab}±0.26	20.60^{b}±0.26	1.54^{b}±0.17	1.02^{b}±0.04
Bargus Bayad	81.22^{a}±0.24	18.81^{bc}±0.24	1.48^{bc}±0.17	0.91^{b}±0.05
Lates niloticus	80.72^{ab}±0.28	19.29^{b}±0.28	1.29^{c}±0.10	0.97^{b}±0.05
Clarias gariepinus	76.20^{c}±1.13	23.80^{a}±1.13	2.28^{b}±0.36	1.32^{a}±0.07
Mormyrus kannume	78.75^{b}±0.43	21.25^{b}±0.34	3.28^{a}±0.35	0.93^{b}±0.03

(n = 25 per sample)

2.2.4 Impact on Human Nutrition

Globally, fish and fish products provide an average of only about 34 calories per capital per day. However, more than as an energy source, the dietary contribution of fish is significant in terms of high quality, easily digested animal proteins and especially in fighting micronutrient deficiencies. A portion of 150g of fish provides about 50 to 60 percent of an adult's daily protein requirement. Fish proteins are essential in the diet of some densely populated countries where the total protein intake is low, and are particularly important in diets in small island developing States (SIDS).

Intermediate Technology Publications wrote in 2023 that "Fish provides a good source of high quality protein and contains many vitamins and minerals". It may be classed as either whitefish, oily fish, or shellfish. Whitefish, such as haddock and seer, contain very little fat (usually less than 1%) whereas oily fish, such as sardines, contain between 10–25%. The latter, as a result of its high fat content, contain a range of fat soluble vitamins (A, D, E and K) and essential fatty acids, all of which are vital for the healthy functioning of the body. Eating oily fish containing long chain omega-3 fatty acids may reduce systemic inflammation and lower the risk of cardiovascular disease. Eating about 140 grams (4.9 oz) of oily fish rich in omega-3 fatty acids once per week is a recommended consumption amount. Increasing intake of omega-3 fatty acids may slightly reduce the risk of a fatal heart attack, but likely has little effect on the overall number of deaths from cardiovascular disease. Fish bone is the most common food related foreign body to cause airway obstruction. Choking on fish was responsible for about 4,500 reported accidents in the United Kingdom in 1998, an overreaction of the immune system and lead to severe physical symptoms from urticaria to angioedema and distributive shock. Allergic reactions can result from ingesting seafood, or by breathing in vapours from preparing or cooking seafood. The most severe allergic reaction is anaphylaxis, a medical emergency requiring immediate attention and is treated urgently with epinephrine.

2.3 Influence of Feed on the Meat Quality of Fish

2.3.1 Traditional vs. Alternative Feeds

Fish is one of the most valuable food products, a source of essential amino acids and high grade protein. Fish is an essential part of the human diet. According to the WHO standards, consumption of fish by one person should make 18.2 kg per year. Balanced and complete feeding is one of the main keys to the intensification of production of fish and fish products. Since fish meat contains a significant amount of biologically active substances (polyunsaturated fatty acids, essential amino acids, enzymes, vitamins), this allows referring it to the group of the most popular food products. This also determines its nutritional and biological value (Morgan, 2022).

Meat of most fish contains a sufficient amount of unsaturated fatty acids, and therefore it can be considered as one of the most important natural components of the anti-sclerotic diet. Thus, the use of fish resources for food, medicinal and other purposes necessitates the study of chemical composition of fish muscle tissue (Iseac, 2023). The chemical composition of fish meat differs radically from that of productive animals. The nutritional value of fish meat is influenced by a set of factors, among which the primary importance is given to breed characteristics. In addition, lifestyle (pelagic, bottom dwelling, passing, semi-passing), habitat (marine, freshwater), metabolic and gender age features, the physiological state of fish and a number of other factors have a pronounced impact .

Fish is the highest quality source of protein. Fish meat has a high nutritional and biological value since its protein contains all essential amino acids. From this viewpoint, fish can be considered as one of the most high quality sources of protein nutrition. Fat content is also one of the most important indicators of nutritional and energy value of fish. However, fat content of most fish breeds is subject to strong fluctuations. The fat content of fish directly depends on age and gender, year season, lifestyle features, and the length of migrations. This variability is usually conditioned by various factors. For the most part, the primary role is played by breeds differences and availability of food resources, as well as features of metabolism, physiological state and other factors (Uajh 2023).

This explains an interesting fact that fish of the same breeds caught in different habitats are characterized by different fat content, due to the peculiarities of food supply and abiotic environmental factors, e.g. water temperature. Temperature is one of the important abiotic factors. A direct relationship between the ambient temperature and the fat content in muscle tissue has been scientifically proven. The lower the ambient temperature, the higher the fat content in fish muscle tissue. Besides, in most cases, the fat content is directly influenced by the morphometric and age characteristics of fish. As the fish grows and ages, the amount of fat in the muscle tissue also increases. Thus, the economic and biological characteristics and chemical composition of fish muscle tissue are influenced by a complex of factors: habitat, food availability, breeding conditions, season, etc. In this regard, organization of a complete and balanced feeding ensuring fish with main nutrients, as well as vitamins and microelements, plays a crucial role in improving the productivity of fish and the quality of the products obtained. The nutritional and biological value of food products is determined by values of their constituent substances. Currently, the range of market feed for fish and aquatic organisms is quite diverse.

At the same time, it should be taken into account that complete and balanced nutrition is the key to productivity, safety and health of fish. It is preferable that fish feed meets the price quality criterion. The Russian technology of feed production must be developed and improved, but this is impossible in the lack of demand from fish farms. Today, the physical and mechanical properties of most Russian feed for various fish breeds do not always meet modern requirements, which causes significant economic damage to the industry. The factors of economic damage include higher feed costs per kilogram of live weight gain and environmental pollution. This is usually determined by low competitiveness of domestic feed as compared to Western counterparts and is one of the reasons for purchasing expensive imported feed from Western companies by Russian fish breeding enterprises (Mallent., 2023).

2.3.2 Nutritional Impacts

A feeding diet is considered complete if it contains all the substances necessary for normal growth and development. Protein, fat and carbohydrates are considered to be energy containing components of fish diet. However, these components are not sufficient for normal life. The diet should also include the necessary micro and macro elements, vitamins, and some other biologically active substances. Protein surely plays an important role in the metabolism of fish. It consists of protein and non-protein components. The protein component is broken down to amino acids used for fish growth and replenishment of protein reserves in the body, as well as performs an energy function. Protein containing non-protein forms of nitrogen is biologically less valuable.

Fish need 2-3 times more protein than productive animals and poultry. This is due to the fact that the excretory function is performed not only by kidneys, but also by gills. Thus, fish do not get poisoned by ammonia, which is formed during the final breakdown of the protein. For example, the optimal protein level for adult fish is 40-45 %, for juveniles 50-60%. It is necessary to take into account the fact that the protein must be complete. Such property as the completeness of protein determines the content of amino acids in it, mainly essential ones (Jaoha 2023).

2.4 How Culture Medium Impacts Water Quality (Plastic Tanks)

2.4.1 Characteristics of Plastic Tanks

Plastic tanks are used extensively in aquaculture for reasons of affordability, durability, and simplicity of maintenance. Their surfaces, being non-porous, allow for ease of cleaning, and there is no chance of pathogen build up. Yet, material properties of plastic can affect parameters of water quality. For example, plastic tanks do absorb and hold heat, which results in high water temperatures, affecting fish metabolism and growth rate. Other plastics can leach chemicals into water in due course of time, which can further affect fish health and water chemistry (Yusoff *et al.,* 2024).

2.4.2 Influence of Water quality on the Meat Quality of Fish

Aquaculture products are popular among consumers because of their low-fat content, tender meat and various nutrients (such as protein, phosphorus, vitamins and inorganic salts). This situation has driven the rapid development of the global aquaculture industry (Li *et al.,* 2022). However, with the strategic needs for efficient supply of high-quality protein and food safety in the world. The traditional aquaculture model (an extensive aquaculture model that relies on manpower) cannot meet this demand Peng *et al.,* (2022). Fortunately, Recirculating Aquaculture

Systems (RAS) are being promoted (Munubi *et al.,* 2022) and developed on a large scale. As RAS is suitable for the concept of sustainable and healthy development of modern fisheries.

Water quality plays a very important role in the survival and development of fish, and it is one of the key factors that determines how successful any aquaculture operation will be. Providing fish with a suitable water environment helps to promote their health and growth, and also ensures that the fish are of good quality for consumption. Several water quality parameters such as temperature, dissolved oxygen, pH, ammonia, and nitrite levels have a strong influence on fish performance, whether in natural waters or in fish farms. For anyone involved in fish farming, it is important to understand how these factors work together, because this knowledge is vital for managing a stable aquatic system and achieving healthy fish production. The following are examples of water quality parameters;

1) pH can be affected by chemicals in the water, pH is an important indicator of water that is changing chemically. pH is reported in "logarithmic units". Each number represents a 10-fold change in the acidity/basicness of the water. Water with a pH of five is ten times more acidic than water having a pH of six.
2) Dissolved Oxygen (DO): Successful fish respiration depends on sufficient DO. DO levels in non-aerated tanks, particularly higher stocking densities, may be lower. All aquatic animals need DO to breathe. Low levels of oxygen (hypoxia) or no oxygen levels (anoxia) can occur when excess organic materials, such as large algal blooms, are decomposed by micro-organisms. During this decomposition process, DO in the water is consumed. Low oxygen levels often occur in the bottom of the water column and affect organisms that live in the sediments. In some water bodies, DO levels fluctuate periodically, seasonally and even as

part of the natural daily ecology of the aquatic resource. As DO levels drop, some sensitive animals may move away, decline in health or even die, usually in conjunction with measurements for temperature and pH while each organism has its own DO tolerance range. Generally, DO levels less than 5mg/L are considered stressful for fish and levels less than 3mg/L are too low to support fish. DO levels below 1mg/L are considered hypoxic and usually devoid of life (Yusoff *et al.,* 2024). For tilapia, the minimum recommended level of dissolved oxygen is 5 (mg/L).

3) Concentration of Ammonia: Ammonia is a product of fish metabolism that can build up within closed systems. Excessive levels of ammonia are harmful to fish and occur due to overfeeding or inefficient filtration. Ammonia is a colorless, corrosive, alkaline gas that has a very pungent odor. The odor detection level ranges from 5 to 53 ppm. Ammonia is used as a compressed gas and in aqueous solutions. The corrosive and exothermic properties of ammonia can result in immediate damage (severe irritation and burns) to the eyes, skin, and mucous membranes of the oral cavity and respiratory tract. In addition, ammonia is effectively scrubbed in the nasopharyngeal region of the respiratory tract because of its high solubility in water. The database for ammonia consisted primarily of case reports, human studies, and experimental studies on lethality and irritation in animals. The case reports were of limited use for quantitative evaluation, but the human and animal studies contained quantitative data useful for deriving AEGL values.

4) Nitrate concentration: Nitrate concentrations are sometimes calculated using the weight of the entire nitrate molecule (usually reported as milligrams per liter (mg/L) of NO_3 or NO_x) and some use the weight of just the nitrogen portion of the nitrate molecule (specified as nitrate nitrogen usually in mg/LNO_3-N or NO_x-N). The nitrogen reported by the Suwannee

River Water Management District (SRWMD) are nitrate nitrogen concentrations. The use of the "x" in NO_x indicates the measurement of nitrite (NO_2) in the analysis. Nitrite is typically present as a very small fraction of NO_x, and is also "bioavailable" and contributes to pollution issues (Yusoff *et al.,* 2024).

5) Temperature: The temperature of the water plays a vital role in how fish metabolize and digest their food. When the water is either too hot or too cold, it can interfere with proper digestion and the way nutrients are absorbed. For instance, in tilapia farming, the most suitable water temperature ranges between 25°C and 30°C to support healthy growth and efficient feeding.

2.4.3 Integration with *Lemna minor*

The inclusion of *Lemna minor* (duckweed) in plastic tank aquaculture systems provides an environmentally friendly strategy for water quality control. *L. minor* has proven to be an effective phytoremediant, being capable of taking up surplus nutrients like nitrogen and phosphorus from within water coliumns. Not only does this enhance water quality, but it also lowers algal blooming and resultant oxygen depletion risks (Chepkirui *et al.,* 2023).

In addition, *L. minor* is an added feed source for specific fish populations, which gives them more protein as well as necessary nutrients. *L. minor*, due to its high growth rate and ease of cultivation, is an effective element used in integrated aquaculture systems. Incorporating *L. minor* has been proved to increase growth efficiency of fish as well as overall sustainability of systems (Chepkirui *et al.,* 2023).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of Study Area

The study was conducted in the Department of Aquaculture and Fisheries Management grow out unit, Faculty of Agriculture, University of Benin, Benin City, Edo state. It is situated east of Isihor in Ovia North-East Local Government Area of Edo state. It is located in the tropical rainforest with evergreen trees, it has a distinct raining and dry season, and an average rainfall of 1500mm- 2500mm. It has two peaks of rainfall (April and October), with relative humidity and temperature of approximately 27°C.

3.1.2 Lab location

This experiment was conducted at the Central Lab in the University of Benin

3.2 Study organisms

Plate 1: *Oreochromis niloticus* (Nile tilapia)

Plate 2: Lemnar minor (duckweed)

3.3 Experimental design

The experiment was designed as 12 weeks culture period × four (4) treatment factorial in complete randomized design. Each treatment was replicated three times.

The treatments include:

T_0: Control, feeding with commercial feed (No duckweed all through the culture period).

T_1: Feeding with 1/2 quantity of commercial feed fed in T_0 with duckweed covering the surface of the tank all through the culture period.

T_2: Feeding with same quantity of commercial feed fed in T_0 with duckweed covering the surface of the tank all through the culture period

T_3: No feeding with commercial feed, only duckweed covering the surface of the tank all through the culture period.

3.4 Experimental procedure

3.4.1 Experimental fish

Fingerlings of *Oreochromis niloticus* was obtained from a reputable farm and acclimatized. The fish was acclimatized by placing the container holding the fish in the plastic tank and allowing it to float for about 15-20mins as this will help the water temperature in the bag equalize with the plastic tank water. Then, open the container containing the fish and add a small amount of tank water of about 10-20%. Wait for another 10-15 minutes and repeat the process, gradually increasing the amount of tank water over time as this will help the fish adjust to the new water chemistry. Tank water was added continually in small amounts every 10-15minutes observing the fish for signs of stress. Once the water in the container is mostly tank water, then the fish was gently released into the tank. Fish was stocked at rate of 50 fish per tank in 12 plastic tanks of carrying capacity of 2000 litres and reared. Fresh *Lemna minor* was harvested from a pond. It was washed and introduced at different concentrations into the culture tanks. The setup includes 3 replicates per treatment. During the culture period, water quality was monitored regularly for temperature, pH, dissolved oxygen, etc using water quality test kits such as thermometer, pH scale, aerators. Water was changed when necessary to ensure optimum condition and reduce pollution. Commercial fish feed with crude protein was used. Fish were fed twice a day.

3.5 Data collection

Samples were collected at the end of the experimental period, and the edible portion of the fish (fillet) was homogenized and used for analysis. A 100g portion of fish was collected from each of the samples as recommended by Ali *et al*., (2024)

3.6 Proximate composition

The proximate composition of *Oreochromis niloticus* meat was determined to evaluate the effect of *Lemna minor* presence in the culture water. The analyses includes moisture, crude protein, crude fat, crude fibre, ash, and nitrogen-free extract (NFE).

3.6.1 Preparation of fish samples for proximate analysis

Samples of *Oreochromis niloticus* were obtained from the culture tank. They were eviscerated, washed and immediately transported to the laboratory in ice containing boxes. Fresh fish was washed with tap water several times to remove adhering blood and slime. The fish was prepared using common household practices such as removing the head, backbone, skin, tail and fin yielding fillets and thereafter taken to the lab for analysis.

3.6.2 Proximate composition analysis

Proximate components of the samples were determined according to the standard described by Association of Official Analytical Chemists AOAC (2010). All analysis were done in triplicate to ensure accuracy. Using a spatula and weighing balance, the required weight of the samples were taken from the homogenized mixture to carry out the following analysis listed below;

3.6.3 Determination of moisture content (AOAC, 2010):

For moisture content determination, samples were taken, weighed into aluminum foil papers labeled appropriately and put into the oven to dry for 24 hours at 105°c, brought out and kept In a desiccator to cool and re-weighed until the weight remains constant. Loss of weight was equated to the moisture. The measurements recorded were used to calculate moisture content. The equation below was used to calculate for the moisture:

$$\text{Moisture content} = \frac{\text{Weight of wet sample (g)} - \text{Weight of dried sample (g)}}{\text{Weight of wet sample (g)}} X\ 100$$

Where;

W_o = Weight of empty foil

W_1 = Weight of wet samples

W_2 = Weight of dry sample + foil paper

Dry matter (DM) = 100- %M

Plate 3: Moisture analyzer (Hot Air Oven)

Model : DHG-9023A (Made in England). It was used for moisture determination by drying at a controlled temperature.

3.6.4 Determination of crude protein (AOAC, 2010)

Crude protein content was determined using the kjeldahl method. 1g of dry samples was collected into a conical flask for crude protein analysis, digestion of the sample was carried out by adding 0.75g of kjeldahl catalyst and 10ml of concentrated H_2SO4 was added to the conical flask. A bent tail funnel was placed on the conical flask and then heated, after digestion, the digest was cooled, filtered and diluted with distilled water up to 50ml. 5ml of water was also taken out of each 50ml to digest into a conical flask. 5ml of 40% NAOH was added, 50ml of water was also added and the sample was then distilled. 5ml of boric acid indicator was used for distillation process. The digested sample was titrated against a standard (0.1 m) hydrochloric acid until change in colour occurs. Nitrogen content was calculated. The crude protein content was then calculated by multiplying nitrogen by 6.25 units.

% Nitrogen content = $\frac{\text{Na} \times \text{Va} \times 14}{1000} X \frac{\text{V1}}{\text{V2}} X \frac{1000}{\text{W} \times 6.25}$

Where;

M= Molarity of acid used (0.1M)

T= Titre value

0.014= Nitrogen constant

V1= Volume of digest (50ml)

V2= Volume of digest used (5ml)

% crude protein= % Nitrogen × 6.25

Plate 4: Kjeldahl apparatus

Bharat scientific, Model number - BSWKJ. It was used to determine crude protein from the samples.

3.6.5 Determination of crude fat (AOAC, 2010)

The crude fat was determined after soxhlet extraction of samples with 75ml of hexane for 6 hours at 40°c . At the end of the extraction, hexane was removed using rotavapour at 40°c, residue was dried at 100°c for 1 hour, cooled and weighed. Fat content was obtained using the formula below;

Fat content (%) = $\frac{W1-W2}{Wo}$ X 100

Plate 5: Soxhlet Extractor

Electrothermal (Made in UK). It was used for the extraction of crude fat from the fish samples based on the method originally developed by Franz Von Soxhlet in 1879

3.6.6 Determination of crude fibre (AOAC, 2010)

Crude fibre analysis was done by placing 2g of sample in conical flask, 100ml of 1.25% of H_2SO4 was added and then boiled for 30 minutes. The solution was filtered and the residue was placed back in a conical flask. 100ml of 1.25% of NaOH was added and boiled for 30 minutes, it was then filtered.

$$\% \text{ crude fibre} = \frac{\text{Ash weight}}{\text{Weight of sample}} \text{ X } 100$$

Plate 6: Fibre Analyzer

Fibretherm, Manufactured by C. Gerhardt GMBH & CO.KG. It was used to determine crude fibre in the samples

3.6.7 Determination of ash (AOAC, 2010)

2g of the sample was added to a pre - weighed crucible and weighed, place in oven and left to ash for 24 hours at 550°c, it was then cooled in a desiccator and re-weighed. The ash content was determined from the values recorded. The percentage ash was calculated by using this equation below;

$$\% \text{ Ash content} = \frac{\text{Weight of ash}}{\text{Weight of sample}} X\, 100$$

Where;

W_o = Weight of empty crucible

W_1 = Initial weight of sample before ashing

W_3 = Final weight of sample after ashing

Plate 7: Muffle Furnace

E. Track Scientific Instrument (Made in England)

Model -S×2.5-10. It is a box type muffle Furnace designed for even heating in a compact chamber and features precise temperature control. It was used to determine the ash content of samples by burning off organic matter at high temperatures leaving behind inorganic residues.

3.6.8. Determination of Nitrogen -free extract (NFE)

Carbohydrates content was determined by subtracting the sum percentage of crude protein, fat, Ash, moisture and fibre from 100%.

N.F. E = 100 - (% moisture + % Ash + % Crude fibre + % Crude fat + % Crude protein)

3.7 Nutrient composition (ISO, 1996 and 1998)

Mineralization of Sample was done using Incineration Method. To prepare the fish tissue samples for mineral analysis, the incineration method was employed to break down the organic matrix and isolate the mineral content. The following steps were duely followed;

3.7.1 Sample Preparation and Ashing:

Approximately 1 gram of the oven dried sample was accurately weighed and placed into a clean, dry porcelain crucible. The crucible containing the sample was then transferred into a muffle furnace, where it was ashed at a temperature range of 500–550°C for 6 hours. This process completely oxidized the organic matter, leaving behind a white or light gray ash, indicating complete incineration.

3.7.2 Acid Digestion:

After the ashing process, the crucible was carefully removed from the furnace and allowed to cool at room temperature. Once cooled, 10 mL of nitric acid solution (1:2) was added directly to the ash in the crucible. This solution was prepared by mixing 1 part concentrated nitric acid (65%) with 2 parts double distilled water and mixing thoroughly.

3.7.3 Heating and Dissolution:

The crucible content was covered with a watch glass to prevent contamination and minimize evaporation, and then placed on a boiling water bath. It was heated gently for 30 minutes, allowing the acid to dissolve the mineral content from the ash residue. The solution was then removed from the heat and allowed to cool at room temperature.

3.7.4 Dilution and Filtration:

The digested sample was quantitatively transferred into a 100 mL volumetric flask using deionized water. The volume was made up to the mark with deionized water and the contents was mixed thoroughly to ensure homogeneity. The resulting solution was then filtered through Whatman No. 42 filter paper (or equivalent). The first 5–10 mL of the filtrate was discarded to eliminate any potential contaminants or residues from the filter paper.

3.7.5 Sample Storage:

The clear filtrate, now containing the mineral elements in a dissolved form, was collected in a clean brown glass bottle to protect it from light induced degradation, especially for light sensitive minerals. The prepared solution was then stored at room temperature (or refrigerated if necessary) until further analysis.

This prepared solution was subsequently used for the determination of macro and micro minerals such as calcium (Ca), magnesium (Mg), Phosphorus (P), sodium (Na), potassium (K), iron (Fe), and zinc (Zn), as recommended by Ali *et al*., (2024) using appropriate analytical techniques such as Atomic Absorption Spectrophotometry (AAS), Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), or Flame Photometry, depending on the mineral .

3. 8 Estimation of macro-elements

3.8.1 Calcium content:

According to procedures of Calcium, OCPC kits "Spectrum-diagnostics, Germany IFUFCC07".

Calcium conc (mg/dl) = $\frac{\text{A sample}}{\text{A standard} \times 10 \times \text{dilution}}$

3.8.2 Phosphorus content:

According to procedures of Phosphorus, Inorganic kits "Spectrum-diagnostics, Germany IFUFCC34".

Phosphorus conc (mg/dl) = $\frac{\text{A sample}}{\text{A standard} \times 5 \times \text{dilution}}$

3.8.3 Magnesium content:

According to procedures of Magnesium kits "Spectrum-diagnostics, Germany IFUFCC94".

Magnesium conc. (mg/dl) = $\frac{\text{A sample}}{\text{A standard} \times 2.5 \times \text{dilution}}$

3. 8.4 Sodium content

According to procedures of Sodium kits "Spectrum-diagnostics, Germany IFUFCC94".

Sodium conc. (mg/dl) = $\frac{\text{A sample}}{\text{A standard} \times 150}\ X\ \frac{\text{dilution} \times 23}{10 ** \times 1 ***}$

Where; *23 = atomic weight of sodium

** 10 = conversion factor (L to dl)

*** 1 = The valence of sodium

3.8.5. Potassium content

According to procedures of potassium kits “Spectrum-diagnostics, Germany IFUFCC53”.

Potassium conc. (mg/dl) = $\frac{\text{A sample}}{\text{A standard} \times 5} \; X \; \frac{\text{dilution}}{10{**} \times 39.098{*}}$

Where; *39.098 = conversion factor which relates to the molecular weight of potassium

**10 = Conversion factor (L to dl)

3.9 Estimation of micro - elements

3.9.1 Iron content

According to procedures of Iron kits “Spectrum-diagnostics, Germany IFUFCC94”

Iron conc. (mg/dl) = $\frac{\text{A sample}}{\text{A standard}} \; X \; \frac{200 \times \text{dilution}}{1000}$

Where ; *1000 = Conversion factor (g to mg)

3.9.2. Zinc content

According to procedures of Zinc kits “Spectrum-diagnostics, Germany IFUFCC94”

Zinc conc. (mg/dl) = $\frac{\text{A sample}}{\text{A standard}} \; X \; \frac{\text{dilution}}{1000{*}}$

Where; *1000 = conversion factor (µg to mg).

The obtained results on a dry-weight basis was converted on wetweight basis using the following equation according to Jurgens and Bregendahl (2007):

Nutrient wet basis%= (nutrient dry basis% X dry matter %)/ (100)

3.10 Statistical analysis

All statistical analysis was performed using Graph Pad Prism version. The data was analyzed using a one way analysis of variance (ANOVA). The findings were shown as a mean SE. Group comparison was conducted using Tukey's multiple range tests when significant differences are detected at $P<0.05$.

CHAPTER FOUR

4.0 Results

4.1 Proximate composition of *Oreochromis niloticus* (Nile tilapia)

Table 1 shows the percentage composition of moisture content, crude protein, ash, crude fat, crude fibre and Nitrogen Free-Extract (NFE) of *Oreochromis niloticus* cultured in water containing different concentrations of *Lemna minor*.

There was significant ($P<0.05$) difference in the moisture content between the four samples. T_3 had the highest (77.86±0.06%) moisture value and T_1 had the lowest value (71.42±0.08%).

There was significant ($P<0.05$) difference in the crude protein content between the four samples. T_1 had the highest (18.99±0.04%) crude protein value and the least value (15.05±0.04%) was seen in T_3.

There was significant ($P<0.05$) difference in the fat content between the four samples. T_1 had the highest (6.36±0.04%) fat value and the least value (3.74±0.04%) was seen in T_3.

There was significant ($P<0.05$) difference in the ash content between the four samples. T_1 had the highest (2.63±0.02%) ash value and the least value (2.32±0.02%) was seen in T_3.

There was significant ($P<0.05$) difference in the fibre content between two samples. The highest values were observed in T_0 and T_3, both having (0.070±0.002%) while the lowest values (0.060±0.002%) were recorded in T_1 and T_2.

There was significant ($P<0.05$) difference in the carbohydrate content between the four samples. T_3 had the highest (0.96±0.02%) carbohydrate value and the least value (0.55±0.02%) was seen in T_1.

There was significant ($P<0.05$) difference in the mean values across the different proximate composition parameters such as (moisture, protein, fat/oil, ash, fibre, carbohydrate). Moisture content had the highest value (74.64) and the least value (0.065) was seen in fibre.

Table 4: Proximate Composition

Treatments	Moisture	Protein	Fat/Oil	Ash	Fibre	Carbohydrate
%						
T_0	73.10±0.05a	17.83±0.03a	5.74±0.03a	2.52±0.02a	0.070±0.002a	0.76±0.04a
T_1	71.42±0.08b	18.99±0.04b	6.36±0.04b	2.63±0.02b	0.060±0.002b	0.55±0.02b
T_2	77.16±0.06c	15.19±0.05c	4.35±0.04c	2.34±0.02c	0.060±0.002b	0.90±0.03c
T_3	77.86±0.06d	15.05±0.04d	3.74±0.04d	2.32±0.02d	0.070±0.002a	0.96±0.02d
Mean	**74.64**	**16.77**	**5.05**	**2.45**	**0.065**	**0.79**

Mean values with the same superscript letter within a column are not significantly different ($p > 0.05$) in One-Way ANOVA using Tukey's Multiple Range Test.

4.2 Mineral composition of *Oreochromis niloticus* (Nile tilapia)

This table shows the concentrations in mg/g of Calcium (Ca), Magnesium (Mg), Phosphorus (P), Sodium (Na), Potassium (K), Iron (Fe), and Zinc (Zn) in the fish under different *Lemna minor* treatments.

There was significant ($P< 0.05$) difference in calcium content between the four samples. T_3 had the highest (3.2065±0.0053 mg/g) calcium value and the least value (0.9619±0.0033 mg/g) was seen in T_2.

There was significant ($P<0.05$) difference in Magnesium content between the four samples. T_3 had the highest (1.1670±0.0036 mg/g) Magnesium value and the least value (0.5835±0.0027 mg/g) was seen in T_2.

There was significant ($P<0.05$) difference in phosphorus content between the four samples. T_3 had the highest (9.8102±0.0053 mg/g) phosphorus value and the least value (4.8614±0.0043 mg/g) was seen in T_0 (control).

There was significant ($P<0.05$) difference in sodium content between the four samples. T_3 had the highest (0.8926±0.0047 mg/g) sodium value and the least value (0.5936±0.0035 mg/g) was seen in T_2.

There was significant ($P<0.05$) difference in Potassium content between the four samples. T_3 had the highest (3.1050±0.0055 mg/g) potassium value and the least value (1.9404±0.0045 mg/g) was seen in T_2.

There was significant ($P<0.05$) difference in Iron content between the four samples. T_3 had the highest (0.0338±0.00008 mg/g) Iron value and the least value (0.0179±0.00007 mg/g) was seen in T_0.

There was significant ($P<0.05$) difference in Zinc content between the four samples. T_3 had the highest (0.0232±0.00004 mg/g) zinc value and the least value (0.0162±0.00004 mg/g) was seen in T_0.

There was significant ($P<0.05$) difference in the mean values across the mineral composition such as (calcium, magnesium, phosphorus, sodium, potassium, iron , zinc) Phosphorus had the highest value (6.7008) and the least value (0.0202) was seen in Zinc.

Table 5: Mineral Composition

Treatments	Calcium	Magnesium	Phosphorus	Sodium	Potassium	Iron	Zinc
				mg/g			
T_0	2.5647±0.0039a	0.9725±0.0025a	4.8614±0.0043a	0.6329±0.0026a	2.3643±0.0041a	0.0179±0.00007a	0.0162±0.00004a
T_1	2.2445±0.0043b	0.7780±0.0026b	6.4818±0.0043b	0.6119±0.0034b	2.0986±0.0044b	0.0201±0.00006b	0.0184±0.00004b
T_2	0.9619±0.0033c	0.5835±0.0027c	5.6496±0.0036c	0.5936±0.0035c	1.9404±0.0045c	0.0254±0.00006c	0.0231±0.00004c
T_3	3.2065±0.0053d	1.1670±0.0036d	9.8102±0.0053d	0.8926±0.0047d	3.1050±0.0055d	0.0338±0.00008d	0.0232±0.00004d
Mean	**2.2444**	**0.8753**	**6.7008**	**0.6828**	**2.3771**	**0.0243**	**0.0202**

Mean values with the same superscript letter within a column are not significantly different ($p > 0.05$) in One-Way ANOVA using Tukey's Multiple Range Test.

CHAPTER FIVE

5.0 DISCUSSION

This chapter discusses the findings obtained from the proximate and mineral composition analyses of *Oreochromis niloticus* cultured under different concentrations of *Lemna minor*. The variations observed among the treatments are explained in relation to the influence of *Lemna minor* on the water quality and the subsequent effect on the nutritional composition of the fish. The results are also compared with findings from previous studies.

The proximate composition of *Oreochromis niloticus* cultured in water treated with *Lemna minor* showed significant variations in moisture, protein, fat, fibre, carbohydrate, and ash contents across treatments. These variations reflect the impact of *Lemna minor* on the nutritional quality of the fish flesh, as proximate composition is strongly influenced by environmental and dietary factors (Pérez-Martín, 2023; Paul and Southgate, 2023).

The mineral composition of *O. niloticus* flesh in this study showed significant variations among the analyzed macro and micro elements (Ca, Mg, P, Na, K, Fe, and Zn). These variations reflect the influence of *Lemna minor* in enhancing mineral bioavailability in culture water.

5.1 Proximate composition

5.1.1 Moisture content

The moisture content obtained in this study fell within the typical range reported by Ali *et al*., (2024), who observed moisture levels around 79.40% in *O. niloticus*. The highest moisture value was recorded in T_3, while the lowest occurred in T_1. The lower moisture content in T_1 suggests better dry matter deposition and muscle firmness compared to other treatments. According to

Navarro (2022), lower moisture in fish fillet is often associated with improved texture and increased nutrient density. Conversely, the higher moisture observed in T_3 may reflect elevated water retention due to reduced protein synthesis and fat accumulation, as also noted by Ali *et al*., (2024), who reported a similar trend in *O. niloticus* exposed to nutrient rich environments.

Moisture variations among treatments could be attributed to differences in metabolism and osmoregulation induced by *Lemna minor*. Pérez-Martín (2023) explained that aquatic plants can alter dissolved oxygen and ionic concentration in culture water, which may affect tissue hydration in fish.

5.1.2 Crude protein

The protein content was significantly affected by *Lemna minor* treatment. T_1 had the highest crude protein while the lowest occurred in T_3. The significant increase in protein content in T_1 indicates that a moderate concentration of *Lemna minor* enhanced nitrogen recycling and amino acid metabolism, leading to improved muscle protein synthesis.

This finding agrees with Cruz and Tsang (2023), who reported that nutrient enriched systems with balanced organic inputs promote protein deposition in fish flesh. The slight decline in protein content in T_2 and T_3 might result from excessive organic load and competition for dissolved oxygen, leading to reduced feed efficiency, as observed by Navarro (2022).

Overall, the protein trend in this study confirms that moderate *Lemna minor* exposure supports protein biosynthesis, while excessive concentration may reduce metabolic efficiency and growth performance. The present study recorded higher protein percentages than those reported by Paul and Southgate (2023), who indicated that most freshwater fish contain between 16–20% protein. The increased protein level may be attributed to the high nutrient recycling potential of *Lemna* *minor*, which releases nitrogenous compounds and trace minerals that promote protein synthesis in fish. This agrees with the findings of Cruz and Tsang (2023), who reported that nutrient enriched culture environments enhance amino acid metabolism and muscle protein formation.

5.1.3 Fat

The fat content in *O. niloticus* cultured with *Lemna minor* also varied significantly. The lipid levels were comparable to the 1.54% reported by Ali *et al*., (2024), and consistent with the range of white fish species described by Intermediate Technology Publications (2023), who stated that white fish typically have less than 2% fat. In this study, T_1 had the highest value while the lowest value occurred in T_3. The results showed that fat accumulation was optimal at moderate *Lemna minor* concentration but declined as exposure increased. This could be due to better feed utilization efficiency and lipid metabolism under optimal nutrient recycling conditions (T_1), while higher *Lemna minor* levels (T_3) possibly reduced oxygen and metabolic energy available for lipid synthesis.

These findings are in agreement with Navarro (2022), who reported that aquatic macrophytes such as *Lemna minor* improve lipid metabolism by supplying essential fatty acids in moderate quantities. Ali *et al*., (2024) also recorded similar lipid values (1.54–6.50%) in *O. niloticus*, suggesting that the observed range is within the normal limit for lean fish species. Furthermore, Pérez- Martín (2023) stated that lipid variation in cultured fish is strongly influenced by dietary quality and water nutrient balance, consistent with the present results.

5.1.4 Crude fibre

The highest Crude fibre was recorded in T_0 and T_3 while the lowest values occurred in T_1 and T_2 . The low values across treatments align with expectations since fibre is generally minimal in fish flesh. The slightly higher fibre in T_3 and control (T_0) could be due to ingestion of suspended plant residues or microbial polysaccharides in the water. Kumar *et al*., (2020) noted that fish cultured in plant based systems exhibit increased indigestible residue content due to water feed interactions. The lower fibre content in T_1 and T_2 suggests better digestive efficiency and nutrient assimilation, which correlates with Pérez- Martín (2023), who found that balanced aquatic vegetation supports healthy gut metabolism in cultured fish.

The fibre levels recorded in the present study were higher than those of wild *O. niloticus* reported by Paul and Southgate (2023), suggesting that the culture water enriched with *Lemna minor* increased the availability of plant derived polysaccharides that may have been assimilated into the fish diet. Crude fibre is essential for proper digestion and intestinal function, and while it is not a major nutrient in fish muscle, its slight presence indicates a balanced metabolic process. Similar observations were reported by Pérez- Martín (2023), who found that dietary fibre from aquatic vegetation can improve gut health and feed utilization efficiency in cultured tilapia.

5.1.5 Carbohydrate (Nitrogen free- extract)

Carbohydrate content in this study also showed a notable trend. The highest carbohydrate was recorded in T_3 while the lowest value was recorded in T_1. This may be due to the assimilation of soluble carbohydrates from decomposed duckweed residues in the culture water. The result supports the observation of Martínez *et al*., (1998), who reported that fish reared in nutrient enriched environments tend to accumulate more glycogen in their tissues. Cruz and Tsang (2023) also stated that carbohydrate levels in fish are affected by feeding regime and environmental nutrient recycling. The higher carbohydrate levels in T_3 might reflect a compensatory response to reduced protein and lipid metabolism, as fish convert more energy into glycogen storage under mild stress or oxygen limitation.

5.1.6 Ash

Ash content represents total mineral matter. The highest ash value was observed in T_1, while the lowest occurred in T_3. This suggests that moderate *Lemna minor* exposure improved mineral accumulation in the fish muscle, likely due to enhanced bioavailability of essential elements in the culture water.

Similar results were reported by Ali *et al*., (2024), who observed increased ash levels in tilapia cultured in nutrient rich water. Martínez *et al*., (1998) also noted that higher mineral content in aquaculture systems enhances ash deposition in fish tissues. The slight decline in ash content in T_3 may be attributed to excessive organic matter, which can reduce mineral uptake efficiency, as stated by Russell *et al*., (2023).

Overall, the proximate composition of *O. niloticus* cultured with *Lemna minor* showed improved nutritional quality, characterized by higher protein, carbohydrate, and mineral contents with moderate fat and moisture levels. This suggests that the integration of *Lemna minor* in aquaculture systems enhances nutrient retention and muscle composition in fish.

5.2 Mineral Composition

5.2.1 Calcium

Calcium concentration was highest in T_3 and lowest in T_2. The increased calcium content in T_3 indicates that a higher concentration of *Lemna minor* enhanced calcium bioavailability and absorption in the fish. *Lemna minor* is known to absorb and release essential minerals such as calcium into the surrounding water, enriching the culture medium and facilitating uptake by fish (Russell *et al*., 2023). Conversely, the lowest calcium content in T_2 could be attributed to competition among ions in the water or sub optimal mineral balance caused by intermediate levels of *Lemna minor*. Martínez *et al*., (1998) and Navarro (2022) reported that calcium deposition in fish tissues depends strongly on environmental mineral concentration and water hardness. The elevated calcium in T_3 therefore suggests improved osmoregulation and bone mineralization due to *Lemna minor* enrichment.

5.2.2 Phosphorus

Phosphorus concentration was highest in T_3 and lowest in T_0. The highest level recorded in the treatment was due to the greatest *Lemna minor* exposure. This trend indicates that *Lemna minor* significantly enhanced phosphorus accumulation in the fish. The plant's high capacity for nutrient absorption and recycling increases phosphate concentration in the water, leading to greater phosphorus uptake by the fish. According to Cruz and Tsang (2023) and Russell *et al*., (2023), phosphorus is essential for skeletal development, energy metabolism, and nucleic acid synthesis. The elevated phosphorus level observed in T_3 supports Martínez *et al*., (1998), who found that fish reared in nutrient rich environments tend to accumulate more phosphorus in their tissues. The Ca/P ratio obtained in this study remained within the ideal physiological range for tilapia growth (1.0–1.5), as also noted by Aranda and Llopis (2023).

5.2.3 Magnesium

Magnesium concentration was highest in T_3 and lowest in T_2. The increased magnesium concentration in T_3 indicates that *Lemna minor* effectively improved magnesium availability in the water. Magnesium is vital for enzymatic activity, muscle function, and protein synthesis (Paul and Southgate, 2023). Navarro (2022) emphasized that magnesium distribution in fish depends largely on water chemistry and diet. The moderate levels observed in T_0 and T_1 may represent balanced absorption, whereas the higher levels in T_3 reflect improved ionic equilibrium and nutrient recycling facilitated by *Lemna minor*. Similar findings were reported by Pérez-Martín (2023), who observed that fish cultured in enriched aquatic systems show elevated magnesium content due to increased mineral uptake efficiency.

5.2.4 Sodium

Sodium concentrations was highest in T_3 and lowest in T_2. The higher sodium level in T_3 could be attributed to the improved ionic balance of the water caused by *Lemna minor*. Sodium plays a critical role in maintaining osmotic pressure, nerve impulse transmission, and acid-base balance in aquatic organisms (Navarro, 2022).

The lower sodium values in T_2 and T_1 might result from competitive ion exchange processes or fluctuations in water salinity. Cruz and Tsang (2023) and Paul and Southgate (2023) highlighted that sodium accumulation in fish is strongly influenced by both dietary input and environmental concentration. Thus, the increased sodium observed in T_3 confirms the positive role of *Lemna minor* in mineral enrichment of aquaculture water.

5.2.5 Potassium

Potassium content was highest in T_3 and lowest in T_2. The elevated potassium in T_3 reflects better mineral cycling and nutrient release from *Lemna minor* into the culture water. Potassium is essential for maintaining cell membrane potential, regulating enzyme activity, and supporting muscle contraction (Paul and Southgate, 2023). According to Navarro (2022), potassium concentration in fish tissues depends on the ionic composition of the culture water. The higher potassium levels in T_3 indicate that *Lemna minor* enhanced the ionic environment, promoting efficient potassium absorption. Pérez-Martín (2023) also reported that aquatic macrophytes improve water mineral content, which subsequently enhances tissue mineralization in fish.

5.2.6 Iron

Iron concentration was highest in T_3 and lowest in T_0, with the highest level found in fish reared under the highest *Lemna minor* concentration. The significant increase in Fe with *Lemna minor* exposure suggests that the plant enhanced the bioavailability of iron through nutrient recycling and water enrichment.

Iron is vital for haemoglobin formation, oxygen transport, and enzyme activation (Prassed, 2022). The result agrees with the findings of Carpenter *et al*., (2023), who reported that nutrient enriched culture systems increase Fe uptake in fish tissues. Navarro (2022) also noted that environmental enrichment influences trace mineral deposition, as observed in this study. The gradual rise in Fe concentration from T_0 to T_3 demonstrates the positive contribution of *Lemna minor* to trace mineral availability.

5.2.7 Zinc

Zinc concentration was highest in T_3 and lowest in T_0 with the highest recorded in the treatment containing the greatest concentration of *Lemna minor*. Zinc plays a key role in enzyme activation, immune defense, and growth regulation (Paul and Southgate, 2023). The higher Zn concentration in T_3 suggests that *Lemna minor* promoted zinc bioavailability and absorption by enriching the water medium.

Prassed (2022) and Carpenter *et al*., (2023) reported similar findings, noting that fish cultured in mineral enriched environments accumulate more Zn in their tissues. Navarro (2022) also explained that dietary and ecological factors influence zinc metabolism in aquatic organisms. The progressive increase in Zn across treatments therefore supports the enhancing effect of *Lemna minor* in water quality and mineral retention.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1. CONCLUSION

This study revealed that the incorporation of *Lemna minor* (duckweed) in the culture water of *Oreochromis niloticus* significantly influenced both the proximate and mineral composition of the fish flesh. The use of *Lemna minor* created an ecologically balanced environment that promoted nutrient recycling, improved water quality, and enhanced the overall nutritional value of the cultured fish.

The proximate composition results revealed notable variations among treatments. Fish reared in water containing *Lemna minor* exhibited higher levels of moisture, carbohydrate, ash, and crude fibre, with a slight reduction in crude protein and lipid content.

In mineral composition, *Lemna minor* markedly improved the concentrations of both macro (Ca, P, Mg, Na, K) and micro minerals (Fe, Zn,) in the fish flesh. This was revealed greatly in T_3 across all the minerals.

The findings revealed that *Lemna minor* serves as a natural bioenhancer in aquaculture systems. Its integration not only improved the proximate composition (notably carbohydrate, fibre, and ash) but also enriched essential macro and micro minerals, in *O. niloticus*. These improvements translate into better meat quality, higher nutritional value, and potential health benefits for consumers, as fish from duckweed integrated systems are richer in essential nutrients.

Therefore, the use of *Lemna minor* in aquaculture presents a sustainable, cost effective, and eco-friendly approach to enhancing fish growth and flesh quality.

6.2 RECOMMENDATION

1. The inclusion of *Lemna minor* (duckweed) in culture water should be encouraged among fish farmers, as it has proven to enhance the proximate and mineral composition of *O. niloticus*. Its ability to improve protein, carbohydrate, and mineral contents particularly calcium, phosphorus, potassium, and sodium demonstrates its potential as a natural and sustainable feed supplement.

2. *Lemna minor* is a fast growing, nutrient rich, and capable of utilizing waste nutrients from culture water. Farmers should incorporate it as part of an eco friendly management system. This approach not only improves water quality but also reduces feed cost and environmental pollution.

3. Future studies should focus on determining the optimal proportion and duration of *Lemna minor* supplementation that yields the best growth performance and flesh quality. Investigations into its effect on fatty acid profiles, amino acid composition, and sensory attributes (texture and taste) would also provide valuable insights.

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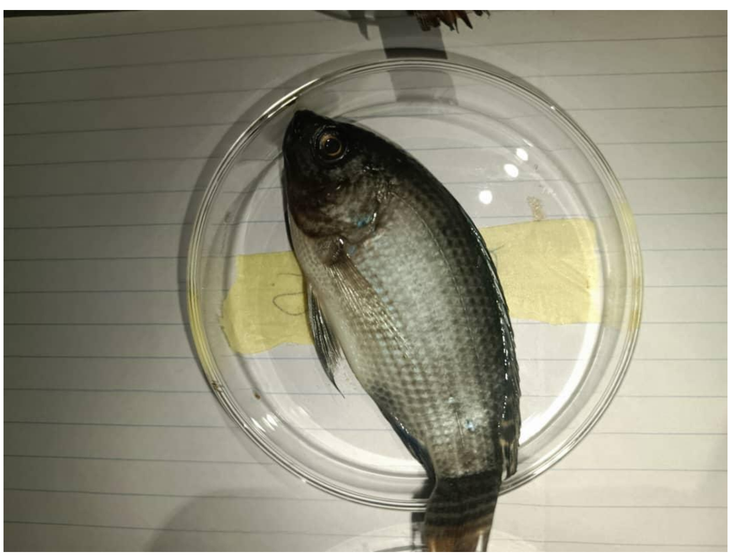
APPENDIX I

Sample of fish from treatment 1

Sample of fish from treatment 2



Sample of fish from treatment 3

Sample of fish from treatment 4

Analyzing *Oreochromis niloticus* for moisture content